Evaluating the efficacy of a GP led pre-diabetes intervention targeting life-style modification

Nicholas Gregory

Thesis submitted in partial fulfilment of the requirements of Aberystwyth University for the degree of Doctor of Philosophy

2019

Candidate's Surname/Family Name	Gregory
Candidate's Forenames (in full)	Nicholas James
Candidate for the Degree of	PhD
Academic year the work submitted for examination	2019-2020

Summary:

The work detailed in this thesis is concerned primarily with preventing the development of Type 2 Diabetes in a population of patients in North Ceredigion considered at increased risk of developing the condition. In collaboration with the Hywel Dda University Health board, this research examined the efficacy of GP led pre-diabetes intervention targeting life-style modification, firstly by means of a service evaluation and secondly by a randomised control trial. This is the first known diabetes prevention service of its kind to be delivered and evaluated in Wales.

Dietary and physical activity behaviours strongly influence the risk of Type 2 Diabetes and therefore work was also undertaken to develop more effective ways of monitoring such lifestyle behaviours in at risk groups, given self-report questionnaires relating to diet and physical activity are prone to bias and reporting inaccuracies. This was achieved by developing a low burden protocol for monitoring diet using urine metabolomics technology. Research has shown metabolites present in urine can be reflective of dietary intake and therefore this methodology was tested in patients at risk of Type 2 diabetes to assess any dietary change following lifestyle advice. Levels of physical activity were also closely monitored using activity trackers in the randomised control trial.

Mandatory Layout of Declaration/Statements

Word Count of thesis: DECLARATION	61634
This work has not previously been accepted in substance for any degree and is not being concurr submitted in candidature for any degree.	
Candidate name	Nicholas James Gregory
Signature:	
Date	

STATEMENT 1

This thesis is the result of my own investigations, except where otherwise stated. Where ***correction services** have been used, the extent and nature of the correction is clearly marked in a footnote(s).

Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

Signature:	
Date	

[*this refers to the extent to which the text has been corrected by others]

STATEMENT 2

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

Signature:	
Date	

Dedication

For Beth, Mum and Dad

Acknowledgements

The production of this thesis would not have been possible without the unwavering support of numerous people, of which there are too many to thank personally here. However, some require special recognition.

To my supervisors, Dr Rhys Thatcher and Dr Simon Payne, thank you for your advice and guidance throughout this process. Thanks also to Dr Thomas Wilson who has provided expert assistance regarding the metabolomics procedures and to all the staff in the Carwyn James building for their input, opinions and thought-provoking lunch time conversations.

Thanks to the Hywel Dda University Health board and the cluster members who have helped to facilitate key aspects of this work.

Thanks to my fellow postgraduates with whom which I've shared offices and laboratories.

Thank you to my family but most especially my Mum and Dad. You have given me everything I've needed to make this dream become a reality, I can't thank you enough.

To my grandparents, who while no longer around, continue to be a source of inspiration.

To Beth, you have been there every step of the way. I simply would not have achieved this without your love, care and support. I will repay you in every way I can and can't wait to spend the rest of our lives together.

Table of Contents

Chapter One

Literature Review: Type 2 Diabetes and Pre-diabetes

1.1. Introduction 1

1.2. Type 2 Diabetes 1

1.3. Pre-diabetes 6

1.4. Combating T2D 9

1.5. Experimental Studies 11

1.6. Health Care Settings 21

1.7. Summary 32

1.8. Thesis Aims and Objectives 35

Chapter Two

The efficacy of a GP-led pre-diabetes intervention targeting lifestyle modification

2.1. Introduction 36

2.2. Method 39

2.2.1. Participants and Ethical Approval 39

2.2.2. Protocol 40

2.2.3. Data Extraction 43

2.2.4. Statistical Analysis 44

2.3. Results 46

2.4. Baseline to 12-month follow up 47

2.4.1. Subjects 47

2.4.2. Baseline to 12-Month HbA_{1c} 47

2.4.3. Baseline to 12-Month Body Mass & BMI 51

2.4.4. Baseline to 12-Month Waist Circumference 52

2.4.5. Baseline to 12-Month Blood Pressure 53

2.4.6. Baseline to 12-Month Correlation Analysis 55

2.4.7. Baseline, 12- and 24-Month Follow Up 57

2.4.8. Baseline, 12- and 24-Month HbA_{1c} 57

2.4.9. Baseline, 12- and 24-Month Body Mass & BMI 62

2.4.10. Baseline, 12- and 24-Month Waist Circumference 63

2.4.11. Baseline, 12- and 24-Month Blood Pressure 64

2.4.12. Baseline and 24 Month Correlation Analysis 65

2.5. Discussion 68

Chapter Three

Literature Review: Dietary Monitoring Methods

3.1. Dietary Monitoring Methods 77

3.2. Limitations of Established Dietary Recording Methods 78

3.2.1. Weighed and Estimated Food Records 78

3.2.2. Twenty-four Hour and Multiple Pass Recall 82

3.2.3. Food Frequency Questionnaire (FFQ) 88

3.2.4. Summary of Established Dietary Recording Methods 90

3.3. Dietary Monitoring Using Urinary Biomarkers 91

3.3.1. Biomarkers for Fruit and Vegetables 93

3.3.2. Biomarkers for Meat and Fish 99

3.3.3. Biomarkers of Wholegrain and Soy 102

3.3.4. Biomarkers for Caffeine, Coffee and Tea 102

3.3.5. Biomarkers of Cocoa, Sweeteners and Sugar 103

3.3.6. Biomarkers for Smoking and Alcohol 104

3.3.7. Dietary Patterns 105

3.4. Summary 107

Chapter Four

General Methods

4.1. Studies 111

4.2. Ethical Approval 111

4.3. Participants and Recruitment 112

4.4. Anthropometry Measures 112

4.5. Blood Collection 112

4.6. HbA_{1c} Analysis 113

4.7. Urine Collection Procedures 114

4.8. Urine sample preparation, extraction and adjustment 115

4.9. Flow infusion electrospray ionisation-high resolution mass spectrometry (FIE-HRMS), multivariate modelling, classification and feature selection 116

4.10. Quantification of known dietary biomarkers 118

4.11. Statistical Analysis 122

Chapter Five

A protocol for accurate monitoring of habitual diet in a free-living environment using multiple spot urine samples

5.1. Introduction 123

5.2. Method 125

5.2.1. Participants 125

5.2.2. Experimental Protocol 125

5.2.3. Sample Preparation and Multinomial Modelling 126

5.3. Results 128

5.3.1. Compliance 128

5.3.2. Sample Collection Times 132

5.3.3. Variability of FMV and BT samples 133

5.3.4. Comparisons between spot, weekly and global pool samples 136

5.3.5 Protocol Feedback 139

5.4. Discussion 143

Chapter Six

The effects of acute moderate exercise on the urinary metabolome

6.1. Introduction 149

6.2. Method 155

6.2.1. Participants 155

6.2.2. Experimental protocol 155

6.2.3. Maximal oxygen uptake test 156

6.2.4. Experimental trials 157

6.2.5. Urine preparation and data analysis 158

6.3. Results 159

6.3.1. Heart rate and RPE 159

6.3.2. Urine Metabolomics 160

6.4. Discussion 164

Chapter Seven

Comparison of the Alere Afinion AS100 Point of Care Analyser and HPLC in the Determination of HbA_{1c}

7.1. Introduction 170

7.2. Method 172

7.2.1. Participants 172

7.2.2. Experimental Protocol 172

7.2.3. Statistical Analysis 173

7.3. Results 174

7.4. Discussion 181

Chapter Eight

The feasibility of conducting a randomised control trial to evaluate the effectiveness of a focussed 15-minute one-to-one consultation to improve blood glucose control in pre-diabetes

8.1. Introduction 184

8.2. Method 186

8.2.1. Participants and Recruitment 186

8.2.2. Experimental Protocol 187

8.2.3. Randomisation 191

8.2.4. Dietary Monitoring by Urine Sampling 191

8.2.5. Physical Activity Monitoring by ActiGraph 192

8.2.6. Blood Treatment 192

8.2.7. Plasma Extraction 193

8.2.8. Plasma Analysis 193

8.2.9. Urine Treatment 193

8.2.10. Physical Activity Analysis 194

8.2.11. Statistical Analysis 195

8.3. Results 196

8.3.1. Participants 196

8.3.2. Personal Diabetes Questionnaire 197

8.3.3. HbA_{1c} 197

8.3.4. Biochemical and Anthropometric Outcomes 201

8.3.5. Baseline to Six Months Correlation Analysis 204

8.3.6. Urine Dietary Biomarkers 214

8.3.7. FIE-MS Urine 216

8.3.8. FIE-HRMS Plasma 218

8.3.9. Food Frequency Questionnaire 220

8.3.10. Physical Activity Outcomes from ActiGraph 222

8.3.11. IPAQ-SF Questionnaire 225

8.3.12. SF36 Questionnaire 227

8.3.13 Feedback Questionnaire 231

8.4. Discussion 232

Chapter Nine

9.1. General Discussion and Future Directions 246

9.2. Realisation of Aims 246

9.3. Future Directions 254

9.4. Conclusion 255

References & Appendices 257

List of Tables

Table 2.1. Summary of practice populations as of 1st April 2016 and the number of patients invited to engage in the programme, 4.5% of patient list 39

Table 2.2A. HbA_{1c}, Body Mass, BMI and Waist Circumference at baseline and 12 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting 48

Table 2.2B. Systolic BP and Diastolic BP at baseline and 12 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting 49

Table 2.3. Correlation Coefficients for change in HbA_{1c} and changes in Body Mass, BMI and Waist Circumference between baseline and 12 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting 54

Table 2.4A. HbA_{1c}, Body Mass, BMI and Waist Circumference at baseline, 12 and 24 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting 59

Table 2.4B. Systolic BP and Diastolic BP at baseline, 12 and 24 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting 60

Table 2.5. Correlation Coefficients for change in HbA_{1c} and changes in Body Mass, BMI and Waist Circumference between baseline and 24 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting 66

Table 3.1. Summary table of urinary metabolites linked to dietary exposure 109

Table 4.1. Known biomarkers of dietary intake measured in research chapters 120

Table 5.1. Total compliance of sample collection across the six-week sampling period 128

Table 5.2. Compliance for AM (FMV) and PM (BT) samples across the six-week sampling period 129

Table 5.3. Compliance for weekday AM (FMV) and PM (BT) samples across the six-week sampling period 130

Table 5.4. Compliance for weekend AM (FMV) and PM (BT) samples across the six-week sampling period 131

Table 5.5. Time of sample collection (in decimal hours) for AM (FMV) and PM (BT) weekday samples across the six-week sampling period 132

Table 5.6. Time of sample collection (in decimal hours) for AM (FMV) and PM (BT) weekend samples across the six-week sampling period 133

Table 5.7. Accuracy, RF Margin and AUC values for FMV samples by week across the six-week sampling period 134

Table 5.8. Accuracy, RF Margin and AUC values for FMV samples by day across the six-week sampling period 135

Table 5.9. Accuracy, RF Margin and AUC values for BT samples by week across the six-week sampling period 135

Table 5.10. Accuracy, RF Margin and AUC values for BT samples by day across the six-week sampling period 136

Table 5.11. Correlation and paired t-test *P* values for three randomly selected spot samples compared to the corresponding weekly pool 137

Table 5.12. Correlation and paired t-test *P* values for different combinations of spot urine samples compared to the overall global urine pool 138

Table 5.13. *P* values from paired t-tests for specific dietary biomarkers from different combinations of spot urine samples compared to the overall global urine pool 138

Table 5.14. Percentage and written feedback questionnaire responses from 14 participants following the six-week sampling period 141

Table 6.1. Mean heart rate (HR; in bpm) and RPE (arbitrary values) in the exercise trial and mean heart values in the rest trial between five and 30 minutes 159

Table 6.2. RF classification margins for FMV, Pre and Post urine samples in exercise and rest trials 160

Table 6.3. Annotated metabolites differing significantly between FMV and post urine samples in rest and exercise trials 160

Table 7.1: Comparisons to be made in HbA_{1c} values as determined by HPLC and Alere Afinion AS100 POC Analyser 173

Table 7.2: HbA_{1c} values from point of care and HPLC analysis 174

Table 8.1. HbA_{1c}, Total, LDL and HDL Cholesterol and Triglycerides at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 199

Table 8.2. Body Mass, BMI, Hip and Waist Circumference, Fat and Muscle%, Systolic and Diastolic BP at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 200

Table 8.3. Correlation coefficients for change in HbA_{1c} and changes in Body Mass, BMI and Waist Circumference between baseline and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 204

Table 8.4. Effects of treatment and time on urinary biomarkers of dietary intake following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 206

Table 8.5. Dietary biomarker concentrations (mmol/L) of metabolites which exhibited significant differences between groups, changes over time or both 210

Table 8.6A. Mean fold change in the concentration of 4-Hydroxyhippuric acid, Ferulic acid-4-O-sulfate and Hippuric acid (where treatment had no effect) between baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 211

Table 8.6B. Mean fold change in the concentration of Caffeic acid and Taurine (where treatment had an effect) between baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 211

Table 8.7. Dietary biomarker concentrations (mmol/L) of metabolites which exhibited significant differences in pre-contemplation participants. 212

Table 8.8. Mean fold change in the concentration of 4-Hydroxyhippuric acid, Dehydroxybenzoic Acid, Ferulic acid, Hippuric acid and Trans-3-Hydroxycotinine in pre-contemplation participants between baseline, three and six months. 213

Table 8.9. Correlation coefficients for change in HbA_{1c} and changes in the concentration of 4-Hydroxyhippuric acid, Caffeic acid, Ferulic acid-4-O-sulfate, Hippuric acid and Taurine between baseline and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 214

Table 8.10. RF classification margins for participant's urine in intervention and control groups between baseline and three months, baseline and six months and three months and six months 216

Table 8.11. RF classification margins for plasma in intervention and control groups between baseline and three months, baseline and six months and three months and six months 217

Table 8.12. AHEI scores from FFQ responses at baseline and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 219

Table 8.13. kCal expenditure, time spent in light, moderate and vigorous exercise intensities and step counts recorded by ActiGraph at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 221

Table 8.14. Weekly walking, moderate, vigorous and total physical activity in met minutes reported in the IPAQ at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 224

Table 8.15A. Physical functioning, role limitations due to physical health, role limitations due to emotional problems and energy/fatigue (a score of 100 represents the highest level of functioning) at baseline, three and six months following a 15-minute one to one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 226

Table 8.15B. Emotional wellbeing, social functioning, pain and general health (a score of 100 represents the highest level of functioning) at baseline, three and six months following a 15 minute one-to one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 227

Table 8.16A. Study feedback responses (%) for recruitment and data collection from 19 participants 228

Table 8.16B. Study feedback responses (%) for urine collection from 19 participants 229

Table 8.16C. Study feedback responses (%) for the 15-minute consultation from 11 participants and physical activity recording from 16 participants 230

List of Figures

Figure 2.1 Eligible patients followed up after 1 and 2 years following a 30-minute one-to-one consultation with a health care professional in a primary care setting. 46

Figure 2.2. Pooled HbA_{1c} data from seven GP surgeries at baseline (M0) and 12 months (M12) following a 30-minute one-to-one consultation with a health care professional in a primary care setting 50

Figure 2.3. Patient numbers in each diabetes classification at baseline (M0) and 12 months (M12) 50

Figure 2.4A/2.4B. Pooled Body Mass (3A) and BMI (3B) data from seven GP surgeries at baseline (M0) and 12 months (M12) following a 30-minute one-to-one consultation with a health care professional in a primary care setting 51

Figure 2.5. Pooled Waist Circumference data from six GP surgeries at baseline (M0) and 12 months (M12) following a 30-minute one-to-one consultation with a health care professional in a primary care setting 52

Figure 2.6. Pooled diastolic blood pressure data from six GP surgeries at baseline (M0) and 12 months (M12) following a 30-minute one-to-one consultation with a health care professional in a primary care setting 53

Figure 2.7. Correlation of pooled body mass change and HbA_{1c} change between baseline and 12 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting 55

Figure 2.8. Correlation of pooled BMI change and HbA_{1c} change between baseline and 12 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting 56

Figure 2.9. Correlation of pooled waist circumference change and HbA_{1c} change between baseline and 12 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting 56

Figure 2.10. Pooled HbA_{1c} data from six GP surgeries at baseline (M0), 12 months (M12) and 24 months (M24) following a 30-minute one-to-one consultation with a health care professional in a primary care setting 61

Figure 2.11. Patient numbers in each diabetes classification at baseline (M0), 12 months (M12) and 24 months (M24) 61

Figure 2.12. Pooled BMI data from five GP surgeries at baseline (M0), 12 months (M12) and 24 months (M24) following a 30-minute one-to-one consultation with a health care professional in a primary care setting 63

Figure 2.13. Pooled Waist Circumference data from four GP surgeries at baseline (M0), 12 months (M12) and 24 months (M24) following a 30-minute one-to-one consultation with a health care professional in a primary care setting 64

Figure 2.14. Correlation of combined body mass change and HbA_{1c} change between baseline and 24 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting 65

Figure 2.15. Correlation of combined BMI change and HbA_{1c} change between baseline and 24 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting 67

Figure 2.16. Correlation of combined waist circumference change and HbA_{1c} change between baseline and 24 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting 67

Figure 4.1. The urine collection kit used by participants in the randomised control trial including; addressed cardboard postage box, collection straws, vacutainers and collection container 115

Figure 4.2. Example of a spectrum fingerprint output following FIE-HRMS 118

Figure 5.1. The urine collection kit used by participants including; collection straw (left), vacutainer (centre) and collection cup (right) used by participants in the home 126

Figure 6.1A/6.1B. Principle Component analysis (PC1; 34.15% of variation, PC2; 23.13% of variation, PC3; 15.8% of variation) of participant's FMV (TO), PRE (PR) and Post (PT) urine samples following exercise (E) and rest (R) 163

Figure 7.1A/7.1B: Spearman's correlation (1A) and Bland Altman limits of agreement plot (1B) for POC analysis (EDTA) vs HPLC 175

Figure 7.2A/7.2B: Spearman's correlation (2A) and Bland Altman limits of agreement plot (2B) for POC analysis (LH) vs HPLC 176

Figure 7.3A/7.3B: Spearman's correlation (3A) and Bland Altman limits of agreement plot (3B) for EDTA vs LH vacutainers using the POC analysis 178

Figure 7.4A/7.4B: Spearman's correlation (4A) and Bland Altman limits of agreement plot (4B) for LH vacutainers vs capillary sampling using POC analysis 179

Figure 7.5A/7.5B: Spearman's correlation (5A) and Bland Altman limits of agreement plot (5B) for EDTA vacutainers vs capillary sampling using POC analysis 180

Figure 8.1. Participant recruitment, randomisation, retention and analysis 196

Figure 8.2. HbA_{1c} at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 198

Figure 8.3. Total cholesterol at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 202

Figure 8.4. LDL cholesterol at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 202

Figure 8.5. Waist circumference at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 203

Figure 8.6. Hip circumference at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 203

Figure 8.7. Correlation of weight change and HbA_{1c} change between baseline and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 205

Figure 8.8. Correlation of BMI change and HbA_{1c} change between baseline and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 205

Figure 8.9. Average step counts at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 223

Figure 8.10. Walking met minutes at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON) 225

List of Abbreviations

ADA	American Diabetes Association
AHA	American Heart Association
AHEI	Alternative Healthy Eating Index
AM	Ante Meridiem
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
ATP-PCr	Adenosine Triphosphate Phosphocreatine
AUC	Area under the curve
BMI	Body Mass Index
BP	Blood Pressure
BT	Bed Time
CHW	Community Health Worker
CON	Control
CRF	Clinical Research Facility
CVD	Cardiovascular Disease
DEPLOY	Translating the Diabetes Prevention Program into the Community
DINE	Dietary Instrument for Nutrition Education
DVD	Digital Versatile Disk
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic Acid
EPIC	European Prospective Investigation into Cancer and Nutrition
ESI	Electrospray Ionization
FFA	Free Fatty Acids
FFQ	Food Frequency Questionnaire
FIE-HRMS	Flow Infusion Electrospray Ionisation High Resolution Mass Spectrometry
FIE-MS	Flow Infusion Electrospray Ionisation Mass Spectrometry
FIN-D2D	National Type 2 Diabetes Prevention Programme in Finland
FINDRISC	Evaluation of the Finnish Diabetes Risk Score
FMV	First Morning Void
FPG	Fasting Plasma Glucose
GLUT4	Glucose Transporter Type 4

GOAL	Good Ageing in Lahti Region
GP	General Practitioner
HbA_{1c}	Glycated Haemoglobin
HDL	High Density Lipoprotein
HDUHB	Hywel Dda University Health Board
HELP-PD	Healthy Living Partnerships to Prevent Diabetes
HEPA	Health Enhancing Physical Activity
HMDB	Human Metabolome Database
HPLC	High-performance liquid chromatography
HR	Heart Rate
HSE	Health Survey for England
IBERS	Institute of Biological, Environmental and Rural Sciences
ID	Identification
IFN-γ	Interferon Gamma
IGT	Impaired Glucose Tolerance
IL-β	Interleukin 1 Beta
INT	Intervention
IPAQ-SF	International Physical Activity Questionnaire: Short Form
IRAS	Integrated Research Approval System
KPCO	Kaiser Permanente of Colorado
LDL	Low Density Lipoprotein
LH	Lithium Heparin
MD	Mean Difference
MET	Metabolic Equivalent of Task
MS	Mass Spectrometry
NERS	National Exercise Referral Scheme
NHS	National Health Service
NHS DPP	National Health Service Diabetes Prevention Programme
NICE	National Institute for Health and Care Excellence
NMR	Nuclear Magnetic Resonance
OGTT	Oral Glucose Tolerance Test
PCA	Principle Component Analysis
PM	Post Meridiem

PO	Power Output
POC	Point of Care
PODOSA	Prevention of Diabetes and Obesity in South Asians
PREDIAS	Prevention of Diabetes Self-Management Program
PREPARE	The Pre-diabetes Risk Education and Physical Activity Recommendation and Encouragement
PUFA	Polyunsaturated Fatty Acids
RAND	Research and Development
RCT	Randomised Control Trial
REC	Research Ethics Committee
RF	Random Forest
ROC	Receiver Operating Characteristic
RPE	Rating of Perceived Exertion
RPM	Rotations per Minute
SD	Standard Deviation
SFA	Saturated Fatty Acids
SLIM	Study on Lifestyle-Intervention and Impaired Glucose Tolerance Maastricht
SPSS	Statistical Package for the Social Sciences
T2D	Type 2 Diabetes
TMAO	Trimethylamine N-oxide
TNF-α	Tumour Necrosis Factor Alpha
TSQ	Triple Quadrupole Mass Spectrometer
UHPLC	Ultra-High-Performance Liquid Chromatography
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
US-DPP	United States Diabetes Prevention Programme
WARU	Wellbeing and Health Assessment Research Unit
WHO	World Health Organisation
YMCA	Young Men's Christian Association

Abstract

This thesis examined the efficacy of a primary care intervention in reducing progression to Type 2 Diabetes (T2D) in patients identified as 'at risk', defined as having a Glycated Haemoglobin (HbA_{1c}) of between 42 and 47 mmol/mol. T2D is a metabolic disorder, which is characterised by high blood glucose, and associated with numerous health complications which are placing an unsustainable financial burden on the NHS.

Study 1, a service evaluation of a GP-led intervention targeting life-style modification, indicated that a 30-minute one-to-one consultation reduced progression to T2D. Five hundred and ninety two pre-diabetic participants received the consultation, 12 months later 17 had progressed to T2D, 217 had reverted to normal blood glucose control while 358 remained in the pre-diabetes range. At 24 months post consultation, data were available on 292 participants, 19 of whom had developed T2D, 119 had reverted to normal glucose control while 154 remained pre-diabetic. While the result of the service evaluation showed great promise, the process identified several challenges which needed to be addressed before an appropriately powered randomised control trial (RCT) could be effectively conducted.

Dietary and physical activity behaviours strongly influence the risk of T2D. Given self-report questionnaires relating to these lifestyle behaviours are prone to bias and reporting inaccuracies, work was undertaken to develop more effective ways of monitoring these behaviours. Research has shown metabolites present in urine can reflect dietary intake, as such Study 2 was undertaken to establish a robust and acceptable protocol for urine collection to monitor dietary behaviour. As physical activity has been shown to influence the urine metabolome at some exercise intensities, Study 3 was undertaken to assess the effects of moderate intensity exercise on the urine metabolome. Glycated Haemoglobin is increasingly quantified by point of care analysers, as such an analyser was to be used to track HbA_{1c} changes during the final study in the thesis, comparisons with high powered liquid chromatography across a range of HbA_{1c} values was conducted during Study 4.

This thesis culminates in Study 5 which examined the feasibility of conducting a RCT to evaluate the effectiveness of a one-to-one consultation delivered through primary care compared to standard care. The RCT revealed the GP-led one-to-one lifestyle consultation to have no additional benefit on measures related to T2D compared to control, with HbA_{1c} concentrations decreasing after six months in both treatments. The urine sampling procedure was effectively implemented in the RCT and successfully quantified 79 metabolites linked to foods and drinks, many of high health importance. Dietary behaviour was unaffected by treatment allocation. Level of physical activity, monitored in part by an electronic activity tracker, was also unaffected by treatment allocation. However, it was demonstrated that the use of such technologies was acceptable and feasible in monitoring healthy behaviours in individuals identified as at risk of developing T2D.

Chapter One:

Literature Review: Type 2 Diabetes and Pre-diabetes

1.1. Introduction

The UK National Health Service (NHS) identifies the current top five diseases responsible for premature death in individuals under the age of 75 years as cardiovascular disease, stroke, cancer, lung and liver disease. Future projections however suggest diabetes will enter the top five leading causes of death by 2030 (Tabak, Herder, Rathmann, Brunner and Kivimäki, 2012). Type II diabetes (T2D) is often a precursor to cardiovascular disease and stroke, as well as other health complications. It is estimated that approximately four million people in the UK are currently suffering with diabetes, 90% of whom have T2D, with Wales having the highest prevalence of the disease among the four UK nations, 7.4% (Diabetes UK). With a new diagnosis of T2D every two minutes, the financial burden placed on the NHS by the condition is becoming unsustainable. Of concern is a statistic reported by the NHS in 2014 stating one in three UK adults were classified as having pre-diabetes, a condition associated with increased risk of developing T2D. Crucially however, development of T2D, and associated diseases, can be prevented with lifestyle modification, including increasing physical activity and improving dietary habits (UK Department of Health, 2014).

1.2. Type 2 Diabetes

T2D is defined as a metabolic disorder of multiple aetiology, characterised by chronic hyperglycaemia with disturbances in carbohydrate, fat and protein metabolism (Alberti and Zimmet, 1998). During development of the condition the homeostasis of fasting and postprandial glucose concentration becomes abnormal, resulting in a chronic hyperglycaemic

state. T2D is associated with numerous complications including cardiovascular disease, stroke, hypertension, nephropathy, retinopathy and neuropathy (Grundy *et al*., 1999; Shlienger, 2013). The causes of T2D are multifaceted and are a result of both genetic and environmental factors (Das and Elbein, 2006) that cause decreased insulin secretion, because of impaired beta cell function, decreased insulin sensitivity, and increased insulin resistance.

Insulin, alongside glucagon, is a major hormone responsible for the maintenance of glucose homeostasis. Insulin is secreted by the beta cells of the pancreas in response to increased levels of circulating glucose following a meal. As well as regulating blood glucose, insulin also influences lipid metabolism by increasing lipid synthesis in fat cells and decreasing the release of fatty acids from adipose tissue. Insulin regulates glucose homeostasis at numerous locations, reducing hepatic glucose production and increasing the rate of glucose uptake into skeletal muscle and adipose tissue (Sesti, 2006). Because of impaired insulin action, glucose transport into the liver, muscle and fat cells is compromised (Olokoba, Obateru and Olokoba, 2012) resulting in the progressive increase in plasma glucose levels which characterises T2D (Scheen, 2003; Cerf, 2013).

It is well established that poor dietary behaviours and physical inactivity increase the risk of T2D. Obesity, and more crucially, body fat distribution is implicated in the development of T2D (Montague and O'Rahilly, 2000). Obese individuals storing most of their fat in visceral adipose depots, in general, suffer greater adverse metabolic consequences compared to those who store similar amounts of fat in subcutaneous sites (Scheen, 2003). Increased visceral depots are associated with increases in the release of non-esterified fatty acids, causing reductions in insulin sensitivity. Work by Fujimoto, Abbate, Kahn, Hokanson and Brunzell (1994) found visceral fat to be strongly correlated with insulin resistance compared to centrally-

located subcutaneous fat. Visceral fat is associated with beta cell dysfunction and is clearly present in individuals with T2D (Kahn, 2003; Malin, Finnegan, Fealy, Filion, Rocco and Kirwan, 2014). Beta cells are responsible for the secretion of insulin and their adequate function is a critical requirement for the fluctuating demand for insulin availability (Cerf, 2013). Butler *et al*. (2003) found a 60% decrease in beta cell mass in individuals with T2D. The decline in beta cell function and mass is a result of several factors which contribute to eventual beta cell exhaustion and subsequently, beta cell demise (Cerf, 2013; Cernia and Dobreanu, 2013).

Long term exposure to adversely high blood glucose (glucotoxicity) has damaging effects on insulin synthesis, secretion and beta cell survival. Numerous mechanisms which impair beta cell function because of chronic hyperglycaemia have been theorised. Prentki and Nolan (2006) suggest the increased insulin secretary request imposed on beta cells inflicts a high demand on endoplasmic reticulum, resulting in cellular stress. In chronic cases of endoplasmic reticulum stress, beta cell death may occur (Eizirik, Cardozo and Cnop, 2008). The prolonged exposure to free fatty acids (FFA) is also proposed to have negative consequences for beta cell function. FFA presence may result in accumulation of toxic fatty acid metabolites within the islet cells of the pancreas, otherwise referred to as lipotoxicity (Cernia and Dobreanu, 2013). Poitout and Robertson (2008) suggest long term exposure to increased fatty acid concentrations results in decreased insulin gene expression. Furthermore, when stressed by hyperglycaemia, pancreatic islets produce an inflammatory response resulting in the increased production of cytokines. Interleukin 1 beta (IL-1β), interferon gamma (IFN-γ), tumour necrosis factor alpha (TNF-α), leptin and resistin are all cytokines that have been demonstrated to adversely affect pancreatic beta cell function (Wang, Guan and Yang, 2010). In contrast, the concentration of the cytokine adiponectin, a modulator of insulin action, is decreased in the presence of obesity

(Scheen, 2003). Low grade inflammation through obesity and high volume of adipose tissue is a primary source of cytokine presence in obese individuals in which inflammatory cytokines activate several kinases that interfere with insulin signalling (Tanti, Ceppo, Jager and Berthou, 2012). Obesity desensitizes glucose receiving organs to the actions of insulin, therefore increasing insulin resistance and exacerbating the impairment in beta cell function. Once beta cell dysfunction is present, insulin secretion becomes impaired, resulting in persistently elevated blood glucose concentrations (Cerf, 2013).

Insulin resistance is defined as a state of reduced responsiveness to normal circulating concentrations of insulin and has a major influence in the development of T2D (Savage, Peterson and Shulman, 2005). Primarily, insulin resistance in skeletal muscle manifests itself because of reductions in insulin stimulated glycogen synthesis. This is due to decreased glucose transport with multiple defects in insulin signalling responsible for impaired glucose metabolism (Sesti, 2006). Insulin's primary targets are skeletal muscle, adipose tissue and the liver. Glucose uptake in skeletal muscle under insulin-stimulated conditions accounts for 75% of glucose disposal in a postprandial state compared to adipose tissue, where less glucose is metabolised (Sesti, 2006). Upon entering the cell, glucose is phosphorylated by hexokinase where it is either stored as glycogen or oxidised to produce adenosine triphosphate (ATP). However, in insulin resistant subjects this process can be interrupted. Abnormalities present in FFA metabolism produce damaging accumulations of lipids within both the muscle and liver, subsequently impairing functionality (Sesti, 2006). Work by Randle, Garland, Hales and Newsholme (1963) and later studies by Roden *et al*. (1996) and Dresner *et al*. (1999) have all demonstrated the presence of elevated FFA concentrations to result in decreased intracellular glucose concentrations because of inhibited glucose transport. The process of glucose transport is initiated by insulin stimulating the translocation of GLUT4, the glucose transporter.

The process of GLUT4 translocation requires the release of GLUT4 from an intracellular space, transport to the plasma membrane before fusion with the plasma membrane (Sesti, 2006); this in turn permits the movement of glucose from the blood into the cell via diffusion (Huang and Czech, 2007). Work by Zierath, He, Gumà, Wahlström, Klip and Wahlberg-Henriksson (1996) found insulin-stimulated glucose transport in skeletal muscle in patients with T2D is down-regulated in the presence of hyperglycaemia. An elevation in serum insulin concentration from 54 to 588 pmol/L resulted in a 1.6-fold increase in GLUT4 in skeletal muscle plasma membranes collected from healthy control subjects. However, no significant increase was found in plasma membranes examined in T2D muscles, despite similar increases in serum insulin concentration. This finding was later confirmed by Garvey, Maianu, Zhu, Brechtel-Hook, Wallace and Barron (1998) who examined GLUT4 translocation following insulin stimulation in insulin-sensitive and insulin-resistant nondiabetic groups, as well as T2D subjects. Their work demonstrated insulin stimulation to result in alterations in the subcellular localization of GLUT4 vesicles within the muscle in insulin-resistant subjects with and without T2D. Specifically, the defect was associated with abnormal accumulations of GLUT4 within dense membrane compartments from which insulin is unable to recruit GLUT4 to the cell surface, therefore demonstrating insulin resistance in both T2D subjects and non-diabetic insulin resistant subjects (Garvey *et al*., 1998). One explanation for insulin resistance in the skeletal muscle is a defect in the insulin-signalling pathway that controls the translocation of GLUT4 or in the molecular processes that are involved in the recruitment of GLUT4 containing vesicles to the plasma membrane, their docking, and their fusion with the membrane (Sesti, 2006).

1.3. Pre-diabetes

Pre-diabetes is a metabolic condition defined as blood glucose higher than considered normal but below the value resulting in a clinical diagnosis of T2D. Pre-diabetes, alternatively referred to as non-diabetic hyperglycaemia or impaired glucose tolerance (IGT) is a rapidly growing health issue which can develop into diagnosed T2D if left untreated (Falade, 2012). Mean blood glucose concentrations are rapidly increasing in both developing and developed countries where mean fasting plasma glucose (FPG) has risen by 0.1 mmol/mol since 1980 when measured in 2.7 million adults (Tabak *et al*., 2012). Evidence from the health survey for England (HSE) demonstrated pre-diabetes prevalence in 2003 was 11.6% which had increased to 35.3% by 2011. The International Diabetes Federation estimated that by 2030, 472 million people worldwide will have pre-diabetes (Tabak *et al*., 2012). Such increases are projected for future years in the absence of concerted interventions (Mainous III, Tanner, Baker, Zayas and Harle, 2014).

Impaired fasting glucose (IFG) and IGT are both conditions which encompass pre-diabetes and may develop in isolation or in conjunction with one another. In the UK, IFG is diagnosed when fasting blood glucose concentration is higher than normal values (between 6.1 mmol/mol and 7 mmol/mol) but does not increase abnormally after consumption of a 75 g glucose challenge (below 7.8 mmol/mol). IGT is diagnosed when blood glucose concentration is higher than the normal range 2 hours after a glucose tolerance test but not high enough to diagnose T2D (between 7.8 mmol/mol and 11.1 mmol/mol; Talking Diabetes, 2012). Pre-diabetes and T2D are simply points on a scale relating to blood glucose concentration with the risk factors for both conditions being; obesity (a body mass index (BMI) > 30 kg/m^2), large waist circumference (> 31.5 inches in females and > 37 inches in men), sedentary lifestyle, high blood pressure (> 140 mmHg systolic, > 90 mmHg diastolic), family history of T2D, age and

ethnicity (Falade, 2012). The identification and diagnosis of pre-diabetes is of critical importance as an estimated 5-10% of people with pre-diabetes will manifest T2D within 12 months while a similar percentage will revert to normal blood glucose levels (Tabak *et al.*, 2012). This percentage estimate is subject to social demographics (low and middle income regions; World Health Organisation, 2016), ethnicity (Bhopal *et al*, 2014) and differing T2D definitions. In unfavourable conditions, the percentage of those who develop manifest T2D will be higher. However, early detection of increased T2D risk can result in complete reversal of the condition if appropriate and timely lifestyle changes are made.

Pre-diabetes and T2D can be diagnosed by recording the level of glycated haemoglobin (HbA_{1c}) present in the blood. HbA_{1c} develops when haemoglobin, the oxygen carrying protein within red blood cells, binds with glucose in the blood, and becomes 'glycated'. Measuring HbA_{1c} provides a more stable indicator of blood glucose concentration over time compared to other methods, such as a fasting blood glucose test or a glucose tolerance test (Falade, 2012). The measurement of HbA_{1c} requires a single non-fasted blood sample and gives a measure of average blood glucose concentration over the preceding 8-12-week period and is considered the gold standard measure in otherwise healthy individuals (Landgraf, 2006). However, while the measurement of HbA_{1c} is commonly used to monitor bloody glucose control and diagnose T2D, certain health complications and conditions can affect its reliability. Campbell, Pepper and Shipman (2019) suggest conditions which affects erythrocyte turnover will alter the age distribution of erythrocytes and subsequently affect HbA_{1c} levels. For example, any condition which shortens the life-span of the red blood cell will cause the associated HbA_{1c} value to be lower than expected for the average blood glucose level (Hardin, Grilley, Baron and Hale, 1999) while in contrast, where the lifespan of the red blood cell is increased, the associated HbA_{1c} is likely to be artificially higher (Radin, 2014). Specifically; anaemia, pregnancy and

liver, thyroid and kidney disease have all been implicated in causing alterations in HbA_{1c} concentration. Therefore, such conditions must be considered by clinicians and researchers prior to monitoring T2D risk and diagnosing T2D using $HbA_{1c.}$ Despite the limitations associated with HbA_{1c} measurement, it remains a widely used method for blood glucose monitoring in countries around the world.

While HbA_{1c} can be used to identify those at high risk of progressing to diabetes, there is uncertainty regarding the specific range of HbA_{1c} that should be used to quantify pre-diabetes. For example, the American Diabetes Association (ADA) recommends a range between 39–46 mmol/mol, while the International Expert Committee recommended 42–46 mmol/mol (Bell *et al*., 2020). However, alongside the health authorities of Australia and Canada, the UK, led by a collaborative group comprised of Public Health England & Wales, Diabetes UK and the NHS, state an HbA_{1c} level of 42–47 mmol/mol is indicative of 'high risk' for T2D (NHS Diabetes Prevention Programme: Revised Guidance, 2017). A glucose tolerance test measures blood glucose following an overnight fast greater than eight hours and another, two hours after 75 g glucose challenge. In the UK, the blood glucose concentration after a glucose challenge for pre-diabetes is 7.8-11.1 mmol/mol. A glucose tolerance test will usually be completed only to confirm a diagnosis of pre-diabetes/T2D or if results from other tests are inconclusive (Falade, 2012).

A significant amount of evidence suggests T2D can be prevented and pre-diabetes reversed with lifestyle intervention. Diabetes UK suggests that "all people diagnosed with pre-diabetes or otherwise at risk of developing T2D should be offered lifestyle interventions that account for their risk of developing T2D". Targets including increased physical activity, weight loss and improved dietary habits should be the primary aims for at risk individuals (Franz *et al*.,

2003; Falade, 2012; Jensen *et al*., 2013).

1.4. Combating T2D

The beneficial effects on T2D of increasing physical activity, improving dietary behaviours and weight loss are all well documented. Physical activity has long been encouraged in maintaining physical health. Numerous early studies have demonstrated that individuals who are regularly active have a greater sensitivity to insulin than sedentary individuals (Lohman, Liebold, Heilmann, Senger and Pohl, 1978; King, Dalsky, Staten, Clutter, Van Houten and Holloszy, 1987; Hardin *et al*., 1995) with recent summary articles supporting these findings (Sigal, Kenny, Wasseman, Castaneda-Sceppa and White, 2006; Colberg *et al*., 2010; Jelleyman *et al*., 2015). Work by Mikines, Sonne, Farrell, Tronier and Galbo (1988) found insulin stimulated glucose uptake increased during, and remained elevated for 16 hours following, exercise. Duncan, Perri, Theriaque, Hutson, Eckel and Stacpoole (2003) reported on the effect of a walking programme on overweight individuals. Insulin sensitivity improved significantly following six months of training, which involved three-seven sessions a week of 30 minutes duration, despite no changes in BMI or waist circumference. Similarly, insulin sensitivity was improved in a group of 19 overweight and obese females following a 12-week aerobic exercise programme independent of changes in body weight and body fat percentage (Nassis *et al*., 2005). Houmard, Tanner, Slentz, Duscha, McCartney and Kraus (2004) examined the effect of different exercise intensities and durations on insulin sensitivity in 154 sedentary, overweight and obese subjects over a six-month period. Groups were categorised by training intensity and duration and included; a low-volume/moderate-intensity group, a low-volume/high-intensity group and a high-volume/high-intensity group. Training volume (miles per week) was achieved by exercising 115 minutes per week (low-volume/high-intensity group) or 170 minutes per week (low-volume/moderate intensity group and high-volume/high-intensity groups). All

exercising groups achieved a significant improvement in insulin sensitivity compared to control although prescribed exercise of 170 minutes per week achieved greater improvements in insulin sensitivity compared to 115 minutes per week, regardless of intensity.

While the above studies reported improved glucose control because of physical exercise independent of weight loss, other studies have demonstrated the importance of reducing body mass in improving metabolic health. Wing, Blair, Bononi, Marcus, Watanabe and Bergman (1994) randomised 93 obese T2D patients into two different calorie restricted groups (1,674 or 4,185 kJ/day; 400 or 1,000 kcal/day) to induce weight loss whilst examining changes in insulin sensitivity. With both groups' achieving weight losses, patients with greater calorie restriction demonstrated lower fasting blood glucose values and greater insulin sensitivity, suggesting that the degree of calorie restriction and resultant weight loss influences fasting blood glucose and insulin sensitivity. The effect of weight loss on insulin sensitivity in obese individuals was also examined by Goodpaster, Kelley, Wing, Meier and Thaete (1999). Thirty-two sedentary individuals completed a four-month weight loss programme with repeated measures of insulin sensitivity. The programme resulted in significant decreases in weight, BMI, total fat mass and percentage body fat. The decreases in anthropometric data correlated with significant improvements in insulin sensitivity as well as decreases in serum leptin, triglycerides and cholesterol. Significant weight loss resulting from nine weeks of calorie restriction contributed to a 30% improvement in insulin stimulated muscle glucose uptake in insulin resistant subjects (Peterson, Dufour, Morino, Yoo, Cline and Shulman, 2012). Other work by Camps, Verhoef and Westerterp (2016) found insulin sensitivity improved significantly eight, 20 and 52 weeks from baseline following eight weeks of energy restriction and 44 weeks of weight maintenance. Insulin sensitivity was greater when weight loss was accompanied by physical activity as measured by a physical activity monitor. The results indicate that maintaining or increasing

physical activity during weight loss is more beneficial for glucose metabolism compared to weight loss alone and provides further support for encouraging combined weight loss and increased physical activity during lifestyle intervention programmes.

Abundant evidence supports the benefits of increased physical activity and weight loss in improving metabolic health in both T2D and pre-diabetes. Such behaviours are of vital importance in the prevention of T2D with studies demonstrating lifestyle intervention programmes to slow or prevent the progression of T2D and do so more effectively than pharmacological intervention, such a metformin (Diabetes Prevention Programme Research Group, 2002). Numerous studies have demonstrated the significant benefits of exercise and improved dietary behaviours in the prevention and control of T2D across different cohorts and in a variety of settings, such as experimental and health care settings.

1.5. Experimental Studies

Controlled experimental studies examining T2D prevention have provided abundant evidence for the use of lifestyle intervention in combating the disease. This evidence has since been translated and applied to clinical 'real world' practice by a variety of methods which have proved both viable and successful in many cases. In this section, studies are discussed if they included an intervention and control group with randomised participant allocation. If RCT studies were conducted within or alongside primary care, they are discussed in a separate section below.

Evidence from the Diabetes Prevention Programme (US-DPP) Research Group (2002) involved a coordinated 27 centre randomised clinical trial to determine the effects of lifestyle intervention and pharmacological therapy on the incidence of T2D in individuals with IGT.

Subjects were randomised into one of three groups: standard lifestyle advice with metformin, standard lifestyle advice with placebo and the intensive lifestyle intervention. The primary aims of the intensive intervention arm, including 1079 subjects, was to achieve an initial 7% weight loss/weight maintenance and complete at least 150 minutes of physical activity each week. The weight loss and physical activity goals were selected as feasible targets which would prevent the onset of T2D. Dietary modification initially focussed on reducing fat intake rather than overall calorie intake. After several weeks, the concept and importance of calorie balance was introduced to subjects. Subjects met regularly with a personal lifestyle coach whose primary role was to deliver lifestyle education, including information on nutrition, physical activity and lifestyle change behaviours, provide motivation and collect subject data. Additionally, subjects were instructed to self-monitor fat and calorie intake daily throughout the first 24 weeks of intervention and record their levels of physical activity. Subjects were followed on average for 2.8 years. Fifty percent of subjects in the intensive intervention group achieved the targeted 7% reduction in weight and 74% had reached 150 minutes per week of physical activity at 24 weeks. Dietary changes assessed at one year by a food frequency questionnaire demonstrated a decreased daily mean energy intake of 249 kcal in the placebo group, 296 kcal in the metformin group and 450 kcal in the intensive lifestyle group. This was accompanied by a 0.8% reduction in fat intake in the placebo and metformin group and a 6.6% reduction in the intensive lifestyle group. Incidence of T2D were 11.0, 7.8, and 4.8 cases per 100 person-years in the placebo, metformin, and lifestyle respectively. Compared to placebo, the lifestyle intervention reduced the incidence by 58% and metformin by 31%. The results demonstrate lifestyle intervention can prevent or delay the onset of T2D in high risk individuals. The effects of the intervention were similar for males and females, all racial and ethnic groups and did not differentiate between ages, demonstrating applicability to diverse populations.

Similar success was achieved by the Study on Lifestyle intervention and Impaired glucose tolerance Maastricht (SLIM) study which examined the effects of a three-year lifestyle intervention programme in individuals with IGT based in the Netherlands (Roumen, Corpeleijn, Feskens, Mensink, Saris and Blaak, 2008). One-hundred and forty-seven subjects were randomised into either the lifestyle intervention group or the control group. The lifestyle intervention was comprised of a physical activity programme and regular dietitian delivered dietary advice. Subjects were encouraged to increase moderate intensity physical activity to at least 30 minutes a day, five days per week. Advice was provided regarding how best to increase levels of physical activity and subjects were given the opportunity to participate in a free exercise programme designed specifically for the intervention which focussed on both aerobic and resistance exercise. Dietary advice was based upon national recommendations and delivered regularly to subjects following a three-day food record assessment of subjects' diet. Target reductions in body weight were set at 5-7%. Control subjects received oral and written information regarding the benefits of a healthy lifestyle but received no personalised information or access to exercise classes. Several measurements were recorded at baseline and every year for three years which included; insulin resistance, body weight, body fat, waist circumference, maximal aerobic capacity, ECG, blood pressure and three-day food records. After one year, mean body weight decreased significantly more in the intervention group (2.7 kg) compared to the control group (0.62 kg) and remained significantly different after three years. Additionally, increases in body fat percentage were significantly smaller in intervention group subjects compared to control. Aerobic capacity improved significantly in the intervention group and was associated with a significant increase physical activity. Conversely, aerobic capacity and physical activity levels declined in the control group. From the dietary measures recorded, fat intake decreased significantly more in the intervention group compared to the control group while fibre intake increased more in the intervention group compared to

control. After one year, plasma glucose levels and insulin resistance decreased in the intervention group while increases in plasma glucose and insulin resistance were observed in control subjects. After three years of follow up the difference between groups in plasma glucose concentration remained as high as 0.8 mmol/mol which was associated with a T2D incidence of 38% in control subjects and 18% in the intervention group, demonstrating 58% less progression towards T2D if treated by lifestyle intervention.

Data from the Diabetes Education and Prevention with Lifestyle Intervention (DEPLOY) were reported by Ackermann, Finch, Brizendine, Zhou and Marrero (2008). This community-based randomised programme enlisted facilities owned by the YMCA to use as sites where the programme identified and educated adults on the risks of developing T2D and promoted the benefits of weight loss and physical activity. From two facilities, one site was used for individuals who received standard advice on healthy eating and physical activity (control) while the other was allocated for the intervention. From the 131 eligible participants, 92 enrolled in the programme. Participants were recruited using a one-page letter which outlined pre-diabetes and the associated risks of developing T2D. Individuals suffering with one or more of the risk factors discussed in the letter were invited to attend a screening event at one of the YMCA facilities. During screening, BMI was assessed and the ADA diabetes risk assessment was completed. A capillary blood glucose sample was collected in people with a BMI greater than 24 kg/m^2 and an ADA risk score greater than 10. Individuals with a capillary blood glucose value between 110-199 mg/dL were eligible to take part in the programme. Measures were collected at baseline (screening event), after 4-6 months and after 12-14 months. Individuals in both groups received information about T2D and advised to begin moderate physical activity and restrict calorie intake to achieve modest weight loss of between 5-10%. Dietary intake was measured using self-report (no information available on self-report method). Individuals in the

diabetes prevention programme group were offered access to the prevention programme; those who participated in this were placed into a group of between 8-12 people who met at a mutually agreed time. Group based activities focussed on goal setting and self-monitoring and took place over 16-20 weeks with a 5-7% weight loss goal targeted. In the intervention group, there was a 6% decrease in body weight, which equated to 5.7 kg loss. The control group was significantly different and recorded a 2% decrease in body weight, equating to a 1.8 kg. Similar results were found at the 12-14 month follow up sessions. The findings of the programme demonstrate that supervised interventions result in significantly positive changes in body weight, lowering the risk of developing T2D in those at increased risk of the disease. It was suggested that increased knowledge of pre-diabetes risk may assist in motivating some individuals to begin lifestyle modification

In other work, a UK based randomised control trial, part of the European Diabetes Prevention Study, was conducted by Penn, White, Oldroyd, Walker, Alberti and Mathers (2009) and targeted T2D prevention in individuals with IGT (n = 102). The intensive behavioural intervention arm promoted increased physical activity and improved dietary habits whereas control provided minimal intervention. Baseline measures for both groups included an oral glucose tolerance test (OGTT), anthropometric (weight, waist circumference, % body fat) and blood measures, diet (photographic food atlas) and physical activity (integer scoring system based on MET scores) diaries and a lifestyle questionnaire (RAND 36). Participants randomised to the intensive behavioural intervention (n = 51) received regular dietary advice from a dietician and further supportive dietary material. Dietary targets included; > 50% total energy intake from carbohydrate, reduced total (< 30%) and saturated fat intake, increase fibre intake and reduce weight to achieve a BMI < 25 kg/m^2. Intervention for physical activity aimed to increase participants' physical activity level to at least 30 minutes moderate intensity each

day and received information and financial discounts for access to physical activity opportunities. Participants were seen by the intervention team five times in the first four months and then every three months thereafter for five years. Control participants received standard leafleted information on healthy eating and physical activity guidelines. Analysis after two years revealed incidence of diabetes was 55% less in the intervention group compared to control with weight significantly decreased in intervention at one year (-2.3 kg compared to 0.01 kg) with small but sustained changes in BMI, dietary behaviours and physical activity associated with reduced T2D risk.

In support, similar results were attained from a German cohort which demonstrated a diabetes prevention self-management programme (PREDIAS) resulted in significantly improved lifestyle factors associated with T2D (Kulzer, Hermanns, Gorges, Schwarz and Haak, 2009). A randomised control trial focussing on weight reduction as well as behavioural, metabolic and psychological factors was conducted in 182 participants with data collected at baseline and 12 months including OGTT, weight, waist circumference, blood pressure, lipids and HbA_{1c}. Measures for physical activity (German Federal Health survey) and nutrition (Three Factor Eating questionnaire) were also collected. Intervention participants received twelve 90-minute lessons covering topics including nutrition and physical activity. Control group participants received only written information detailing the topics. Significant improvements in body weight, BMI and waist circumference were observed in the intervention group compared to controls. While physical activity and dietary behaviours improved in both groups, changes were greater in intervention participants. However, despite improvements in factors associated with T2D risk, HbA_{1c} and OGTT values remained similar at 12 months between treatments.

Healthy-Living Partnerships to Prevent Diabetes (HELP PD) by Katula *et al*. (2011), aimed to

find innovative, cost effective systems to deliver lifestyle interventions targeting patients at risk of T2D within the local community. This study used community health workers (CHW) to implement lifestyle interventions designed to prevent diabetes. Data were collected at baseline and then every six months and up to 24 months of follow up. Recruitment was accomplished primarily through mass mailings to targeted post codes. Interested individuals contacted a study telephone number and were provided basic information about participation including steps in the screening and randomization process and the time commitment and expectations associated with participation before completing a telephone screening questionnaire. Fasting blood glucose, insulin and anthropometry were assessed at baseline and at the six- and 12-month visits. Community health workers were responsible for conducting the intervention group sessions, managing participants and collecting data on participant body weight during group sessions. The lifestyle weight loss intervention targeted decreased calorie intake and increased calorie expenditure through moderate physical activity. The primary objective was to produce a total weight loss of 5-7% during the first 6 months of treatment. During the second six months, participants were encouraged to continue to meet or maintain their weight loss goal providing BMI did not fall below 20 kg/m^2. Participants met weekly for CHW led group sessions during the first six months. Fourteen different groups of 8-12 participants were conducted at various community sites. Participants also received three personalised consultations with a registered dietician during months one, three and six. During months 7-12, participants received two scheduled contacts with the CHW each month, one group session and one telephone contact. Interventions were supported by a DVD series developed by a research team to cover nutrition and physical activity basics, energy balance, healthy eating, goal setting and problem solving. The enhanced usual care consisted of two individual sessions with a nutritionist during the first three months involving healthy eating and physical activity education to support weight loss.

Results were gathered from 301 participants with an average BMI of 32.8 kg/m^2. Lifestyle weight loss participants lost a net of 6% of their body weight and 5 cm in waist circumference, similar results were observed for BMI. Fasting glucose decreased by 4.3 mg/dL in the lifestyle weight loss intervention versus a decrease of 0.4 mg/dL in the usual care participants. This intervention demonstrated that the HELP PD project was successful in reducing blood glucose, insulin, body weight, waist circumference and BMI over 12 months in overweight and obese individuals with pre-diabetes. While healthy eating advice was provided to participants in this study, no specific analysis of dietary change was undertaken.

In another study, the 'Pre-diabetes Risk Education and Physical Activity Recommendation and Encouragement' (PREPARE) programme, based in the UK, aimed to improve glucose regulation in 98 overweight or obese individuals with IGT. The PREPARE programme was designed to assess the effectiveness of a group based structured education programme targeting individuals with pre-diabetes, promoting the benefits of physical activity, specifically walking (Yates, Davies, Sehmi, Gorely and Khunti, 2011). Subjects were randomised into one of three groups. Group one (control) subjects received an advice leaflet containing information relating to T2D. Group two subjects (education) received a structured three-hour education programme which was delivered by trained educators and provided information regarding the benefits of at least 150 minutes of physical activity each week in regulating glucose, action planning and goal setting. Group three subjects (education with pedometer) received the same three-hour education programme but were also provided with a pedometer and steps per day targets to assist with goal setting. Subjects in group two and three both received one-to-one counselling at three and six months. An OGTT was carried out at three, six and 12 months as well as measurements for body weight and waist circumference. Post two-hour glucose reduced significantly in group three subjects although no significant difference was observed in the

education only group. Six control participants, four education only participants and two education-with-pedometer participants were diagnosed with T2D after 24-months; this equated to a 19 and 61% reduction in the relative risk of developing diabetes in the education and education-with pedometer groups, respectively. These data provide support for the implementation of lifestyle interventions targeting high risk individuals while also demonstrating the potential use of wearable technology in combating T2D development. The feasibility of using such technology will be explored in a later chapter of this thesis.

In contrast to the beneficial findings of other studies, a 16-week randomised control trial found no benefit of additional T2D preventative support compared to modest standard care (Nilsen, Bakke and Gallefoss, 2011). This work examined the effects of individual counselling compared to individual counselling with additional group education on lifestyle behaviours related to T2D in at risk participants (n = 182). These included; 5% weight reduction, 5 cm waist circumference reduction, improvement in exercise capacity and improved dietary behaviours. Participants receiving individual counselling met a physician at baseline, six, 12 and 18 months whereas the additional group based educational programme participants attended six workshops covering diabetes avoidance, nutrition, physical activity, behaviour change, lifestyle planning and an element of physical training. At each visit, fasting blood, blood pressure, waist circumference and weight were recorded and an OGTT, dietary questionnaire (Smart Diet Score Questionnaire) and physical fitness assessment completed. Follow up at 18 months revealed significant improvements in anthropometric measures, aerobic capacity and dietary behaviour although diastolic BP increased. Significantly however, values for HbA_{1c} remained similar after 18 months. Additional group intervention had no further benefit on the results, suggesting modest contact time with health professionals may provide enough stimuli to improve measures related to T2D risk.

Another community-based lifestyle intervention was administered at a community centre to low-income individuals at risk of T2D in the USA (Ockene *et al*., 2012). Three-hundred and twelve individuals were randomised to receive either lifestyle intervention or usual care. Lifestyle intervention participants received three individual and 13 group sessions focussing on T2D prevention, positive attitudes towards physical activity and dietary change, self-monitoring and goal setting. Individual sessions were completed in participant's homes and group sessions at a community site. Sessions were delivered by trained members of the community with experience of nutrition education, although none were dieticians. Following one year, intervention participants lost significantly more weight which was accompanied by a significant decrease in HbA_{1c} and insulin resistance. Additionally, intervention participants demonstrated significantly greater reductions in percentage fat intake. Weight change was associated with session attendance with individuals attending more than seven sessions losing on average 4.75 lb compared to those attending less than seven sessions losing 0.13 lb. Changes in HbA_{1c} were not associated with changes in dietary behaviour with the authors suggesting dietary analysis through a recall questionnaire was insufficiently sensitive to allow for meaningful analysis. Improved analysis techniques may be able to demonstrate, for example, associations between improved dietary behaviours with reductions in HbA_{1c}. Such inaccuracies in dietary monitoring methods will be discussed and examined in latter chapters of this thesis.

Individuals of south Asian origin have a well-established susceptibility to T2D, greater than other ethnic backgrounds, and therefore the Prevention of Diabetes and Obesity in South Asians (PODOSA) study tested the effectiveness of a three-year intervention targeting weight loss and increasing physical activity in this UK cohort (Bhopal *et al*., 2014). One-hundred and seventy-one participants were randomised to receive either the intervention, consisting of 15

visits from a dietician over three years, or control where participants received four dietician visits over the same period. All visits were co-ordinated by an academic research facility but took place in participant's homes. Dietician visits in the intervention group focussed on T2D prevention, weight loss, increasing physical activity and improving dietary behaviours. Additionally, intervention participants received pedometers to monitor physical activity. Control group subjects received standardised written and verbal advice on T2D prevention, healthy eating, physical activity and how to access weight control and physical activity services. All participants were visited by a dietician after one, two and three years for monitoring. After one year, intervention participants lost more weight compared to control and this was sustained over three years. Control participants lost weight in year one but had gained weight by year three. Changes in body weight over three years were related to changes in BMI, waist and hip circumference. OGTT values decreased in both groups after three years although decreases were not significant. Progression to T2D however was lower in intervention participants while blood pressure remained similar across treatment groups. Financial analysis in the intervention group revealed a significant cost per person of £1126 to achieve modest changes in weight and other variables related to T2D development.

1.6. Health Care Settings

Compared to experimental settings, where resources are plentiful and often well-funded, T2D prevention in health care or 'real world' settings pose several challenges. Lifestyle intervention programmes often require considerable resources including; staff expertise, specialised equipment, appropriate facilities and time availability which are often unavailable or restricted in front line health care practice. Further to this, resource and time intensive intervention in clinical settings may decrease access for socioeconomically deprived and geographically isolated groups. Increasing financial burdens imposed on health services, caused in part by an

increasing and ageing population and reduced healthcare budgets, mean less time intensive and low-cost interventions are required to help tackle the increasing T2D prevalence. Therefore, several research studies have examined the effectiveness and viability of health care led prevention schemes on T2D incidence in at risk cohorts.

Absetz *et al.* (2007) reports on the effectiveness of the Good Ageing in Lahti Region (GOAL) Lifestyle Intervention Trial conducted in health care centres in Finland. Three-hundred and fifty-two subjects at increased risk of T2D were contacted based on pre-identified risk factors including obesity, high blood pressure and impaired blood glucose values. To measure the success of GOAL, five key lifestyle changes were implemented including; < 30% of total energy intake from fat, < 10% of total energy intake from saturated fat, at least 15 g of fibre per 1000 kcal, at least four hours a week of moderate physical activity and > 5% reduction of body weight. It was hypothesised that the adoption of at least four of these lifestyle changes would be enough to prevent the onset of T2D and demonstrate that successful adoption of these objectives would indicate the intervention as a success for that individual. To support lifestyle change, individuals received six counselling sessions, five of which were conducted during an eight-week period and the final session taking place after eight months. During each counselling session, individuals were provided information on T2D prevention which was supported by group discussions, methods for effective goal setting and self-behavioural monitoring. Additionally, individuals received access to a dietician during one of the sessions and introduced to sports facilities by a sports officer. All lifestyle measures were collected by a practice nurse following recruitment and then after 12 months of the intervention. Dietary intake was measured using a three-day food diary. Compared to baseline measures, significant differences were found in the attainment of the lifestyle measures stipulated by the intervention after one year. The highest success rates were identified in individuals who already met the

lifestyle objectives during baseline (20% of total group). This is identified as a limitation, with the study suggesting the reason for this is due to recruitment occurring among healthcare patients who may already have had access to lifestyle counselling and therefore already having made positive changes to their lifestyle behaviours. The intervention decreased T2D risk factors which included marked reductions in diastolic blood pressure, weight and BMI (males) and waist circumferences (males and females) but did record a statistically significant increase in FPG in females and a non-significant increase in males. Despite increases in FPG, the values remained within a range considered to be normal.

However, improved glucose values were demonstrated in a T2D prevention programme conducted in Australian primary health care using 237 participants at moderate and high risk of T2D, identified by FPG and/or an OGTT (Laatikainen *et al*., 2007). Participants received six 90-minute group sessions run by trained nurses covering topics including T2D education, diet, physical activity and goal setting techniques. Targets included reductions in body weight, total and saturated fat consumption, increase fibre consumption and to exercise more regularly. Measures including; height, weight, waist and hip circumference, blood pressure, fasting glucose and lipid profile were collected at baseline, three months and one year. From the 79 patients with impaired glucose values at baseline, 42 reverted back to normoglycemia at 12 months while five developed T2D. After three months, significant improvements in weight, waist circumference, total and LDL cholesterol were recorded and were sustained at 12-month follow up. Fasting glucose, HDL cholesterol, triglycerides and diastolic blood pressure were significantly improved after 12 months. Furthermore, questionnaire responses regarding bodily pain, physical functioning and mental health showed significant improvements after 12 months. This study demonstrates the applicability of such a method to 'real world' clinical settings and suggests future implementation could be generalizable to wider primary health

care locations.

A similar study by Payne *et al*. (2008) assessed the effectiveness of a low resource lifestyle modification programme targeting socioeconomically deprived and geographically isolated groups at risk of T2D in Australian primary care based on T2D risk factors. This work examined the effects of resistance training in the home compared to a gymnasium accompanied by appropriate clinical support. The 12-month intervention included goals to reduce weight by > 5%, achieve 150 minutes of weighted resistance exercise each week and an additional five sessions of moderate intensity exercise. Dietary goals included < 30% of total energy intake from fat and < 10% from saturated fat. Six 90-minute educational sessions covering topics including self-management, self-monitoring, goal setting, physical activity and dietary habits were completed by all participants before randomisation into 11 weeks of either home-based or gymnasium-based resistance exercise followed by a 33-week maintenance programme. Assessments on plasma glucose, blood pressure, blood lipids, BMI, waist circumference, physical activity level (Active Australia Survey) and dietary behaviours (Food Frequency Questionnaire) were made at baseline and after one year. 122 participants (4.9 % IFG and 32 % IGT) were recruited of which 98 attended a one year follow up. Significant reductions were recorded in the proportion of participants with IFG or IGT while significant improvements were recorded in participants reaching dietary and body composition targets. Interestingly, home and gym-based resistance training resulted in similar changes across all the measured variables, except for systolic BP, which decreased significantly more in the gymnasium trained group. The effectiveness of a low resource cost effective T2D intervention led by primary care is demonstrated here and provides viable evidence that such an approach, where financial and resource restrictions exist, can help prevent T2D in deprived, isolated groups and may have potential to be applied to similar groups in other countries.

The Kaiser Permanente Colorado Organisation (KPCO) aimed to develop a weight loss programme effective in preventing the onset of T2D in primary health care settings with modest or limited resources. This study, by Almeida, Shetterly, Smith-Ray and Estabrooks (2010) had access to 14,379 individuals classified as having IFG. Individuals were eligible if fasting glucose was measured between 100 – 125 mg/dL. From the 12,468 individuals eligible based on the above guidelines, 1030 attended a single group meeting which gave advice and planning on healthy nutrition, physical activity and weight loss. Each intervention participant was matched with one or two control subjects who were not participating. Matching of control subjects to participating subjects was based on the month and year of the fasting glucose sample, sex, age (within five years) and BMI (within two units). Analysis after 12 months of the intervention revealed body weight had decreased significantly compared to matched controls. Individuals participating in the small group classes lost at least 5% of their body weight and were 1.5 times more likely to lose 5% of their body weight in comparison to control subjects. This study demonstrated a single session which provided advice on healthy eating and physical activity was beneficial in reducing weight in individuals considered to be pre-diabetic and at risk of T2D. The work also showed the programme's capability to be delivered within a primary health care setting of limited resources although does concede the intervention did not broadly reach all areas of the community at risk of diabetes and may suggest an element of self-selection. Despite this limitation, this work suggests a similar model could be applied to T2D prevention in the UK where financial and time resources are limited.

In Finland, the National Program for the Prevention of T2D (FIN-D2D) was implemented in five hospital districts with the aim of identifying individuals at increased risk of T2D (Saaristo *et al*., 2010). Those identified were provided with lifestyle change support designed to reduce future risk of T2D development. High risk individuals were identified using the Finish Diabetes

Risk Score which was used opportunistically within health centres, pharmacies and health fayres. Individuals with risk scores > 15 were considered at increased risk of T2D and referred to FIN-D2D. Individuals were also considered eligible for referral if they had a history of IFG, IGT, cardiovascular disease or gestational diabetes. Measures including height, weight, waist circumference, blood pressure, and an OGTT and lipid profile were collected at baseline and follow up. The intervention visits comprised of individual counselling sessions as well as group-based meetings led by nurses. Sessions focused on body weight, meal frequency, fat intake, alcohol consumption, benefits of physical activity and smoking cessation. Group lectures regarding T2D and lifestyle behaviour change were also provided. The frequency and duration of visits varied between practices depending on local resources and circumstances. Baseline and one year follow up data from 2798 nondiabetic at risk individuals revealed a mean decrease in body weight of 1.3 kg in men and 1.1 kg in women with the reductions associated with a mean 1.3 cm decrease in waist circumference. Blood pressure decreased alongside total cholesterol, LDL cholesterol and triglyceride levels which decreased by 5-8% and 2-5% in men and women respectively. Seventeen and a half percent of individuals lost > 5% body weight, 16.8% lost between 2.5 - 4.9% body weight and 46.1% maintained body weight. 19.6% of individuals gained > 2.5% body weight. The greatest decreases in blood pressure were observed in individuals who lost > 5% body weight. Incidence of T2D was 2% and 1.2% in men and women with normal baseline glucose tolerance, 13.5% and 7.4% in those with IFG and 16.1% and 11.3% in IGT respectively (Saaristo *et al*., 2010). Greater than 5% decreases in body weight were associated with a 69% reduction in T2D risk after one year and demonstrate moderate weight loss is effective in reducing the risk of T2D development.

In 32 primary care centres in Japan existing resources were used to deliver lifestyle intervention to individuals at risk of T2D (Sakane *et al*., 2011). Three-hundred and four participants were randomised to detailed bi-annual T2D support, delivered by practice nurses covering topics

including general T2D information, T2D prevention, healthy eating and physical activity with additional regular contact delivered to support behaviour chance. Nutrition and physical activity were assessed by food frequency questionnaire and the compendium of physical activities, respectively. Control group participants received one group session on healthy lifestyle behaviours and T2D prevention at baseline although no additional support was given. Anthropometric and blood measures were collected regularly from both groups. Results after one year demonstrated significantly improved body weight, energy expenditure and insulin sensitivity in the intervention group compared to control. T2D incidence was lower in the intervention group compared to control but was not significant.

As part of the Diabetes in Europe – Prevention using Lifestyle, Physical Activity and Nutritional intervention health care professionals in 18 primary health care facilities in Spain conducted T2D risk evaluation (Costa *et al*., 2012). Screening took place in the form of questionnaires which calculated individual diabetes risk scores and gathered further information such as age, sex, weight, height, waist circumference, use of medications, history of high blood glucose, physical activity, family history of diabetes and daily consumption of fruits and vegetables. On a second occasion an OGTT was performed to confirm eligibility, a blood glucose of > 7.8 mmol/mol and < 11.1 mmol/mol two hours post glucose load. Eligible participants also completed a basic questionnaire which was designed to offer baseline and future indications of dietary and physical exercise habits (FINDRISC). The study consisted of two separate interventions, the standard care intervention and the intensive intervention (group and individual). For all groups, targets were set for lifestyle intervention which included; consuming a maximum of 30% of daily energy from fat, a maximum of 10% of daily energy from saturated fat and a minimum of 3.6 g/1000 kj of fibre, completing a minimum of 30 minutes of moderate intensity exercise a day, and achieving at least a 3% weight reduction.

Participants randomised into the standard care group received information on nutrition, cardiovascular health and T2D but received no additional support other than optional future visits to health care centres. Intensive group participants received a group based six-hour educational programme and additional training materials, and were provided with in-depth information on T2D risk, diet, physical exercise and tobacco use. Participants in the intensive 'individual' group received similar information which was provided on a one-to-one basis. Regular phone contact was made with participants to ensure motivation was maintained throughout the intensive intervention. 168 individuals were placed into the standard care group, 171 into the 'individual' intensive group and 162 into the 'group' intensive group. It is notable that there was a large dropout rate during the intervention process. In total, 228 individuals ceased their participation, 102 in the standard care group and 126 in the intensive interventions (breakdown of withdrawals from individual and group intensive interventions not provided). Reasons for ceasing involvement ranged from time commitments, illness and death. The 12-month follow up found a modest 24.7% of individuals in the intensive group and 18.8% of individuals in the standard care group reduced their weight by at least 3% with the study also demonstrating the risk of developing diabetes was reduced by 36.5% in the intensive intervention group when compared to the standard care group. Subsequently, the study recommended all individuals attending health care centres should be screened for T2D with those at risk being referred to academically tested intervention programmes.

In another European study, a comparison between T2D lifestyle intervention and usual care in Dutch general practice was investigated by Vermunt *et al*. (2012). Nine-hundred and twenty-five participants from 14 practices were randomised to receive either intervention or usual care. Intervention participants received eleven 20-minute consultations focussing on behaviour change, five group meetings addressing diet and physical activity and an additional one-hour

consultation with a dietician. Control group participants received only oral and written information about T2D at baseline. Both groups attended follow up appointment after six, 18 and 30 months. Despite significantly higher contact time in the intervention group, there was no significant difference in effects between intervention and control with both groups demonstrating modest changes in body weight, blood glucose concentration, physical activity and dietary intake. The authors suggest one possible explanation may be due to control participants becoming motivated to change behaviour by the prospect of annual check-up visits. This suggests time intensive intervention in primary care may not be necessary and only limited visits to primary care centres with subsequent monitoring are needed to achieve modest changes in variables associated to T2D.

Despite previous studies consistently demonstrating the success of primary care lifestyle interventions reducing incidence of T2D, evidence from the UK (translating diabetes prevention research into clinical practice) remained to be elucidated, therefore leading to the UK based intervention 'Let's Prevent Diabetes' (Gray *et al.*, 2012). This work targeted evidence regarding the long-term effectiveness of a structured, educational diabetes prevention programme targeting lifestyle and behaviour change, conducted within primary care settings. The intervention programme consisted of two phases. Firstly, a screening phase to identify patients at risk of T2D using an automated validated risk tool and secondly, the intervention phase. The automated risk score used data routinely stored on practice computer databases. The top 10% of scorers from surgeries were considered at highest risk and invited for further screening. Patients were classified as pre-diabetes if fasting blood glucose ranged between 6.1-6.9 mmol/mol and between 7.8-11 mmol/mol two hours following an OGTT. Height, weight, waist circumference, blood pressure, seven-day step count, T2D family history, smoking status, alcohol consumption, occupation, sleeping habits were all recorded alongside level of social

deprivation. Fat and fibre intake were assessed using the DINE food frequency questionnaire.

Eligible patients were randomised at GP practice level by an independent researcher into either the control arm or intensive arm intervention. Control patients received an information booklet detailing the risk factors associated with T2D and how changes to physical activity levels and lifestyle can be used to prevent or delay progression of T2D. Control arm patients attended follow up appointments at the same time points as the intensive arm patients. Patients in the intensive arm intervention received the same information booklet as the control arm patients as well as an invitation to 'Let's Prevent', a six-hour structured education programme, nursing support phone calls every three months and a yearly three-hour updated structured education programme to review key messages and personal action plans. The goals from the programme were to achieve > 5% reduction in body weight, total fat intake < 30% of total energy intake, total saturated fat intake < 10% of total energy intake and increase fibre intake to > 15g for every 1000 calories consumed. For physical activity, patients were to increase their daily walking to 45 minutes per day or complete 4,500 steps. Patients received a pedometer to allow self-monitoring of physical activity to help promote goal setting and encouraged to increase their total by 500 steps per day, every two weeks. Throughout the three-year study, intervention arm patients were supported by three monthly phone calls from a nurse trained to support patients in behaviour change. Data were collected at six, 12, 24 and 36 months.

A total of 880 patients were recruited into the study from 43 general practices with 447 patients and 433 patients randomised into the intervention and control arms respectively. Patients attending the initial educational session and at least one further session (retainers) were less likely to develop T2D in comparison to patients in the control arm. Retainers were associated with improved glucose control and daily step count and reduced $HbA_{1c,}$ weight, waist

circumference and anxiety. The results from the 'Let's Prevent Diabetes' study demonstrate that high risk patients who engage with and are retained in a T2D prevention programme are at less risk of developing T2D in comparison to patients who received standard care. The study highlights the importance of engaging with patients who are classified as non-engagers and non-retainers as these hard-to-reach groups exhibit high risk T2D characteristics. Furthermore, the likelihood of study participation based upon socio-economic status demonstrates the requirement to test such intervention programmes across a range of different cohorts where intervention success may vary.

Given the relative success of Gray *et al's* findings in combating T2D in the UK, NHS England, in partnership with Public Health England and Diabetes UK, implemented the NHS Diabetes Prevention Programme (NHS DPP), an intensive lifestyle management intervention aimed at individuals at high risk of T2D. Launched in June 2016, patients are screened at primary care level with those considered at risk, based upon elevated HbA_{1c} (42-47 mmol/mol) and/or BMI > 25 kg/m^2, offered 13 educational group sessions over a nine-month period. Each session lasts between one and two hours and focuses on education and exercise with the aims of improving dietary behaviours, increasing physical activity and targeting weight loss. The programme is designed primarily to decrease the current and future workload burden on the NHS by providing an end to end behaviour change intervention outside of immediate primary care to reduce the future prevalence of T2D through the delivery of an evidence-based intervention. The NHS DPP intends to have the programme available across the entirety of England by 2019 with 100,000 places available each year for patients to access. As a recently implemented and ongoing programme, full analyses of the intervention have yet to be undertaken. However, early indications suggest the programme is exceeding targets for both referrals into and patient engagement in the programme, resulting in a mean weight loss of 3.7 kg after 1 year of

participation (NHS DPP, 2018) and a decrease in HbA_{1c} of 1.26 mmol/mol (Valabhji *et al*., 2019). Data detailing the characteristics of participants engaging with the DPP demonstrate it is successfully reaching groups who are most at risk of T2D. In year one of the programme for example, 25% of patients were from black and ethnic minority groups, and 45% were under the age of 65 years. Data also found that 44% of those taking up the programme are male; a significantly higher percentage than typically attend commercial weight loss programmes. Significantly though, this programme is only available to patients in England, with no such access available for the remaining countries within the UK, including Wales, where T2D incidence is highest.

1.7. Summary

Abundant evidence demonstrates the significant impact lifestyle interventions, which promote and facilitate increased physical activity and improving dietary behaviours, have on T2D development in at-risk groups. Improvements in measures related to T2D risk have been demonstrated across groups of different nationality, ethnicity and socio-economic background, suggesting strong applicability to all populations, although such assumptions must be examined in untested cohorts. A common theme however in many of the above studies and interventions is the volume of resources required for successful T2D prevention.

For example, while numerous intervention programmes have shown health benefits, some are time intensive, resource heavy and financially expensive, with Bhopal *et al*. (2014) demonstrating significant costs of over £1000 per participant. In other cases, resource heavy programmes did not always result in additional benefit to at risk persons. A 16-week randomised control trial found no benefit of additional T2D prevention support (Nilsen, Bakke and Gallefoss, 2011) when the effects of short individual counselling sessions were compared

to individual counselling with additional group education on lifestyle behaviours relating to T2D. Additional group intervention had no further benefit on T2D related outcomes, suggesting modest contact time with health care professionals provided sufficient stimuli for patients to improve measures related to T2D risk, including anthropometric measures, aerobic capacity and dietary behaviours. In support of this conclusion, the work by Vermunt *et al*. (2012) found participants in the intervention group, who received eleven 20-minute consultations focussing on behaviour change, five group meetings addressing diet and physical activity and an additional one-hour consultation with a dietician, demonstrated no significant changes in body weight, blood glucose concentration, physical activity and dietary intake in comparison to control participants who received only written T2D advice. The authors suggest a possible explanation may be because control participants became motivated to change behaviour by the prospect of annual check-ups with a health care professional. This could suggest time intensive intervention in primary care may not be necessary and only limited visits to primary care locations with subsequent monitoring are needed to achieve changes in variables associated to T2D. Almeida, Shetterly, Smith-Ray and Estabrooks (2010) support this notion in their findings where it was demonstrated that a single session providing advice on healthy eating and physical activity was beneficial in reducing weight in individuals considered to be pre-diabetic and at risk of T2D. The work also showed the programme's capability to be delivered within a primary health care setting of limited resources and suggests a similar design could be implemented in other health authorities and targeted towards pre-diabetic groups where effective treatments are required which can be feasibly implemented within health board budgets. Furthermore, findings by Gray *et al*. (2012) suggest that participation in the 'Lets Prevent Diabetes' study was dependent upon socio-economic status and therefore demonstrates the requirement to test intervention programmes across a range of different cohorts and locations where intervention success may vary.

Based on the evidence of increasing T2D prevalence in the UK, intervention in some form is crucial in preventing T2D. However, the degree to which healthcare services should intervene and to what depth interventions should go to achieve a balance between effectiveness and resource cost, is debatable. As stated by Dunkley *et al*. (2014), who reviewed the effectiveness of lifestyle interventions in preventing T2D, more research is needed to establish the best strategies for maximizing both cost-effectiveness and longer-term maintenance of weight loss and diabetes prevention effects. An intervention design, based on upon a single appointment with a healthcare professional where information on T2D risk and ways to combat T2D development is given, may provide enough benefit for pre-diabetes patients in some cohorts as well as limit the time, resource and financial pressures often experienced within healthcare settings in trying to combat non-communicable diseases, such as T2D. Given the alarming increase in incidence of T2D in the UK, the implementation of lifestyle interventions that are effective in preventing the development of T2D in those at increased risk is critical. This is especially crucial for both patients and UK healthcare providers in locations where no T2D prevention pathways are currently in place. While T2D prevention programmes have been implemented and evaluated successfully in countries across the world, there is a lack of lifestyle intervention programmes for people at risk of T2D living in Wales. Despite significant support being provided for T2D prevention in England, a similar model for the remaining areas of the UK has yet to be implemented, nor has significant funding been made available to address the issue. This is despite the prevalence of the disease being highest in Wales compared to the remaining areas of the UK (Diabetes UK, State of the Nation, 2015) and there being a considerably portion of the Welsh population at increased risk of T2D. Given the health complications associated with T2D as well as financial implications involved in treating the condition, it is of extreme importance that an effective pathway is implemented within NHS

Wales' healthcare structure which has been thoroughly evaluated and proven to be effective in reducing population T2D risk.

1.8. Thesis Aims and Objectives

The overall aims and objectives of this thesis are:

Aim:

Evaluate the efficacy of an educational consultation delivered through primary care in reducing progression to T2D in a north Ceredigion pre-diabetic population.

Objectives:

- Complete service evaluation of the North Ceredigion GP cluster pre-diabetes intervention.
- Develop and validate methods to quantify dietary changes over time.
- Establish the feasibility of conducting a randomised control trial to establish the efficacy of a diabetes prevention programme delivered through primary care.

Chapter Two:

The efficacy of a GP-led pre-diabetes intervention targeting lifestyle modification

2.1. Introduction

The cost of the care and treatment associated with diabetes across the UK is currently estimated at £23.7 billion per year (includes both the direct and indirect costs; Diabetes UK, State of the Nation, 2015). By 2035 it is predicted that this will increase to £39.8 billion by which time it is estimated 4.9 million people will have T2D. In Wales the incidence of T2D is increasing at an alarming rate, in 2015 it was estimated that 182,600 people had T2D and by 2025 that number will increase to 300,000 (Diabetes UK, State of the Nation, 2015). The financial implications for NHS Wales are huge, with approximately 10% of the annual budget spent on treating the condition and its complications, a figure that equates to approximately £500 million spent treating a highly preventable disease (Diabetes UK, State of the Nation, 2015). Of great concern are the estimated 540,000 individuals in Wales currently at risk of developing T2D as approximately 10% of unaddressed cases will progress to T2D (Tabak *et al*., 2012). In the absence of effective treatments and interventions these estimations are projected to increase (Diabetes UK, State of the Nation, 2015). Given the increasing rate of T2D cases in Wales and projected future cases, there is an urgent need for effective intervention programmes which can delay or prevent the onset of T2D.

In England the NHS, in partnership with Public Health England and Diabetes UK, have implemented the NHS Diabetes Prevention Programme (NHS DPP), an intensive lifestyle management intervention aimed at individuals at high risk of T2D. Patients are offered at least

13 sessions, each lasting between one and two hours, which focus on education and exercise with the aims of improving dietary behaviours, increasing physical activity and targeting weight loss. The NHS DPP intends to have the intervention available across England by 2019 with 100,000 places available each year for patients to access. In Wales however, no such programme is available. Given the success of other interventions in preventing T2D (DPP Research Group (2002), Almeida, Shetterly, Smith-Ray and Estabrooks (2010), Yates, Davies, Sehmi, Gorely and Khunti, (2011), Gray *et al*., (2012) and Piper, Marossy, Grffiths and Adegboye (2017)), there is an evidence-base for the implementation of a similar preventative model within Wales where the incidence of T2D is increasing year on year.

The Hywel Dda University Health Board (HDUHB) covers approximately 25% of the landmass of Wales, containing the local authorities of Pembrokeshire, Carmarthenshire and Ceredigion. The HDUHB has an estimated patient population of 375,000, of which 5.9% (22,000) have diabetes (Diabetes Delivery Plan, 2013-2016). Patients in north Ceredigion are served by seven GP surgeries which make up the North Ceredigion GP cluster. Three of these surgeries are in Aberystwyth: Church, Padarn and Ystwyth with the remaining four located in Borth, Tregaron, Llanilar and the Tanyfron surgery located in Aberaeron. In 2015 the cluster initiated a GP-led pre-diabetes intervention targeting lifestyle modification, aimed at reducing the number of patients developing T2D by identifying, educating, monitoring and signposting patients to lifestyle support services. The intervention consisted of a 30-minute one-to-one patient consultation delivered through general practice with opportunity for exercise (NERS) and dietary (FoodWise) referral. Previous work has shown that a single educational session focussing on improved lifestyle behaviours can be successful in reducing incidence of T2D in healthcare (Almeida, Shetterly, Smith-Ray and Estabrooks, 2010). Additionally, significant

reductions in T2D risk following referral to lifestyle groups outside of primary care have been demonstrated (Piper, Marossy, Grffiths and Adegboye, 2017).

Aim:

• Evaluate the efficacy of a T2D prevention programme with a 30-minute educational consultation aimed at reducing the risk of patients developing T2D in the North Ceredigion population by proactively identifying, monitoring and signposting patients to healthy lifestyle support services.

Objective:

• Compare HbA_{1c}, body mass, BMI, waist circumference and blood pressure, measured during the initial 30-minute one-to-one consultation and subsequent follow up appointments at 12 and 24 months.

2.2. Method

2.2.1. Participants and Ethical Approval

The seven surgeries which make up the North Ceredigion cluster were serving a total of 47,107 patients when the programme commenced (Table 2.1). Each surgery reviewed patient records and identified up to 4.5% of their patient list for invitation into the programme.

Inclusion criteria:

- Registered patient at a participating surgery
- Between the ages of 18-75 years old
- HbA_{1c} between 42-47 mmol/mol

GP professional judgement, using National Institute for Health and Care Excellence (NICE) guidance, was used for any patient where eligibility was unclear.

Exclusion Criteria:

- Existing comorbidities which would make exercise contraindicated

Table 2.1. Summary of practice populations as of 1st April 2016 and the number of patients invited to engage in the programme, 4.5% of patient list

Surgery	Practice populations (1st April 2016)	4.5% of practice population
Borth	2,664	120
Church	11,150	501
Llanilar	3,215	144
Padarn	11,577	520
Tanyfron	7,426	334
Tregaron	3,553	160
Ystwyth	7,516	338
TOTAL	47,107	2,118

The programme was provided under a Service Level Agreement and was classified as a service evaluation.

2.2.2. Protocol

The pre-diabetes intervention programme was developed by Aberystwyth University (IBERS) in conjunction with the seven participating surgeries. Specifically, the structure of the 30-minute one-to-one consultation was developed by Aberystwyth University and Church Surgery. During the developmental phase, the consultation was designed and suitable information leaflets and questionnaires relating to pre-diabetes were identified (detailed below). Information regarding the structure and contents of the consultation was then disseminated to the remaining six surgeries to begin the initial stages of the intervention.

The information included in the consultation and supporting documentation was based upon the latest NICE guidelines regarding physical activity and healthy eating behaviours. Eligible patients received an information pack via the postal service to their home address outlining their T2D risk and inviting them to attend an appointment at their registered surgery for a 30-minute, one-to-one consultation with a health care professional (this could be a practice nurse or GP and varied from surgery to surgery). Patients were encouraged to book their consultation either by phone call or in person at the surgery.

Within the information pack (see appendix; A, B, C, D) patients received; an invitation letter; a GP designed leaflet providing general information on pre-diabetes and the risks associated with T2D, a dietary intake questionnaire (Starting the Conversation) and the International Physical Activity Questionnaire - Short Form (IPAQ-SF). The IPAQ-SF is a seven-point validated questionnaire designed primarily for physical activity surveillance in adults and represents a feasible approach for conducting physical activity surveillance in large groups (van der Ploeg *et al*., 2010). The IPAQ-SF collects data on the amount of time respondents complete vigorous and moderate physical activity, the amount of time spent walking and sitting, with

responses for all questions based upon a typical week. The cumulative amount of time spent engaging in each activity is used to categorise respondents as either inactive, minimally active or HEPA (Health Enhancing Physical Activity) active. Work by Craig *et al*. (2003) demonstrated the IPAQ-SF to correlate reasonably well ($P = 0.4$) with accelerometer data in a UK cohort. The Starting the Conversation questionnaire is an eight-point validated screening method designed for clinical practices to quickly assess patient dietary habits. It identifies dietary patterns and was derived from a validated 54-item instrument (Paxton, Strycker, Toobert, Ammerman and Glasgow, 2011). The questionnaire scores respondents on a scale between zero and 16. A score of zero indicates a healthy, balanced diet whereas a score of 16 indicates an unhealthy diet. Both questionnaires were completed by patients prior to the consultation. Responses from the Starting the Conversation and IPAQ-SF questionnaires were used to help identify goals for patients and provide appropriate conversational direction during the second half of the consultation (data from questionnaires were not forwarded by the surgeries for analysis).

On arrival at the surgery, patients were met by a health care professional and the questionnaire responses were returned and scored. During the first phase of the consultation, baseline data were collected including; body mass (kg), height (cm; to calculate BMI), waist circumference (cm) and BP (mmHg). If a HbA_{1c} had not been recorded during the preceding two months a blood sample was taken; analysis took place at Bronglais Hospital Pathology Laboratory, Aberystwyth. All data collected were added to the patient's medical record.

During the second phase of the consultation patients were engaged in conversation, during which information regarding the benefits of physical activity and healthy eating in relation to T2D risk were discussed; all recommendations were based upon NICE guidelines. Physical

activity advice included the recommended completion of at least 150 minutes of moderate intensity activity each week, such as 30 minutes per day on five days of the week, or alternatively, 75 minutes of vigorous intensity activity spread across the week or combinations of moderate and vigorous intensity activity. Patients were also advised to undertake resistance exercise to improve muscle strength on at least two days a week as well as minimise the amount of time spent being sedentary for extended periods. Summarised advice regarding healthy dietary behaviours included eating at least five portions of a variety of fruit and vegetables every day, basing meals on wholegrain carbohydrates, choosing lower fat and lower sugar diary options, consuming beans, pulses, fish, eggs, lean meat and other proteins, choosing unsaturated oils and spreads, drinking six-eight cups/glasses of fluid a day and limiting the consumption of alcohol to no more than 14 units per week.

Based on the professional judgement of the health professional, patients were signposted to local physical activity groups (walking clubs/parkrun UK/University exercise classes). Referrals were also made to the nutrition education initiative, Foodwise for Life, an eight-week evidence-based approach to weight management designed by public health dietitians in Wales and delivered by Public Health Network Cymru. Foodwise for Life focuses on adopting improved dietary habits, increasing levels of physical activity and employing behaviour change strategies to help facilitate weight loss. Each session consists of structured discussions, practical learning and group activities where participants enhance their understanding in relation to food portion sizes, food labelling, the benefits of regular physical activity and weight management planning. Patients requiring supervised exercise were referred to the National Exercise Referral Scheme (NERS). NERS is a structured 16-week supervised exercise programme overseen by a qualified exercise professional and includes the following:

1. An initial consultation collecting physiological data including resting heart rate, blood pressure, BMI and waist circumference, completion of lifestyle questionnaires, introduction to local leisure facilities and goal setting.
2. Access to individualised and/or group exercise classes.
3. Discounted rates for exercise activities.
4. Four- and 16-week reviews with an exercise professional, evaluating targets set and goals achieved.
5. Health check upon completion of 16 weeks and signposted to exit routes.
6. Eight-month progress check conducted by a health professional and 12-month review including a repeated health check.

Patients attended follow up appointments at their surgery after approximately 12 and 24 months with the same data collected as per the initial 30-minute consultation. In some cases, data relevant to the intervention were added to patient records during other GP appointments and therefore not collected during the initial consultation or follow up appointments. In such instances, data were only included in statistical analysis if they were collected within two months of the initial consultation or the follow up appointments. The primary outcome measure for evaluation of the intervention was HbA_{1c}. Secondary outcomes were anthropometric measures and blood pressure.

2.2.3. Data Extraction

Data extraction for 12 months follow up took place in February 2017. Extraction for 24 months follow up took place between April and July 2018. Data were only included for analysis if returned during these extraction time points. Examination of 12-month data revealed inconsistencies in data extraction search terms, with some surgeries providing more data than

others. Therefore, pilot work prior to 24-month data extraction was conducted alongside Church surgery. This ensured a standardised data extraction method and meant the delivery of anonymised data from general practice to Aberystwyth University was tested prior to the remaining surgeries extracting and returning their 24-month data. Pilot data extraction by Church surgery took place in October 2017. To generate relevant patient data on the variables associated with the intervention database search terms were confirmed by Church surgery and distributed to the other practices to ensure all patient data were produced from identical search criteria before returned for analysis.

2.2.4. Statistical Analysis

All statistical analyses were performed using SPSS (version 17.0 for windows, SPSS Inc, Chicago, IL). Normally distributed data are presented as mean ± SD. For patients who attended a baseline and 12-month follow up appointment, normally distributed 12-month follow up data were analysed using paired t-tests. Non-normally distributed data are presented as median (range). Non-normally distributed 12-month follow up data were analysed using Wilcoxon signed rank test. For patients who attended a baseline, 12- and 24-month follow up appointment, normally distributed data were analysed using one-way repeated measures ANOVA. Significant differences were identified using post-hoc analysis with paired t-tests. Non-normally distributed data were analysed using Friedman's test with significant differences in the data identified by post-hoc analysis with Wilcoxon signed rank test. Correlation analysis was conducted to identify relationships in the data after the intervention by analysing change in body mass, BMI and waist circumference with change in HbA_{1c} using Pearson's (parametric data) and Spearman's (non- parametric data) correlation test. Data from the seven surgeries were pooled and analysed as a total patient cohort. In addition, data from each of the seven surgeries were analysed independently. Statistical significance was accepted at $P < 0.05$. All

data are presented tabularly. Where statistically significant findings are observed, the data are also presented in figures.

2.3. Results

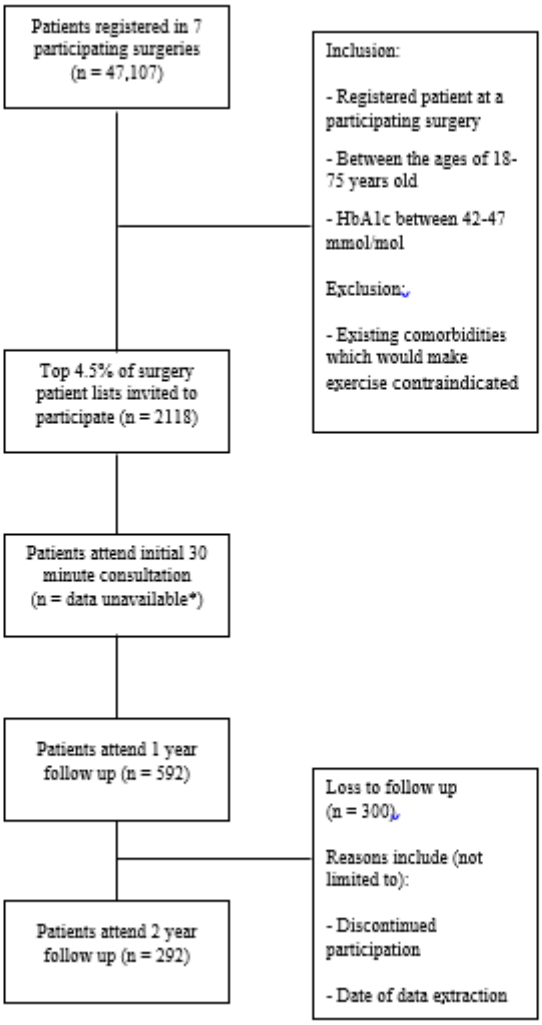


Figure 2.1 Eligible patients followed up after 1 and 2 years following a 30-minute one-to-one consultation with a health care professional in a primary care setting. *Data not provided by surgeries for patients who only attended a consultation but no further follow up visits.

2.4. Baseline to 12-month follow up

2.4.1. Subjects

In February 2017 analysis of preliminary data took place, data on patients who had attended the initial 30-minute one-to-one consultation and a 12-month follow up appointment (n= 129) were analysed. The results from this analysis formed part of an interim report which was distributed to the participating surgeries, the HDUHB and presented at the Welsh Endocrine and Diabetes Society Spring conference, 2017 (Thatcher and Gregory, 2017). Between April and July 2018 data were extracted from the patient records for all surgeries, data for baseline and 12-month follow-up were available for 592 patients.

2.4.2. Baseline to 12-Month HbA_{1c}

Analysis of HbA_{1c} revealed significant decreases in six surgeries, due to limited data from Padarn surgery for HbA_{1c} (n = 2) analysis was not conducted independently. HbA_{1c} decreased in Borth surgery (P = 0.00), Church surgery (P = 0.00), Llanilar surgery (P = 0.00), Tanyfron surgery (P = 0.00), Tregaron surgery (P = 0.00) and Ystwyth surgery (P = 0.00). When the data were pooled across surgeries a significant decrease in HbA_{1c} was observed (P = 0.00) (Table 2.2A and Figure 2.2). Of the 592 patients identified as pre-diabetic at baseline, 408 patients' HbA_{1c} decreased, 96 increased and 88 remained the same. Of the 408 patients who had a reduction in HbA_{1c}, 217 were below 42 mmol/mol, taking them below the threshold for pre-diabetes, 358 patients remained in the 'at risk' range while 17 patients developed T2D (Figure 2.3).

Table 2.2A. HbA_{1c}, Body Mass, BMI and Waist Circumference at baseline and 12 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting. Data are represented as median (range). Statistical significance* is accepted at $P < 0.05$. (**An HbA_{1c} of 112 mmol/mol provided by Ystwyth surgery at 12 months).

	Borth	Church	Llanilar	Padarn	Tanyfron	Tregaron	Ystwyth	Total
HbA_{1c} (mmol/mol)								
Number of Patients	93	106	99	2	54	115	123	592
Baseline	43 (5)	43 (5)	44 (5)		44 (5)	43 (5)	44 (5)	44 (5)
12 Months	43 (18)	42 (10)	42 (16)		42 (90)	42 (14)	43 (81)	42 (85)**
Significance	0.00*	0.00*	0.00*		0.00*	0.00*	0.00*	0.00*
Body Mass (kg)								
Number of Patients	65	103	92	8	55	94	94	511
Baseline	79.6 (88.5)	78 (93)	80.8 (73)	99.8 (69.4)	82 (98.5)	86.1 (87)	83 (100)	82 (114.6)
12 Months	79.2 (82.7)	75 (98)	81 (73)	100.6 (79.5)	83 (95)	85 (87)	81 (100)	81 (110)
Significance	0.03*	0.00*	0.98	0.44	0.13	0.33	0.11	0.00*
BMI (kg/m^2)								
Number of Patients	62	103	92	8	55	93	93	506
Baseline	28.1 (26.3)	28.3 (29)	28.9 (30.3)	38 (17.2)	28.6 (28.7)	30 (31.5)	29.5 (29)	29.0 (34.6)
12 Months	28.5 (25.9)	27.6 (29.4)	29.1 (28.6)	38.2 (21.3)	28.3 (26.8)	30 (24.2)	29.2 (32.6)	28.8 (36.3)
Significance	0.58	0.00*	0.96	0.31	0.30	0.40	0.89	0.00*
Waist Circumference (cm)								
Number of Patients	23	99	69	7	55	53		306
Baseline	104 (47)	99 (64)	100 (55)	124 (27)	104 (73)	101 (61)		100 (73)
12 Months	104 (46)	97 (69)	97 (66)	124 (37)	104 (74)	102 (51)		100 (81)
Significance	0.76	0.00*	0.00*	0.68	0.56	0.87		0.01*

Table 2.2B. Systolic BP and Diastolic BP at baseline and 12 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting. Data are represented as median (range). Statistical significance* is accepted at $P < 0.05$.

	Borth	Church	Llanilar	Padarn	Tanyfron	Tregaron	Ystwyth	Total
Systolic BP (mmHg)								
Number of Patients	58	97	62	8	55	84	112	476
Baseline	132 (108)	130 (60)	135 (56)	135 (45)	127 (52)	132 (90)	140 (93)	133 (110)
12 Months	130 (73)	130 (50)	132 (87)	143 (48)	132 (61)	134 (83)	137 (87)	132 (97)
Significance	0.72	0.41	0.98	0.39	0.90	0.15	0.36	0.81
Diastolic BP (mmHg)								
Number of Patients	58	97	62	8	55	84	112	476
Baseline	70 (48)	80 (53)	78 (43)	75 (51)	78 (34)	78 (50)	75 (46)	77 (70)
12 Months	70 (64)	80 (52)	79 (49)	80 (49)	78 (44)	78 (52)	80 (52)	78 (74)
Significance	0.15	0.76	0.02*	0.06	0.42	0.51	0.01*	0.01*

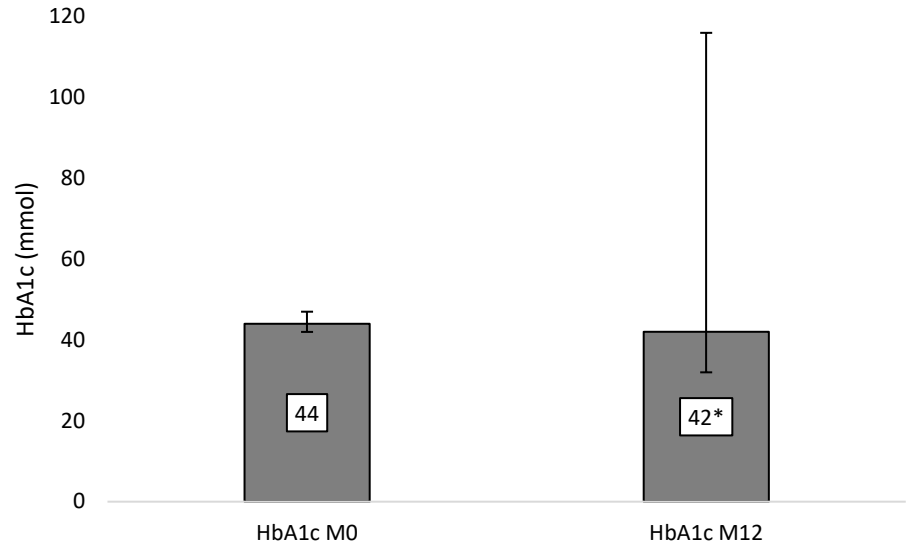


Figure 2.2. Pooled HbA_{1c} data from seven GP surgeries at baseline (M0) and 12 months (M12) following a 30-minute one-to-one consultation with a health care professional in a primary care setting. *Significantly different ($P < 0.05$) from baseline. Data are represented as median and range.

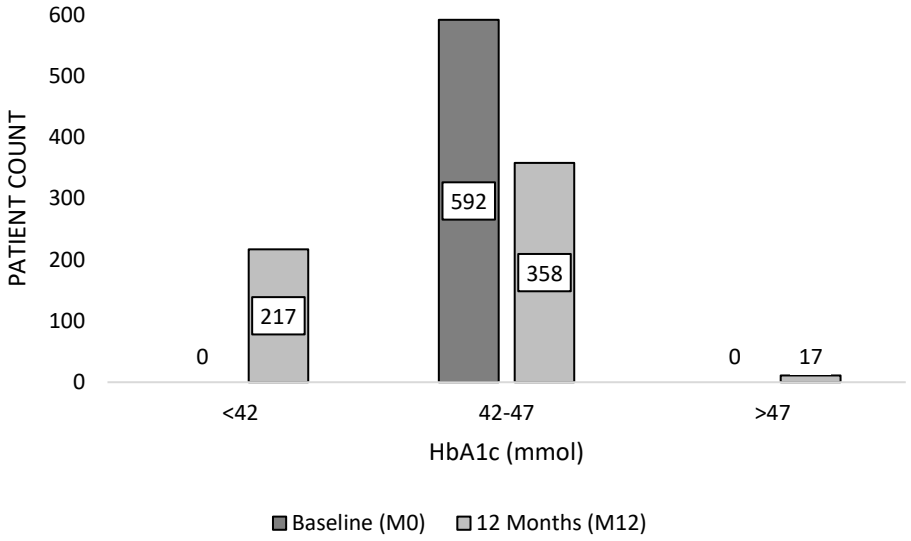


Figure 2.3. Patient numbers in each diabetes classification at baseline (M0) and 12 months (M12).

2.4.3. Baseline to 12-Month Body Mass & BMI

Body mass and BMI decreased significantly at 12 months in Church surgery (P = 0.00) and Borth surgery (P = 0.03) with no significant difference found in the remaining five surgeries (P > 0.05). Pooled body mass and BMI data from the seven surgeries revealed a significant change at 12 months (P = 0.00) (Table 2.2A and Figure 2.4A/B).

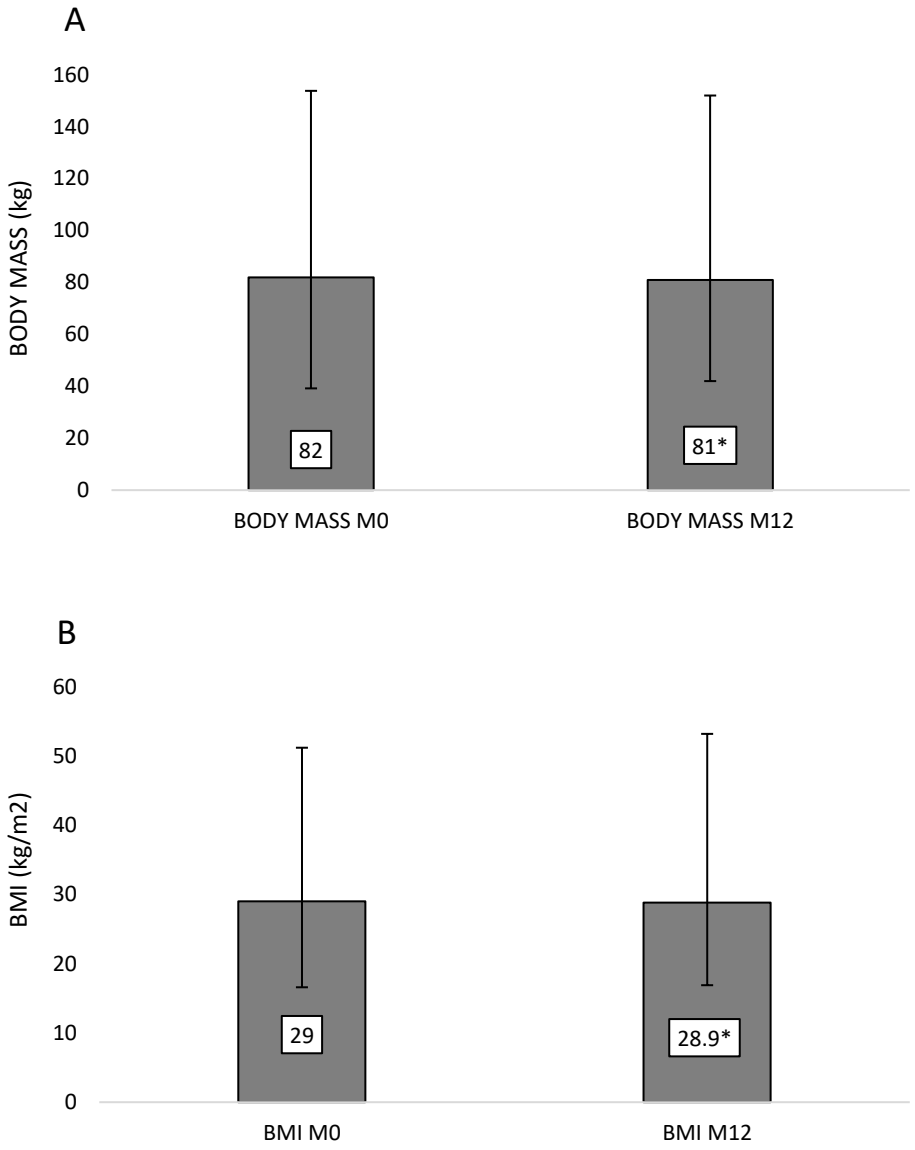


Figure 2.4A/2.4B. Pooled Body Mass (3A) and BMI (3B) data from seven GP surgeries at baseline (M0) and 12 months (M12) following a 30-minute one-to-one consultation with a health care professional in a primary care setting. *Significantly different (P < 0.05) from baseline. Data are represented as median and range.

2.4.4. Baseline to 12-Month Waist Circumference

Data for waist circumference from Ystwyth surgery was not provided and therefore analysis was not possible. Waist circumference decreased significantly in Church and Llanilar surgeries ($P = 0.00$) although no significant changes were found in the remaining four surgeries ($P > 0.05$). Analysis of pooled data for waist circumference revealed a significant difference ($P = 0.01$) (Table 2.2A and Figure 2.5).

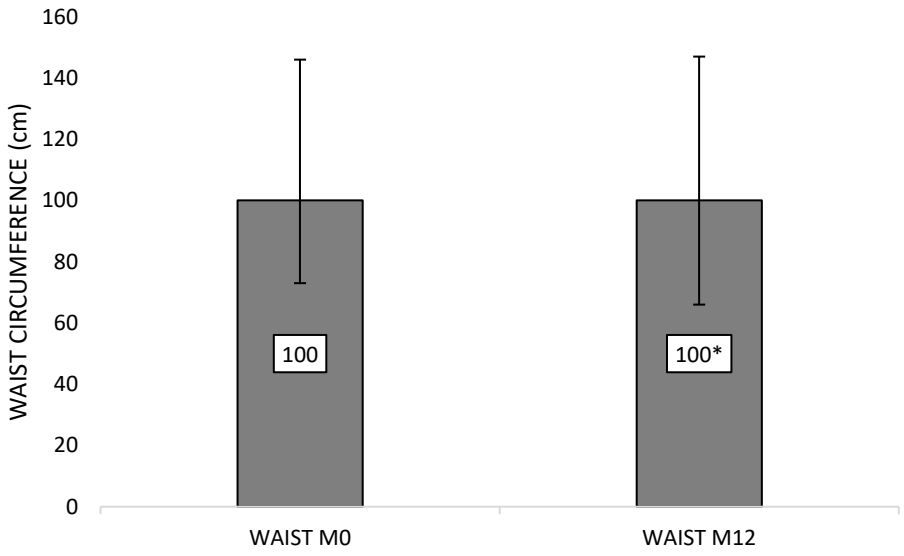


Figure 2.5. Pooled Waist Circumference data from six GP surgeries at baseline (M0) and 12 months (M12) following a 30-minute one-to-one consultation with a health care professional in a primary care setting. *Significantly different ($P < 0.05$) from baseline. Data are represented as median and range.

2.4.5. Baseline to 12-Month Blood Pressure

Systolic BP remained unchanged across all surgeries and when the data were combined (P > 0.05). Diastolic BP increased significantly in Llanilar (P = 0.02) and Ystwyth (P = 0.01) surgeries although remained unchanged in the remaining five (P > 0.05). Pooled data for diastolic BP demonstrated a significant increase (P = 0.01) (Table 2.2B and Figure 2.6).

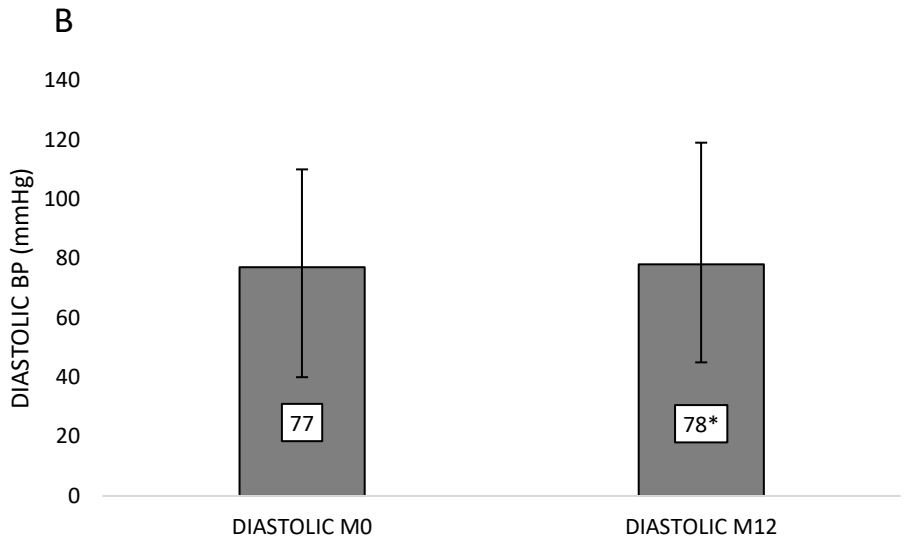


Figure 2.6. Pooled diastolic blood pressure data from six GP surgeries at baseline (M0) and 12 months (M12) following a 30-minute one-to-one consultation with a health care professional in a primary care setting. *Significantly different (P < 0.05) from baseline. Data are represented as median and range.

Table 2.3. Correlation Coefficients for change in HbA_{1c} and changes in Body Mass, BMI and Waist Circumference between baseline and 12 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting. [1] Pearson's Correlation Test; [2] Spearman's Correlation Test. Statistical significance* is accepted at $P < 0.05$.

	Borth	Church	Llanilar	Tanyfron	Tregaron	Ystwyth	Total
Change in Body Mass / Change in HbA_{1c}							
Correlation Coefficient	0.31 [1]	0.18 [2]	0.03 [2]	0.57 [1]	0.31 [2]	0.39 [2]	0.26 [2]
Significance	0.01*	0.06	0.72	0.00*	0.00*	0.00*	0.00*
Change in BMI / Change in HbA_{1c}							
Correlation Coefficient	0.18 [2]	0.21 [2]	0.04 [2]	0.54 [1]	0.30 [2]	0.36 [2]	0.26 [2]
Significance	0.16	0.03*	0.64	0.00*	0.00*	0.00*	0.00*
Change in Waist Circumference / Change in HbA_{1c}							
Correlation Coefficient	0.09 [2]	0.19 [2]	0.05 [2]	0.29 [1]	0.00 [2]		0.13 [2]
Significance	0.65	0.05	0.64	0.02*	0.96		0.02*

2.4.6. Baseline to 12-Month Correlation Analysis

Significant correlations were found between body mass changes and HbA_{1c} changes in Borth (r = 0.31, *P* = 0.01), Tanyfron (r = 0.57, *P* = 0.00), Tregaron (r = 0.31, *P* = 0.00) and Ystwyth surgeries (r = 0.39, *P* = 0.00) and when analysed as a total cohort (r = 0.26, *P* = 0.00) (Table 2.3 and Figure 2.7). Significant correlations between changes in BMI and changes in HbA_{1c} were found in Church (r = 0.21, *P* = 0.03), Tanyfron (r = 0.54, *P* = 0.00), Tregaron (r = 0.30, *P* = 0.00), Ystwyth (r = 0.36, *P* = 0.00) and when analysed as a total cohort (r = 0.26, *P* = 0.00) (Table 2.3 and Figure 2.8). Lastly, a significant correlation between change in waist circumference and change in HbA_{1c} was found in Tanyfron surgery (r = 0.29, *P* = 0.02) and when the data were combined (r = 0.13, *P* = 0.02) (Table 2.3 and Figure 2.9).

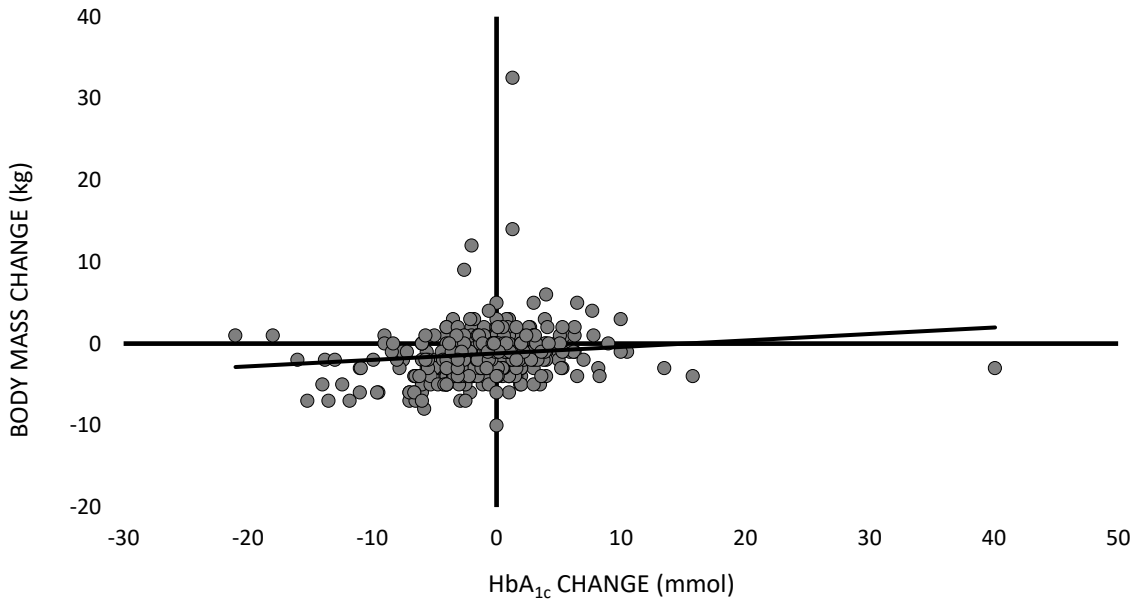


Figure 2.7. Correlation of pooled body mass change and HbA_{1c} change between baseline and 12 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting.

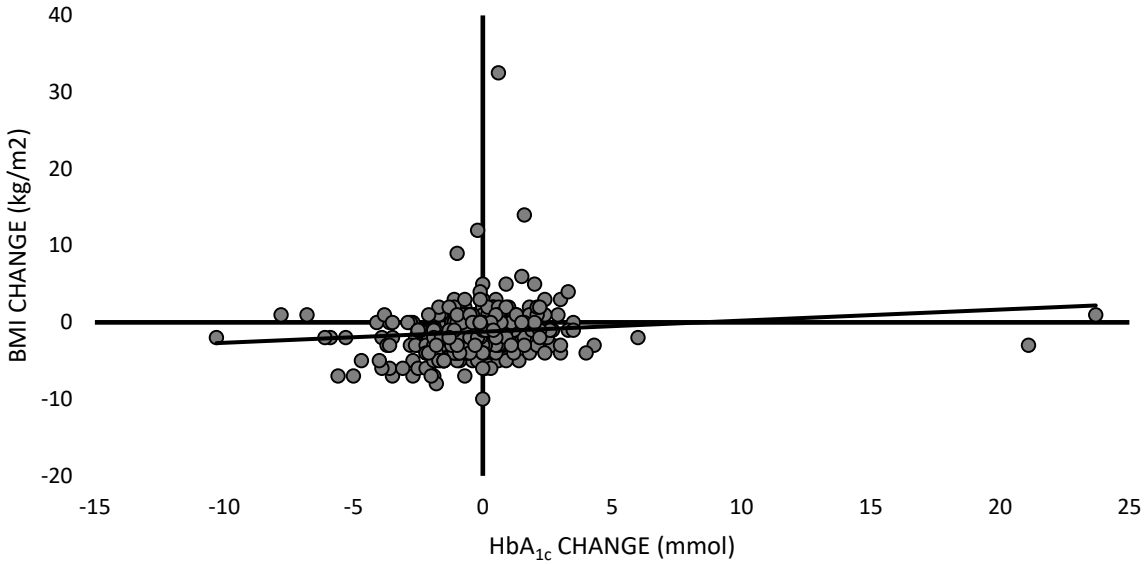


Figure 2.8. Correlation of pooled BMI change and HbA_{1c} change between baseline and 12 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting.

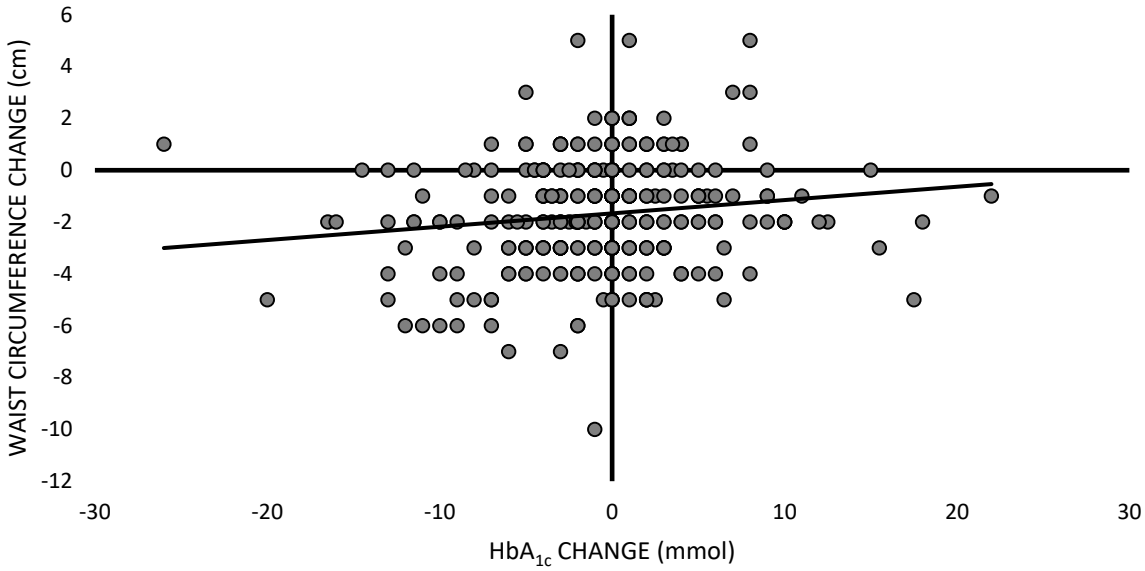


Figure 2.9. Correlation of pooled waist circumference change and HbA_{1c} change between baseline and 12 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting.

2.4.7. Baseline, 12- and 24-Month Follow Up

Between April and July 2018 data were extracted from the patient records for all surgeries, data for baseline, 12- and 24-month follow-up were available for 292 patients, Padarn surgery did not provide 24-month data.

2.4.8. Baseline, 12- and 24-Month HbA_{1c}

There were significant changes in HbA_{1c} across the 24-month follow-up period in all surgeries except for Tanyfron. Significant differences in HbA_{1c} were found in Borth surgery ($P = 0.01$), with post-hoc analysis revealing decreases between baseline and 12 months ($P = 0.00$) but no significant difference between baseline and 24 months ($P = 0.21$), HbA_{1c} between 12 and 24 months remained similar ($P = 0.20$). HbA_{1c} decreased significantly in Church surgery ($P = 0.00$), post-hoc tests revealed decreases between baseline and 12 months ($P = 0.00$) and baseline and 24 months ($P = 0.00$), values between 12 months and 24 months remained similar ($P = 0.73$). Analysis of Llanilar surgery patients revealed significant changes in HbA_{1c} ($P = 0.00$), post-hoc testing showed HbA_{1c} decreased from baseline to 12 months ($P = 0.00$) and baseline to 24 months ($P = 0.00$), values did not change between 12 and 24 months ($P = 0.17$). Values from Tregaron surgery demonstrated an overall change in HbA_{1c} ($P = 0.00$) decreasing between baseline and 12 months ($P = 0.00$) and baseline and 24 months ($P = 0.00$) with values remaining similar between 12 and 24 months ($P = 0.94$). HbA_{1c} values in patients from Ystwyth surgery changed significantly ($P = 0.03$) also, a decrease between baseline and 24 months was found ($P = 0.04$) although differences between baseline and 12 months ($P = 0.14$) and 12 and 24 months ($P = 0.93$) were not significant. When HbA_{1c} was analysed as a total cohort, analysis indicated a significant change in the data ($P = 0.00$), pooled HbA_{1c} differed significantly between baseline and 12 months ($P = 0.00$), and between baseline and 24 months ($P = 0.00$) but remained unchanged between 12 and 24 months ($P = 0.13$) (Table 2.4A and Figure 2.10).

At 24 months following the one-to-one consultation 119 of the 292 patients had reverted to normal HbA_{1c} (41%), 154 remained categorised as pre-diabetic (53%) and 19 developed T2D (6%) (Figure 2.11).

Table 2.4A. HbA_{1c}, Body Mass, BMI and Waist Circumference at baseline, 12 and 24 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting. Parametric data are represented as mean ± standard deviation. Non-parametric data are represented as median (range). Statistical significance* is accepted at $P < 0.05$. (**An HbA_{1c} of 112 mmol/mol provided by Ystwyth surgery at 12 months).

	Borth	Church	Llanilar	Tanyfron	Tregaron	Ystwyth	Total
HbA_{1c} (mmol/mol)							
Number of Patients	21	75	80	20	56	40	292
Baseline	44 (5)	44 (5)	44 (5)	44 (5)	42 (5)	45 (5)	44 (5)
12 Months	43 (5)	42 (10)	42 (15)	43 (8)	42 (10)	43 (78)	42 (84)**
24 Months	43 (27)	42 (17)	42 (12	44 (24)	42 (14)	44 (27)	42 (35)
Significance	0.01*	0.00*	0.00*	0.20	0.00*	0.03*	0.00*
Body Mass (kg)							
Number of Patients	12	67	67		39	31	216
Baseline	81.6 ± 22.6	76 (65)	83 (73)		86 ± 15.5	79.1 (73.4)	81.7 (94.2)
12 Months	80.2 ± 22.3	74 (63)	82 (73)		86.4 ± 14.1	78.6 (81.8)	81 (94.7)
24 Months	79.8 ± 24.2	74 (64)	83.5 (75)		87.1 ± 14.3	77.5 (81.3)	81.9 (100.9)
Significance	0.36	0.00*	0.50		0.28	0.00*	0.07
BMI (kg/m^2)							
Number of Patients	11	67	68		39	31	216
Baseline	29.9 ± 5.8	28.4 (23.7)	28.5 (28.1)		29.5 ± 4.6	32.0 ± 5.5	29.3 (32)
12 Months	29.7 ± 5.2	27.1 (23)	29 (26.1)		29.4 ± 4.2	31.3 ± 5.9	29.0 (27.9)
24 Months	30.0 ± 6.6	27.6 (23.5)	28.8 (28.2)		29.6 ± 4.4	30.8 ± 5.6	28.9 (32.8)
Significance	0.93	0.00*	0.51		0.23	0.00*	0.00*
Waist Circumference (cm)							
Number of Patients		57	26	16	17		116
Baseline		96.9 ± 10.4	102.5 ± 12.5	104.6 ± 20.6	102 (53)		99 (73)
12 Months		95.7 ± 10.7	98.4 ± 13.2	106.6 ± 21.7	105 (47)		98 (74)
24 Months		94.7 ± 10.9	98.8 ± 14.0	104.0 ± 20.0	102 (50)		97 (78)
Significance		0.00*	0.03*	0.18	0.05		0.00*

Table 2.4B. Systolic BP and Diastolic BP at baseline, 12 and 24 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting. Parametric data are represented as mean ± standard deviation. Non-parametric data are represented as median (range).

	Borth	Church	Llanilar	Tanyfron	Tregaron	Ystwyth	Total
Systolic BP (mmHg)							
Number of Patients	11	66	44	20	36	34	211
Baseline	136 ± 16	130 (50)	135 (86)	128 ± 15	132 (60)	141 ± 15	132 (76)
12 Months	136 ± 9	130 (50)	132 (87)	132 ± 12	135 (57)	139 ± 14	133 (87)
24 Months	135 ± 15	130 (89)	131 (38)	133 ± 13	136 (54)	137 ± 15	132 (89)
Significance	0.91	0.24	0.51	0.39	0.34	0.55	0.80
Diastolic BP (mmHg)							
Number of Patients	11	66	44	20	36	34	211
Baseline	72 (16)	80 (53)	76 ± 9	79 ± 7	76 ± 8	77 ± 9	78 (54)
12 Months	80 (44)	79 (52)	79 ± 9	78 ± 7	76 ± 8	80 ± 10	80 (57)
24 Months	80 (31)	75 (40)	77 ± 7	76 ± 9	77 ± 9	79 ± 11	78 (52)
Significance	0.26	0.11	0.09	0.25	0.65	0.25	0.33

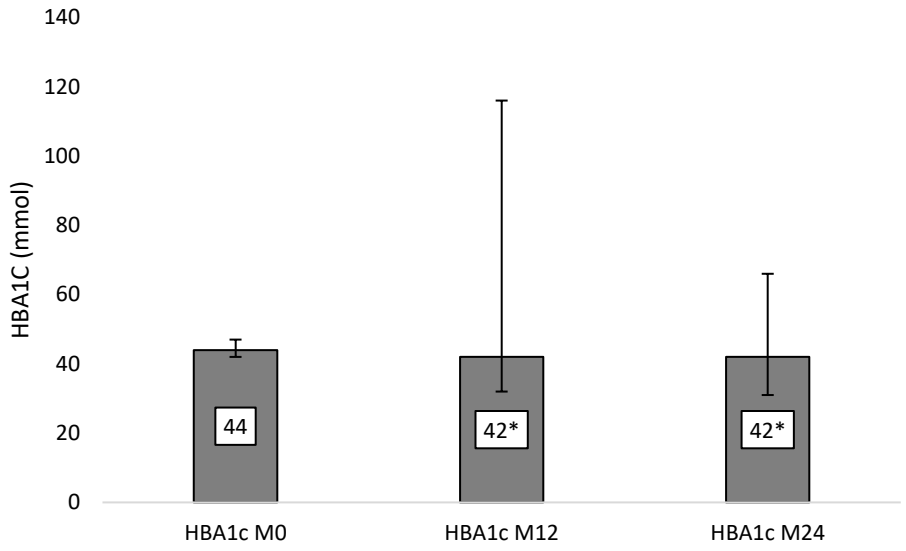


Figure 2.10. Pooled HbA_{1c} data from six GP surgeries at baseline (M0), 12 months (M12) and 24 months (M24) following a 30-minute one-to-one consultation with a health care professional in a primary care setting. *Significantly different ($P < 0.05$) from baseline.

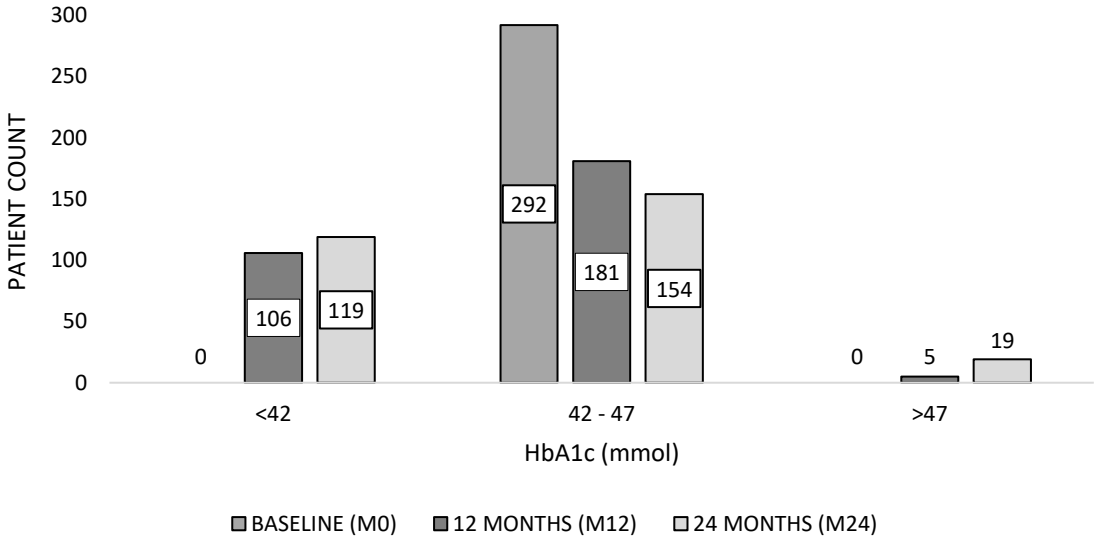


Figure 2.11. Patient numbers in each diabetes classification at baseline (M0), 12 months (M12) and 24 months (M24).

2.4.9. Baseline, 12- and 24-Month Body Mass & BMI

Data from Church surgery patients demonstrated a significant decrease in body mass (P = 0.00), with post-hoc analysis indicating body mass decreased between baseline and 12 months and baseline and 24 months (P = 0.00) while values between 12 and 24 months were unchanged (P = 1.00). A significant decrease was present in Ystwyth surgery patients (P = 0.00) where values decreased between baseline and 12 months (P = 0.04) and baseline and 24 months (P = 0.00) while remaining similar between 12 and 24 months (P = 0.15). No significant changes in body mass were found in Borth, Llanilar and Tregaron surgeries or when the data were pooled (P > 0.05) (Table 2.4A). Twenty-four-month body mass data were not provided by Tanyfron surgery.

In Church surgery patients BMI changed significantly (P = 0.00) with a significant decrease between baseline and 12 months and baseline and 24 months (P = 0.00) and no difference between 12 and 24 months (P = 0.76). Data from Ystwyth surgery indicated a significant decrease in BMI between baseline and 24 months (P = 0.00). No significant changes in BMI were found in Borth, Llanilar or Tregaron surgeries (P > 0.05). Twenty-four-month BMI data were not provided by Tanyfron surgery. Pooled BMI data indicated a significant difference (P = 0.00) with a significant decrease in BMI between baseline and 12 months (P = 0.01) and baseline and 24 months (P = 0.00), no significant differences were found between 12 and 24 months (P = 0.89) (Table 2.4A and Figure 2.12).

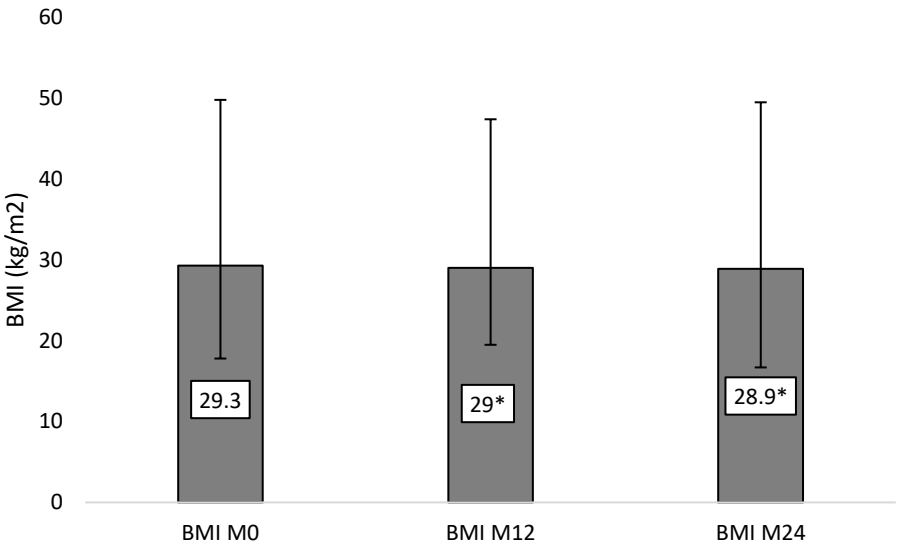


Figure 2.12. Pooled BMI data from five GP surgeries at baseline (M0), 12 months (M12) and 24 months (M24) following a 30-minute one-to-one consultation with a health care professional in a primary care setting. *Significantly different ($P < 0.05$) from baseline.

2.4.10. Baseline, 12- and 24-Month Waist Circumference

Waist circumference differed significantly in patients from Church surgery ($P = 0.00$) with a significant decrease from baseline to 24 months ($P = 0.00$) although differences between baseline and 12 months and 12 and 24 months were insignificant ($P > 0.05$). Similarly, waist circumference decreased in Llanilar surgery patients ($P = 0.03$), with a significant decreased from baseline to 12 months ($P = 0.02$) and from baseline and 24 months ($P = 0.03$), values between 12 and 24 months were similar ($P = 1.00$). No significant changes in waist circumference were found in Tanyfron or Tregaron surgeries ($P > 0.05$). Borth and Ystwyth surgeries did not provide 24-month waist circumference data. Analysis on pooled data for waist circumference revealed a significant overall decrease between baseline and 24 months ($P = 0.00$). Values decreased, albeit insignificantly, between baseline to 12 months ($P = 0.11$) and continued to decrease between 12 and 24 months ($P = 0.01$) (Table 2.4A and Figure 2.13).

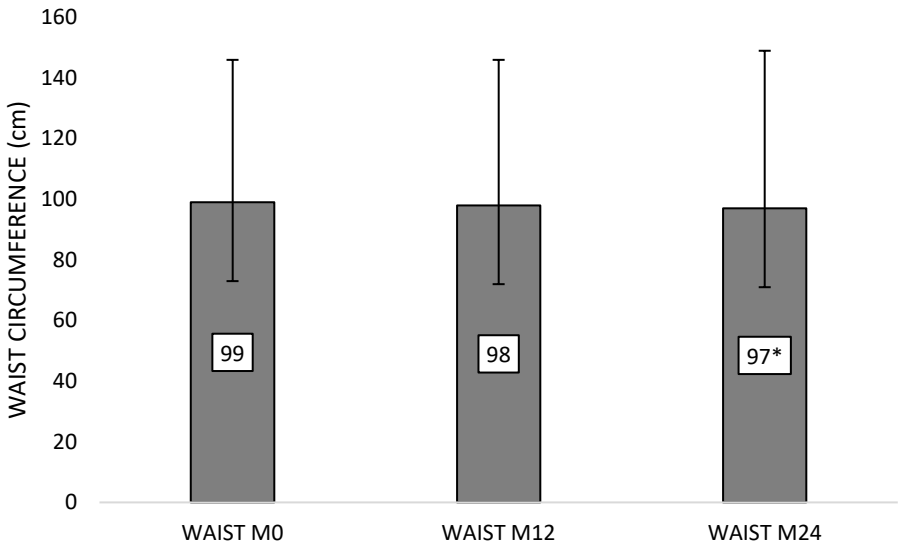


Figure 2.13. Pooled Waist Circumference data from four GP surgeries at baseline (M0), 12 months (M12) and 24 months (M24) following a 30-minute one-to-one consultation with a health care professional in a primary care setting. *Significantly different ($P < 0.05$) from baseline.

2.4.11. Baseline, 12- and 24-Month Blood Pressure

Across 24 months, systolic and diastolic BP remained unchanged across all surgeries and when analysed as a total cohort ($P > 0.05$) (Table 2.4B).

2.4.12. Baseline and 24 Month Correlation Analysis

Significant correlations between change in body mass and change in HbA_{1c} were found in Borth ($P = 0.00$), Church ($P = 0.01$) and Ystwyth ($P = 0.00$) surgeries and when analysed as a total cohort ($P = 0.00$) (Table 2.5 and Figure 2.14). For change in BMI and change in HbA_{1c}, significant correlations were found in Church surgery ($P = 0.02$) and in the total cohort ($P = 0.00$) (Table 2.5 and Figure 2.15). Similarly, significant correlations were found in change in waist circumference and change in HbA_{1c} in Church surgery ($P = 0.03$) and as a total cohort ($P = 0.02$) (Table 2.5 and Figure 2.16).

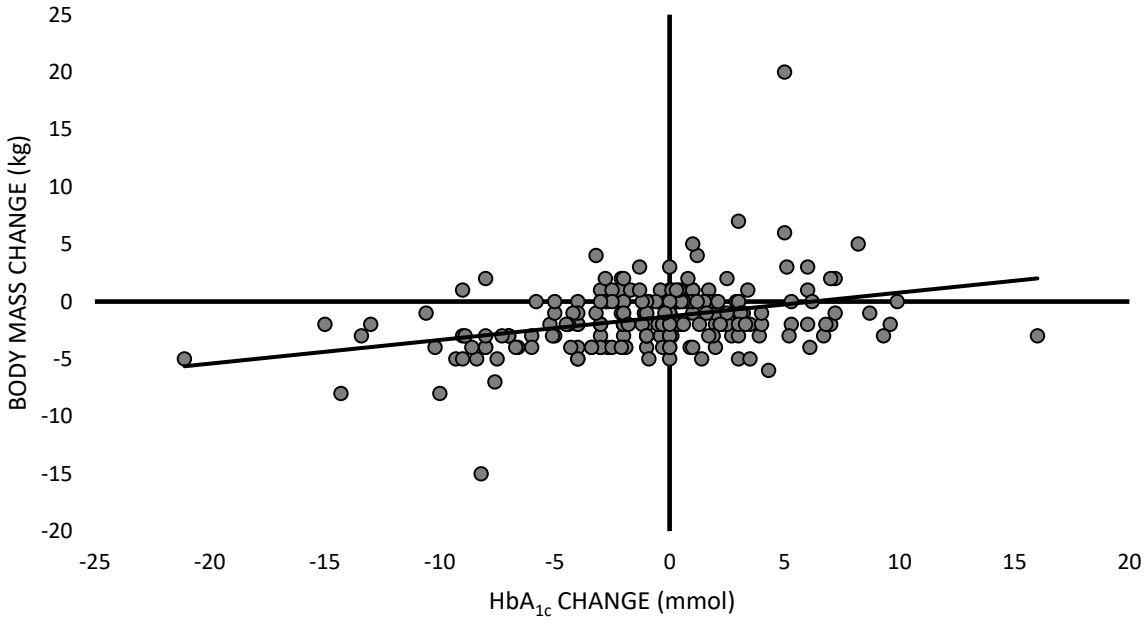


Figure 2.14. Correlation of combined body mass change and HbA_{1c} change between baseline and 24 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting.

Table 2.5. Correlation Coefficients for change in HbA_{1c} and changes in Body Mass, BMI and Waist Circumference between baseline and 24 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting. Pearson's Correlation Test represented by [1] and Spearman's Correlation Test represented by [2]. Statistical significance* is accepted at $P < 0.05$.

	Borth	Church	Llanilar	Tanyfron	Tregaron	Ystwyth	Total
Change in Body Mass / Change in HbA_{1c}							
Correlation Coefficient	0.74 [2]	0.30 [2]	0.20 [2]		0.19 [2]	0.47 [2]	0.30 [2]
Significance	0.00*	0.01*	0.09		0.24	0.00*	0.00*
Change in BMI / Change in HbA_{1c}							
Correlation Coefficient	0.48 [2]	0.28 [2]	0.20 [2]		0.10 [2]	0.25 [2]	0.23 [2]
Significance	0.05	0.02*	0.09		0.54	0.18	0.00*
Change in Waist Circumference / Change in HbA_{1c}							
Correlation Coefficient		0.27 [2]	0.04 [2]	0.49 [2]	0.01 [1]		0.19 [2]
Significance		0.03*	0.80	0.05	0.94		0.02*

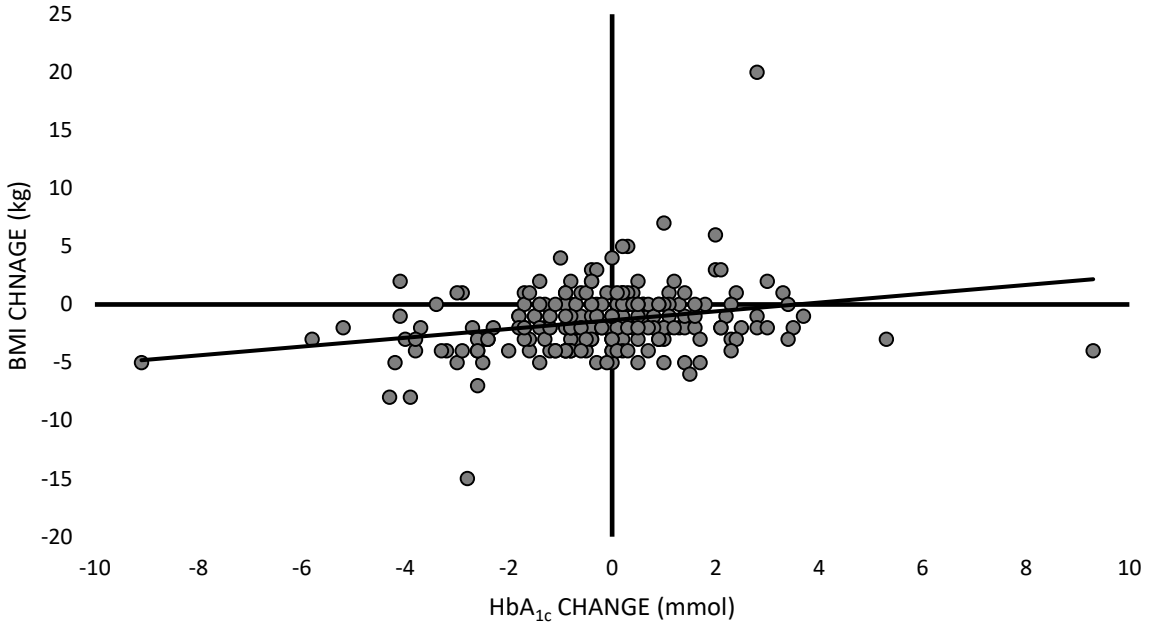


Figure 2.15. Correlation of combined BMI change and HbA_{1c} change between baseline and 24 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting.

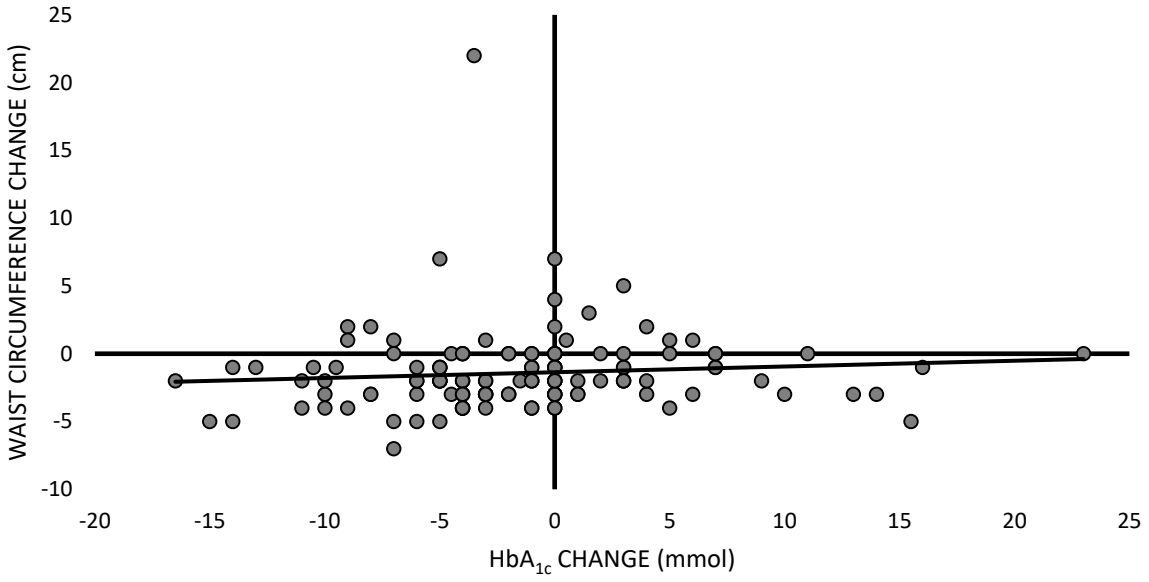


Figure 2.16. Correlation of combined waist circumference change and HbA_{1c} change between baseline and 24 months following a 30-minute one-to-one consultation with a health care professional in a primary care setting.

2.5. Discussion

The purpose of the one-to-one consultation was to reduce the onset of T2D in a group of at-risk individuals through their engagement in a GP-led pre-diabetes intervention in seven surgeries in Ceredigion, Wales. Analysis of follow up data at 12 and 24 months in patients exposed to the intervention demonstrated a significant overall decrease in HbA_{1c}, a key variable associated with increased risk of T2D. The findings compare favourably to other published work which has examined the effects of lifestyle intervention on T2D development in individuals at increased risk of the condition. Previous studies examining the role of lifestyle intervention in managing T2D incidence, for example the DPP Research Group (2002), Almeida, Shetterly, Smith-Ray and Estabrooks (2010), Yates, Davies, Sehmi, Gorely and Khunti, (2011), Gray *et al*. (2012) and Piper, Marossy, Grffiths and Adegboye (2017) have all successfully demonstrated significant health outcomes following lifestyle intervention. Previous reviews state lifestyle intervention to be at least as effective as drug treatment in combating progression to T2D (Gillies *et al*., 2007). Additionally, Sheng *et al*. (2019) found lifestyle modification, involving improved nutrition, increases in exercise level and weight loss contributed to health by reducing BMI, body weight, waist and hip circumference, systolic and diastolic pressure, fasting, and two-hour postprandial blood glucose, total cholesterol and increasing HDL. Given the significant impact of lifestyle intervention on a wide range on health outcomes related to T2D as well as other health complications associated to obesity, physical inactivity and poor dietary behaviour including cardiovascular disease, cancer, lung and liver disease, it is credible to argue that lifestyle intervention has a wider ranging impact on health compared to pharmacological intervention alone when targeting T2D. While numerous lifestyle intervention programmes have been conducted previously, this is the first study to evaluate the efficacy of a GP-led pre-diabetes intervention, which involves a 30-minute one-to-one consultation targeting lifestyle modification, in primary care.

The primary outcome measure of this evaluation was HbA_{1c}, which is an indication of the amount of glucose present in the blood over the previous eight to 12-week period and provides a more stable indication of blood glucose over time compared to other methods such as a fasting blood glucose test or an OGTT. An HbA_{1c} between 42–47 mmol/mol is indicative of 'high risk' for T2D and therefore the intervention's primary aim was to identify individuals within this range and reduce their HbA_{1c} through lifestyle modification, which has previously been demonstrated to be effective. The data presented demonstrate significant decreases in HbA_{1c} when analysed as individual surgeries as well as when the data were pooled. In many cases, participation in the intervention led to HbA_{1c} decreasing to values below the range considered to increase the risk of T2D development. The data for HbA_{1c} recorded post intervention reached values considered to be healthy by NHS Wales, Public Health Wales and Diabetes UK in 37% of cases after 12 months (217 of 592 participants) and 41% of cases after 24 months (119 of 292 participants). Conversely, 3% (17 of 592 participants) and 6.5% (19 of 292 participants) had HbA_{1c} values which fell in the diabetes range after 12 and 24 months, respectively. These numbers compare favourably with Tabak *et al*. (2012) who report that approximately 5-10% of unaddressed cases of pre-diabetes will progress to T2D within 12 months, while a similar percentage will revert to normal control.

The primary risk factors associated with pre-diabetes and increased HbA_{1c} are; obesity (BMI > 30 kg/m^2), large waist circumference (> 94 cm males / > 80 cm females) and high blood pressure (> 140 / 90 mmHg; Falade, 2012). Therefore, these variables were recorded during the initial 30-minute one-to-one consultation and after 12 and 24 months to evaluate the intervention's effect on each risk factor and identify if any changes were associated with decreases in HbA_{1c}. Significant overall decreases in pooled data for body mass, BMI and waist circumference after 12 months and BMI and waist circumference after 24 months support other

work in the literature with similar findings, although the clinical significance of BMI changes in particular is debateable. Examples include significant decreases in body mass (Absetz *et al*., 2007; Ackermann, Finch, Brizendine, Zhou and Marrero, 2008; Almeida, Shetterly, Smith-Ray and Estabrooks, 2010), BMI (Absetz *et al*., 2007) and waist circumference (Katula *et al*., 2011) 12 to 24 months following intervention and baseline data collection. In five practices, and in pooled data, correlations were evident between changes in HbA_{1c} and anthropometric measures after 12 months, demonstrating the potential effectiveness between reductions in body mass, BMI and waist circumference with decreases in HbA_{1c} with a similar trend evident at 24 months in three surgeries and in the pooled data. Excess body fat is strongly associated with impaired glycaemic control due to ineffective insulin signalling (Klein *et al*., 2004) while weight loss has been demonstrated to lessen insulin resistance and improve glycaemic control (Peterson, Dufour, Morino, Yoo, Cline and Shulman, 2012; Camps, Verhoef and Westerterp, 2016). Evidence in a review produced by Gummesson, Nyman, Knutsson and Karpeors (2017) shows a relationship between weight loss and HbA_{1c} reduction, finding HbA_{1c} values decreased by 0.1 percentage points for every 0.1 kg lost when studies focussing on weight loss and HbA_{1c} were examined. Similar findings suggest weight loss and decreased waist circumference are associated with improved HbA_{1c} values in obese patients who underwent an intensive weight management programme (Rothberg *et al*., 2017).

Decreases in HbA_{1c} were positively correlated with decreases in body mass, BMI and waist circumference in individual surgeries and pooled data. In other cases, however, decreases in HbA_{1c} occurred independently of changes in anthropometric measures. A possible explanation for this finding may be attributed to increased levels of physical activity undertaken by the patient. The benefits of physical activity in improving health outcomes were discussed as part of the 30-minute one-to-one consultation. Patients were encouraged to increase their level of

weekly physical activity to 150 minutes of moderate intensity exercise, 75 minutes of vigorous intensity exercise or a combination of both intensities plus additional resistance exercise. In many cases, patients were also referred to classes which specialised in supervised exercise provision, such as NERS. Previous work has demonstrated significant improvements in insulin sensitivity following exercise, independent of significant changes in body mass (Duncan, Perri, Theriaque, Hutson, Eckel and Stacpoole, 2003; Nassis *et al*., 2005). The effects of increased exercise induced GLUT4 translocation through increased skeletal muscle contraction support this notion (Lund, Holman, Schmitz and Pedersen, 1995). The acute effects of exercise on glucose metabolism are also well documented. Duncan, Perri, Theriaque, Hutson, Eckel and Stacpoole (2003) found insulin sensitivity improved significantly following six months of walking training in previously sedentary individuals with the improvement in insulin sensitivity occurring in the absence of weight loss. Ross (2003) supports this finding, suggesting modest exercise is associated with significant improvements in glucose and lipid metabolism and should be performed on a regular basis. Further evidence found 12 weeks of aerobic training improved insulin sensitivity in overweight and obese subjects without changes in body mass or percentage body fat (Nassis *et al*., 2006).

While the present intervention aimed to significantly reduce anthropometric values across all surgeries, this data may demonstrate the importance of increasing levels of physical activity in patients with pre-diabetes, even in the absence of weight loss. The data may also suggest that patients engaged more in physical activity compared to improving dietary habits given HbA_{1c} values improved despite lack of body weight decreases in some surgeries. While patients were asked to provide an indication as to their physical activity and dietary habits during the initial consultation visit, this information was not followed up at 12 and 24 months by surgeries, nor were the initial questionnaire responses provided for analysis. To gain a thorough

understanding as to the specific reasons why HbA_{1c} values improved, further work within this cohort must collect data on change in physical activity and dietary behaviours. Accurate methods for recording physical activity can be achieved using electronic activity trackers while modern methods for dietary recording, including analysis of biological samples (e.g. urine and/or plasma), have been developed (Favé *et al*., 2011, Beckmann *et al*., 2013 and Lloyd *et al*., 2013). Assessment of both behaviours can also utilise self-report questionnaire although issues of reliability of such practices may be a limiting factor (Wrieden *et al*., 2003). While the 'Starting the Conversation' questionnaire for diet and IPAQ-SF for physical activity were easily administered to patients and provided some insight into patient behaviour, the information provided is both limited in depth and prone to respondent bias (Lee, McDowell, Leung, Lam and Stewart, 2011). Accurate recording of dietary and physical activity behaviours using enhanced methodologies will contribute knowledge as to the primary reasons for improved glycaemic control following lifestyle intervention initiatives and may help to inform future directions for primary care lifestyle modification programmes in pre-diabetes.

This work demonstrates a feasible and potentially effective method for lifestyle modification in pre-diabetes resulting in decreased HbA_{1c} values; however, there are some limitations within the present study which require discussion and further development prior to future research being undertaken. Primarily, whilst efforts were made to standardise the 30-minute one-to-one consultation as much as possible to ensure all patients received the same level of intervention and care, it is likely individual GP surgeries, and specifically the health professionals delivering the consultations, adopted different approaches when consulting with patients. While it is acknowledged that consultations will vary from one another depending on the patient, some surgeries demonstrated greater changes in HbA_{1c} and anthropometry in comparison to others with the differences in values possibly represented by altered approaches to the consultation

by the health professional. For example, patients from Church surgery demonstrated significant decreases in values for HbA_{1c}, weight, BMI and waist circumference whereas patients from Llanilar surgery only recorded reductions in HbA_{1c} and waist circumference. These results may infer conflicting focusses during the consultation and possible uses of motivational language and techniques by a particularly inspiring health professional. This may suggest a more robust structure for the consultation is required to achieve similar results across the entire patient cohort. Borelli (2011) states treatment fidelity is key to making conclusive statements about treatment effects. The health practitioners delivering the consultation must be of equal ability to ensure a consistent delivery of the T2D intervention across all participating surgeries. Should the responsibility of consultation delivery be shared between general practitioners and nurse practitioners, both should have specific and appropriate training in delivering T2D lifestyle interventions. Standardised protocols for pre-diabetes discussion should be developed to help counter this limitation in the future as well as co-ordinated training days for all health professionals involved in consultation delivery.

Inconsistencies in data collection and delivery were also an issue. Despite protocols being developed and agreed between the cluster GP surgeries and the research team prior to the initiation of the intervention, some surgeries failed to collect all the data requested by the research team and in the case of one surgery, did not deliver the results as requested and were therefore not included in large sections of data analysis. Furthermore, lack of data regarding attendance to the National Exercise Referral Scheme and Foodwise for Life programme makes it difficult to understand the potential benefits of lifestyle referral in pre-diabetes within this cohort. While the research team repeatedly sought this information from the cluster, it was not possible to receive for reasons outside of the researcher's control. Improved systems are required which communicate patient attendance between lifestyle referral programmes and

primary care which can be placed on patients' medical records in the same way other data are (e.g. HbA_{1c} results, body mass, BMI etc.). Whilst one surgery provided information on referral of patients to lifestyle programmes, confirmed attendance figures were not provided and therefore statistical analysis of such data were not possible.

In addition, while a number of significant results were demonstrated in the current work, it is important to consider the clinical relevance of these findings also. Following analysis of year one follow up data, a number of significant results were observed including HbA_{1c} and body mass in Borth surgery. Despite these data providing a *P* value lower than 0.05, it is clear they are clinically insignificant findings. For HbA_{1c} in Borth surgery, values remained similar between baseline and one year (43 mmol/mol) while body mass only decreased by 0.4 kg. Similarly, combined totals for BMI between baseline and one year only decreased by 0.2 kg/m^2. In addition, after year two follow up, Tregaron surgery observed a significant result for HbA_{1c} when in fact values remained at 42 mmol/mol throughout the intervention. Clinical significance should reflect the extent of change, whether the change makes a real difference to patient lives and how long the effects last (Ranganathan, Pramesh and Buyse, 2015). When considering this statement, it is clear the results discussed here do not meet the definition of clinically significant and should be treated with caution in the wider evaluation of the intervention.

Lastly, a key factor in the future implementation of this intervention is its cost effectiveness. Currently, no formal financial assessment has been undertaken to determine the cost of the intervention per patient, with only estimated costs available. Should further work demonstrate this intervention to be a success, it is crucial a financial assessment is conducted to support the intervention's implementation at a national level. Demonstrating cost effectiveness will show the wider implications of lifestyle intervention on health outcomes and associated costs

compared to pharmacological-only approaches, such as metformin. For example, Herman *et al*. (2009) found a lifestyle intervention targeting T2D prevention was cost-effective in all age groups while a metformin intervention did not represent good use of resources for persons older than 65 years of age. A similar cost effectiveness finding for the current programme would be advantageous in promoting the use of the current intervention in future primary care settings. The results of this work demonstrate promise in reducing T2D prevalence in at risk groups, however, given the design of the study as a cohort observation, it is difficult to identify the primary reasons for reduction in HbA_{1c} and associated risk factors. Potential drivers of behaviour change may have included; the initial letter confirming T2D risk and accompanying information and/or the information received during the one to one consultation. Future work must include a randomised control trial to assess the effectiveness of the consultation in comparison to current practice in pre-diabetes care in Wales (written information only). Greater understanding of physical activity and dietary practices in at risk groups also needs to be explored to assess the effects of the consultation and/or standard care on these lifestyle behaviours. Therefore, the latter chapters of this thesis will explore methods of dietary monitoring using urine sampling as a robust method for quantifying consumption of foods of importance to health using biomarker identification. The feasibility of implementing a randomised control trial will then be developed and delivered within primary care to compare the effectiveness of the consultation to current standard practice and assess if a large-scale trial can be conducted successfully across the HDUHB. Physical activity recording (using activity trackers) and dietary monitoring by urine and plasma sampling will be utilised within the randomised control trial to assess the consultation's effect on physical activity and nutritional behaviours.

In conclusion, this chapter has demonstrated the potential effectiveness of a 30-minute one-to-

one consultation with a healthcare professional in lowering HbA_{1c} values in patients at increased risk of developing T2D. Future work needs to establish the precise mechanisms driving behaviour change in pre-diabetes using robust study designs and data collection processes.

Chapter Three:

Literature Review: Dietary Monitoring Methods

3.1. Dietary Monitoring Methods

The findings from Chapter two suggest engagement in a GP-led lifestyle modification intervention can result in a reduction in HbA_{1c} and improvement in other outcomes related to risk of developing T2D. However, one limitation identified during the evaluation of the intervention was the lack of accurate monitoring of healthy behaviours, specifically diet. While information on participants' dietary intake was sought at the start of the intervention by the healthcare professional delivering the consultation, this information was not followed up at 12 or 24 months. Additionally, the questionnaire used (Starting the Conversation) was both short and broad in its line of questioning and therefore unable to examine dietary behaviour in persons at risk of T2D in detail. While questionnaires such as 'Starting the Conversation' are easily and quickly administered and simply analysed, they lack the depth and detail required to provide accurate and specific information on dietary behaviour and are prone to reporting bias. It is important to obtain reliable information on dietary intake as there is a robust evidence base which links diet with the risk of developing a number of chronic medical conditions, including T2D (Bingham, 2002). Good dietary behaviours, including intake of antioxidant vitamins, fat and plant polysaccharides, vegetables and fruit, are linked with a decreased incidence of chronic conditions (Bingham, 2002). To understand the risk of disease development in free living individuals accurate measures of habitual dietary intake are necessary, something that is described by Bingham (2002) as one of the most difficult and challenging problems in human nutrition.

The use of dietary monitoring to assess habitual diet or dietary change following intervention is beneficial for both the participant and the researcher. Dietary monitoring, of which there are several well-established methods, was originally derived from disease surveillance and is designed to build a greater understanding of nutrition which can lead to improvements in nutritional behaviours in different populations (Mason and Mitchell, 1983). Information from dietary monitoring can help improve the understanding of problems associated with malnutrition in developing countries, to assist in the long-term planning of health and development and, most pertinently here, to provide information on programme management and evaluation (Mason and Mitchell, 1983). National policies, public health and nutrition programmes and timely warning and prevention programmes can all benefit from robust dietary monitoring (Mason and Mitchell, 1983). Recording dietary behaviours following lifestyle intervention provides an opportunity to assess dietary adherence and if necessary, provide ongoing support to patients who require further nutritional guidance to prevent future disease development. Established methods of dietary monitoring include; weighed food records, estimated food records, 24-hour recall, multiple pass recall and food frequency questionnaires (Wrieden *et al*., 2003). However, while each method has beneficial features, these forms of dietary monitoring contain limitations which result in data inaccuracies and misreporting, which ultimately leads to misinterpretation.

3.2. Limitations of Established Dietary Recording Methods

3.2.1. Weighed and Estimated Food Records

A weighed food record requires an individual to record every food and drink item prior to consumption (Wrieden *et al*., 2003). Individuals provide a detailed description of the food item, and its weight, in a specifically designed booklet. Leftover food is also recorded so an accurate

calculation can be made on the amount of food consumed in a diet (Wrieden *et al*., 2003). This method is described as the "gold standard" when compared to other forms of dietary monitoring (Hamer, Mchnaughton, Bates and Mishra, 2010; Johnson, 2002; Wrieden *et al*., 2003) although as reported by Trabulsi and Schoeller (2001), not free from limitation. It has been acknowledged previously that weighed food diaries are subject to measurement error, both random and systematic (Trabulsi and Schoeller, 2001). While random error such as writing mistakes can occur, Trabulsi and Schoeller (2001) focus their attention mainly on systematic errors, primarily the under reporting of food intake which can lead to misleading conclusions relating to diet and disease.

Under reporting of food intake is the failure of respondents to record all the food items they consume during the recording period or incorrectly weighing the food consumed and is defined as a discrepancy between reported energy intake and measured energy expenditure without any change in body mass (Poslusna, Ruprich, de Vries, Jakubikova, van't Veer, 2009; energy expenditure is often determined using the doubly labelled water method[1]). In addition, previous work has also reported that weighed dietary recording can influence an individual's habitual food choices, resulting in altered food consumption and in some cases, a loss in body weight (Goris, Westerterp-Plantenga and Westerterp, 2000; Prentice *et al*., 1986). Furthermore, Gersovitz, Madden and Smiciklas-Wright (1978) suggest that as the number of dietary monitoring days increase, recording inaccuracies also increase. The study reports a decrease in validity on the fifth, sixth and seventh day of recording, demonstrating a high respondent burden, a weakness reported in previous work (Wrieden, *et al*., 2003). Reporting inaccuracies

[1] Individuals are provided with a weighed oral dose of $^{2}H_{2}{}^{18}O$ and then required to provide urine samples at specific points over a time course. Carbon dioxide output is recorded as the difference in the water pool, measured by $^{2}H_{2}$, and the bicarbonate plus water pool, measured by ^{18}O, and energy expenditure estimated. The recorded energy expenditure should be equal to energy intake when changes in mass have been considered (Bingham, 2002).

have also been identified in several other studies including percentage underestimations of 13 and 32%, in women and men, respectively (Goran and Poehlman, 1992), 27% (Reilly *et al*., 1993), 20% (Tomoyasu, Toth and Poehlman, 2000) and 11% (Tomoyasu, Toth and Poehlman, 2000). Other work has found obese subjects to underestimate dietary intake (Prentice *et al*., 1986; Litchman *et al*., 1992; Buhl, Gallagher, Hoy, Matthews and Heymsfield, 1995; and Black, Bingham, Johansson and Coward, 1997). Subject motivation is another potential reason for under reporting, with the weighed food record perceived as tedious and time consuming (Hill and Davies, 2002). Lack of motivation is an important factor to consider when analysing weighed food diaries and should be considered with other methods of dietary recording also. Participant experience in the method can also influence the accuracy of data recording. Champagne *et al*. (2002) reported a lack of experience in the use of weighed dietary recording resulted in reduced recording accuracy when compared to data from dieticians trained in dietary recording. Given that this method is designed to be used by the general population, most of whom are inexperienced in dietary monitoring, it is perhaps unsurprising that under reporting is so prevalent in many studies.

The estimated food record is similar in design to a weighed food record, however, the quantification of food and drink consumed by an individual is estimated using common household food measures and photographs, as opposed to being weighed (Wrieden *et al*., 2003). While the estimated food record is a widely used form of dietary monitoring and carries a lower respondent burden in comparison to weighed food diaries (Wrieden *et al*., 2003) it again has limitations. Poslusna *et al*. (2009) reviewed 11 studies which employed estimated food records, they found the number of participants who under reported to range from 11.9 to 44% while the percentage of over reporters ranged from 3.5 to 7%. Individual studies have shown significant underestimations of food intake when using estimated food diaries. For

example, Samaras, Kelly and Campbell (1999) demonstrated an overall under reporting of energy intake by 32% of participants, with 18% of individuals of a healthy weight, 39% of overweight individuals and 44% of obese individuals classed as under reporters. Similarly, Lafay *et al*. (2000) found under reporting of food compared to energy expenditure (16% in this study) was not the result of a systematic underestimation of meal size containing all food items but rather associated with food items which are considered bad for an individual's health. A similar finding was reported by O'Loughlin *et al*. (2013) who used an estimated food diary when studying the efficacy of a wearable camera in increasing the accuracy of dietary analysis. Participants were provided with a wearable camera and asked to complete a one day estimated food diary. The results demonstrated that out of the 34 participants in the study, only one participant returned a food diary which closely matched the images obtained from the wearable camera. The study highlighted a significant under reporting of food intake compared to energy expenditure, ranging from 10-18%, when using the estimated food diary alongside the camera. A lack of ability to accurately report portion size, despite clear written and verbal instruction, was the primary reason why significant under reporting was prevalent in the study's sample.

Given the significant burden both weighed and estimated food diaries impose, where respondents are required to record all daily food and beverage consumption, they are both inappropriate and ineffective methods for monitoring habitual diet in numerous groups and in longitudinal studies where mis-reporting has shown to increase over time. While still limited, other methods which provide a less burdensome indication of long-term dietary behaviour, where intake is recalled as opposed to immediately recorded, can give more informative data within large epidemiological studies.

3.2.2. Twenty-four Hour and Multiple Pass Recall

Twenty-four-hour recall requires an individual to list, to a trained interviewer, all the food and drink they have consumed in the past 24 hours (Wrieden *et al*., 2003) and is another regularly utilised method (Castell, Serra-Majem and Ribas Barba, 2015). Twenty-four-hour recall relies on an accurate memory (a limitation in the elderly and subjects 12 years of age and under; Castell *et al*., 2015) and the ability to accurately report food intake (Wrieden *et al*., 2003). To assist the respondent, the interviewer may use photographic prompts to help with portion size estimation. The information provided to the interviewer should describe the type of food consumed and that food's characteristics, such as whether the food is fresh, frozen, pre-prepared, canned or preserved (Castell *et al*., 2015). While this method of dietary recording carries a lower respondent burden (compared with weighed and estimated diet records) and is suitable for large scale surveys (Wrieden *et al*., 2003; Raina, 2013), it does not provide an accurate representation of an individual's long-term dietary exposure (Raina, 2013). Therefore, it is difficult to develop associations between chronic disease development (T2D for example) and habitual food intake based upon the results of a 24-hour recall (Raina, 2013).

Previous work demonstrates that one 24-hour recall is inadequate in representing habitual dietary intake. Seventy-nine female participants completed seven 24-hour recalls over a period of two weeks whilst also measuring energy expenditure using the doubly labelled water method (Yungsheng *et al*., 2009). The aim of this study was to establish how many 24-hour recalls were required to provide an accurate indication of individual dietary habits. Analysis revealed mean energy expenditure of the participants was 2115 kcal per day. The first 24-hour recall revealed a mean intake of 1501 kcal per day. The second and third recalls were significantly higher (2246 and 2315 kcal per day, respectively). Averaging the first and second recall provided a better estimate of energy intake compared to the first recall alone. Averaging of the

three recalls provided an even greater energy intake approximation (Yungsheng *et al*., 2009) although any further recalls did not improve energy intake estimation. The results led to the conclusion that a single 24-hour recall is an inadequate measure of energy intake and demonstrated that three recalls were necessary to provide an accurate indication of habitual dietary exposure (Yungsheng *et al*., 2009). This is supported in another study which suggests a minimum of five recalls may be necessary to establish an individual's habitual intake (Castell *et al*., 2015). The collection of numerous 24-hour recalls can cause increased complication in field work, requiring a greater time commitment from both the participant and interviewer to collect the dietary data (Castell *et al*., 2015).

A validation of the 24-hour dietary recall method reported a large over estimation of protein intake in a group of 244 participants when compared to protein intake measured by a 24-hour urine sample (Kahn *et al*., 1995). Twenty-four-hour urine samples had previously demonstrated to be a useful indicator of protein consumption three to four days following intake (Kahn, 1987). Participants returned their 24-hour urine specimen on the same day as a 24-hour recall being recorded. The urine was collected on a day within the week prior to the recall interview taking place. Protein intake was reported to be higher via 24-hour recall when compared to 24-hour urine, specifically, lightweight men tended to exaggerate protein intake compared to other groups. This led the authors to suggest that misinformation on dietary intake provided by participants may lead to incorrect conclusions regarding diet-disease relationships (Kahn *et al*., 1995). Similarly, work by Novotny *et al*. (2003) measured a range of participant characteristics to identify possible reasons for under reporting of diet when using 24-hour food records. Ninety-eight healthy adult volunteers provided two 24-hour recalls to a registered dietician, the second recall three to ten days after the first. Energy expenditure was measured using either

the doubly labelled water method (44 participants) or the intake balance method[2] (54 participants). All participants completed questionnaires measuring social desirability, memory (to identify if memory was associated with misreporting) and lifestyle behaviours. For all participants, the answers provided in the two 24-hour dietary recalls were averaged and compared against energy expenditure. As a group, energy expenditure differed significantly from reported energy intake by 294 kcal per day. The difference between energy intake and expenditure was significantly different for women but not for men (Novotny *et al*., 2003). Eighty-five percent of women under reported their intake while 15% over reported compared to 61% of men who under reported intake while 39% over reported. For both sexes, under reporting of intake was associated to increased body fat. Under reporting was also associated with various factors related to body image, which included a weight loss attempt in the previous 12 months, weight gain over the previous ten years and difference in reported weight from a participant's 'ideal' weight (Novotny *et al*., 2003). The memory test for men and the social desirability score for women, as well as increased body fat, provided indications of misreporting of energy intake. The need for social desirability which led women to misreport their energy intake significantly more so than men has been reported in previously mentioned studies regarding other forms of dietary recording (Hill and Davies, 2002).

Comparison of the 24-hour recall method and a food frequency questionnaire was made by Schatzkin *et al*. (2003) demonstrating a food frequency questionnaire to under report energy intake by 30-40% compared to a 24-hour recall which underestimated by 10-20%. While the 24-hour recall method in this study under reported energy intake to a lesser extent compared to a food frequency questionnaire, the results provided by a 24-hour recall were still inaccurate

[2] The intake balance method required participants to consume a pre-prepared diet which provided an energy content equal to average energy expenditure, estimated by a registered dietician and altered weekly based upon the participants weight (Novotny *et al*., 2003)

when compared to energy expenditure. Four-hundred and eighty-four healthy volunteers completed two food frequency questionnaires and two 24-hour recalls, separated by approximately three months. Participants received a dose of doubly labelled water during visit one and returned two weeks later for the doubly labelled water assessment (Schatzkin *et al*., 2003). Despite the results demonstrating the 24-hour recall method to be more reliable than a food frequency questionnaire, the study did suggest that a single 24-hour recall is ineffective in measuring energy intake and multiple recalls are required, supported by Yungsheng *et al*. (2009). While increasing the number of 24-hour recalls may increase the accuracy of estimated energy intake, the monetary expense of conducting this method of dietary recording in a large cohort, due to high administrative costs, would not provide an effective solution to dietary monitoring (Schatzkin *et al*., 2003).

Multiple pass recall shares similarities with the 24-hour recall method in that individuals are required to recall their dietary intake from the past 24-hour period. 'Multiple pass' relates to the steps involved in the interview process (Wrieden *et al*., 2003) and was designed to minimise under reporting (Reilly, Montgomery, Jackson, MacRitchie and Armstrong, 2001). The first pass records all the foods that have been consumed, the second records information on the meals and snacks that have been consumed as well as the time and place of consumption. The third pass then prompts for foods and snacks which the individual may have forgotten to recall previously. This information is then followed up by a final stage which records any further food items as well as recording portion sizes (Wrieden *et al*., 2003).

Energy intake measured by multiple pass recall was compared to energy expenditure by Reilly *et al*. (2001). This study used 41 children between the ages of three and four to assess a multiple pass recall. Recall interviews were completed over the telephone following an interview with

the primary carer of the child. The multiple pass recalls were completed over three days within the seven days of energy expenditure measurement via the doubly labelled water method, encompassing two weekdays and a weekend day. The selection of days for the recalls was suggested to provide an adequate summation of energy intake in children (Reilly *et al*., 2001). Extending the recall further than three days was considered, but it was concluded that additional days would add a considerable burden to the primary carers (Reilly *et al*., 2001) and arguably not applicable in realistic practice. Despite no significant difference between energy intake and expenditure when comparing sexes, intake was significantly different to expenditure by 600 kj (143 kcal) per day when the group was analysed. Despite advantages such as a relatively modest average total interview time of 32 minutes over three days and a well-tolerated recall process, the multiple pass recall resulted in significant over estimation of energy intake compared to energy expenditure. While the authors suggest that an increase dietary assessment phase may have increased precision, they argued that increasing this phase would be at the expense of participant compliance (Reilly *et al*., 2001).

Under controlled conditions, in a study conducted in a group of 42 men, Conway, Ingwersen and Moshfegh (2004) examined the accuracy of a multiple pass recall when compared to actual energy intake measured over one day. In the first week of the two-week study, BMI and body fat percentage were collected. During one day within the second week, participants consumed three meals at a human research facility. Participants were free to select from a wide assortment of pre-weighed food, independent of other participants and were instructed to only consume foods provided by the investigators during the day. The food selection made by participants was observed inconspicuously by a dietician. Any food left over was weighed and recorded with adjustments then made for energy intake. Participants were free to take home weighed snacks to consume in the evening but were requested to return any uneaten items the following

day. A multiple pass dietary recall was completed the day after the controlled food consumption protocol and compared to actual energy intake as observed by the study's investigators. There was no significant over or under reporting of carbohydrate, protein or fat demonstrating in this study that a multiple pass recall is an effective method for assessing energy intake. However, the research was conducted under controlled conditions, and therefore it is difficult to use the results to conclude that multiple pass recall is reliable method when used in a 'real world' environment, as demonstrated by other work.

In support of this statement, disease related malnutrition in over 85's (a group in which 18% are at medium or high risk of malnutrition but often excluded from many studies because of age; Davies *et al*., 2014), was examined by Mendonca *et al*. (2016) using multiple pass recall. Seven-hundred and ninety-three participants, including both males and females, completed two multiple pass recalls with a trained nurse on non-consecutive days. Following the exclusion of missing data, 192 of the remaining 731 participants, 26.3% of the entire group, were identified as mis-reporters. Specifically, 158 participants (21.6%) were classed as under reporters whereas 34 participants (4.7%) were considered over reporters. Due to the age of the participants involved, it is possible that under and over reporting of energy intake may be due to reduced memory capacity, a limitation mentioned above (Castell *et al*., 2015) and should be considered a weakness in the multiple pass method when using an elderly sample.

If dietary information is recorded accurately, recall methods can provide useful information on quantifying acute dietary intake. However, as stated by Raina (2013), recalls cannot provide an accurate representation of an individual's long-term dietary pattern which is required to provide information on habitual diet and chronic disease associations and intervention effectiveness. Conducting multiple recalls to gather greater information on dietary behaviour

causes increased complication in field work and requires a greater time commitment from both the participant and interviewer (Castell *et al*., 2015), meaning such a method is unrealistic in studies aiming to capture habitual diet data.

3.2.3. Food Frequency Questionnaire (FFQ)

The FFQ comprises a list of foods and several options relating to the frequency of consumption such as times a day, week or month. The aim of collecting food intake information over various time frames is to gain a greater understanding of habitual diet (Wrieden *et al*., 2003). The FFQ can be self-administered or completed via interview, making it a relatively simple and cost-effective method for monitoring diet (Shim, Oh and Kim, 2014). Like other methods however, the FFQ can be subject to both under and overestimation of food intake.

Schaefer *et al*. (2000) demonstrated the FFQ to underestimate the intake of fat and protein whilst overestimating carbohydrate consumption. Results derived from the FFQ were significantly different compared to actual intakes for saturated fat, monounsaturated fat and protein in individuals fed a high-fat diet and for polyunsaturated fat, saturated fat, fibre, and cholesterol in the lower-fat diet participants and like the findings by Schatzkin *et al*. (2003; discussed above). Similarly, Kowalkowska, *et al*. (2013) demonstrated protein intake to be overestimated by 34.7 g and fat intake by 48.6 g per day when measured using the FFQ. In another study, mean nutrient intake was found to be significantly overestimated with FFQ by both sexes, but more so by women (Paalanen *et al*., 2006). Women overestimated intake of 17 out of 21 nutrients including polyunsaturated fatty acids, long chain *n* - 3 fatty acids, carotenoids and vitamin E while vitamin C was overestimated in both sexes. Men significantly overestimated all foods with the FFQ except for wheat, butter, margarine, cheese, poultry, sausages and coffee when compared to a standard three-day food record (Paalanen *et al*., 2006).

The FFQ is however a widely used instrument for dietary monitoring and its use is supported by Sun (2017) who suggests FFQs should be considered as the best approach to measuring habitual diet in large populations given their cost, representativeness, and the extent of participant involvement compared to other methods. The validity of the FFQ is supported by the ability of dietary intakes to predict long-term disease risk with FFQ assessments of trans fat intake alongside plasma biomarkers, demonstrating trans-fat intake to be detrimental to cardiovascular health (Sun, 2017). Other recent work by Guasch-Ferré, Bhupathiraju, and Hu (2018) state the FFQ to be easily administered with a low respondent burden which provide multiple-week records on dietary intake. While the studies above have demonstrated limitations in using the FFQ, its application to large populations makes it a useful tool for measuring dietary intake in epidemiological studies. Liu *et al*. (2013) found statistically significant correlations between FFQ and 24-hour recalls (except for male protein intake) although correlations did vary depending on the nutrient measured (r = 0.13 to 0.61). Limits of agreement (Bland Altman plots) however demonstrated no serious systematic bias between the two dietary instruments over the range of mean intakes.

Other work by Khalesi, Doshi, Buys and Sun (2017) found the FFQ to correlate reasonably well with three-day food diaries (r = 0.39 to 0.69) with Bland Altman plots demonstrating acceptable agreement when dietary intake was recorded in a population of Australian adults. In support, further work by Whitton *et al*. (2017) found the FFQ to correlate reasonably when assessed against 24-hour recall, fasting plasma and urine biomarkers. The correlation between FFQ and 24-hour recall responses was 0.40 while significant associations were also observed between the FFQ and several biomarkers within plasma and urine including; isoflavones and soy protein intake (r = 0.46), carotenoids and fruit and vegetable intake (r = 0.34), plasma eicosapentaenoic acid and docosahexaenoic acid and fish/seafood intake (r = 0.36), and plasma

odd chain saturated fatty acids (SFA) and dairy fat intake (r = 0.25).

The unique challenges posed in the complex assessment of the human diet means the development of different dietary assessment tools in epidemiological studies has been a focus of research for a significant period. While each method discussed has both strengths as well as limitations, the FFQ is considered here to be the most appropriate way to measure habitual diet in large populations. Compared to other methods, the FFQ is relatively inexpensive, provides a broad representation of dietary behaviour over time, and does not require intense participant involvement. Despite the FFQ being considered a usable methodology for long-term dietary assessment, validation by other methods should be utilised to confirm FFQ data, which can be prone to systematic error. However, on the basis that participants are provided with appropriate instruction and guidance, the FFQ can feasibly be used for the purpose of ranking individuals' dietary exposures in epidemiological studies if later validated.

3.2.4. Summary of Established Dietary Recording Methods

A review of established methods for dietary monitoring demonstrate a range of limitations which effect their reliability, making it difficult to accurately understand habitual diet. High respondent burden, influences on food consumption (Goris, Westerterp-Plantenga and Westerterp, 2000; Prentice *et al*., 1986), lack of previous user experience (Champagne *et al*., 2002) and decreases in recording accuracy over time (Gersovitz, Madden and Smiciklas-Wright, 1978) all negatively impact the weighed food record while over and underestimation of food intake (Poslusna *et al*., 2009; Schaefer *et al*., 2000) and reliance on memory (Castell *et al*., 2015; Wrieden *et al*., 2003) are limitations of other methods such as the estimated food diary, 24-hour recall, multiple pass recall and the FFQ. Additionally, perceptions of body image and social desirability also appear to negatively influence the above methods (Hill and Davies,

2002). Furthermore, such methods are inappropriate for use with certain groups such as the elderly (Searle *et al*., 2002) or obese individuals (Weber *et al*., 2001) who often underestimate energy intake. These issues are especially pertinent when trying to establish dietary behaviours in clinical groups where dietary behaviour change may be crucial in preventing future chronic disease development. While acknowledging that some dietary recording methods are more feasible than others in monitoring dietary status, it is challenging to quantify individuals' dietary behaviour precisely and accurately using questionnaires alone, especially when collecting data in large-scale epidemiological studies. Therefore, the opportunity to validate responses to dietary questioning should be explored as a matter of importance.

3.3. Dietary Monitoring Using Urinary Biomarkers

Due to the limitations associated with conventional dietary monitoring tools, measurement of urinary biomarkers using metabolite profile techniques, offers both an alternative approach for measuring dietary exposure as well as the opportunity to validate existing dietary recording methods (Favé *et al*., 2011). Urine contains a high number of individual metabolites which act as biomarkers reflective of dietary exposure. The development of dietary biomarkers can more accurately classify an individual's dietary intake in comparison to questionnaires and as a result, improve the understanding between diet and disease (Gorman, Gibbons and Brennan, 2013; Perez 2017) as well as confirm adherence to dietary recommendations (Heinzmann *et al*., 2010).

Metabolomics is the study of small molecules present in biofluids with one key aim being to enhance knowledge of metabolic pathways relevant to human nutrition (Gibney, Walsh, Brennan, Roche, German and Van Ommen, 2005). As advancing chemical technologies, mass spectrometry (MS) and nuclear magnetic resonance (NMR) provide opportunity for

widespread analysis of metabolites, nutrients and other compounds which are present in urine and other biofluids (Gibney, Walsh, Brennan, Roche, German and Van Ommen, 2005). Targeted analytical approaches focus on specific subsets of predefined metabolites and follow a hypothesis-lead approach of metabolites of known identity while untargeted metabolomics detect thousands of unknown metabolites that may provide novel information on biological pathways with clinical relevance and can be conducted within cohort studies to validate other forms of dietary monitoring (Guasch-Ferré, Bhupathiraj and Hu, 2018).

Foods contain thousands of non-nutritive secondary metabolites which once digested and metabolised, result in the presence of metabolites in bodily fluids, including urine and blood (Favé, Beckman, Draper and Mathers, 2009) with more than 6800 fully annotated metabolites listed on the Human Metabolome Database relevant to diet, as well as other metabolic intermediates (Guasch-Ferré, Bhupathiraj and Hu, 2018). Metabolite profiling of serum and plasma can be used for dietary analysis as demonstrated in studies including: El-Sohemy *et al*. (2002); Pollard *et al*., (2003); Floegel *et al*. (2013); Schmidt *et al*. (2015); Esko *et al*. (2017). Urine concentration is modulated primarily by a person's fluid intake and micturition pattern, which creates additional challenges, compared to homeostatic controlled plasma. Large metabolite concentration ranges can be caused by a number of other factors too, including age, gender, genetic background, diurnal variation, health status and physical activity level (Bouatra *et al*., 2013). This can be problematic given the concentration of urine influences the concentration of urinary biomarkers (Ralib *et al*., 2012), therefore, to account for differences in urine concentration between individuals, all samples must normalized in an additional step prior analysis to ensure all measurements are made within the same dynamic range to avoid misleading results. Furthermore, urine biomarkers undergo biotransformation often resulting in multiple derivatives of single compound; which adds to the challenge of using urine

biomarkers for measuring dietary intake. However, urine as a bio specimen is less invasive than blood collection, less expensive to obtain, and affords greater collection volumes, thus making it a highly useful biological resource for large clinical and population studies. Additionally, Favé *et al*. (2009) demonstrated urine composition remained consistent in a time window of two to four hours after meal consumption, consequently affording a flexible time window for postprandial urine sampling.

Previous work has demonstrated urine to be as useful as plasma in identifying metabolites representative of habitual diet (Playdon *et al*., 2016). Numerous studies have been conducted to both identify and validate dietary biomarkers using urinary metabolomic approaches. While it is unlikely that biomarker assessment will replace traditional dietary recording methods entirely, the use of metabolomics in identifying novel and robust biomarkers of dietary exposure and intake can both enhance and validate such methods (Gorman, Gibbons and Brennan, 2013).

Generally, the adoption of urinary metabolomics to classify dietary biomarkers has been achieved either by acute dietary intervention to identify specific food biomarkers, analysis of dietary patterns and metabolic profiles to identify biomarkers, or identifying biomarkers from cohort studies (Gorman, Gibbons and Brennan, 2013). Several studies have been conducted which have successfully identified biomarkers of foods with links to health importance including meat, fish, vegetables, fruits, wholegrain, tea, coffee and sugar as well as biomarkers of unhealthy behaviours, such as smoking and alcohol consumption.

3.3.1. Biomarkers for Fruit and Vegetables

Fruit and vegetable intake is a highly important component of a healthy diet. Conversely,

decreased fruit and vegetable intake is associated with poor health and increased risk of developing non-communicable diseases (Slavin and Lloyd, 2012). Therefore, the identification of biomarkers for fruit and vegetable consumption is an important step in developing enhanced dietary monitoring using urine metabolomics. Mennen *et al*. (2006) studied correlations between the intake of polyphenol-rich foods and beverages and the urinary concentrations of 13 polyphenols and metabolites from a population of free-living individuals. Participants (n = 53) completed a two-day dietary record within which 24-hour urine was collected throughout the second day and a spot urine sample collected on the morning of the third day. Correlations were discovered between apple consumption and coumaric acid, isorhamnetin, kaempferol and pholertin; red fruit consumption with kaempferol; orange consumption and hesperetin; citrus fruit and fruit juice consumption with hesperetin, caffeic acid and naringenin and total fruit and fruit juice intake with naringenin, gallic and 4-*O*-methylgallic acid, isorhamnetin, kaempferol, hesperetin, naringenin and pholertin. Fruit juice consumption also resulted in increased concentrations of quercetin in an earlier study by Young *et al*. (1999). Furthermore, positive correlations were identified between vegetable intake and enterolactone concentrations. This work demonstrates several correlations between the intake of polyphenol-rich foods and beverages and the excretion of polyphenols in urines of free-living individuals and suggests phenolic compounds in spot urine samples may be biomarkers indicative of polyphenol consumption. In support, ferulic acid and its derivatives are also suggested as general markers of polyphenol rich foods by Hodgson *et al*. (2004).

Urinary biomarkers of citrus fruit consumption were investigated by Heinzmann *et al*. (2010), eight participants consumed standardised meals for three days as well as an additional mixed fruit meal on day two with urine samples collected four times each day. Analysis revealed significant metabolic changes between pre and post fruit meals with an observed increase in

the excretion of proline betaine, tartaric acid and hippuric acid, of which proline betaine demonstrated the strongest correlation. Consequently, citrus fruit and citrus fruit juice samples were analysed for concentrations of proline betaine. All citrus samples contained proline betaine but varied depending on the fruit (orange > lime > satsuma > grapefruit > lemon; Heinzmann *et al*., 2010). These findings were then applied to a large cohort of participants (n = 499) who completed two multiple pass dietary recalls and provided a 24-urine sample across the same period with the procedure repeated after three weeks. Analysis revealed significantly greater concentrations of proline betaine in participants who reported citrus fruit consumption compared to those that recorded no citrus fruit intake. Subsequently, the findings of this work led to the conclusion that proline betaine can be regarded as a specific and sensitive biomarker of citrus fruit consumption.

Additionally, the role of proline betaine as a biomarker for citrus fruit consumption was also investigated by Lloyd, Beckmann, Favé, Mathers and Draper (2011) across two studies. Study one; subjects (n = 12) attended a clinical research facility (CRF) on two occasions, separated by several months. Subjects collected all urine after the consumption of a standardised evening meal up until and including the first morning void sample, collected prior to attending the clinical research facility. Following an overnight fast, urine was collected before subjects received a standardised breakfast including 200 ml of orange juice (Lloyd *et al*., 2011). Further urine samples were collected two, four and six hours after breakfast consumption. Study 2; eleven subjects attended the CRF for six identical test days, separated by a minimum of one week, over the duration of 14 months. A non-targeted metabolomics approach using flow infusion electrospray ionisation mass spectrometry (FIE-MS) successfully discovered and structurally identified biochemical markers of citrus exposure in urine in the study one subjects. This finding was confirmed in the study two subjects across 14 months, demonstrating the

presence of proline betaine to be a strong indicator of both acute and habitual consumption of citrus containing foods.

The discovery of a broader range of metabolites present in urine that act as biomarkers in foods of high health importance was investigated by Lloyd *et al*. (2011). Twenty-four subjects consumed a standardised evening meal before attending the test day following a minimum 12-hour overnight fast with fasting urine samples collected on arrival. Subjects visited the CRF six times over a period of eight months and were provided with either a standardised breakfast including cornflakes with milk or with a breakfast in which the cornflakes and milk were replaced with one of four test foods. The test foods consisted of; a cruciferous vegetable (200 g of steamed broccoli), a berry fruit (200 g raspberries), oily fish (20 g salmon) or a whole grain wheat cereal with 125 ml of ultra-high temperature treated semi-skimmed milk (Lloyd *et al*., 2011). Urine samples were collected one and a half, three, and four and a half hours after breakfast consumption. Consumption of each of the four breakfasts was randomised and interspersed with consumption of two standardised breakfasts over the six visits. Analysis found consumption of broccoli and raspberries resulted in increases in ascorbate, which is abundant in both fruit and vegetables, evidenced by Stella *et al*. (2006).

The consumption of cruciferous vegetables is inversely correlated to many human diseases including cancer (breast, lung, and bladder), diabetes, and cardiovascular and neurological disease and therefore work examining potential biomarkers of cruciferous vegetable was undertaken by Edmands *et al*. (2011), using 20 healthy male participants. Separated into three stages of 14 days, the study included: a period of restricted cruciferous vegetable consumption (stage one); a phase of high cruciferous vegetable intake where participants consumed 250 g per day of both broccoli and brussel sprouts (stage two) followed by a wash-out period with

restricted cruciferous vegetable consumption (stage three). Each participant provided all cumulative urine over 48 hours on completion of each stage. Urine samples analysed after each phase demonstrated significant discrimination between stages of cruciferous vegetable consumption with the concentration of S-methyl-l-cysteine sulfoxide significantly elevated following high cruciferous vegetable intake.

In a later study, Lloyd, Beckmann, Haldar, Seal, Brandt and Draper (2013) developed a data driven strategy to identify urinary biomarkers indicative of habitual exposure to different food items described in a typical FFQ without a long-term food intervention. A standardised evening meal was provided to subjects (n = 68) on the evening prior to each visit to the CRF. Twenty-four-hour urine samples were collected the day before each CRF visit at weeks four, eight and twelve. Subjects provided a fasting spot urine sample at the CRF before biometric measurements were recorded. The assessment of habitual exposure to different food groups was assessed using a validated FFQ and was available for subjects to estimate their diet on four occasions over a 12-week period. The FFQ used in this study was a simplified 58 food item version of the original 139 component version. Average consumption of each food category was assessed, and each subject given a consumption score. The strategy for biomarker discovery depended on the ability to define differential dietary exposure classes for each dietary component (containing > eight individuals; Lloyd *et al*., 2013). Subjects were categorized in terms of their intake for the 58 dietary components on a scale comprised of: <one time/week, one time/week, two-four times/week, five-six times/week, one time/day and >one time/day. In total, 38 dietary components were considered suitable for biomarker classification based on substantial differential exposure (Lloyd *et al*., 2013). The study found good separation of metabolite profiles between subjects reporting low and high citrus fruit consumption which confirms the finding in an earlier study by Lloyd *et al*. (2011) examining proline betaine as a

biomarker of citrus exposure. Discrimination between exposure levels improved linearly when low consumers of citrus were compared to subjects reporting increasingly higher intakes. This finding was similar for all foods examined, with consumption frequency and food distinctiveness proving influential in whether differential dietary exposures could be detected (Lloyd *et al*., 2013). Biomarkers for single distinctive foods were also examined in each of the consumption frequency classes. Consumption of tomatoes was associated with the presence of hippuric acid, hydroxyhippuric acid, caffeic acid sulfate and hydroxyphenyl-valerolactone sulfate derivative, the presence of which are generally associated with colonic fermentation of dietary polyphenols (Lloyd *et al*., 2013). Other work examining biomarkers of plant-based polyphenol rich foods by Vetrani *et al*. (2014) found dihydroxybenzoic acid to be another metabolite indicative of polyphenol exposure when measured in urine profiles of participants consuming diets with different levels of polyphenol content. Similar studies examining the identification of biomarkers for plant-based foods include rhamnitol as a biomarker of apple consumption (Tomnita *et al*., (2015) and dopamine sulfate as a biomarker for banana intake (Wang *et al*., 2018).

Quantification of dietary biomarkers in foods of high health importance in the UK collected from urines of free-living individuals (n = 6) was investigated by Lloyd *et al*. (2016). Metabolite levels in urine collected before sleep or upon waking revealed a group of biomarkers which correlated with reported dietary intake. Fruit and vegetable intake correlated with hippuric acid, citrus fruit intake with proline betaine, cruciferous vegetable intake with *D*, *L*-Sulforaphane-*N*-acetyl-*L*-cystine and grapes with tartrate. Tartaric acid was later identified as a dose-responsive urinary biomarker of grape intake and subsequently quantified in volunteers following several four-day dietary interventions including 0 g, 50 g, 100 g, and 150 g per day of grapes in standardized diets (Garcia-Perez *et al*., 2016). Resveratrol is also stated

to be abundant in grapes and excreted following intake (Meng, Maliakal, Lu, Lee and Yang, 2004). Furthermore, work concerning metabolite profiles following ingestion of foods and diets of high health importance has investigated the urinary metabolome of individuals exposed to range of diets based upon WHO dietary guidelines (Garcia-Perez *et al*., 2017). The analysis of dietary patterns as opposed to specific foods provided an opportunity to examine relationships between different diets and potential development of non-communicable diseases. Nineteen participants attended a research facility for a period of 72 hours on four occasions, consuming four different diets in a randomised crossover design. Visits were separated by at least five days. Each dietary intervention was developed in accordance with WHO guidelines for healthy nutrition. High energy density is associated with non-communicable diseases and therefore the diets had a range of densities. Diet-one most closely matched WHO guidelines and was least energy dense whereas diet-four was least matched and most energy dense. During each inpatient visit, urine was collected across three periods (0900-1300, 1300-1800 and 1800-0900) and pooled to create a 24-hour sample. Analysis of samples revealed clear separation in metabolic profiles of 24-hour urines between diets one and four with the presence of 19 metabolites significantly higher in diet-one compared to diet-four, reflecting higher intake of fruits including; rhamnitol, 4-hydroxyhippurate, hippurate, tartrate, and glycolate and vegetables; *N*-acetyl-S--propenyl-cysteine sulfoxide, *N*-acetyl-S-methyl-cysteine sulfoxide, and S-methylcysteine sulfoxide. Nine metabolites were significantly higher after consumption of diet-four in comparison to diet-one (Garcia-Perez *et al*., 2017).

3.3.2. Biomarkers for Meat and Fish

Given the reported association between meat consumption, chronic disease and mortality (Larsson and Orsini, 2013) numerous studies have examined biomarkers reflective of meat intake, while biomarkers of fish, associated with a healthy diet (Hosomi, Yoshida and

Fukunaga, 2012), have also been examined. Myint *et al*. (2000) examined the correlation between reported meat consumption by FFQ and urinary methylhistidine excretion. The study found moderately strong correlations between reported meat intake and excretion of methylhistamine in urine alongside strong discrimination between vegetarian and omnivore diets while a review by Dragsted (2010) suggests potential biomarkers of dietary meat intake include creatine, creatinine, carnitine, carnosine, anserine and 3-methylhistidine.

Cross, Major and Sinha (2011) examined creatinine, taurine, 1-methylhistidine, and 3-methylhistidine as potential biomarkers for red meat consumption. In a randomised crossover feeding design, 24-hour urine samples were collected from 17 individuals who consumed four different controlled diets including; vegetarian, low red meat (60 g per day), medium red meat (120 g per day) and high red meat (420 g per day). When compared to the vegetarian and low red meat diets, all four metabolites were significantly higher in the medium and high red meat diets. Concentrations for 1-methylhistidine and 3-methylhistidine were significantly different for every diet type, increasing in urine concentration as the amount of meat consumed in the diet increased, therefore suggesting both may be good biomarkers for red meat consumption.

The study by Lloyd *et al*. (2011) which examined foods of high health importance (discussed above) found dietary exposure to smoked salmon associated with 1-melthylhistidine and anserine, which are known to be highly abundant in the skeletal muscle of salmon and other oily fish and is supported by previous work (Abe, 1983; Abe, Okuma, Sekine, Maeda and Yoshiue, 1993). Urinary concentration of trimethylamine N-oxide (TMAO), a metabolite of trimethylamine and a degradation product formed from carnitine, also increased following smoked salmon consumption, concentrations of which are associated with fish consumption and supported by Stella *et al*. (2006). Similarly, methylhistidine was identified as a biomarker

of oily fish consumption in the later work by Lloyd *et al*. (2013) while 1- and 3- methyl-histidine (chicken and oily fish) and dimethylamine and TMAO (fish) were identified as meat and fish biomarkers in the work by Garcia-Perez *et al*. (2017).

Markers of general protein as opposed to specific food items have also been proposed with Patel *et al*. (2012) suggesting higher urinary concentrations of p-Cresol sulfate may be indicative of protein intake when compared to vegetarian diets, where concentrations were markedly lower. Similarly, work by Poesen *et al*. (2015) found indoxyl sulfate, a metabolite of dietary tryptophan, was significantly increased in urinary concentration in subjects consuming high protein compared to low protein diets. Dietary intake of protein sources was further examined by Cheung *et al*. (2017) in which four groups of ten subjects consumed increasing quantities of either chicken, red meat, processed meat or fish over a three-week period. Identical breakfasts, lunches and evening meals were served at 0800, 1200, and 1900, respectively, on each of the three consecutive days in the same week. Meat or fish was served at midday and evening meals for each group. Subjects consumed their habitual diet during the four remaining days of each week. All meals provided similar intakes of energy and fibre, but the amounts of proteins, fats, and carbohydrates varied each week. For example, week one: 13% protein, 30% fat, 57% carbohydrate, week two: 20% protein, 35% fat, 45% carbohydrate; and week three: 30% protein, 40% fat, 30% carbohydrate. The amount of meat or fish consumed therefore increased with the percentage of protein in the diet during each week. Twenty-four-hour urine samples were collected from the second void of day two until the first void of day three (Cheung *et al*., 2017). Metabolites including 3-methylhistidine, anserine, carnosine, 3-acylcarnitines and trimethylamine-N-oxide associated with meat or fish intake. Specifically, 3-methylhistidine and anserine was associated with chicken intake with anserine presence specific to chicken consumption. Trimethylamine-N-oxide correlated with fish intake

while carnosine and 3-acylcarnitines was associated more generally with meat intake and meat and fish intake respectively.

3.3.3. Biomarkers of Wholegrain and Soy

Evidence suggests that wholegrain consumption plays an important role in chronic disease prevention by facilitating antioxidant defence systems and moderating the risk factors of cancer, cardiovascular diseases, and other chronic diseases (Zhu, Wang, Sha and Sang, 2016). Biomarkers reflective of long-term wholegrain rye and wheat intake include dihydroxyphenylpropionic acid (Soderholm, Koskela, Lundin, Tikkanen and Aldercreutz, 2009) and benzoxazolinone (Koistinen *et al*., 2018). In soy studies, a randomized controlled study was used to compare a high and low-soy diets in 76 healthy adults over ten weeks with results demonstrating concentrations of isoflavones and their gut microflora metabolites in urine were significantly higher in the participants with high soy intake compared to those who consumed low amounts of soy (Wiseman *et al*., 2004). Daidzein has been suggested as a biomarker of soy intake, with work by Ahn-Jarvis, Clinton, Riedl, Vodovotz and Schwartz, (2012) demonstrating increased excretion of daidzein following soy consumption. Morimoto, Beckford, Franke and Maskarinec (2014) support this, suggesting urinary isoflavonoid excretion, of which daidzein forms part of its class, is an excellent biomarker for discriminating soy consumption following a randomised control trial involving low and high soy diets.

3.3.4. Biomarkers for Caffeine, Coffee and Tea

Consumption of coffee and tea has been associated with reduced mortality (Van den Brandt, 2018) with previous research substantiating such associations (Khan and Mukhtar, 2013; Grosso *et al*., 2014; Petta and Marchesini, 2017) while intake of caffeine is associated with both positive and negative health outcomes (Ruxton, 2009). Research by Crews, Oliver and

Wilson (2001) demonstrated that 24-h urine after caffeine intake allowed for quantification of caffeine as a metabolite representative of caffeine consumption. Rechner, Spencer, Kuhnle, Hahn and Rice-Evans (2001) suggest chlorogenic acid presence in urine is mainly attributed to coffee intake (amongst other dietary items) while Hodgson *et al*. (2004) suggests that ferulic acid and its derivatives may also provide usefulness as a biomarker for coffee consumption, as well as polyphenol rich foods. Vanillic acid is also proposed as a possible coffee biomarker by Rechner *et al*. (2001) and trigonelline by Slow *et al*. (2004). In the work by Mennen *et al*. (2006), correlations were identified between coffee consumption and caffeic acid. Additionally, research by Sang, Lee, Yang, Buckley and Yang (2008) found consumption of tea resulted in the presence of metabolites associated with tea polyphenols in human urine and included; epigallocatechin, glucuronide, methylated epigallocatechin glucuronide, methylated epigallocatechin sulfate, epicatechin glucruronide, epicatechin sulfate and methylated epicatechin sulfate.

3.3.5. Biomarkers of Cocoa, Sweeteners and Sugar

Excessive intake of rapidly absorbed sugars and other sweetened items is a major health concern given sugar's association with health complications, including pre-diabetes, T2D and cardiovascular disease. Work by Wilson *et al*. (1999) examined acesulfame-k as a biomarker of sweetener intake. Analysis of 48-hour food diaries and 24-hour urines found good agreement between reported intake of artificial sweeteners and acesulfame-k presence in 24-hour urine. More recently, Beckmann *et al*. (2016) examined the metabolic effects of acute sucrose exposure with the aim of identifying metabolites which correlate with sucrose intake in a randomised control design. Standard meals low in carbohydrate content and no extrinsic sugars were provided to subjects (n = 90) on the evening prior to the test day, at lunch and on the evening of the test day. Subjects collected all their urine following consumption of the evening

meal up until the first morning void prior to arrival at the test facility. Aside from the test drink, subjects avoided sucrose intake throughout the study protocol. Subjects fasted for 12 hours before arriving at the test facility where a spot urine and blood sample were collected. The test drink, containing 0, 50 or 100 g sucrose in water, was provided before a further urine sample and blood sample was collected after three hours. Subjects consumed a standard lunch, similar in content to the breakfast, before urine samples were collected after six and nine hours. A standard evening meal was consumed before urine was collected for the rest of the evening, overnight and first morning void. Subjects fasted for 12 hours following the evening meal before returning to the test facility to provide a final, post 24-hour urine sample and blood sample (Beckmann, *et al*., 2016). This work demonstrated ingestion of sucrose to influence over 120 metabolites present in urine and plasma which discriminated between sucrose treatments. Metabolites directly correlated to sucrose intake included sucrose and fructose.

In the Garcia-Perez *et al*. (2017) study clear discrimination was observed in urinary glucose between diet one (diet most closely matching WHO guidelines) and diet four (diet least matching WHO guidelines) demonstrating increased sugar intake was detectable in urine samples across varying diets. Furthermore, recent work by Michielsen, Almanza-Aguilera, Brouwer-Brolsma, Urpi-Sarda and Afman (2018) propose methylxanthines as candidate biomarkers for cocoa consumption following a systematic review of the literature regarding cocoa and liquorice intake studies.

3.3.6. Biomarkers for Smoking and Alcohol

The negative health consequences of smoking and alcohol consumption are extremely well established in the literature with both contributing to the development of non-communicable chronic disease, and subsequently, the significant economic health burden imposed on health

services (Scarborough *et al*., 2011). Therefore, the establishment of biomarkers which can monitor smoking status and alcohol consumption can prove beneficial in tracking unhealthy behaviours. In a study using 1580 smokers and 3126 non-smokers, analysis found concentrations of both nicotine and cotinine varied considerably in urine depending on smoking status (Heinrich *et al.*, 2005). Significantly, intake of food containing nicotine was not identified as source of nicotine and cotinine presence in urine with tobacco smoking the only source for the urinary presence of these biomarkers. In support of this work, the use of cotinine as a biomarker for cigarette smoking was confirmed by Petersen, Leite, Chatkin and Thiesen (2010) while 2-cyanoethylmercapturic acid is advocated as a biomarker of cigarette smoking by Scherer *et al*. (2010) and 3-hydroxypropylmercapturic acid by Gregg, Minet and McEwan (2013). Research examining alcohol exposure, by Mackus *et al*. (2017), used 36 healthy social drinkers as participants. Comparison of urine samples between an alcohol consumption and alcohol-free day demonstrated concentrations of ethyl glucuronide to be significantly elevated following alcohol intake and correlated significantly with urinary ethanol, which is abundant in alcohol, as well as individual headache scores, a symptom traditionally associated with alcoholic induced illness. While ethyl glucuronide concentration did not correlate with the number of alcoholic drinks consumed, the authors concluded their findings to demonstrate ethyl glucuronide as a potential biomarker of alcohol consumption.

3.3.7. Dietary Patterns

Assessment of dietary patterns as opposed to identification of biomarkers associated to specific foods is another line of research undertaken in urinary metabolomics. As already discussed, work by Lloyd *et al*. (2013) developed a data driven strategy to identify urinary biomarkers indicative of habitual exposure to different food groups, finding significant differences in markers for citrus consumption between high and low intake patterns. Additionally, the study

by Garcia-Perez *et al*. (2017) successfully identified metabolic differences in urine profiles between contrasting diets of high and low quality, based on WHO guidelines, as well as identifying biomarkers of specific foods. This work is supported by other literature which has focussed on habitual dietary patterns and metabolomic profiles.

O'Sullivan, Gibney, and Brennan (2011) conducted a study involving 160 volunteers finding dietary intakes could be separated into three distinct clusters. Cluster one was represented by a high energy contribution from whole-meal bread, milk, fish, confectionary, and desserts and a lower contribution from energy drinks. Cluster two was characterised by a high energy contribution from foods including savoury snacks, low-fat milk, yogurts, fruit, poultry, and sauces and a lower contribution from energy drinks. Cluster three contributions included white bread, sugars and preserves, butter and spreads, red meat, red-meat dishes, meat products, and alcohol and a lower contribution from vegetables compared with the cluster one and two. Significantly, analysis of participant urine allowed the identification of metabolites associated with different dietary patterns. Several of the metabolites identified were associated with the consumption of specific food groups, demonstrating habitual diet to be represented in metabolic data.

Other work by Vazquez-Fresno *et al*. (2015) examined the urinary profiles of 98 participants consuming either a Mediterranean diet, Mediterranean diet with olive oil, a Mediterranean diet with nuts or a controlled low-fat diet. Urine analysis revealed significant separation in urine profiles between the Mediterranean diets and the low-fat diet. Analysis revealed that the most prominent hallmarks concerning the Mediterranean diet groups were related to the metabolism of carbohydrate, creatine, creatinine, amino acids, lipids and microbial cometabolites (Vazquez-Fresno *et al*., 2015). Conversely, hippurate, trimethylamine-N-oxide, histidine,

methylhistidine, carnosine, anserine and xanthosine were prominent in urines of low-fat diet consumers, demonstrating the ability of urinary metabolomics to classify individuals based upon the dietary intake pattern. Further to these findings, work by Gibbons *et al*. (2017) found urinary metabolomic data could be used to classify individuals into either "healthy" (e.g. intake of breakfast cereals, low fat and skimmed milks, potatoes, fruit and fish, fish dishes) or "unhealthy" diets (e.g. intake of chips/processed potatoes, meat products, savoury snacks and high-energy drinks) in nutrition data from 567 participants. Table 3.1 presents a summary of the major food groups and the urinary metabolites associated with consumption in the diet.

3.4. Summary

Notwithstanding the limitations associated with traditional forms of dietary monitoring, it is likely questionnaire-based methods will continue to be utilised in both primary care and within academic research for the foreseeable future. This is because for many foods and nutrients, specific biomarkers are not yet available. Additionally, for foods which do have established biomarkers, they are unable to quantify the amount of food and drink consumed by an individual. However, through the successful identification of biomarkers of foods of high health importance encompassing fruit, vegetables, meat, fish, wholegrain, tea, coffee, sugar, successful discrimination of metabolic profiles derived from different diets as well as biomarkers for smoking and alcohol consumption, it is now possible to both improve and validate traditional dietary monitoring methods with urinary metabolomics to develop an improved understanding of diet and nutrition status. Urine can be easily and non-invasively collected, is less expensive to obtain than other biofluids and can be collected in large volumes, therefore making it a highly useful biological resource for large clinical and population studies where dietary recall is often used but is limited by a reliance on memory and accurate reporting.

Evidence of questionnaire validation using urinary biomarkers has been demonstrated in several studies including Lloyd *et al*. (2013) and more recently by Fraser *et al*. (2016) who found good correlation between several urinary biomarkers and reported food intake by repeated 24-hour recalls and FFQ. However, a limitation of many of the studies discussed is the requirement for participants to either provide inconvenient 24-hour urine samples at home or attended regular and lengthy visits to research facilities to provide urine samples for metabolomic assessment. Such limitations suggest the development of simplified protocols to monitor habitual diet in free-living environments using metabolomic technologies is a prudent next phase of research. The potential of such advances will allow for greater confidence in results derived from questionnaire based dietary monitoring as responses from such can be validated. This is especially useful in groups known to misreport dietary intake and those at increased risk of non-communicable disease where habitual dietary monitoring can be used to assess the efficacy of lifestyle interventions with regards to dietary advice. The challenge therefore is to determine how best, robust and reliable urinary data can be collected from free living individuals in the community, without causing inconvenience and disruption to everyday life or relying on intensive laboratory studies to collect biological data for metabolic analysis.

Table 3.1. Summary table of urinary metabolites linked to dietary exposure

Dietary Component	Biomarker
Fruits and Vegetables	
General fruit and fruit juice intake	naringenin, gallic and 4-*O*-methylgallic acid, isorhamnetin, kaempferol, hesperetin, naringenin, pholertin, rhamnitol, 4-hydroxyhippurate, hippurate, tartrate, glycolate, quercetin, ferulic acid
General vegetable intake	hippuric acid, N-acetyl-S--propenyl-cysteine sulfoxide, N-acetyl-S-methyl-cysteine sulfoxide, S-methylcysteine sulfoxide, ferulic acid
Apple	coumaric acid, isorhamnetin, kaempferol, pholertin, rhamnitol
Red fruit	Kaempferol
Citrus fruit and fruit juice	hesperetin, caffeic acid, naringenin, proline betaine
Cruciferous vegetables	S-methyl-l-cysteine sulfoxide, *D*, *L*-Sulforaphane-*N*-acetyl-*L*-cystine
Tomatoes	hippuric acid, hydroxyhippuric acid, caffeic acid sulfate and hydroxyphenyl-valerolactone sulfate
Potato	Calystegine
Banana	dopamine sulfate
Grapes	tartaric acid, resveratrol
Legumes	pyrogallol
Protein, Meat and Fish	
General meat intake	methylhistamine, creatine, creatinine, carnitine, carnosine, anserine, taurine
General fish intake	trimethylamine N-oxide
General protein intake	p-Cresol sulfate, indoxyl sulfate, tryptophan
Red meat	1-methylhistidine, 3-methylhistidine
Oily Fish	1-melthylhistidine, anserine
Chicken	anserine
Wholegrain and Soy	
Wholegrain rye and wheat	dihydroxyphenylpropionic acid, benzoxazolinone
Soy	Daidzein

Caffeine, Coffee and Tea	
Caffeine	Caffeine
Coffee	chlorogenic acid, ferulic acid, caffeic acid, vanillic acid
Tea	epicatechin (and derivatives)
Sugar and Cocoa	
Sweetener	acesulfame-k
General sugar intake	sucrose, fructose, glucose
Cocoa	methylxanthines
Smoking and Alcohol	
Smoking	nicotine, cotinine, 2- cyanoethylmercapturic acid, 3-hydroxypropylmercapturic acid
Alcohol	ethyl glucuronide

Chapter Four:

General Methods

4.1. Studies

This thesis is comprised of five studies including; Study one: The efficacy of a GP-led pre-diabetes intervention targeting lifestyle modification; Study two: A protocol for accurate monitoring of habitual diet in a free living environment using multiple spot urine samples; Study three: The effects of acute moderate exercise on the urinary metabolome; Study four: Comparison of the Alere Afinion AS100 point of care analyser and HPLC in the determination of HbA_{1c}; Study five: The feasibility of conducting a randomised control trial to evaluate the effectiveness of a focussed 15-minute one-to-one consultation to improve blood glucose control in pre-diabetes. In instances where methods are used in more than one study, they are detailed in this chapter. Where methods are used only once, they are detailed in respective research chapters. Given study one's design as a service evaluation which informed later work within this thesis, methods are described previously in chapter two and not included here.

4.2. Ethical Approval

Necessary approval for all studies was granted prior to any subject recruitment or testing procedures taking place. Ethical approval for studies two, three and four was granted by Aberystwyth University Research Ethics Committee using an online application system. Study five required NHS ethical approval by a Wales Research Ethics Committee (REC Seven). A full application was submitted through the Integrated Research Application System (IRAS), an online application that creates application forms for relevant parties, including the National Health Service (NHS) REC and Hywel Dda University Health Board Research. The application

was reviewed in full by an ethics committee comprised of both health professionals and lay members. The review was attended in person by both the chief investigator and academic supervisor. The application received immediate favourable opinion without protocol revision and was subsequently registered with www.ClinicalTrials.gov.

4.3. Participants and Recruitment

Participants were given written and verbal information on the purpose and protocol of studies before giving their consent to proceed. All participants who took part in studies were informed their participation was voluntary and they were free to withdraw at any point without giving prior notice or reason for withdrawal which would not affect their future participation in research or their right to healthcare. Participants wishing to confirm their interest or to receive further information did so by telephone, email or visiting the University research facility in person. Recruitment for studies two, three and four was by social media, University email bulletins, fliers and word of mouth. Recruitment for study five was achieved by letter of invitation via surgery patient records.

4.4. Anthropometry Measures

For descriptive and analytical purposes, height (Holtain Ltd, Crymych, UK) and body mass (wearing loose fitting clothing; Seca 899, Hamburg, Germany) were recorded by the same investigator.

4.5. Blood Collection

Participants were seated in a phlebotomy chair and the process of blood sampling was explained in full. Following sample site selection, the skin at the sampling site was cleaned with an alcohol swab and allowed to dry. For venous blood, samples were obtained by

venepuncture from an antecubital vein using a 21-gauge needle and drawn into a six ml heparinised vacutainer with minimal stasis (BD Vacutainer Systems, Plymouth, UK). For capillary blood, a sample was collected from the middle or index finger using a lancet (Accu-Chek Safe-T-Pro Plus Lancet, Roche Diabetes Care Limited, UK). All blood samples were collected by the investigator.

4.6. HbA_{1c} Analysis

For analysis of venous blood, separate 1.5 µl samples of whole blood were transferred from a heparinised vacutainer into separate HbA_{1c} and lipid test cartridges and analysed respectively by an automated point of care (POC) blood analyser (Alere Afinion AS100, Abbot, Cheshire, UK). For capillary blood, 1.5 µl of whole blood was collected directly into the HbA_{1c} test cartridge from the puncture site. Control samples containing different target HbA_{1c} concentrations were assayed by the POC analyser according to the manufacturer's instructions on the morning of each participant's visit to ensure the detection range was operating between 20-140 mmol/mol. Test samples involved taking 1.5 µl of whole blood, collected either directly from the capillary puncture site or from the EDTA/LH vacutainer before loading in to the POC analyser. The blood sample was automatically diluted and mixed with a solution resulting in the release of haemoglobin from the erythrocytes. The sample solution was then transferred to a blue boronic acid conjugate, which binds to the cis-diols of glycated haemoglobin. The reaction mixture was soaked through a filter membrane with all precipitated haemoglobin, glycated and non-glycated, remaining on the membrane, the excess conjugate was removed using a washing reagent. The POC analyser then evaluated the precipitate on the membrane. By measuring the reflectance, the blue (glycated haemoglobin) and the red (total haemoglobin) colour intensities were quantified with the ratio between them proportional to the percentage of HbA_{1c} in the sample. Following sample loading, the POC analyser produced an HbA_{1c}

percentage within three minutes, displayed on the digital screen. An identical process was followed for lipid samples.

4.7. Urine Collection Procedures

In study two, the urine collection kit consisted of; 14 cm non-sterile urine collection straws (Vacutest Kima, Rome, Italy), four ml additive free evacuated vacutainer tubes (Vacutest Kima, Rome, Italy) and 125 ml transparent polypropylene collection containers (Praxisdienst, Longuich, Germany) (Figure 5.1). Collection straws and vacutainer tubes were contained in a 650 ml plastic container (Poundland, Wednesbury, UK). The same equipment was used in study three. In study five, participants received three urine collection kits for each collection phase. The kits consisted of; three 14 cm non-sterile urine collection straws, six 4 ml additive free evacuated vacutainer tubes, housed within absorbent pouches and sealed plastic bags, and a 125 ml collection container. Collection straws, vacutainer tubes and collection container were contained within addressed cardboard boxes suitable for human biofluid postage (Figure 4.1). All urine storage equipment and instructions were fully compliant with the UN3373 standard for Category B biological substances. In all studies where urine was collected, participants received instructions to fill a collection container with a mid-stream urine sample to minimise the possibility of bacterial contamination from urethral contaminants (Gilbert, 2006). The tip of the transfer straw was submerged into the urine and a vacutainer tube was inserted into the transfer straw to draw the urine into the vacutainer. Any remaining urine in the container was deposited within the lavatory. The collection container was rinsed with tap water and reused for further samples. Collection straws were returned and disposed of safely in laboratory-based sharps bins. Following their return to the investigator, samples were frozen at -80°C to await analysis.

Figure 4.1. The urine collection kit used by participants in the randomised control trial (chapter 8) including; addressed cardboard postage box, collection straws, vacutainers and collection container**.**

4.8. Urine sample preparation, extraction and adjustment

To account for differences in fluid intake between participants, all urine samples were normalized by refractive index before analysis. This ensured all measurements were made within the same dynamic range. Refractive index normalization takes the complete composition of the sample into account when normalising, not just the concentration of a single analyte. Samples were defrosted overnight in a 4 °C refrigerator. Once thawed, samples were centrifuged (14000 RPM for five min at 4 °C), placed on ice and aliquots of thawed urine (1000 µl) were transferred into two ml labelled Eppendorf tubes. The remaining sample volume was returned for freezing at −20°C. A hand-held Refractometer (Bellingham Stanley™ Brix 54 Model) was calibrated with de-ionised water (dH2O) and dried with tissue according to the manufacturer's instruction. 200 µl of urine was transferred onto the refractometer dish and a

value recorded when the refractometer returned three identical values consecutively. The refractometer was rinsed with dH2O between samples and dried prior to the next recording. Specific gravity correction factors were calculated per participant as the fold change of individual sample specific gravity to the specific gravity of the sample in the whole sample set that recorded the minimum value (Wilson *et al*., 2019). Based on refractometer values, aliquots of the required amounts of urine from centrifuged two ml Eppendorf tubes and dH2O were transferred into new two ml Eppendorf tubes on ice to make up 500 µl for extraction. 500 µl of pre-chilled (−20°C) H2O: MeOH (3:7) was added to each adjusted sample and vortexed. Samples were returned for freezing at -80°C to await analysis. 24 hours prior to analysis, samples were moved to a 4°C refrigerator and thawed fully.

4.9. Flow infusion electrospray ionisation-high resolution mass spectrometry (FIE-HRMS), multivariate modelling, classification and feature selection

This phase of analysis was completed by colleagues within the Institute of Biological, Environmental and Rural Sciences (IBERS; Aberystwyth University) who have specialised expertise in FIE-HRMS, multivariate modelling, classification and feature selection (see acknowledgements). Methods used within this phase of analysis have also been utilised in other work within the IBERS laboratory (Lloyd *et al*., 2019).

High resolution flow infusion fingerprinting was acquired on either an Exactive Orbitrap (Thermo Finnigan, San Jose, CA) or a Q-Exactive + (Thermo Finnigan, San Jose, CA) mass spectrometer. The Exactive Orbitrap mass spectrometer (Thermo Finnigan, San Jose, CA), was coupled to an Accela (Thermo Finnigan, San Jose, CA) ultra-performance liquid chromatography system front end. Twenty µl of sample was injected into the electrospray ionisation (ESI) source via a flow solvent (mobile phase) of pre mixed HPLC grade MeOH and

ultra–pure H2O (7:3) with a total acquisition time of three minutes. Flow rate was maintained at 200 µl·min^{-1} for zero to one and half minutes and then 600 µl·min^{-1} for one and half to three minutes. Positive and negative ionisation modes were acquired simultaneously using fast polarity switching. For each ionisation mode, one scan event was used to acquire all mass spectra (positive; 55.00 – 1000.00 m/z, negative; 63.00 – 1000.00 m/z), with a scan rate of 1.0 Hz. The resolution was 100,000 with an automatic gain control target of 5 x 10^5 and a maximum injection time of 250 ms. The Q- Exactive Plus Orbitrap mass spectrometer (Thermo Finnigan, San Jose, CA), coupled with a Unimate 3000 liquid chromatography tower (Dionex, Thermo Scientific) front end. Twenty µl of sample was injected into the ESI source via a flow solvent (mobile phase) of pre-mixed HPLC grade MeOH and ultra–pure H2O (7:3) with a total acquisition time of three minutes. Flow rate was maintained at 200 µl minutes^{-1} for zero to one and half minutes and then 600 µl·min^{-1} for one and a half to three minutes. Positive and negative ionisation modes were acquired simultaneously using fast polarity switching. For each ionisation mode, one scan event was used to acquire all mass spectra (positive; 80.00 – 1200.00 m/z, negative; 80.00 – 1200.00 m/z) with a scan rate of 1.0 Hz. The resolution was 280,000 with an automatic gain control target of 5 x 10^5 and a maximum injection time of 250 ms.

Dimensionality reduction of the mass spectra was performed by taking each m/z value from scans about the apex of the infusion profile and binning the m/z and intensity values for direct comparison of urine fingerprints, before signal annotation. For feature selection, supervised random forest (RF) classification was conducted using the random forest package using R software (R Core Team, 2013). RF classification margins were used to evaluate the performance of classification models (Enot *et al*., 2008) which is defined as the proportion of votes for the correct class minus the maximum proportion of votes for the other classes—the larger the margin, the more confidence in the classification (Fave *et al*., 2011). Models were

acceptable if RF margins were >0.3. Other classification performance identifiers included: Accuracy, defined as the proportion of correctly classified data points among all data points where accuracy values ranged from zero to one (one equates to perfect classification); the area under the Receiver Operating Characteristic (ROC) curve (AUC) which aggregates performance across the entire range of trade-offs between true positive rate and false positive rate and ranges from 0.5 (indicating random performance) to 1.0 (denoting perfect classification; Fave *et al*., 2011). For metabolite signal annotation, accurate m/z values were extracted to allow identification of metabolites at one–five ppm and queried using MZedDB, an accurate mass annotation database used to annotate signals by means of neutral loss and/or adduct formation rules (Draper *et al*., 2009; Lloyd *et al*., 2019).

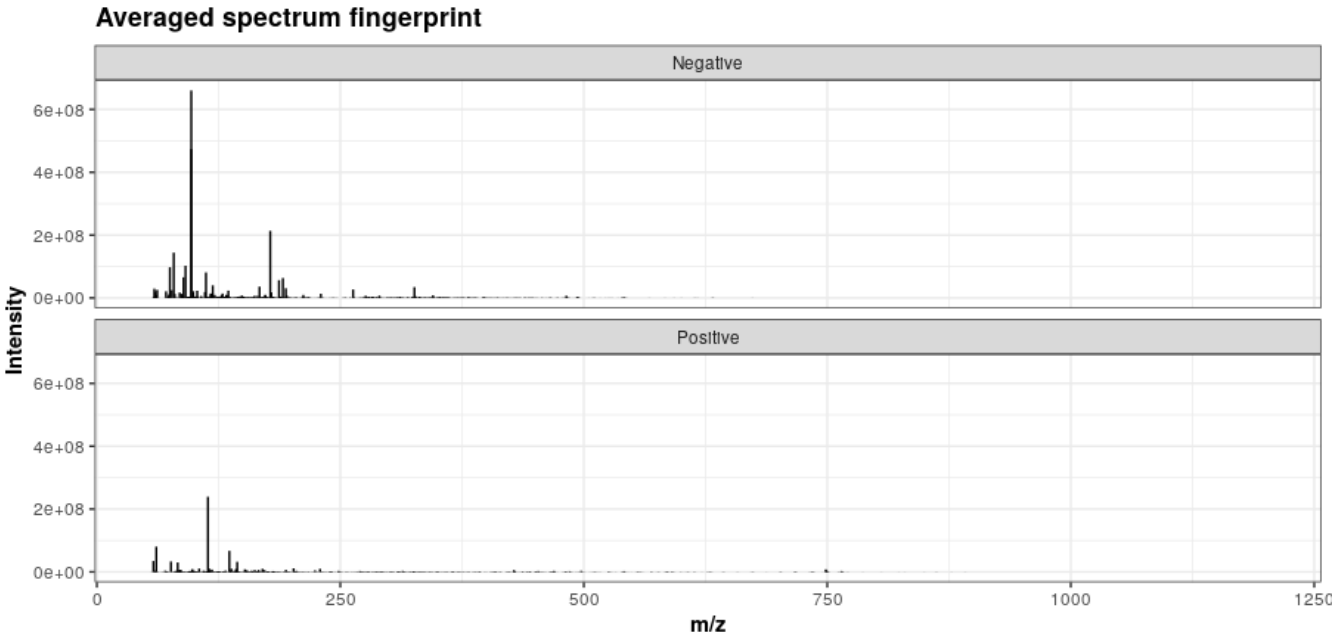


Figure 4.2. Example of a spectrum fingerprint output following FIE-HRMS

4.10. Quantification of known dietary biomarkers

Quantification of known biomarkers was performed on a TSQ Quantum Ultra triple quadrupole (QQQ) mass spectrometer (Thermo Scientific), equipped with a heated electro-spray ionization source and coupled to an Accela UHPLC system (Thermo Scientific; Lloyd *et al*., 2019). For

Hydrophilic Interaction Liquid Chromatography analysis, chromatographic separation was performed on a ZIC-pHILIC column. The mobile phase consisted of 10 mm ammonium acetate in water: acetonitrile (95:5) and 10 mm ammonium acetate in water: acetonitrile (5:95). HPLC was carried out in low pressure mode with zero and 650 bar as minimum and maximum pressures, respectively. For reverse phase (RP) analysis, chromatographic separation was performed on Hypersil Gold (1.9 μm, 200×2.1 mm^2) (Thermo Scientific). The mobile phase consisted of 0.1% formic acid in H_2O and 0.1% formic acid in MeOH. For RP analysis the flow rate was maintained at 400 $\mu l \cdot min^{-1}$. The high-performance liquid chromatography was carried out in high pressure operating mode with zero and 1000 bar as minimum and maximum pressures, respectively. For both chromatographic analyses, column oven and autosampler tray were maintained at 60 and 14°C, respectively. To ensure consistent sample delivery, 20 μl were injected using a 20 μl loop and partial loop injection mode. After each injection, syringe and injector were cleaned using a 10% HPLC grade MeOH solution in ultra-pure water (1 ml flush volume) to avoid sample carryover. Spectra were collected at a scan speed of 0.010 and 0.003 seconds for HILIC and RP analysis, respectively. A scan width of 0.010 u and peak width of 0.7 FWHM were used for both HILIC and RP analyses. Mass spectra were acquired in multiple reaction monitoring mode, in positive and negative ionization mode simultaneously using optimized values of shimmer offset, collision energy, and tube lens for each MRM transition. Absolute concentrations were calculated using a nine-point calibration curve (0.006561 to 100 $\mu g \cdot ml^{-1}$ for each biomarker). Xcalibur (V3.0.63, Thermo Fisher Scientific) was used for peak integration, calibration, and quantification (Lloyd *et al*., 2019).

Table 4.1. Known biomarkers of dietary intake measured in research chapters

Biomarker	Dietary Component
2-Cyanoethylmercapturic acid	Tar (Smoking)
2,3-Dihydroxyphenylpropionic acid	Wholegrain
3-Hydroxyhippuric acid	Fruit & Vegetables
3-Hydroxypropylmercapturic acid	Tar (Smoking)
3-Methyl-xanthine	Cocoa
3,4-Dihydroxybenzoic acid	Fruit & Vegetables
3,5-Dihydroxybenzoic acid	Fruit & Vegetables
3,5-Dihydroxybenzoic acid-3-sulfate	Fruit & Vegetables
4-Hydroxyhippuric acid	Fruit & Vegetables
7-Methyl-xanthine	Cocoa
Acesulfame-K	Sweetened Drinks
Benzoxazolinone	Wholegrain
Caffeic acid	Fruit & Vegetables
Chlorogenic acid	Fruit, coffee, green tea, vegetables
Cotinine	Nicotine
D, L-Sulforaphane-glutathione	Cruciferous Vegetables
D, L-Sulforaphane-L-cysteine	Cruciferous Vegetables
Daidzein	Soy
Dihydrocaffeic acid	Cocoa
Epicatechin	Apple, cocoa, coffee, green/black tea, fruit, vegetables
Ferulic acid	Coffee, polyphenol-rich foods
Ferulic acid-4-O-sulfate	Coffee, polyphenol-rich foods
Feruloylglycine	Coffee, polyphenol-rich foods
Furaneol	N/A
Hippuric acid	Fruit and Vegetables
Isoferulic acid	Coffee, polyphenol-rich foods
m-Coumaric acid	Coffee, olives
N-(2-Furoyl) glycine	Strongly heated foods

Naringen	Citrus
Norfuraneol	N/A
Quercetin	Fruits, vegetables, tea
Quercetin-3-O-beta-D-glucuronide	Fruits vegetables, tea
Resveratrol	Red wine, grapes
Vanillic acid	Coffee, cocoa, wholegrain, herbs, olive
p-Cresol Glucuronide	Protein
1-Methyl histidine	Poultry
2,3-Dihydroxyphenylpropionic acid-3-sulfate	Wholegrain
3-Methyl histidine	Poultry
4-Hydroxyproline betaine	Citrus
5-Hydroxy-L-tryptophan	Protein
5-Methoxyindole-3-acetic acid	Poultry
5-Oxo-L-prolyl-L-proline	Processed Cheese
B-Alanine	Protein
Calystegine A3	Potatoes
Calystegine B	Potatoes
Creatinine	Meat
D-Sucrose	Sugar
Dopamine 3-O-Sulfate	Bananas
Dopamine-4-O-sulfate	Bananas
Ethyl-beta-D-glucuronide	Alcohol
Gallic acid	Fruits & vegetables, Wine/grape, tea
Indoxyl Sulfate	Protein
L-Anserine	Poultry
L-Carnitine	Red Meat
L-Carnosine	Meat
L-Histidine	Protein
L-Nicotine	Nicotine
L-Tartarate	Grape

p-Cresol sulfate	Protein
Phenyl-acetyl-L-glutamine	Protein
Phenylalanine	Protein
Proline betaine	Citrus
Rhamnitol	Apple
Taurine	Striated muscle meat
Trans-3'-Hydroxycotinine	Nicotine
Trigonelline	Beans, soya, peanuts, almonds, coffee, peas
Trimethylamine-N-oxide	Fish
Tryptophan	Protein

4.11. Statistical Analysis

Two computerized statistical packages were used to analyse data (SPSS version 17.0, SPSS inc., Chicago, IL; R Core Team (2013; for metabolomics data)). Specific statistics employed are outlined in each experimental chapter.

Chapter Five:

Developing a protocol for accurate monitoring of habitual diet in a free-living environment using multiple spot urine samples

5.1. Introduction

An important feature in effective implementation of public health programmes is the requirement for validated, population-level dietary intake screening tools to establish the effectiveness of healthy eating interventions to change dietary behaviour (Lloyd *et al*., 2019). Limitations associated with traditional forms of dietary monitoring include; respondent burden (Goris, Westerterp-Plantenga and Westerterp, 2000; Prentice *et al*., 1986), lack of previous user experience (Champagne *et al*., 2002), decreased recording accuracy over time (Gersovitz, Madden and Smiciklas-Wright, 1978) over and underestimation of food intake (Poslusna *et al*., 2009; Schaefer *et al*., 2000), reliance on memory (Castell *et al*., 2015; Wrieden *et al*., 2003) and perceptions of body image and social desirability (Hill and Davies, 2002). Notwithstanding such limitations, it is likely these methods will continue to be utilised for dietary monitoring within both health care and academic research environments for the foreseeable future, given they are relatively cheap and simple to deliver. However, a significant body of research demonstrates the use of urine metabolomic profiling as an alternative approach for measuring dietary exposure as well as providing the opportunity to validate existing dietary recording methods (Favé *et al*., 2011). Urine dietary biomarkers are developed based on the notion that urinary excretion of specific metabolites quantitatively reflects intake of a corresponding food or nutrient over a fixed time period. In some instances, multi-metabolite biomarker panels can offer a more reliable estimation of dietary exposure than a single-biomarker approach as well as other dietary monitoring protocols (Lloyd *et al*., 2019). Many studies examining urinary

metabolomics for habitual dietary monitoring have thus far involved participants collecting 24-hour urine samples, which while robust and accurate, places a significant burden on participants due to its adverse impact on normal daily life as well as significant processing costs for the researcher (John *et al*., 2016). Given this limitation, it is unlikely the use of such could be applied to a 'real world' setting. Therefore, it is of interest to determine whether habitual dietary behaviour can be monitored within a free-living environment without intrusive or disruptive protocols being administered.

Metabolic profiling of a single spot urine sample can provide comprehensive quantitative data of dietary exposure for 12 to 24 hours prior to urine collection (Fave *et al*., 2011). While a spot urine sample is not representative of an individuals' habitual dietary exposure, a series of spot samples collected over a prolonged period may give a robust overview of an individual's nutritional behaviour and benefit from reduced logistical costs (Wilson *et al*., 2019). Work by Lloyd *et al*. (2013) demonstrated spot fasting urine samples to adequately discriminate between exposure class for several dietary items, similarly to 24-hour urines. The challenge is selecting spot samples that can give the most reliable representation over a sustained dietary exposure period whilst maintaining a low burden protocol. Therefore, determining the appropriate number of spot urine samples required and the time and frequency of collection which provides an accurate and reproducible measurement of dietary exposure, will provide essential information on improved and acceptable methods for dietary monitoring. Once established, such methods can be applied to individuals within free living environments requiring dietary monitoring following lifestyle intervention and/or disease diagnosis.

Aims:

• Determine sample collection compliance over a six-week sampling period

• Determine the number urine samples and at what collection times are needed to provide a reliable measure of dietary exposure for a six-week period.

• Gather acceptability feedback from participants on the ease of urine collection in the home.

5.2. Method

5.2.1. Participants

16 Participants; six female and 10 males volunteered to take part. Two male participants withdrew from the study before completion. The characteristics of the remaining participants were: mean age 34 ± 13 (SD) years; height 172 ± 8 cm, body mass 72 ± 13 kg and BMI 24.1 ± 3.4 kg/m^2.

5.2.2. Experimental Protocol

Before commencing the sampling phase, participants visited the laboratory to provide informed consent, record their height and body mass, receive the urine collection kits and a demonstration and written instructions (see appendix; E, F, G) on how to operate the kit correctly (see Chapter Four, General Methods, 4.7.). Participants were instructed to collect two urine samples a day for a total of six weeks. The urine samples were collected in the morning immediately after participants woke (first morning void; FMV) and before bed time (BT) in the evening. The collection straws and vacutainer tubes were contained in a 650 ml plastic container. A second 650 ml plastic container was provided to keep used vacutainer tubes in whilst being refrigerated in the participant's home.

Figure 5.1. The urine collection kit used by participants including; collection straw (left), vacutainer (centre) and collection cup (right) used by participants in the home.

Following the FMV sample, the collection straw was rinsed under tap water and left to dry, ready to be used again for the evening sample. Participants used one transfer straw per day. The sample was labelled with the sample number (e.g. one for sample one / 84 for sample 84) and stored in a refrigerator at 4ºC for a maximum of 10 days before the sample was returned to the laboratory in person for freezing at –80ºC to await analysis. Participants recorded the collection details of their urine samples onto a sample collection sheet which noted the sample number and the date and time of collection. Participants were permitted to eat and drink freely, without restriction, for the entire data collection period. Participants completed online diet diaries (FatSecret) however due to technical issues this data was not analysed. At the end of the collection period, participants were sent a questionnaire designed by the investigator detailing the acceptability of the urine sampling and storage protocols (see appendix H).

5.2.3. Sample Preparation and Multinomial Modelling

24 hours prior to treatment, individual samples were transferred from -80°C to a refrigerator at

4°C to thaw. Once defrosted, samples were normalised by refractive index using a handheld refractometer, extracted and adjusted as described previously (see Chapter Four, General Methods, 4.8.). For the creation of weekly AM and PM pools, raw individual urine samples were vortexed and centrifuged for five minutes at 14,000g. On ice, 50 µl of supernatant was transferred to a 2 ml Eppendorf, diluted with 450 µl of -20ºC pre chilled methanol and vortexed. Individualised volumes (in µl depending on urine concentration) of extracted urine were then transferred into 2 ml labelled Eppendorfs representing weekly AM and PM pools, diluted with individualised volumes of distilled water and vortexed. For the creation of global AM and PM pools representing the entire six-week collection period, individualised volumes of weekly pooled urine were transferred to labelled Eppendorfs, diluted with individualised volumes of distilled water and vortexed. For both weekly and global pools, 80 µl of supernatant was transferred to glass vials containing a 200 µl micro glass insert. Vial caps were crimped and stored at 4ºC ready for same day FIE-MS analysis (see Chapter Four, General Methods, 4.9.).

Models were created using multinomial classification. Multinomial classification has the advantage of evaluating the explanatory potential of a model across a series of classes, instead of focusing on selected binary comparisons. For example, instead of modelling binary combinations of each weekday pair, the question 'Does weekday have an effect?' can be asked, where the day of the week is the response variable. Model performance was evaluated using model accuracy and Hand & Tills (2001) extension of multi class AUC. For each model combination, dimensionality was reduced using principle component analysis (PCA). The PCA components were then reduced to a single representative vector using multi-dimensional scaling (MDS). This allowed for the entire urinary metabolome to be represented in a single dimension. Dimensionality reduced components were compared between sample combinations using Pearson correlation and students t-test.

5.3. Results

5.3.1. Compliance

Total compliance over the six-week sampling period, including both FMV and BT samples, demonstrated percentage compliance to range from 90% - 100%. Seven participants (50% of the cohort) successfully provided all 84 samples (Table 5.1.). Comparison between collection times showed compliance was higher for FMV samples (93% - 100%) than BT samples (86% - 100%; Table 5.2.). Weekday compliance ranged between 87% - 100% (Table 5.3.) while weekend compliance decreased modestly in comparison (75% - 100%; Table 5.4.).

Table 5.1. Total compliance of sample collection across the six-week sampling period

Participant Number	Total Collected	% Collected
1	84	100
2	84	100
3	81	96
4	84	100
5	83	99
6	84	100
7	84	100
8	79	94
13	77	92
16	81	96
17	84	100
19	76	90
20	83	99
21	84	100
Median:	**84**	**100**
Range	**8**	**10**

Table 5.2. Compliance for AM (FMV) and PM (BT) samples across the six-week sampling period

Participant	Sample	Total Collected	% Collected	Sample	Total Collected	% Collected
1	AM	42	100	PM	42	100
2	AM	42	100	PM	42	100
3	AM	41	98	PM	40	95
4	AM	42	100	PM	42	100
5	AM	42	100	PM	41	98
6	AM	42	100	PM	42	100
7	AM	42	100	PM	42	100
8	AM	42	100	PM	36	86
13	AM	39	93	PM	38	90
16	AM	41	98	PM	40	95
17	AM	42	100	PM	42	100
19	AM	40	95	PM	36	86
20	AM	42	100	PM	41	98
21	AM	42	100	PM	42	100
Median		**42**	**100**		**42**	**99**
Range		**3**	**0.7**		**6**	**0.14**

Table 5.3. Compliance for weekday AM (FMV) and PM (BT) samples across the six-week sampling period

Participant	Sample Type	Total Collected	% Collected
1	AM	30	100
	PM	30	100
2	AM	30	100
	PM	30	100
3	AM	29	97
	PM	30	100
4	AM	30	100
	PM	30	100
5	AM	30	100
	PM	30	100
6	AM	30	100
	PM	30	100
7	AM	30	100
	PM	30	100
8	AM	29	97
	PM	27	90
13	AM	28	93
	PM	27	90
16	AM	29	97
	PM	29	97
17	AM	30	100
	PM	30	100
19	AM	29	97
	PM	26	87
20	AM	30	100
	PM	29	97
21	AM	30	100
	PM	30	100
Median		**30**	**100**
Range		**4**	**13**

Table 5.4. Compliance for weekend AM (FMV) and PM (BT) samples across the six-week sampling period

Participant	Sample Type	Total Collected	% Collected
1	AM	12	100
	PM	12	100
2	AM	12	100
	PM	12	100
3	AM	12	100
	PM	10	83
4	AM	12	100
	PM	12	100
5	AM	12	100
	PM	11	92
6	AM	12	100
	PM	12	100
7	AM	12	100
	PM	12	100
8	AM	12	100
	PM	9	75
13	AM	11	92
	PM	11	92
16	AM	12	100
	PM	11	92
17	AM	12	100
	PM	12	100
19	AM	11	92
	PM	10	83
20	AM	12	100
	PM	12	100
21	AM	12	100
	PM	12	100
Median		**12**	**100**
Range		**3**	**25**

5.3.2. Sample Collection Times

For weekday samples (in decimal time), the mean FMV sample was 7.17 ± 0.35 (07.10 hours ± 21 minutes) and the mean weekday BT sample was collected at 10.28 ± 1.48 (22.16 hours ± 1 hour 28 minutes; Table 5.5.). The mean weekend FMV sample was collected at 7.70 ± 0.90 (07.42 hours ± 54 minutes) and the mean weekend BT sample at 10.13 ± 1.95 (22.07 hours ± 1 hour 57 minutes; Table 5.6.).

Table 5.5. Time of sample collection (in decimal hours) for AM (FMV) and PM (BT) weekday samples across the six-week sampling period

Participant	Sample Type	Mean	SD	Sample Type	Mean	SD
1	AM	5.17	0.69	PM	10.39	0.40
2	AM	7.99	0.73	PM	10.27	0.37
3	AM	7.48	0.99	PM	10.00	3.41
4	AM	7.34	0.62	PM	10.76	0.65
5	AM	6.37	0.42	PM	10.07	0.53
6	AM	7.77	0.84	PM	10.74	3.44
7	AM	7.74	0.85	PM	10.34	1.89
8	AM	6.92	1.70	PM	9.71	3.66
13	AM	7.48	0.83	PM	11.24	0.46
16	AM	7.82	1.01	PM	9.64	4.09
17	AM	5.70	0.56	PM	11.55	0.35
19	AM	7.61	1.41	PM	9.48	3.39
20	AM	7.83	0.99	PM	10.43	1.45
21	AM	7.23	0.47	PM	9.35	0.81
Mean		**7.17**	**0.35**		**10.28**	**1.48**

Table 5.6. Time of sample collection (in decimal hours) for AM (FMV) and PM (BT) weekend samples across the six-week sampling period

Participant	Sample Type	Mean	SD	Sample Type	Mean	SD
1	AM	5.13	0.68	PM	10.30	0.53
2	AM	8.32	1.01	PM	10.30	0.54
3	AM	8.54	1.01	PM	8.63	6.83
4	AM	7.63	0.62	PM	10.67	0.64
5	AM	6.84	0.47	PM	10.15	0.53
6	AM	8.56	1.02	PM	11.58	0.71
7	AM	7.64	0.79	PM	10.72	0.69
8	AM	7.83	2.09	PM	9.94	1.00
13	AM	8.54	0.53	PM	11.33	0.48
16	AM	8.67	1.01	PM	9.01	6.55
17	AM	5.97	0.83	PM	11.65	0.45
19	AM	8.18	1.14	PM	8.37	6.22
20	AM	8.35	0.76	PM	9.87	1.52
21	AM	7.58	0.67	PM	9.33	0.54
Mean		**7.70**	**0.90**		**10.13**	**1.95**

5.3.3. Variability of FMV and BT samples

Accuracy, RF margins and AUC values were calculated for FMV and BT samples to assess the variability of participant's diet across the six-week sampling period by conducting multiclass comparisons between week and day combinations. While RF margins are useful for classifying significant discriminations between two classes, their utility is not as effective for multiclass analysis and therefore any significant discrimination between weeks and/or days was identified using AUC and accuracy values. Previous work by Enot *et al*. (2008), and more recently in two studies by Lloyd and colleagues (2019), have used AUC thresholds of >0.8 to identify significant discriminations between metabolic patterns. Tables 5.7. to 5.10. represent week and day variability for FMV and BT, and show that overall, diet did not vary considerably between weeks or by day, except for participant five where an AUC >0.8 and an accuracy of 0.54 was demonstrated. All remaining AUC values were <0.8 and not considered discriminatory in either

FMV or BT samples which suggest dietary intake remained similar across the six-week sampling period in most participants.

Table 5.7. Accuracy, RF Margin and AUC values for FMV samples by week across the six-week sampling period

Participant	Accuracy	RF Margin	AUC
1	0.27	-0.06	0.69
2	0.17	-0.09	0.64
3	0.24	-0.03	0.71
4	0.26	-0.05	0.67
5	0.57	0.01	0.85
6	0.12	-0.12	0.63
7	0.31	-0.06	0.64
8	0.16	-0.07	0.64
13	0.13	-0.12	0.64
16	0.25	-0.06	0.68
17	0.28	-0.06	0.67
19	0.25	-0.07	0.68
20	0.33	-0.03	0.76
21	0.24	-0.05	0.69

Table 5.8. Accuracy, RF Margin and AUC values for FMV samples by day across the six-week sampling period.

Participant	Accuracy	RF Margin	AUC
1	0	-0.11	0.76
2	0.07	-0.10	0.69
3	0.09	-0.13	0.66
4	0.14	-0.11	0.71
5	0.02	-0.09	0.71
6	0.14	-0.12	0.69
7	0.02	-0.14	0.67
8	0.19	-0.08	0.73
13	0.08	-0.11	0.72
16	0.02	-0.12	0.74
17	0.21	-0.07	0.76
19	0.03	-0.13	0.73
20	0.02	-0.09	0.66
21	0.09	-0.09	0.68

Table 5.9. Accuracy, RF Margin and AUC values for BT samples by week across the six-week sampling period.

Participant	Accuracy	RF Margin	AUC
1	0.33	-0.06	0.65
2	0.31	-0.03	0.74
3	0.3	-0.04	0.72
4	0.24	-0.06	0.71
5	0.41	-0.01	0.78
6	0.19	-0.09	0.68
7	0.19	-0.11	0.67
8	0.12	-0.10	0.74
13	0.05	-0.09	0.75
16	0.05	-0.09	0.65
17	0.07	-0.09	0.66
20	0.29	-0.07	0.69
21	0.17	-0.07	0.70

Table 5.10. Accuracy, RF Margin and AUC values for BT samples by day across the six-week sampling period.

Participant	Accuracy	RF Margin	AUC
1	0.07	-0.09	0.67
2	0.17	-0.08	0.72
3	0.12	-0.11	0.65
4	0.05	-0.11	0.78
5	0.12	-0.09	0.68
6	0.12	-0.13	0.70
7	0	-0.13	0.73
8	0.06	-0.12	0.70
13	0.19	-0.09	0.69
16	0.05	-0.11	0.76
17	0.10	-0.08	0.68
19	0.03	-0.12	0.74
20	0.05	-0.1	0.69
21	0.07	-0.09	0.68

5.3.4. Comparisons between spot, weekly and global pool samples

To establish the degree of correlation and pairwise differences between multinomial spot and pooled samples, the data was scaled to two dimensions through multidimensional scaling to allow for direct comparison between sample types. For weeks one-six, three randomly selected days (FMV samples only) were selected and compared to the weekly pool by means of a Spearman's correlation and paired sample t-tests (Table 5.11.). Further analysis was conducted to compare specific times and phases of the week to the global urine pool to establish how frequently samples need to be collected and at what points during the week which most resemble the entire 6-week urine metabolome (Table 5.12.) Data presented in Table 5.11. demonstrate relatively strong correlations between spot and weekly pool samples in five out of six weeks ($r > 0.45 < 0.81$). Paired t-tests conducted on scaled data demonstrated no statistically significant difference between spot and weekly pooled samples in any week ($P > 0.05$).

Table 5.11. Correlation and paired t-test *P* values for three randomly selected spot samples compared to the corresponding weekly pool. Statistical significance* is accepted at $P < 0.05$.

Week	R	*P*
1	0.81	0.76
2	0.40	0.64
3	0.52	0.75
4	0.63	0.99
5	0.45	0.85
6	0.04	0.57

To determine which specific sampling time points best represented habitual dietary behaviour whilst maintaining a low burden sampling protocol, a series of sampling combinations were created and compared to the global urine pool which comprised of all urine samples collected across the entire sampling period. These combinations included; all Saturdays, all Saturdays & Sundays, all Weekdays, x3 Weekdays (randomly selected), x3 Weekdays (randomly selected), x2 Weekdays + Weekend Day (randomly selected; 6 weeks), x2 Weekdays + Weekend Day (randomly selected; 3 weeks) and x2 Weekdays + Weekend Day (randomly selected; 3 weeks; Table 5.12.).

Data presented in Table 5.12. demonstrates Spearman correlation values to range from 0.42 to 0.68, suggesting the correlations between spot sample combinations and the overall global urine pool to be moderately strong. The strongest correlation was found when correlating three randomly selected weekdays to the global pool ($r = 0.68$). The weakest correlation was seen in samples from Saturdays and Sundays ($r = 0.42$). No significant differences were found between the selected combinations of spot samples and the global pool ($P > 0.05$).

Table 5.12. Correlation and paired t-test *P* values for different combinations of spot urine samples compared to the overall global urine pool. Statistical significance* is accepted at $P < 0.05$.

Combination	Weeks Used	Total Days	R	*P*
All Saturdays	6	6	0.44	0.97
Saturday & Sunday	6	12	0.42	0.99
All Weekdays	6	30	0.58	0.86
x3 Weekdays	6	18	0.68	0.84
x3 Weekdays	6	18	0.53	0.69
x2 Weekdays + Weekend Day	6	18	0.65	0.75
x2 Weekdays + Weekend Day	3	9	0.66	0.81
x2 Weekdays + Weekend Day	3	9	0.65	0.83

Table 5.13. *P* values from paired t-tests for specific dietary biomarkers from different combinations of spot urine samples compared to the overall global urine pool. 1. All Saturdays, 2. Saturday & Sunday, 3. All Weekdays,4. x3 Weekdays, 5. x3 Weekdays, 6. x2 Weekdays + Weekend Day, 7. x2 Weekdays + Weekend Day, 8. x2 Weekdays + Weekend Day. Statistical significance* is accepted at $P < 0.05$.

Biomarker	1	2	3	4	5	6	7	8
N-Methylhistidine	0.08	0.11	0.01	0.01	0.01	0.01	0.01	0.01
Hippuric acid	0.12	0.06	0.02	0.01	0.02	0.02	0.02	0.03
Proline Betaine	0.23	0.28	0.96	0.87	0.93	0.98	0.84	0.76
P-Cresol Glucoronide	0.71	0.85	0.84	0.78	0.81	0.87	0.90	0.75
3-Methylglutarylcarnitine	0.01	0.00	0.40	0.87	0.51	0.41	0.01	0.74
Sucrose	0.11	0.11	0.76	0.87	0.59	0.87	0.48	0.54
TrimethylamineÂ N-oxide	0.05	0.04	0.00	0.00	0.00	0.00	0.00	0.00
beta-Alanine	0.59	0.68	0.31	0.28	0.32	0.35	0.31	0.32
Creatinine	0.39	0.25	0.03	0.03	0.03	0.03	0.04	0.03
Acesulfame	0.53	0.21	0.58	0.61	0.58	0.54	0.50	0.53
N-Methylxanthine	0.19	0.08	0.55	0.75	0.55	0.18	0.16	0.22
Tartaric acid	0.04	0.02	0.25	0.22	0.16	0.23	0.85	0.27
Citric acid	0.33	0.30	0.05	0.07	0.07	0.06	0.07	0.03

A series of dietary biomarkers associated with the intake of specific foods were analysed using the same combinations of days presented in Table 5.12. The biomarkers displayed in Table 5.13 were selected for analysis based on their links to foods of health importance and to establish how different combinations of spot samples affects specific dietary related metabolites in comparison to the global urine pool. Significant differences in the concentrations of diet specific metabolites were found in all combinations of days when compared to the global pool. Combination one (all Saturdays) demonstrated the fewest significant differences (3-Methylglutarylcarnitine, P = 0.01; Tartaric acid, P = 0.04) while combination seven (x2 Weekdays + Weekend Day; randomly selected; 3 weeks; N-Methylhistidine, P = 0.01; Hippuric acid, P = 0.02; 3-Methylglutarylcarnitine, P = 0.01; TrimethylamineÂ N-oxide, P < 0.00; Creatinine, P = 0.04) and combination eight (x2 Weekdays + Weekend Day; randomly selected; 3 weeks; N-Methylhistidine, P = 0.01; Hippuric acid, P = 0.03; TrimethylamineÂ N-oxide, P < 0.00; Creatinine, P = 0.03; Citric acid, P = 0.03) demonstrated the largest number of metabolites showing a significant difference between spot combinations and the global urine pool.

5.3.5 Protocol Feedback

Feedback from participants regarding the urine sampling procedure was largely positive (Table 5.14.). One hundred percent of participants suggested the urine collection kit was easy to use with 86% stating they successfully used the kit every time they were required to provide a urine sample. Seventy nine percent of respondents felt the urine sampling procedure did not cause excessive disruption to their usual daily routine whilst the same percentage of participants felt procedures for storing their samples was easy. Of interest, 93% of participants suggested they would be happy to use a similar urine collection kit for future research purposes. This

demonstrates the urine sampling procedure to be wholly acceptable to study participants and can feasibly be implemented in future work.

Table 5.14. Percentage and written feedback questionnaire responses from 14 participants following the six-week sampling period

Question	Yes	No	Comments
Did the time of day you were required to provide a urine sample cause disruption to your usual daily routine?	21%	79%	**Participant 19**: *"Yes but only due to the fact that I often work nights"* **Participant 21**: *"A minor hindrance as it took a few minutes"*
Was the urine sampling kit easy to use?	100%	0%	No additional comments
Did you use the urine sampling kit successfully every time?	86%	14%	**Participant 5:** *"only one malfunction"* **Participant 12:** *"Sometimes I forgot to put the end of the tube/end of the needle all the way into the urine and the tube wasn't filled up all the way"*
Was it easy to store your urine samples once they had been collected?	79%	21%	**Participant 2**: *"Most of the time this wasn't a problem. It was more tricky if we were away especially when camping but even staying with family was more difficult"* **Participant 9:** *"Inconvenient when going away on holidays/weekends away/staying at girlfriends house"* **Participant 19**: *"There were some occasions when I was unable to store the samples in the fridge due to being away and having access to a fridge. However, when at home, storage was really*

			simple" **Participant 20**: *"Would have been yes - but when away I didn't always have access to a fridge"*
Having completed this process and knowing what it involves, would you be prepared to use the urine sampling kit for research purposes in the future?	93%	7%	No additional comments
Further comments			**Participant 3**: *"As a woman, collecting urine wasn't much of an issue as I thought it would be which is a positive. Fell easily into the routine. I believe it was an effective method* **Participant 4**: *"The process of using the sampling kit was relatively straight forward"* **Participant 5**: *"No problems with the urine kit or exchange/resupply arrangements"* **Participant 9**: *"Every day sampling is inconvenient, but if it was only a couple each week, it would be hard to remember when to take sample (you get into a routine)"*

5.4. Discussion

Given the limitations associated with traditional forms of dietary monitoring, the use of urinary metabolomics as an alternative dietary recording method is a growing area of research which can provide useful insights into the effectiveness of dietary change initiatives. Most studies examining urinary metabolomics as a dietary monitoring method have thus far involved participants collecting 24-hour urine samples, which while being able to represent an entire dietary intake phase, places a significant burden on participants due to the method's adverse impact on normal daily life (John *et al*., 2016) as well as resulting in significant processing costs for the researcher (Wilson *et al*., 2019). Provision of a single spot urine sample can give a comprehensive snapshot of dietary exposure for 12 to 24 hours prior to urine collection (Favé *et al*., 2011) although is not representative of an individuals' habitual dietary intake. However, a series of spot samples collected over a prolonged period may provide a robust overview of an individual's nutritional behaviour. For example, spot urine samples have been shown to adequately discriminate between exposure class for several dietary items, similarly to 24-hour urines (Lloyd *et al*., 2013). Therefore, the challenge of this work was to identify spot urine samples which can give the most reliable representation of dietary behaviour over a sustained exposure period. This was in addition to maintaining a low burden protocol that could be completed within a 'free living' environment, required fewer processing resources and therefore making it cheaper and subsequently more viable for future application to large scale epidemiological research.

To have utility in monitoring habitual free-living dietary behaviour, a urine sampling protocol must be easily administered and acceptable for participants. The first aim of this work was to establish participant's adherence to the urine sampling protocol. This included calculating total compliance, collection time compliance and weekday and weekend compliance. Participants

were instructed to collect an FMV and BT sample every day for a six-week period. Results demonstrated total collection compliance to range between 90% to 100%. When separated by collection time, compliance for FMV samples was greater than BT samples, ranging between 93% - 100% and 86% - 100%, respectively. When analysed by day, compliance was higher for weekday samples (87% - 100%) compared to weekend samples (75% - 100%). A reduced adherence to sample collection on weekend days is significant given dietary behaviour of the participants likely changed when compared to week days. Previous work by An (2016) has shown significant alterations in dietary intake on weekends including increased intakes of sugar-sweetened beverages, alcohol, total fat, saturated fat, sugar and sodium whilst decreasing intakes of fruit, vegetables and fibre. Similarly, a study by Jahns *et al*. (2017) found intake of alcohol increased and intakes of fruit, vegetables, poultry, nuts, seeds and whole grains decreased on weekends compared to weekdays. Weekend sample collection compliance was reduced in the present work, compared to week day compliance and therefore future research should not only include a weekend sample to capture dietary behaviour but also emphasise the importance of adhering to weekend sampling to future participants and patients. With the data demonstrating FMV samples to be collected more compliantly compared to BT samples, it may be beneficial for future research to focus solely on FMV samples to ensure increased compliance while further reducing participant burden by removing the BT sample. The shorter time frame in which FMV samples were collected by participants also provides a more controlled window of sample collection compared to BT samples where the range of collection times was wider. Furthermore, findings in work by Wilson *et al*. (2019) suggests biomarkers derived from colonic fermentation may not be highly represented in BT urine samples and so therefore recommends the use of FMV urine sampling to ensure full dietary metabolite coverage is achieved. An example of such in Wilson and colleagues' work was the skewed distribution towards lower concentrations measured in post dinner spot urines for D-L-

sulforaphane (a biomarker of exposure to cruciferous vegetables).

The values presented in Tables 5.7.-5.10. demonstrate the composition of both FMV and BT samples remained similar across the six-week sampling period and suggests that participants dietary behaviour was habitual, with data for only one participant being suggestive of a change in dietary intake. The large variation between weeks, and the days within, is unsurprising given participants were free to consume any food and drink of their choosing and were not required to follow a structured diet plan or intervention during the sample collection period. This suggests participants maintained consumption of a habitual diet throughout the sampling phase with the primary driver of variation in urinary metabolome models being inter-individual as opposed to intra-individual. Given a short temporal phase was used (i.e. six weeks) for sample collection, lack of intra-individual differences was not unexpected. Seasonal changes in dietary behaviour are a primary factor that can influence a persons' habitual diet in the absence of an intervention (Ma *et al*., 2006) with consumption of several fruits and vegetables, for example, demonstrating significant variation depending on the season (Capita and Alonso-Calleja, 2005). Therefore, future work should ensure a dietary monitoring protocol using urinary metabolomics, as well as other dietary recording protocols, covers the entire investigative period following lifestyle intervention to ensure any seasonal variation in dietary behaviour is captured.

Given collection compliance was greater for FMV samples in comparison to BT samples, as well as evidence suggesting FMV samples will capture a greater depth of dietary metabolites (Wilson *et al*., 2019), comparisons between spot sample combinations and weekly and global urine pools were conducted using FMV samples only. The data presented in Table 5.11. shows three randomly selected spot samples compared favourably to their corresponding weekly pool

in five weeks out of the six-week sampling period, with moderate to strong correlations demonstrated (> 0.4). This provides a reasonable argument to suggest that three urine samples a week can adequately describe a person's dietary intake, in comparison to the weekly pool, without imposing a daily and burdensome urine sampling protocol on the participant. In support, the data presented in table 5.12. show moderate to strong correlations between combinations of spot samples and the global urine pool. Of interest, three randomly selected weekday samples showed the highest correlation between spot sampling and the global pool (r = 0.68) while a paired samples t-test suggested no significant difference between the metabolite content of the spot samples and the pool ($P = 0.84$). While three weekday samples were shown to correlate the strongest, evidence discussed above also highlights the importance of capturing dietary behaviour on weekend days. Therefore, combinations to include two randomly selected weekdays and a weekend day were included to understand their performance compared to the globally pooled urine. When analysed across the entire six-week period, two weekday and a weekend day sample correlated well to the global pool (r = 0.65) while no significant difference was found in metabolite profiles ($P = 0.75$). However, given a key aim of this work was to establish a low burden urine sampling protocol, an identical combination of samples, encompassing two weekdays and a weekend sample, was examined against the global pool, only across a three-week period as opposed six weeks. Interestingly, a similar correlation was discovered despite reducing the number of samples in the correlation from eighteen to nine (r = 0.66) and again finding no significant difference between the spot combinations and the global pool ($P = 0.81$). This shows that three urine samples collected per week for three weeks, including two-week day samples and a weekend sample, can provide similar dietary information compared to the global pool, which includes FMV samples collected once a day for a six-week period. This finding means a significantly reduced sampling protocol can be implemented in future work which can both capture useful dietary intake data without

excessively burdening the participant, therefore making it more likely sampling adherence will be maintained.

While this work has shown promise in developing a low burden protocol for accurate monitoring of habitual diet in a free-living environment, this method does have some limitations. The data presented in Table 5.11. shows that in week six, three randomly selected days did not correlate well with the week six pool, suggesting the spot sampling protocol was ineffective in successfully identifying dietary intake when compared to daily sampling. In addition, the urine concentration of several metabolites which are associated to specific dietary items were shown to be significantly different to the global urine pool across all spot sample combinations. This was most apparent for spot sample combinations seven and eight which both showed five metabolite concentrations to be significantly different between two weekday and a weekend day sample in comparison to the global pool. This is important given it is this specific spot sampling protocol (2 x weekday/ 1 x weekend day) that has been suggested as the most appropriate in capturing dietary intake information whilst maintaining a low burden sampling protocol. However, it must be argued that while the two weekday/one weekend day protocol does contain limitations, a balance must be found between capturing highly accurate dietary information and the burden imposed by a lengthy and demanding sampling procedure.

This work has successfully demonstrated a sampling protocol encompassing two weekdays and a weekend day to correlate well to the overall urine pool, which includes all FMV samples over the six-week urine collection period and means an overall quantitative snapshot of dietary intake can be accessed through this methodology. The growing use of urine metabolomic technology for the purposes of dietary monitoring was and is not designed to fully replace more established forms of dietary monitoring, such as dietary recalls and FFQs, but to supplement

and validate such methods. While a three per week/three-week sampling procedure may not capture every individual metabolite change as demonstrated in Table 5.13., it has been shown to provide reasonable agreement with spot samples collected every morning for six weeks (Table 5.12.) which can give an acceptable overall impression of dietary pattern. In addition, feedback collected from participants demonstrates the urine sampling protocol completed within the home setting is highly acceptable, with only a small number of negative comments made (Table 5.14.). Such comments mostly related to the inconvenience of every day sampling and the difficulty storing samples when away from home. This feedback therefore supports the proposal of limiting sampling to three days per week for three weeks given daily sampling increased the difficulty of storing samples when away from home and resulted in an increased burden generally.

In conclusion, this work has shown that three FMV urine samples collected over three weeks, encompassing two-week days and a weekend day, correlates well to daily urine sampling over a six-week period for measuring metabolite dietary patterns. This finding, alongside data for sampling adherence and acceptability, suggests this protocol can successfully be implemented in future work to both monitor dietary behaviour and assist in validating other dietary monitoring methods.

Chapter Six:

The effects of acute moderate exercise on the urinary metabolome

6.1. Introduction

Metabolomic profiling of human urine is a developing method for monitoring habitual diet. Given the increase in diagnoses of chronic diseases associated with poor dietary habits, methods for improved dietary monitoring are becoming more important. The work detailed in chapter five has demonstrated a feasible community-based method for collecting urine samples in the home across a six-week period using a simple urine collection kit. This method of urine collection provided a robust overview of dietary behaviour, was methodologically acceptable to the participants and did not cause excessive disruption to participant's daily life. This method will continue to be developed to provide a more accurate way of monitoring diet in the community by validating traditional methods of dietary recording, including people at risk of chronic disease, such as T2D.

While community-based urine sampling demonstrates a successful and non-disruptive method for monitoring diet, several studies have also examined the effects of different modes of exercise on the biological metabolome. Exercise has been shown to cause significant metabolic disruptions which alter the metabolites present in human bio fluids. This has been demonstrated in plasma (Lewis *et al*., 2010; Chorrel *et al*., 2012) and in serum (Pohjanen *et al*., 2007; Yan *et al*., 2009) using a metabolomic approach. More recently, some studies have focussed attention on how exercise affects the urinary metabolic fingerprint including; high intensity exercise (Enea *et al*., 2010; Pechlivanis *et al*., 2010; Pechlivanis *et al*., 2015; Siopi *et al*., 2017), submaximal exercise (Enea *et al*., 2010; Ali *et al*., 2016) and resistance exercise (Siopi *et al*.,

2017). Most of these studies demonstrate significant changes in the urinary metabolome following different forms of exercise which vary in duration and intensity. For example, work by Pechlivanis *et al*. (2010) analysed urine samples following two repeated sprint protocols, differing in the rest duration between exercise efforts. Analysis revealed clear distinction between pre and post exercise samples caused mainly by the presence of lactate in the latter. Multivariate analysis found a further 22 metabolites changed significantly from pre to post in both groups. When the effect of the rest period was examined, 12 metabolites were found to respond differently depending on the amount of rest provided. Sprint exercise resulted in large increases in the products of anaerobic carbohydrate degradation, namely lactate and pyruvate, both of which were clearly separated by rest duration. The results of this work demonstrate repeated high intensity sprints to result in significant changes in the urine metabolome with post exercise urines demonstrating different profiles to pre-exercise samples.

In another study, Enea *et al*. (2010) explored the extent of metabolic changes following both short, intense exercise and prolonged exercise. Urine samples were collected before and 30 minutes after both exercise regimens. Marked differences in signals between pre and post samples were demonstrated within the short intensive exercise group. The major identifiable signals were attributed to lactate and creatinine. Other metabolites responsible for differences between pre and post samples were alanine, acetate, acetoacetate, pyruvate, succinate, hippurate and hypoxanthine. Prolonged exercise caused no significant distinctions between pre and post exercise urine samples, suggesting prolonged exercise does not significantly alter urinary metabolite profiles. In later work examining high intensity exercise, Pechlivanis *et al*. (2015) examined changes in the urinary metabolome in a two-hour period following two identical sprint sessions. Sample analysis revealed significant changes in several metabolites within post exercise urines, demonstrating short, high intensity sprint exercise to result in

significant metabolic disturbance which persists for at least one hour following exercise cessation before most metabolites return to baseline values.

The effects of one-hour aerobic activity on the urine metabolome was investigated in a pilot study by Daskalaki, Blackburn, Kalna, Zhang, Anthony and Watson (2015). Three physically active males underwent a 37-hour protocol with urine samples collected at regular time points both before and after one hour of prolonged exercise in which the exercise intensity was controlled by the participants. Data analysis revealed changes in the purines; hypoxanthine, xanthosine, inosine and guanine which reflected the acute impact of exercise, with peak concentrations observed within the first post exercise samples. Metabolites associated to tryptophan metabolism were also significantly affected by exercise and included 3-hydroxy tryptophan, hydroxyindole pyruvate, hydroxytryptophol, pyridone carboxamide and to a lesser extent, kynurenate. Pantothenic acid also increased in the first post exercise sample which the authors suggest may be indicative of decreased demand for Acetyl coenzyme A (Acetyl-CoA) and a subsequent shift to glycolysis in the place of fat metabolism. Increases in pyruvate support this. Given fat oxidation decreases with increasing exercise intensity, this may well be a plausible explanation. However, participants were free to control the exercise workload themselves and therefore it is difficult to determine the predominant energy system usage without specific values for exercise intensity being provided.

In a follow up study, Ali, Burleigh, Daskalaki, Zhang, Easton and Watson, (2016) recruited 10 participants to complete a standardised light-moderate intensity exercise protocol. Analysis revealed clear separation between pre and post exercise samples on the day of exercise. The metabolites responsible for separation were hypoxanthine, guanine, deoxyinosine, inosine and xanthosine all of which are metabolites within the purine pathway. Changes in these

metabolites were not present within samples collected the previous day and suggest the 'purine response' requires physical activity to result in observable changes. Additionally, an increase in nonanoyl carnitine, decanoyl carnitine and ketodecanoyl carnitine, increases which were not observed during the rest day, was attributed to a decreased reliance on fatty acid metabolism during exercise which represents a shift towards glycolysis to maintain ATP synthesis. The results of this work demonstrate the impact of light-moderate exercise on metabolites such as hypoxanthine and inosine, both of which are markers of exercise as demonstrated from other studies.

In a recent investigation comparing the acute effects of different modes of exercise on the urinary metabolic fingerprint was conducted by Siopi *et al*. (2017). Three forms of exercise were investigated including high intensity interval exercise, continuous moderate intensity exercise and resistance exercise. In a randomised crossover design, 23 male participants completed four trials including high intensity interval exercise, moderate intensity continuous exercise, resistance exercise and rest. When comparing the three modes of exercises employed, resistance exercise resulted in the greatest and most sustained effect on the metabolome, followed by high intensity interval exercise and lastly continuous moderate exercise which had little effect. The modes of exercise employed in this study were designed to encompass the full energy system with the expectation that different exercise intensities will result in distinct variations in the metabolic fingerprint. Previous work has demonstrated such differences in plasma (Lewis *et al*., 2010; Chorrel *et al*., 2012) and results from this study examining urine support those previous findings. The impact of exercise on the urinary metabolome continued for several hours following exercise completion with metabolites gradually returning to baseline levels after 24 hours. The changing metabolome was indicative of both increased lipid oxidation and glycogen synthesis which is synonymous with exercise. The changes occurring

in the metabolome over this time were attributed to degradation of purines, carbohydrates, proteins and amino acids (Siopi *et al*., 2017).

Many changes in metabolite concentrations brought about through short duration, high intensity exercise and resistance exercise are the result of changes in the compounds related to the ATP-PCr system and the glycolytic system. These are the predominant energy pathways associated with this high intensity exercise. A clear metabolic theme is present in the above studies with several metabolites regularly appearing in urine samples following high intensity exercise which provides a clear distinction in the urinary metabolome. Metabolites of the purine pathway are a common presence in post exercise samples and represent increased ATP utilisation and breakdown with the presence of purines most common following intense physical activity. Similarly, the frequent presence of lactate and pyruvate after an intense exercise protocol represents a shift in energy metabolism to anaerobic mechanisms. In addition, changes in amino acids are attributed to muscle protein breakdown followed by metabolism of the released amino acids. However, studies focussed on moderate intensity exercise and its effects on urine metabolites have produced mixed findings. Enea *et al*. (2010) found submaximal exercise resulted in no distinctions between the pre and post urine metabolic profiles. This study however only used female subjects and therefore may not be representative of a general population's metabolic response to exercise as previous research has demonstrated metabolic signatures in urine can be distinguished based on gender (Slupsky *et al*., 2007). Similarly, work by Siopi *et al*. (2017) also found moderate intensity activity to have little metabolic effect. Conversely, Ali *et al*. (2016) demonstrated several metabolic changes in urine following 45 minutes of continuous light and moderate exercise on a cycle ergometer using both male and female participants. This study revealed a significant impact of moderate intensity exercise on established markers for exercise including purine metabolites

hypoxanthine and inosine. Given the disagreement between these studies regarding the effects of moderate/submaximal exercise on urine metabolites, it appears prudent to further investigate the changes in urine profiles following this mode of exercise and develop a firm understanding on the effects of moderate intensity exercise on the urinary metabolome.

The present study is concerned primarily with moderate intensity exercise as this level of physical activity is recommended by national health guidelines and therefore most likely undertaken by individuals attempting improved lifestyle behaviours following clinical advice. Moderate exercise, such as continuous jogging, swimming or cycling, is defined by the NHS as aerobic exercise hard enough to increase heart rate and sweat rate above resting values. Thirty minutes of moderate intensity exercise, five times a week, is recommended by Public Health England & Wales and the National Health Service (in line with recommendations by the four UK chief medical officers; UK Chief Medical Officers' Physical Activity Guidelines, 2019) to maintain and/or improve general health and is easily accessible in community settings. It is suggested by these organisations that these modes of exercise at a moderate intensity are most applicable to individuals requiring lifestyle modification. It is therefore of interest to determine whether clear distinction between urine samples before and after moderate intensity exercise is present and to what extent does the urinary metabolome change. Determining the effects of moderate exercise on the urinary metabolome will prove useful for a later chapter within this thesis where urine samples (for dietary monitoring purposes) will be collected from individuals at risk of T2D who are undergoing lifestyle modification. If moderate exercise does result in urinary metabolome change, knowing which metabolites are likely to be present in samples will be advantageous.

Aim:

• Determine change in the urinary metabolome following a 30-minute moderate intensity

exercise protocol.

6.2. Method

6.2.1. Participants

Participants completed a health questionnaire before commencing the study to identify any reason that would prevent the completion of 30 minutes moderate exercise. 17 participants, 10 female and seven males, volunteered and were recruited to the study. The physical characteristics of the participants were: mean age, 41 ± 14 (SD) years; height, 171 ± 9 cm; body mass, 75 ± 10 kg; BMI 25.8 ± 3.9 kg/m^2 and peak oxygen uptake ($\dot{V}O_2$peak) 2.9 ± 0.9 L·min^{-1}.

6.2.2. Experimental protocol

Participants attended the laboratory on three separate occasions, separated by at least seven days, having refrained from the consumption of alcohol and any strenuous or unaccustomed physical activity for 24 hours prior to each visit. Visit one commenced between the hours of 0700 to 1700 while visits two and three commenced between the hours of 0700 to 0900. Participants recorded their diet for 24 hours prior to visits two and three using a simple diet diary designed specifically for the study (see appendix K) and were asked to ensure dietary intake was similar for visits two and three. Dietary adherence was confirmed by the researcher inspecting each food diary prior to testing. Participants visited the laboratory after an overnight fast of > eight hours (not including the $\dot{V}O_2$peak test, see below) but were instructed to consume at least 500 ml of plain water, before arriving at the lab for each visit. The first visit required participants to complete a ramp incremental exercise test on a cycle ergometer (Lode Corival, Groningen, Netherlands) to establish $\dot{V}O_2$peak, using an online breath by breath system (Quark PFT, Cosmed, Rome, Italy). Participants completed the remaining two visits in a randomised,

crossover design. The order of visits was randomised using an open source software package (PEPI for Windows, Brixton Health). During one of the visits, participants exercised on a cycle ergometer for 30 minutes at a fixed exercise intensity. The other visit required participants to sit quietly for 30 minutes in the laboratory. Participants provided a first morning void (FMV) sample prior to arriving in the lab for both experimental visits. Further urine samples were collected before and after the exercise and rest trials.

6.2.3. Maximal exercise test

A 30 $W \cdot min^{-1}$ ramp test to volitional exhaustion on a cycle ergometer was used to establish $\dot{V}O_2peak$. Prior to each test, the breath-by-breath analyser was calibrated using a standard three L volume cylinder (Hans Rudolf, Kansas, USA) and gases of known concentration, assuming accuracy of the concentration certificates provided by the supplier (BOC, Guildford, UK). Participant's height and body mass were recorded before being fitted with a radio telemetry heart rate monitor (Polar, FS2C, Kempele, Finland). Once seated on the cycle ergometer, seat height and handle bar position were adjusted into the appropriate position for each participant. Participants were fitted with a sterilised silicon face mask (Hans Rudolf, Kansas, USA), which was held in place via a head cap (Hans Rudolf, Kansas, USA), and sat quietly on the ergometer for one minute to regulate their breathing. The first three minutes of exercise were unloaded (0 W) and then the power output increased by one W every two seconds until participants reached volitional exhaustion. Information regarding revolutions per minute (RPM) and power output (PO) was visible to the participant and strong verbal encouragement was provided throughout the test.

On completion of the $\dot{V}O_2peak$ test, the data were averaged over six second periods and exported to Microsoft Excel (Microsoft Excel 2010, Computer Software, USA). Each

participant's gas exchange threshold (defined as an increase in the rate at which $\dot{V}CO_2$ rises versus $\dot{V}O_2$, accompanied by an increase in $\dot{V}E/\dot{V}O_2$ while $\dot{V}E/\dot{V}CO_2$ continued to decrease or levelled off) was determined using the modified version of the v-slope method (Beaver *et al*., 1985). Data for $\dot{V}O_2$ and $\dot{V}CO_2$ were plotted against time for the gas exchange threshold to be visually identified and were confirmed by a graph of ventilatory equivalents versus time. Identification of the gas exchange threshold for each subject was confirmed by an additional investigator. The power output equivalent to 10% below the lactate threshold was calculated individually for each subject from the gas exchange data and adopted for the constant-load, submaximal exercise visit.

6.2.4. Experimental trials

On arrival at the laboratory, participants returned their 24-hour diet diary alongside their first morning void (FMV) urine sample which was frozen immediately at -80ºC for later analysis. Participants provided a mid-stream urine sample (PRE) into a 600 ml Pyrex beaker (Fisher Scientific UK Ltd., Loughborough, UK) in private. Urine was transferred from the beaker into a four ml additive free evacuated vacutainer tube before freezing. A radio telemetry heart rate monitor was fitted on to the chest and baseline heart rate recorded. Participants either exercised for 30 minutes on a cycle ergometer at 10% below the lactate threshold (78 ± 22 W) or sat quietly in an upright position for the equivalent amount of time. The cycle ergometer was set in hyperbolic mode which allowed the required PO to be maintained irrespective of the subject's RPM. Heart rate and ratings of perceived exertion using a 15-point scale (RPE; Borg, 1982) was recorded every five minutes of exercise. Information regarding RPM and PO was visible, but participants were given no feedback on time elapsed. During the rest trial, participants sat quietly in a phlebotomy chair within the laboratory. Heart rate was recorded every five minutes. Upon completion of the 30 minutes exercise or rest, participants

immediately provided a second urine sample in private (POST; Sample treated the same as FMV & PRE). Participants were then free to use the shower and changing facilities before leaving the laboratory.

6.2.5. Urine preparation and data analysis

Random forest classification margins were generated to identify discriminations between exercise and rest samples, where the greater the margin demonstrates increased confidence in the sample classification. RF margins > 0.3 were used to indicate adequate sample classification. Feature selection of discriminatory signals was conducted where acceptable margins were reached (Enot *et al*., 2008). For metabolite signal annotation, accurate m/z values were extracted from the mass spectra to allow direct identification of metabolites. Signals were identified using MZedDB, an interactive accurate mass annotation tool which can directly annotate signals (Draper *et al*., 2009; Lloyd *et al*., 2017).

Heart rate and RPE data were tested for normality using skewness and kurtosis tests. Heart rate data were analysed by two-way repeated measures ANOVA and RPE data analysed by one-way repeated measures ANOVA. Data are presented as mean ± standard deviation. Statistical significance was accepted at $P < 0.05$.

6.3. Results

6.3.1. Heart rate and RPE

Mean heart rate during exercise was 121 ± 17 beats per minute, this equated to 68% of maximum heart rate values during exercise, which agrees with other work examining moderate exercise (Siopi *et al*., 2017). Heart rate was significantly different between the exercise and rest trials ($P < 0.00$), increasing significantly between five and 30- minutes during the exercise trial (114 ± 15 to 126 ± 16 bpm; $P < 0.00$). Heart rate remained similar between five and 30-minutes during rest (65 ± 12 to 65 ± 11 bpm; $P = 0.71$). Mean RPE for the exercise trial was 11 ± 1 arbitrary units. RPE increased significantly between five and 30- minutes of exercise (9 ± 2 arbitrary units to 11 ± 2 arbitrary units; $P < 0.00$). Values reported by participants ranged between six and 15 arbitrary units which represent perceived workloads of "No exertion at all" to "Hard" exertion, suggesting most participants were between values in which moderate exertion falls. No participants reported values which represent workloads of "very hard" and "maximum exertion" (Williams, 2017).

Table 6.1. Mean heart rate (HR; in bpm) and RPE (arbitrary values) in the exercise trial and mean heart values in the rest trial between five and 30 minutes.

Exercise					
HR5	**HR10**	**HR15**	**HR20**	**HR25**	**HR30**
114 ± 15	120 ± 14	121 ± 15	124 ± 14	123 ± 16	126 ± 16
RPE5	**RPE10**	**RPE15**	**RPE20**	**RPE25**	**RPE30**
9 ± 2	10 ± 2	11 ± 2	11 ± 2	11 ± 2	11 ± 2
Rest					
HR5	**HR10**	**HR15**	**HR20**	**HR25**	**HR30**
65 ± 12	63 ± 12	64 ± 12	64 ± 12	64 ± 11	65 ± 11

Table 6.2. RF classification margins for FMV, Pre and Post urine samples in exercise and rest trials.

Pairwise	RF Margin	Accuracy	AUC
FMV Rest- Post Rest	0.4	0.83	0.93
FMV Exercise – Post Exercise	0.32	0.84	0.93
FMV Rest – Pre-Rest	0.11	0.64	0.73
Pre-Exercise- Post Exercise	0.07	0.63	0.64
FMV Exercise – Pre-Exercise	0.03	0.56	0.6
Pre-Rest – Post Rest	0.03	0.53	0.57

Table 6.3. Annotated metabolites differing significantly between FMV and post urine samples in rest and exercise trials.

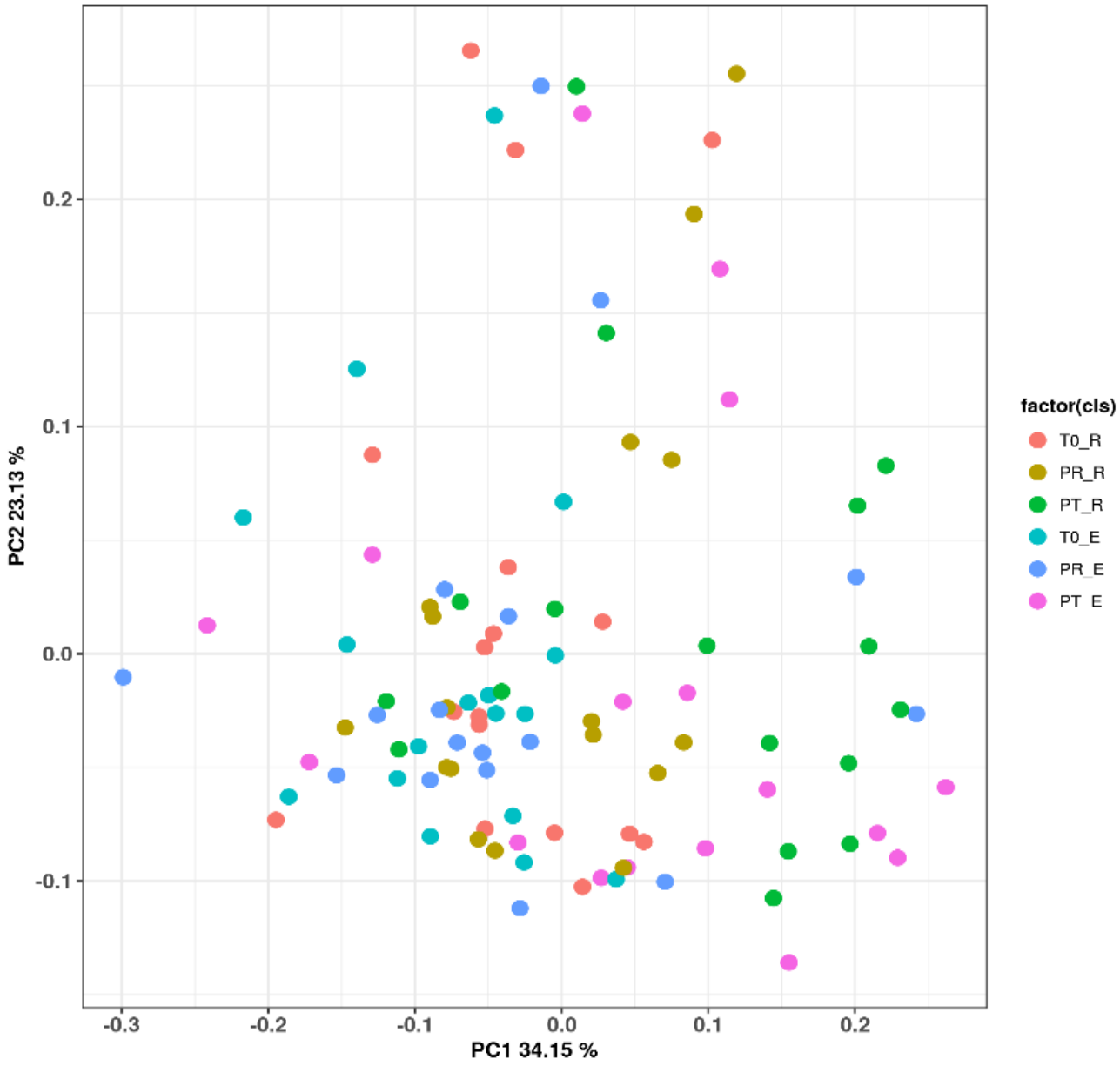
Polarity	m/z	Metabolite	*P* value
		Rest Trial	
-	199.0972	(1R,2R,3S,1'R)-Nepetalinic acid	< 0.00
-	89.0238	2-hydroxypropanoic acid	< 0.00
-	539.24585	Polyethylene glycol 500	0.03
		Exercise Trial	
-	332.17761	1,8-Epoxy-p-menthan-4-ol glucoside	0.01
-	152.04294	2',3'-Dihydroxyacetophenone	0.03
-	89.0238	2-hydroxypropanoic acid	0.03

6.3.2. Urine Metabolomics

Examination of RF classification margins demonstrated 30 minutes of moderate exercise on a cycle ergometer resulted in no urinary metabolome changes. An RF margin of 0.07 strongly suggests an untargeted metabolomic approach was unable to detect significant metabolic changes which distinguish pre and post exercise urines. Similarly (and expected) no distinct classification of samples was detected in resting urine samples (RF margin; 0.03). Lack of sample classification is evidenced further by PCA plots (Figures 6.1A and 6.1B) which demonstrate no clear visual separation between sample time points. Given no separation was

found between metabolites in pre and post samples in both exercise and rest, no feature selection of discriminatory signals was required. RF margins between FMV and post urine samples in both exercise and rest trials exceeded the 0.3 classification threshold. Feature selection was conducted to determine discriminatory signals between time points which successfully identified a small group of metabolites contributing towards sample classification. In rest, changes between FMV and post rest samples were found in (1R,2R,3S,1'R)-nepetalinic acid (P = 0.00), 2-hydroxypropanoic acid (P = 0.00) and polyethylene glycol 500 and (P = 0.03). In the equivalent exercise samples, 1,8-Epoxy-p-menthan-4-ol glucoside (P = 0.01), 2',3'-Dihydroxyacetophenone (P = 0.03) and 2-hydroxypropanoic acid (P = 0.03) changed significantly between FMV and post exercise samples.

6.1A


PC2 23.13 %
0.2
0.1
0.0
-0.1
-0.3
-0.2
-0.1
0.0
0.1
0.2
PC1 34.15 %
factor(cls)
T0_R
PR_R
PT_R
T0_E
PR_E
PT_E

6.1B

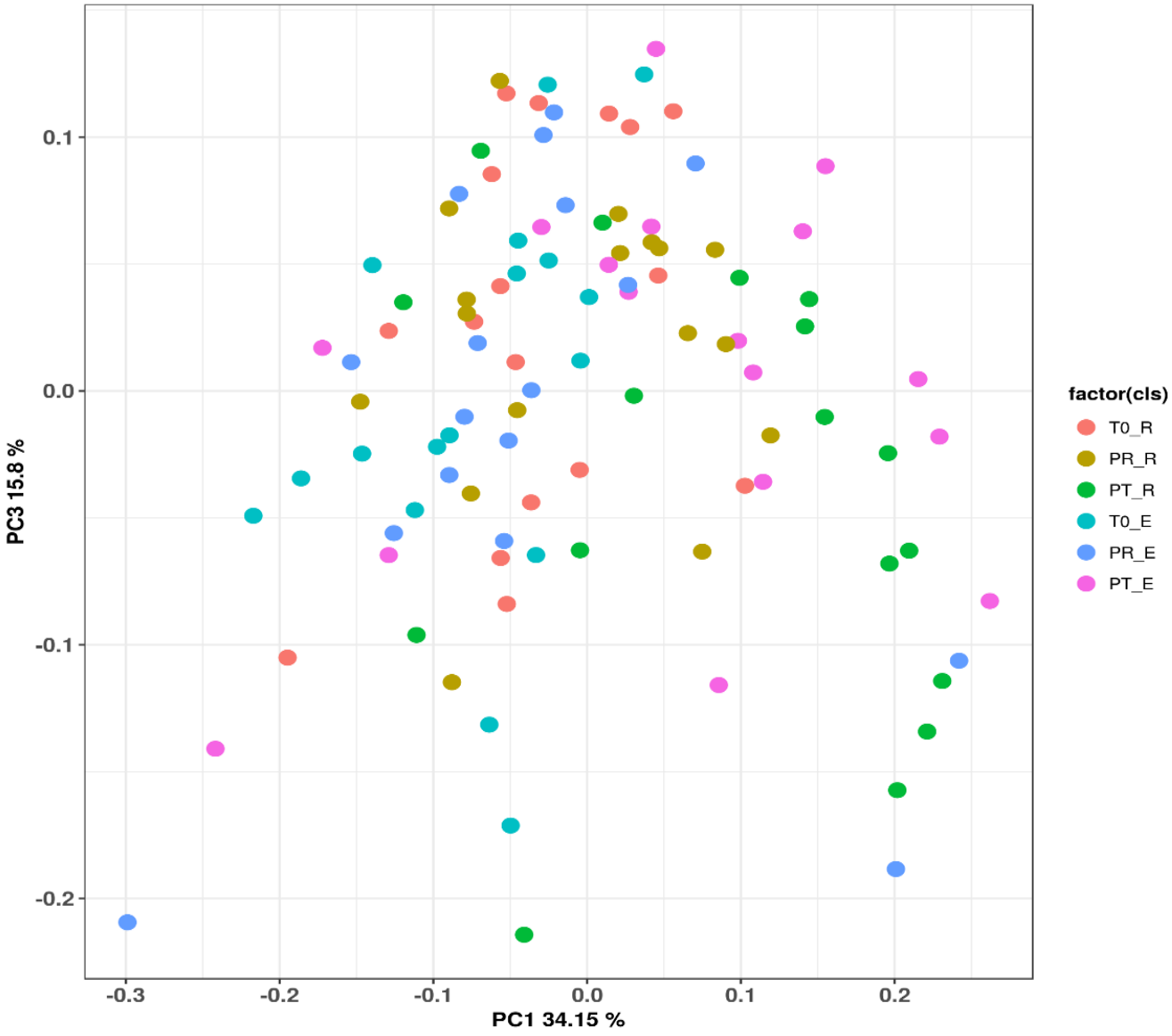


Figure 6.1A and 6.1B. Principle Component analysis (PC1; 34.15% of variation, PC2; 23.13% of variation, PC3; 15.8% of variation) of participant's FMV (TO), PRE (PR) and Post (PT) urine samples following exercise (E) and rest (R).

6.4. Discussion

The aim of this study was to examine urinary metabolome change following 30-minutes of moderate intensity exercise on a cycle ergometer. Previous work has demonstrated the effects of both high intensity exercise and resistance exercise on urinary metabolite changes (Enea *et al*., 2010; Pechlivanis *et al*., 2010; Pechlivanis *et al*., 2015; Siopi *et al*., 2017). However, the effects of moderate and submaximal exercise are less established with disagreements in the literature regarding the extent of metabolic disturbance following this form of physical activity (Enea *et al*., 2010; Ali *et al*., 2016). This exercise mode is of interest as UK national activity guidelines state all adults should undertake 30 minutes of moderate intensity exercise, five days per week. Such guidance is especially encouraged in patients requiring lifestyle modification for the prevention of non-communicable diseases, such as those who participated in the pre-diabetes intervention, detailed in chapter two as well as being applicable to participants in a future study, outlined later in this thesis. In chapter five, work was undertaken to develop a simple and non-disruptive method for urine collection in the home to monitor habitual diet. As discussed previously, metabolites within urine can reflect both individual food intake as well differentiate between dietary patterns. Prior to testing the above urine collection procedure in a population at risk of T2D, this work aimed to determine metabolic change in urine following both 30 minutes of standardised moderate exercise on a cycle ergometer, an intensity based on national exercise guidelines, compared to rest. Determining such change provides useful information to researchers regarding what metabolites may be present in the urine of individuals who are providing samples for dietary monitoring and those who engage in moderate physical activity as per advice from lifestyle intervention.

This work demonstrated 30 minutes of standardised moderate intensity exercise to have no significant impact on the urinary metabolome. This is evidenced by an RF margin of 0.07 and

is further supported by PCA plots which demonstrate no separation between pre and post exercise urine samples. The findings from this study agree with Enea *et al*. (2010) who examined both short term intense exercise and prolonged exercise. Despite a significant metabolic shift in urines post short-term intense exercise, no changes were found following 30 minutes of continuous exercise on a cycle ergometer. Following the intense exercise protocol, the major identifiable signals responsible for class separation were attributed to lactate and creatinine. Other metabolites responsible for separation between pre and post samples were alanine, acetate, acetoacetate, pyruvate, succinate, hippurate and hypoxanthine, all of which were excreted significantly more following completion of the short intensive exercise test. The changes in the urinary metabolite profile following short intense exercise were attributed to changes in muscle bioenergetics and specifically compounds related to the ATP-PCr and glycolytic systems The authors suggested that given the prolonged exercise test was performed at a moderate intensity, predominant energy usage was derived from aerobic metabolism, including lipid and carbohydrate oxidation, which has consistently shown to be elevated during moderate intensity activity and is well established (Horowitz and Klein, 2000; Achten, Gleeson and Jeukendrup, 2001). The authors concluded a lack of urinary metabolome change following prolonged moderate exercise was not unexpected as metabolites associated to lipid and carbohydrate oxidation are not routinely discovered in urine if the kidneys work effectively (Enea *et al*., 2010).

While the findings from the present work and that of Enea *et al*. (2010) are similar, the conclusions by Ali *et al*. (2016) are in significant contrast. This study examined metabolome changes following 45 minutes of moderate intensity exercise on a cycle ergometer. During the submaximal exercise protocol, participants cycled at 50 watts (W) for 5 minutes, 15 minutes at 40% work rate max (WR_{Max}), 15 minutes at 50% WR_{Max} and 10 minutes at 60% WR_{Max}.

Analysis revealed clear separation between pre and post exercise samples on the day of exercise. The metabolites responsible for separation between the exercise day FMV and the first post exercise sample were hypoxanthine, guanine, deoxyinosine, inosine and xanthosine all of which are metabolites within the purine pathway. Changes in these metabolites were not present within samples collected the previous non-exercise day and suggest the 'purine response' requires physical activity to result in observable changes. Additionally, an increase in nonanoyl carnitine, decanoyl carnitine and ketodecanoyl carnitine, increases which were not observed during the rest day, was attributed to a decreased reliance on fatty acid metabolism during exercise, which represented a shift towards glycolysis to maintain ATP synthesis. Furthermore, panthothenic acid increased following exercise with this change suggested to indicate an inhibition of CoA biosynthesis, which is tightly controlled by pantothenate kinase. Acetyl CoA inhibits pantothenate kinase by allosteric binding so an increase in urine panthothenic acid indicates a decrease in CoA synthesis (Ali, Burleigh, Daskalaki, Zhang, Easton and Watson, 2016). Fatty acid oxidation relies on CoA and therefore a decrease in CoA concentration would suggest a shift to glycolysis in the Krebs cycle. An increase in lactate after exercise compared to no change during rest is evidence of this. An increase in microbial products was also observed with an elevation in testosterone sulphate recorded in the first sample following exercise. Similarly, tetrahydroaldosterone increased after exercise with the suggestion that increases in both these metabolites are the result of increased energy metabolism. Neither metabolite changed during rest. The results of this work demonstrated the impact of submaximal exercise on metabolites such as hypoxanthine and inosine, both of which are markers of exercise as demonstrated from other studies. This is in addition to many other metabolites which changed significantly following submaximal exercise. Whilst samples from the rest day demonstrated clear separation at the same time points, many metabolites were affected only by the exercise protocol. However, the significant increases in lactate post

exercise suggests participants may have been working in an exercise intensity greater than considered 'moderate' which may explain why the work by Ali *et al*. (2016) was able to demonstrate significant metabolomic changes in urine following exercise considered to be submaximal. Furthermore, the authors state it was likely there was a decreased demand for fatty acids during exercise, resulting in a switch towards glycolysis to maintain ATP levels under conditions of increased oxygen demand. Rather than cycling at a fixed intensity for the duration of the test, participants were required to work at increasing power outputs (five min warm up at 50 W, 15 min at 40% of maximal cycling work rate (WRMax), 15 min at 50% WRMax, and 10 min at 60% WRMax. It is possible the increasing workload resulted in a shift in substrate metabolism and therefore caused alterations in the urine metabolome which have been demonstrated in other studies examining exercise above moderate intensities. Mean heart rates at each respective WRMax of 115 bpm, 156 bpm and 169 bpm suggest participants were exercising at greater intensities compared to those in the present work and in Enea *et al*. (2010), although heart rate data for the latter was not published.

Although distinct sample classification was not achieved between pre and post exercise samples, RF margins between FMV and post samples for both exercise and rest trials reached accepted values (> 0.3; Enot *et al*., 2008; Table 6.2). From the signals demonstrating a significant change between FMV and post sampling time points, a small number were identified using MZedDB, an interactive accurate mass annotation tool which can directly annotate signals (Draper *et al*., 2009). In the rest trial, the metabolites; (1R,2R,3S,1'R)-nepetalinic acid, 2-hydroxypropanoic acid, polyethylene glycol changed significantly. The Human Metabolome Database (HMDB) suggests both (1R,2R,3S,1'R)-nepetalinic acid and polyethylene glycol are associated with lipid transport and metabolism while 2-hydroxypropanoic acid is more commonly referred to as lactic acid. Presence of 2-

hydroxypropanoic acid was also identified as a distinguishable feature between FMV and post samples in the exercise trial, alongside 1,8-Epoxy-p-menthan-4-ol glucoside and 2',3'-Dihydroxyacetophenone. The HMDB states 1,8-Epoxy-p-menthan-4-ol glucoside is found in citrus while 2',3'-Dihydroxyacetophenone is listed as a flavouring ingredient. It is unclear as to the reasons why such metabolites were distinguishable between FMV and post-exercise samples. It is possible that changes between FMV and post-trial urine samples following exercise were the result of 'washout' from the previous evening meal. While subjects were asked to repeat identical eating habits prior to both experimental trials, this was not tightly controlled, nor was a specific control meal provided the evening prior to each trial. Another explanation, encompassing both trials, is simply the cyclical nature in the concentration of urinary metabolites throughout the day (Slupsky *et al.*, 2007). Given there was no acceptable classification between pre and post urine samples, suggesting exercise specifically resulted in no metabolome shifts, this is a possibility.

Moderate intensity exercise was studied as it is likely to be adopted by participants undergoing healthcare led lifestyle intervention, based on UK national exercise guidelines, and is applicable to participants involved in a later study in this thesis. These findings therefore suggest that metabolome changes and metabolite presence in urines provided by lifestyle intervention participants are unlikely to be caused by 30 minutes of moderate intensity physical activity. However, it would be unreasonable to suggest that some individuals undergoing lifestyle intervention would solely undertake moderate intensity exercise as recommended to them by a health care professional with some assuming higher intensity exercises as part of lifestyle modification. As discussed above, evidence suggests high intensity exercise results in significant metabolome changes which are associated to the purine pathway, anaerobic metabolism and amino acid degradation. With this evidence for high intensity exercise, coupled

with evidence for moderate intensity exercise (from this work and others) and resistance exercise, there is a reasonably strong collection of data sources for linking urinary metabolome changes and metabolite presence to physical exercise. In a future study within this thesis, examining lifestyle modification in pre-diabetes, participants will provide regular urine samples for the purposes of dietary monitoring. Participants in this study will also receive advice to undertake 30 minutes of moderate intensity activity, five days a week, as per the UK national guidelines for physical activity. While this study has demonstrated that this exercise intensity does not cause any significant urinary metabolome change, should metabolites present themselves in urines which may be suggestive of higher intensity exercise, previous data from other work is available to inform such findings. Cross referencing potential urinary metabolites with activity tracker data and physical activity questionnaire data can further inform such findings in urine samples if necessary. Furthermore, previous work by Pechlivanis *et al*. (2015) found many metabolites affected by exercise returned to baseline values within two hours of exercise cessation. This therefore suggests FMV urine samples are best placed to limit any urinary metabolic disturbance caused by exercise while at the same time containing rich dietary information, as demonstrated in chapter four. Dietary monitoring using urine sampling and activity tracking in individuals at risk of T2D will be explored in greater detail in chapter seven.

In conclusion, this study demonstrated 30 minutes of standardised moderate intensity exercise to have no effect on the urinary metabolome. This finding helps provide a better understanding of the effects of this form of exercise in a field or previously mixed results. With reference to a future study within this thesis examining lifestyle modifications with urinary dietary monitoring, there is little evidence to suggest exercise, based on UK national guidelines, will result in significant metabolome disturbance in urines.

Chapter Seven:

Comparison of the Alere Afinion AS100 Point of Care Analyser and HPLC in the Determination of HbA_{1c}

7.1. Introduction

In chapter two, patients at risk of T2D attended a 30-minute one-to-one consultation with a healthcare professional. During this consultation information on the risks of T2D was provided as well as discussions on the benefits of increasing physical activity and improving dietary behaviours for improving blood glucose control. Eligibility for the study included an HbA_{1c} test result from the preceding four weeks of between 42 and 47 mmol/mol. Analyses of patient's HbA_{1c} was conducted at Bronglais Hospital Pathology Laboratory, Aberystwyth, using high-performance liquid chromatography (HPLC). HPLC is considered the gold standard for analysing HbA_{1c} and is a reference method to standardize other analytical techniques (Maesa *et al*., 2014). However, hospital based HPLC is costly, time consuming and requires trained personnel to operate the machinery (Karami and Baradaran, 2014). As a result, the use of automated point of care (POC) analysers for immediate HbA_{1c} analysis in primary care and research is becoming more common. Use of such systems means those at risk of T2D can be tested and receive their HbA_{1c} value within minutes, have a discussion with a health professional about the implication of their result and, if necessary, be signposted to relevant support services, all within the same appointment and without the lengthy waiting times and financial costs associated with HPLC analysis. One such POC device is the Alere Afinion AS100, which uses the boronate affinity method which detects attachment of glucose at both the non-N-terminal sites and N-terminal sites of the beta chain of the haemoglobin molecule.

As the use of POC analysers becomes more widespread it is of interest to determine how closely the results agree with those from HPLC. Wood *et al.* (2012) reported a mean relative difference of + 0.5% to + 3.9% when using an Afinion POC analyser compared to an HPLC method across a range of values from < 7% (53 mmol/mol) to > 10% (86 mmol/mol), however all samples were taken from those with a clinical diagnosis of diabetes. It is not clear from the literature what the agreement is between the Alere Afinion AS100 POC analyser and HPLC in values between 30 and 60 mmol/mol, which includes individuals from across the healthy, pre-diabetic and diabetic ranges. It is also not clear from the literature what, if any, impact on the result different methods of blood sampling (e.g. venous and capillary blood sampling) will have on HbA_{1c} values. While Mailankot, Thomas, Pravenna, Jacob, Benjamin and Vasudevan (2012) and Sharma, Sarmah and Sonker (2013) both report stability in HbA_{1c} values irrespective of anticoagulant both studies used HPLC in the determination of HbA_{1c}. As such this study will examine agreement between Alere Afinion AS100 POC analyser and HPLC across a range of values spanning healthy, pre-diabetic and diabetic individuals. The study will also examine different blood sampling methods and any impact of the anticoagulant used on HbA_{1c} result.

Primary Aim:

• Compare, and establish the limits of agreement, in HbA_{1c} (mmol/mol) values between HPLC and Alere Afinion AS100 POC analyser in venous blood samples collected via ethylenediaminetetraacetic acid (EDTA) and lithium heparin (LH) vacutainers (Aim 1).

Secondary Aims:

• Compare, and establish the limits of agreement, in HbA_{1c} (mmol/mol) values between venous blood samples collected via EDTA and LH vacutainers when analysed by Alere Afinion AS100 POC analyser (Aim 2).

• Compare, and establish the limits of agreement, in HbA_{1c} (mmol/mol) values between venous blood, collected via EDTA and LH vacutainers, and capillary blood when analysed by Alere Afinion AS100 POC analyser (Aim 3).

7.2. Method

7.2.1. Participants

Twenty-five participants volunteered and were recruited to the study; two participants were later diagnosed as anaemic following HPLC sample analysis and were withdrawn from the study. Data for 23 participants (16 male and seven female) with a mixture of healthy (n = 18, 35 ± 2 mmol/mol), pre-diabetic (n = 2, 45 ± 4 mmol/mol) and diabetic (n = 3, 54 ± 5 mmol/mol) HbA_{1c} values were used for statistical analysis.

7.2.2. Experimental Protocol

Participants attended the laboratory on a single occasion. On arrival the procedures and purpose of the study were explained before participants gave their informed consent to continue (see Appendix; L, M). Participants were seated in a phlebotomy chair prior to a blood sample being obtained from the antecubital vein and collected into vacutainer tubes (2 x 4 ml EDTA tube and 1 x 6 ml into a LH tube). 1.5 µl of whole blood was transferred directly from one of the EDTA vacutainers into the HbA_{1c} test cartridge and assayed immediately on the Alere Afinion AS100 POC analyser. The same process was then repeated using the LH vacutainer, the EDTA

and LH vacutainers were then disposed of via standard clinical practice. A capillary blood sample was then collected from the middle or index finger using a lancet. 1.5 µl of whole blood was collected directly into the HbA_{1c} test cartridge from the puncture site and assayed immediately. The remaining EDTA vacutainer was sent to Bronglais Hospital pathology laboratory for HPLC analysis (Tosoh G8, Tessenderlo, Belgium). All samples were delivered to the hospital and analysed by HPLC within eight hours of sample collection.

7.2.3. Statistical Analysis

Comparison between methods was achieved by calculating the mean difference (MD) between HbA_{1c} values with normally distributed mean differences analysed using a one sample t-test and non-normally distributed mean differences analysed using a one-sample Kolmogorov-Smirnov test. One sample analysis was used to determine if the difference between values attained from each method varied significantly from zero. Relationships between methods were analysed using Spearman's correlation and Bland Altman plots (Bland and Altman, 1986) were used to determine limits of agreement. Statistical significance was accepted at $P < 0.05$. A summary of all comparisons made can be seen in Table 7.1.

Table 7.1: Comparisons to be made in HbA_{1c} values as determined by HPLC and Alere Afinion AS100 POC Analyser

		Alere Afinion AS100 POC			
		HPLC	EDTA	LH	Capillary
Alere Afinion AS100 POC	HPLC		Aim 1	Aim 1	
	EDTA			Aim 2	Aim 3
	LH				Aim 3
	Capillary				

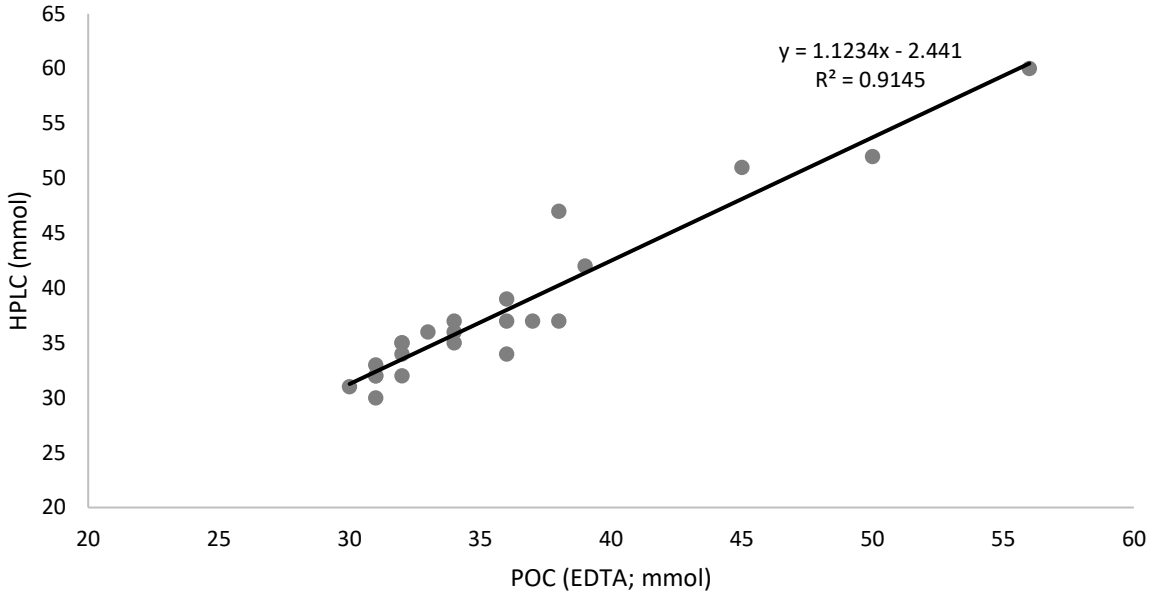
7.3. Results

Values for HbA_{1c} attained from HPLC and the Alere Afinion AS100 POC analyser can be seen in Table 7.2. Statistical analysis indicated HbA_{1c} values attained from HPLC differed significantly from POC analysis when samples were collected in both EDTA (MD -2.0 mmol/mol) and LH (MD -1.7 mmol/mol) vacutainers ($P = 0.01$) with HPLC on average giving higher values. Spearman's test found significant positive correlations between POC analysis (EDTA) and HPLC (r = 0.92, $P = 0.00$, Figure 7.1A) and POC analysis (LH) and HPLC (r = 0.93, $P = 0.00$, Figure 7.2A). The 95% limits of agreement between HPLC and POC analysis with sample collected in EDTA were 2.21 to -6.61 mmol/mol (Figure 7.1B), while the 95% limits between HPLC and POC with sample collected in LH were 2.82 to -6.21 mmol/mol (Figure 7.2B).

Table 7.2: HbA_{1c} values from point of care and HPLC analysis. Data are represented as median (range)

	Alere Afinion AS100 POC analyser			**HPLC**
	EDTA	**LH**	**Capillary**	
HbA_{1c} (mmol/mol)	34 (26)	34 (27)	34 (24)	36 (30)

A



B

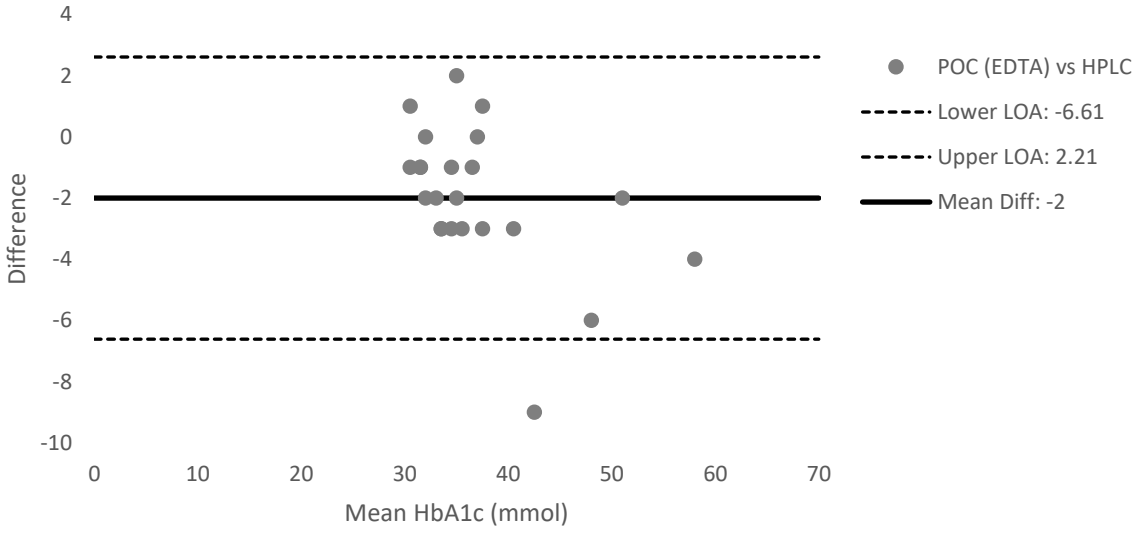
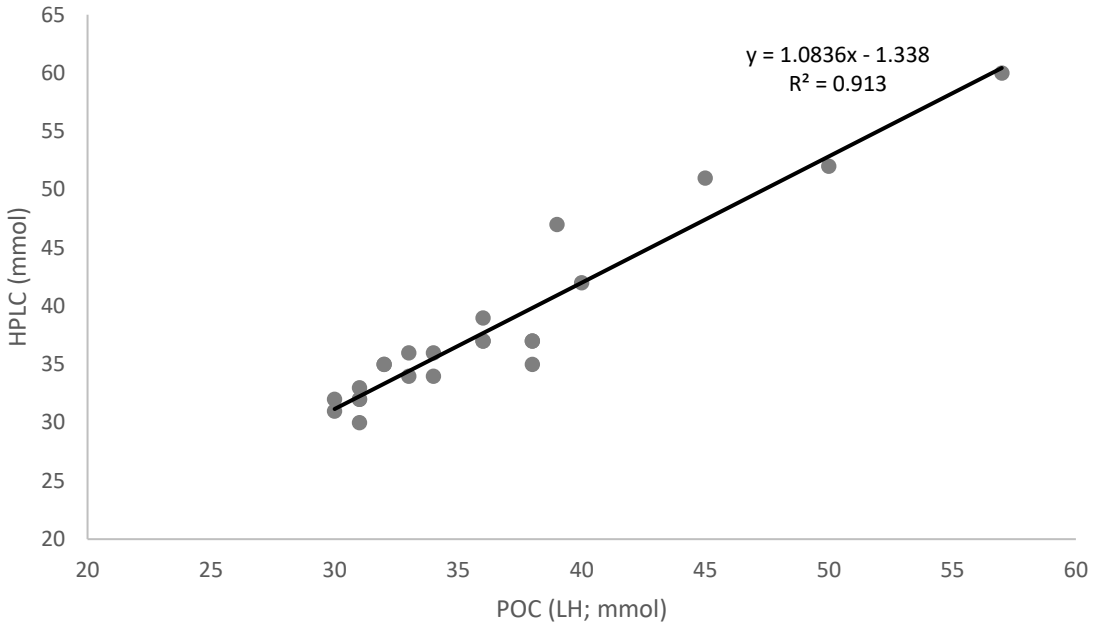


Figure 7.1A/7.1B: Spearman's correlation (1A) and Bland Altman limits of agreement plot (1B) for POC analysis (EDTA) vs HPLC

A



B

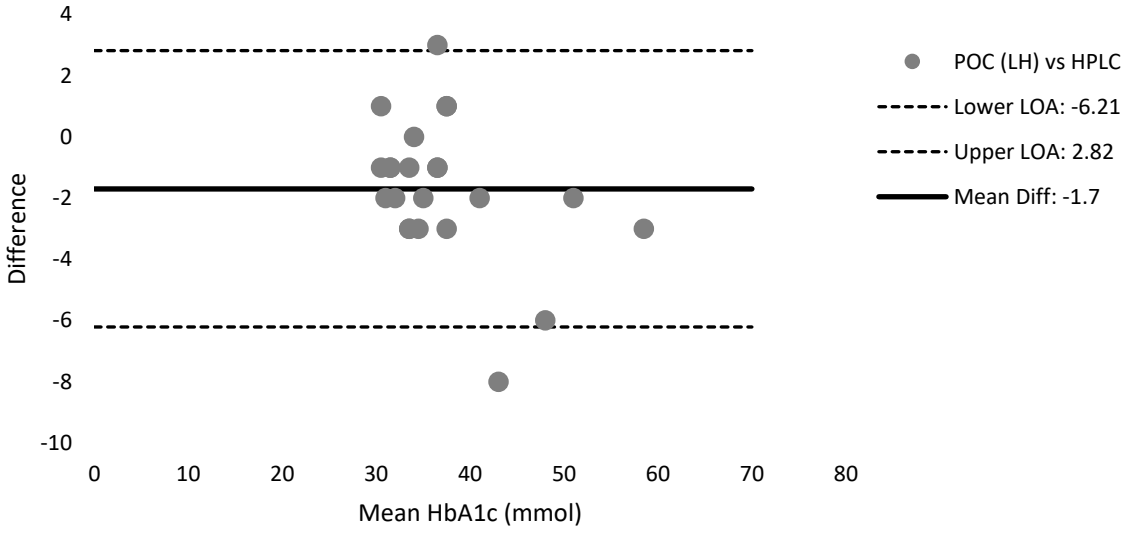
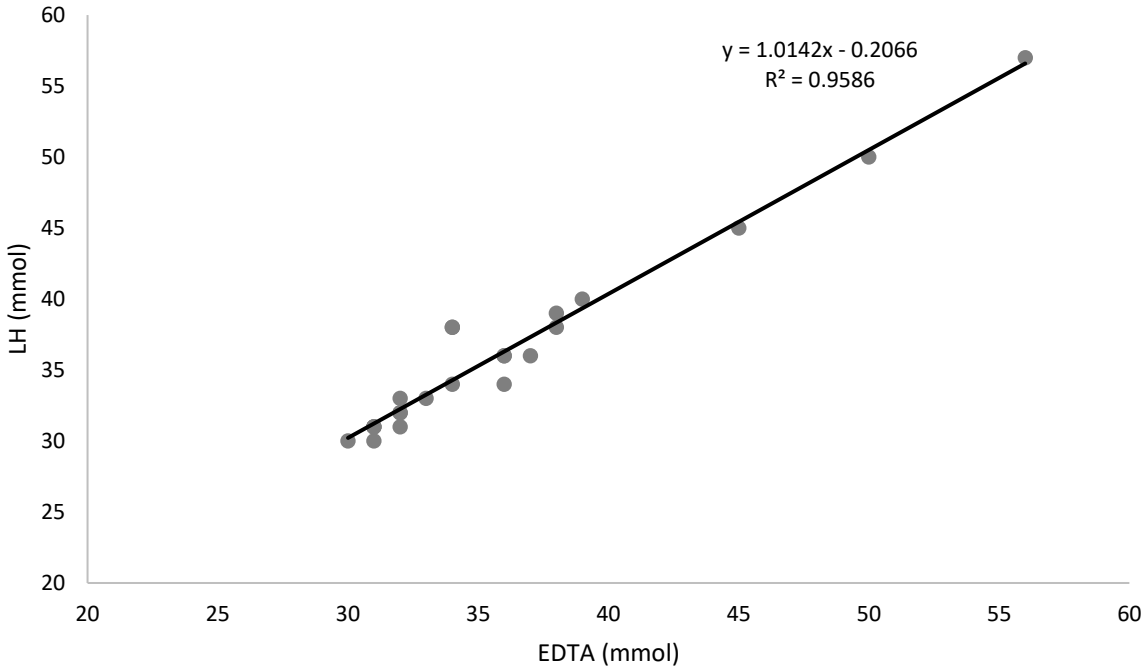


Figure 7.2A/7.2B: Spearman's correlation (2A) and Bland Altman limits of agreement plot (2B) for POC analysis (LH) vs HPLC

A significant difference in HbA_{1c} was found between EDTA and LH vacutainers (MD = -0.3, *P* = 0.00) with LH vacutainers giving higher values. Results obtained from the two vacutainer types were correlated (r = 0.95, *P* = 0.00, Figure 7.3A) and the 95% limits of agreement between the two types of vacutainer were 2.37 to -2.98 mmol/mol (Figure 7.3B).

When capillary samples were compared to venous samples there was a significant difference between LH vacutainers and capillary samples (MD = -0.5, *P* = 0.00) with LH vacutainers giving higher values. No significant difference between EDTA vacutainers and capillary samples (0.2 MD, *P* = 0.38) although EDTA vacutainers gave higher values. Spearman's test found significant positive correlations between LH vacutainers and capillary samples (r = 0.93, *P* = 0.00, Figure 7.4A) and EDTA vacutainers and capillary samples (r = 0.93, *P* = 0.00, Figure 7.5A). The 95% limits of agreement between LH vacutainer and capillary samples were 2.18 to -3.2 mmol/mol (Figure 7.4B), while the 95% limits between EDTA vacutainer and capillary samples were 2.5 to -2.07 mmol/mol (Figure 7.5B).

A



B

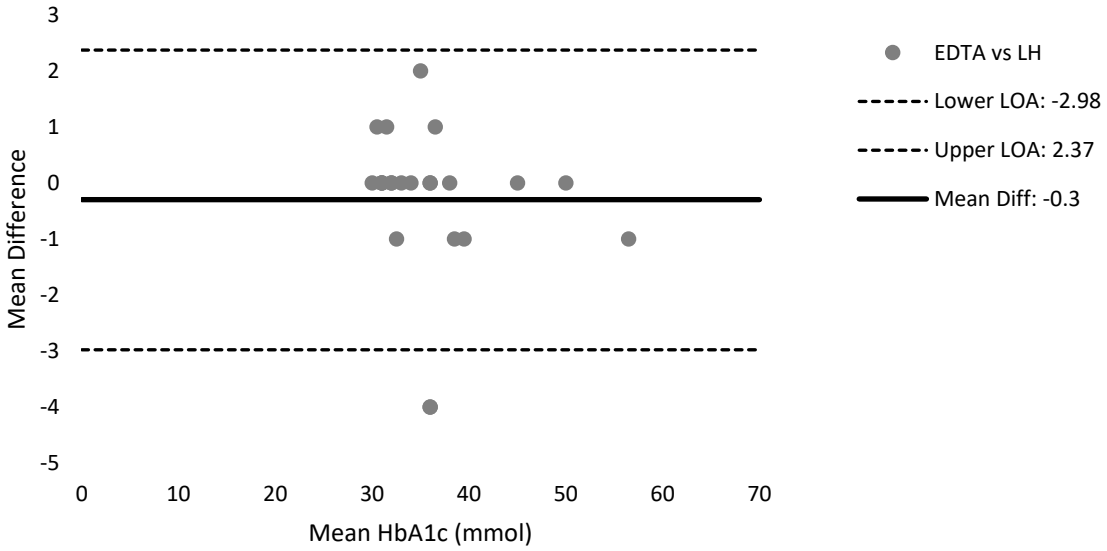
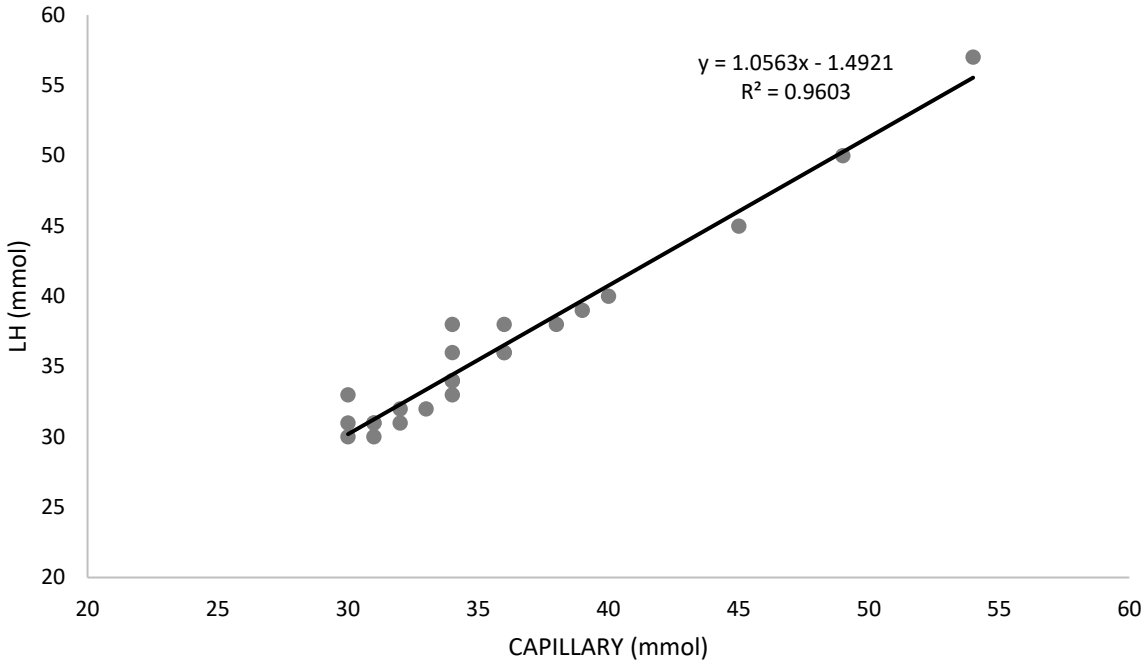


Figure 7.3A/7.3B: Spearman's correlation (3A) and Bland Altman limits of agreement plot (3B) for EDTA vs LH vacutainers using the POC analysis.

A



B

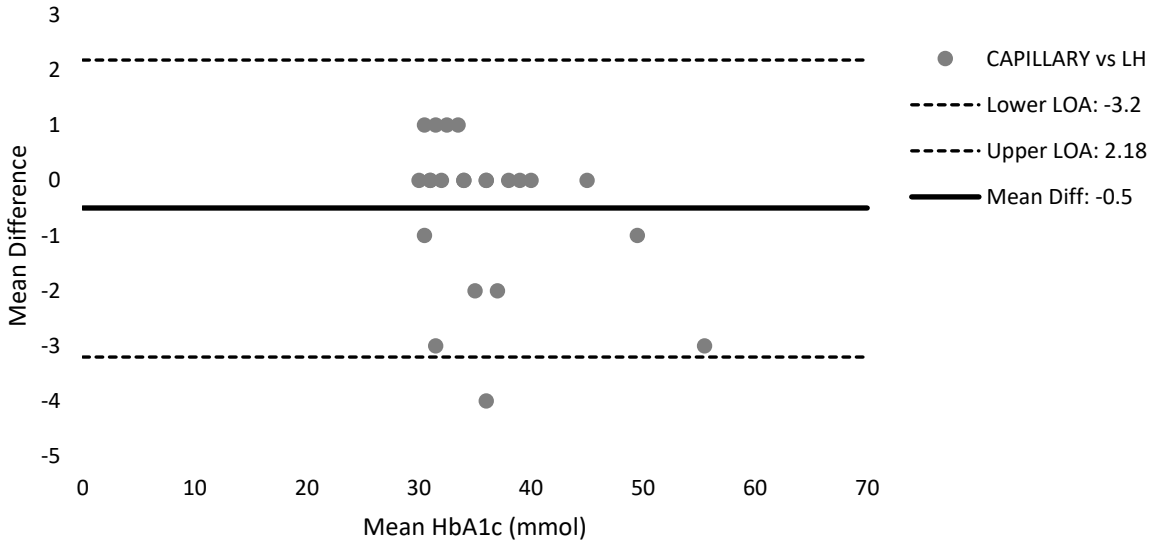
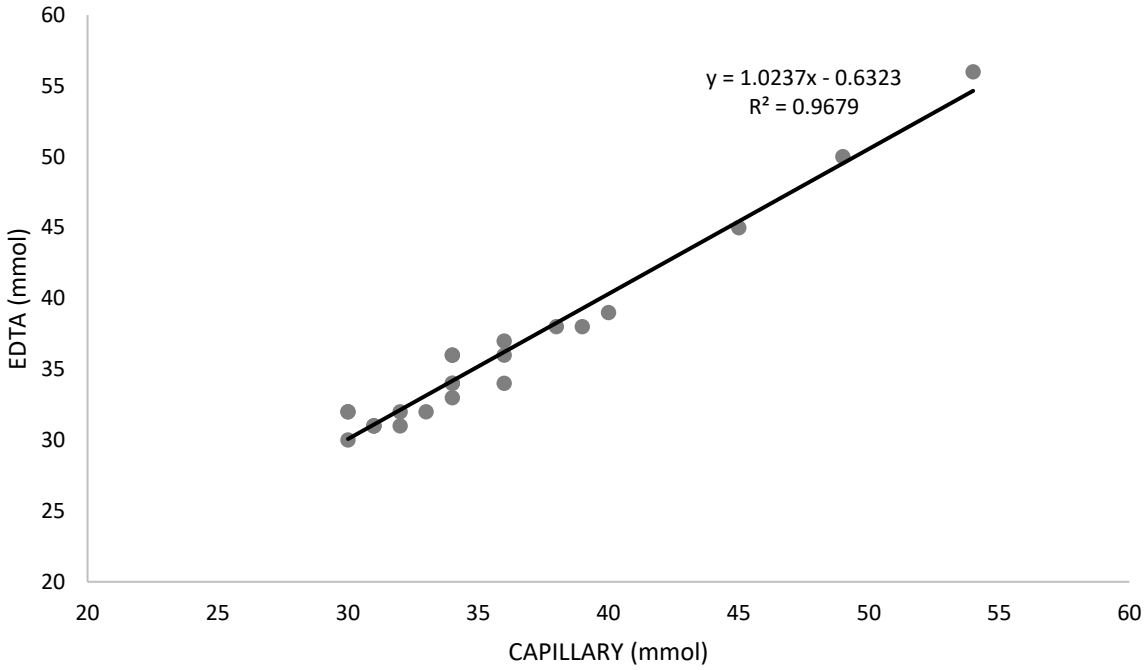


Figure 7.4A/7.4B: Spearman's correlation (4A) and Bland Altman limits of agreement plot (4B) for LH vacutainers vs capillary sampling using POC analysis.

A



B

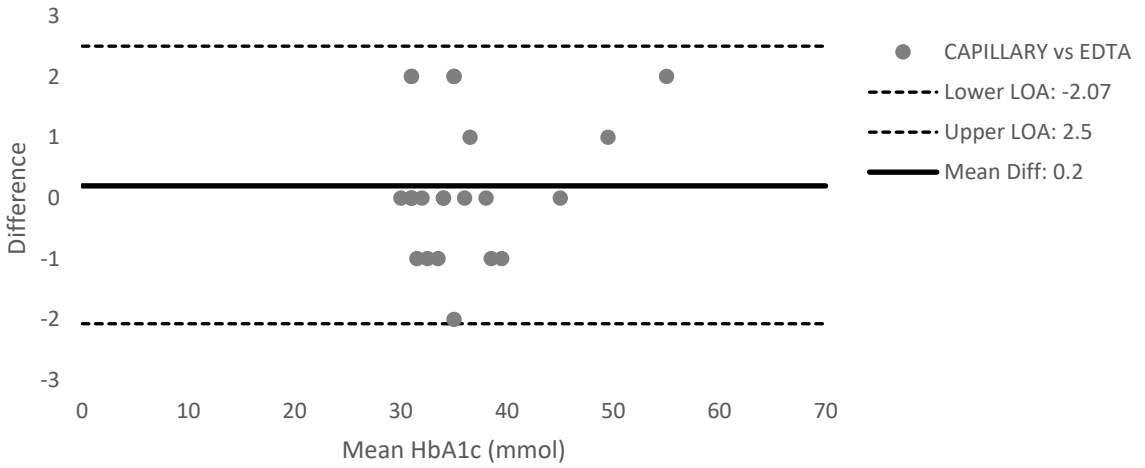


Figure 7.5A/7.5B: Spearman's correlation (5A) and Bland Altman limits of agreement plot (5B) for EDTA vacutainers vs capillary sampling using POC analysis.

7.4. Discussion

The primary aim of this study was to compare the Alere Afinion AS100 Point of Care Analyser and HPLC in the determination of HbA_{1c} prior to using the former as part of an RCT targeting patients at increased risk of T2D. The main finding was that HbA_{1c} values from the two methods varied significantly; compared to HPLC the POC analyser underestimated HbA_{1c} by two mmol/mol when using an EDTA collection vacutainer and by 1.7 mmol/mol when using a LH vacutainer. The 95% limits of agreement indicate that when using EDTA the POC analyser gives values in a range of between +2.21 to -6.61 mmol/mol of that obtained from HPLC (Figure 7.1B) while POC samples obtained via LH vacutainer give values of between +2.82 and -6.21 mmol/mol of those obtained via HPLC (Figure 7.2B). In three cases, the POC analyser misclassified participants, placing them into the pre-diabetes range while HPLC analysis categorised the participant as T2D. Such misclassification of categories suggests the POC analyser is not suitable for accurate monitoring of HbA_{1c} values at a clinical level and demonstrates the importance HPLC in confirming pre-diabetes and T2D diagnoses. The findings from this study are however similar to work by Grant, Dunseath, Churm and Luzio (2017) who found a median two mmol/mol difference in HbA_{1c} values between HPLC and POC analysis using boronate fluorescence quenching technology. Notwithstanding the difference, Grant *et al.* (2017) concluded that the POC analyser could be used for HbA_{1c} monitoring, however they recommended that HPLC analysis was undertaken for diagnoses.

Despite significant mean differences, the positive correlation between methods suggests that while the POC analyser underestimated HbA_{1c} values compared to HPLC, the results from both POC analysis (EDTA (Figure 7.1A) and LH (Figure 7.2A)) and HPLC increased in a similar manner to one another. As such, the POC analyser may be used, albeit cautiously, to help track trends in HbA_{1c} over time and provide useful information for the patient and/or researcher

regarding the effectiveness of lifestyle interventions in combatting T2D. Should an HbA_{1c} value approaching 47 mmol/mol be obtained from a POC analyser a strong recommendation should be made to get a confirmatory analysis via HPLC.

The significant difference and lack of agreement between LH vacutainers and EDTA vacutainers (Figure 7.3B) and capillary sampling (Figure 7.4B) was somewhat surprising. Previous work has reported no difference in HbA_{1c} values when collected into vacutainers containing different anticoagulants, including EDTA and LH (Mailankot, Thomas, Pravenna, Jacob, Benjamin and Vasudevan, 2012), as well as the POC analyser manufacturer's own recommendation that numerous anticoagulants can be used for sample collection. The reasons for the significant disagreement among vacutainer types are unknown. In contract to this finding venous sampling using EDTA vacutainers and capillary sampling demonstrated no significant difference when analysed by the POC analyser and the 95% limits of agreement indicated that the two methods differed by +2.5 to -2.07 mmol/mol (Figure 7.5B). Similarities in results from venous, collected via EDTA vacutainer, and capillary sampling methods will allow flexibility in HbA_{1c} measurement in participants where venous sampling is challenging.

A limitation of this study lies in the relatively low number of participants recruited. General recommendations made by Simundic (2016), and as demonstrated by Grant *et al*. (2017), suggest method comparison studies should recruit a minimum of 40 but up to 100 participants to cover the whole clinically meaningful measurement range to reduce random error. While the present study ensured participants were recruited from healthy (18), pre-diabetes (2) and T2D (3) categories, the pre-diabetes and T2D categories are underrepresented.

In conclusion, the results from this study demonstrate a laboratory POC analyser for the determination of HbA_{1c} underestimates results by a median of two mmol/mol and in some cases misclassifies diabetic state. Based on these results, the POC analyser it is not suitable for clinically diagnosing T2D. However, the significant positive correlations between the POC analyser and HPLC analyses suggest that the former can be used, with caution, to assess intervention effectiveness by monitoring longitudinal changes without the waiting time, additional costs and expertise associated with HPLC analysis.

Chapter Eight:

The feasibility of conducting a randomised control trial to evaluate the effectiveness of a focussed 15-minute one-to-one consultation to improve blood glucose control in pre-diabetes

8.1. Introduction

In Chapter two, a GP-led 30-minute one-to-one consultation targeting lifestyle modification in patients at risk of T2D was associated with significant decreases in HbA_{1c} and other health outcomes associated with the condition including body mass, BMI and waist circumference. Despite this success, the design of the intervention did not include a control group, and as such, direct comparisons with usual care cannot be made. Patients in Chapter two received both written information regarding their T2D risk and ways to improve lifestyle as well as the opportunity to attend a 30-minute one-to-one consultation with a healthcare professional. It is possible that the written information given to this cohort provided the appropriate stimulus to initiate behaviour change and that the one-to-one consultation did not provide any additional benefit.

In order to establish the benefit of an intervention, a control group with appropriate comparison and randomisation is needed. Given the complexity and logistical challenges of conducting an appropriately powered Randomised Control Trial (RCT) in primary care, it is prudent to first assess the feasibility of initiating such a design with a smaller cohort of patients. Dawes *et al*. (2015) examined the feasibility of conducting a cluster randomised control trial in pre-diabetes in a Canadian population which compared a primary care led lifestyle intervention to usual care, with 95% of participants remaining engaged in the research after six months. Those

receiving lifestyle intervention within primary care reduced body mass, BMI and waist circumference significantly compared to control participants, with the authors concluding it would be feasible to implement a larger trial with greater follow up duration to assess intervention effectiveness in diabetes prevention. The current study was undertaken to establish the feasibility of conducting an RCT in mid-Wales to evaluate the effectiveness of a one-to-one consultation to improve blood glucose control in pre-diabetes. The design of the RCT meant the consultation did not involve the collection of baseline data, which was the case in Study one (Chapter two), and subsequently the time required to deliver the intervention was reduced from 30 minutes to 15 minutes. The findings from studies two and three (Chapters 5 and 6) informed the development of a urine collection kit and collection procedures for monitoring dietary intake in a free-living population and represents the first time such processes have been trialled within a clinical population in Wales.

Aims:

The study's primary aims were to determine:

• The percentage of patients eligible to participate who gave consent to be part of the study.

• The percentage of patients who completed baseline; three-month; and six-month testing.

Secondary aims are to determine:

• The impact of the consultation on participant biochemistry, anthropometry, physical activity and dietary intake.

8.2. Method

8.2.1. Participants and Recruitment

Patients registered with Church Surgery, Aberystwyth, who were at increased risk of developing T2D, based on a body mass index (BMI) > 25 kg/m^2 or an existing glycated haemoglobin (HbA_{1c}; recorded within the last 12 months) of between 42 and 47 mmol/mol, were identified on the surgery's patient record system. Database searches were completed by trained surgery employees. Patients who received the 30-minute one-to-one consultation detailed in Chapter two were not eligible for inclusion. Patients identified as at risk of T2D based on BMI (without a <12-month HbA_{1c}) were invited by letter (see appendix N) to the surgery for a blood test to determine their HbA_{1c}. Patients with HbA_{1c} between 42 mmol/mol and 47 mmol/mol or patients approaching the HbA_{1c} risk threshold and considered at increased risk of T2D, based on clinical judgement by a GP, were informed of the study by their surgery via an information pack (see appendix; B, O, P). The pack contained; a letter outlining the patient's increased risk of T2D, a T2D information leaflet and the participant information sheet detailing the study. The T2D information leaflet was the same document sent to patients participating in Study one and included key information relating to T2D and steps that can be taken to avoid developing T2D. The participant information sheet included material on the purpose and procedures of the research study and contact details for the research team. For patients confirming their involvement, verbal and written informed consent was sought in person at the start of their first visit to the Well-being Assessment Research Unit (WARU) before being inducted into the study (> 24 hours between receipt of the study invite letter & participant information sheet and signing informed consent; see appendix Q). Individuals who declined involvement received usual care from their GP.

8.2.2. Experimental Protocol

Participants visited the WARU on three separate occasions for baseline, three-month and six-month testing. At the start of each visit; measures for height, body mass, waist and hip girth (Seca 201, Seca Gmbh & Co, Hamburg, Germany) were recorded. Body composition was analysed with participants in a supine position using bioelectrical impedance (Bodystat 1500, Bodystat Ltd, Douglas, British Isles) and a blood sample was collected for the determination of HbA_{1c} and lipid profile (See Chapter Four, General Methods, 4.5 & 4.6). WARU informed Church Surgery if a participant's HbA_{1c} was above 47 mmol/mol. Finally, participants sat quietly in an upright position for 10 minutes in the laboratory before BP was recorded from the upper non-dominant arm (Omron M3, Omron Healthcare Ltd, Milton Keynes, UK). Blood pressure was measured in duplicate (recordings separated by five minutes) and the lowest value was recorded.

Next, participants completed several validated questionnaires, these included; components of the Personal Diabetes Questionnaire (baseline), IPAQ-SF (see Chapter Two, 2.2.2.), and SF-36 (baseline, three- and six- months) and FFQ (baseline and six-months; see appendix; R, S, T). The Personal Diabetes Questionnaire is a validated 19-point questionnaire taken from the original Personal Diabetes Questionnaire (Stetson, Shlundt, Rothschild, Floyd, Rogers and Mokshagundam, 2011) and addressed the following behaviours relating to diabetes; body mass change readiness, dietary change readiness, dietary barriers and exercise barriers. Answers provided to questions relating to body mass and dietary change readiness categorised respondents into the; pre-contemplation, contemplation, preparation or action group. Answers provided to questions relating to dietary and exercise barriers provided respondents with a numerical score with higher scores representing greater barriers to change. The SF-36 is a 36-point validated health questionnaire designed to assess perceptions of physical functioning,

bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, general mental health, social functioning, energy & fatigue, and general health (Hays, Sherborne and Mazel, 1993). Questions were scored on a scale from zero to 100, with 100 representing the highest level of functioning. Scores from questions that addressed each specific area of functional health status were averaged together to calculate a final score within each of the eight categories measured.

The FFQ used was derived from the EPIC-Norfolk version (Bingham *et al*., 2001) and consists of a list of foods (grouped by type) and beverages with response categories to indicate usual consumption frequency over the previous six months. Using a consumption frequency score between one and nine, respondents can be categorised into high, medium and low consumption classes of different foods and nutrients based upon the Alternative Healthy Eating Index (AHEI; Varraso *et al*., 2015). The AHEI is based on foods and nutrients predictive of chronic disease risk. Higher scores on the AHEI are strongly associated with lower risk of major chronic disease as well as risk of CVD, diabetes, heart failure, colorectal and breast cancer, and total and cardiovascular mortality (Chiuve *et al*., 2012). Food frequency questionnaire responses were scored based on a protocol by Chiuve *et al*. (2012). 1-Vegetables: five servings per day was considered ideal, which reflects the upper range of current dietary guidelines. 2-Fruit: four servings per day was considered ideal, which is consistent with the upper range of current dietary guidelines. 3-Wholegrains: 75 g per day was considered optimal (five servings per day) for women and 90 g per day (six servings per day) considered optimal for men based on current guidelines for total grains. 4-Sugar-sweetened beverages: One serving per day was considered least optimal based on the associations in the literature. 5-Nuts, legumes, and vegetable protein: One serving per day considered ideal based on the AHEI recommendations and the current literature. 6-Red meat and processed meat: Less than one serving per month

was ideal. 7-Trans-fats: Cut-offs are consistent with original AHEI cut-offs for trans-fat. 8-Fish: Two to four servings of fish per week is considered optimal, which is consistent with current guidelines. 9-Polyunsaturated Fats: Highest score given to individuals with 10% of total energy intake from PUFA based on current guidelines from the USDA and the AHA. 10-Sodium: Cut-offs were based on deciles of distribution in the population, due to lack of brand specificity in the FFQ to accurately estimate absolute intake. Values in lowest decile were 1112 mg per day in women and 1612 mg per day in men and in highest decile were 3337 mg per day in women and 5271 mg per day in men. 11-Alcohol: The highest score was assigned to moderate, and the worst score to heavy, alcohol consumers. Non-drinkers received a score of 2.5. All components of the AHEI were scored from zero (representing a bad diet) to 10 (representing a perfect diet) based upon consumption frequency. The total AHEI score ranged from zero (nonadherence to dietary recommendations) to 110 (perfect adherence to dietary recommendations).

The purpose of, and completion process for, each questionnaire was explained to participants and the opportunity to ask questions was provided before questionnaires were completed. Questionnaires were completed unassisted although the chief investigator was available to answer any further questions the participant had. Lastly, participants received instructions on the use of the urine collection kit for dietary monitoring and the ActiGraph for physical activity monitoring. Participants were then free to leave the laboratory.

An additional acceptability questionnaire designed by the chief investigator was given to participants at the end of the six-month visit and included questions relating to the recruitment procedures, WARU visits, 15-minute consultation (if received), urine collection and ActiGraph use (see appendix X). Visits lasted between 75 minutes and 120 minutes depending on

participant mobility and the time taken for participants to complete the questionnaires.

Following completion of the baseline visit, participants were randomised into either the intervention group (INT) or the control group (CON). Church surgery were informed of the participants who were randomised to receive the intervention with referral arrangements made between the chief investigator and surgery staff. Participants in INT received a 15-minute one-to-one consultation with a healthcare professional at Church Surgery. The consultation derived from the original 30-minute protocol employed during the study outlined in Chapter two. The first half of the 30-minute consultation in Chapter two collected data including; height, body mass, waist circumference and blood pressure and the second half provided patients with lifestyle advice on preventing diabetes. The consultation used in the present study did not require general practice to collect baseline data and therefore the consultation time was reduced to 15 minutes.

The consultation provided the participant with one-to-one information regarding the benefits of physical activity and healthy eating, based upon NICE guidelines. Physical activity advice included the recommended completion of at least 150 minutes of moderate intensity activity each week, such as 30 minutes per day on five days of the week, or alternatively, 75 minutes of vigorous intensity activity spread across the week or combinations of moderate and vigorous intensity activity. Participants were also advised to undertake resistance exercise to improve muscle strength on at least two days a week and minimise the amount of time spent being sedentary for extended periods. Summarised advice regarding healthy dietary behaviours included eating at least five portions of a variety of fruit and vegetables every day, basing meals on wholegrain carbohydrates, choosing lower fat and lower sugar diary options, consuming beans, pulses, fish, eggs, lean meat and other proteins, choosing unsaturated oils and spreads,

drinking six-eight cups/glasses of fluid a day and limiting the consumption of alcohol to no more than 14 units per week. Furthermore, participants had the opportunity to discuss pre-diabetes with the health professional and ask questions. Participants randomised to CON received written T2D information and attended visits to the WARU only.

8.2.3. Randomisation

Randomisation was achieved using a minimisation software package (Minim; Evans, Royston and Day, 2017). Minimization is a dynamic approach and assigns treatment based on previous allocations and ensures balance in important factors (Pandis, 2011). Participant details recorded after the first visit were inputted into the randomisation package. Following randomisation of the first participant, remaining participants were randomly allocated into either the intervention or control based upon age (ages 60 + in both groups) and gender. Randomisation was conducted by the primary academic supervisor and a laboratory technician. Information on participant allocation was then provided to the chief investigator who informed Church surgery which participants required a consultation. Appointment bookings for participants randomised to the intervention were co-ordinated by the chief investigator. Participants randomised to control were not contacted regarding allocation and were only contacted to book follow up visits to the research facility.

8.2.4. Dietary Monitoring by Urine Sampling

After each WARU visit, participants were asked to collect three First Morning Void (FMV) samples each week on non-consecutive days for a three-week period, encompassing two-week day samples and one weekend sample. Duplicate samples were collected at each time point. Once the sample was successfully collected, the vacutainer tubes were returned to the absorbent pouch, placed within a sealed leak-proof plastic bag and kept in the participants' refrigerator

for storage at 4°C. Vacutainer pouches were labelled anonymously with information including participant number, phase, week and sample number. Participants recorded the collection details of their urine samples onto a sample collection sheet which noted the sample number and the date and time of collection (see appendix U). Samples were stored for a maximum of 10 days before being placed into an addressed box and returned to the laboratory either in person or by free post, see Figure 4.1. Samples were refrigerated temporarily upon arrival at the laboratory before processing and freezing at -80ºC. In total, participants collected 27 urine samples during their participation in the study.

8.2.5. Physical Activity Monitoring by ActiGraph

Physical activity was recorded using ActiGraph (ActiGraph LLC, Pensacola, FL, USA) following baseline, three and six-month WARU visits. Participants received written and verbal instructions (see appendix V) to wear the belt around their waist with the monitoring device positioned on the right-hand side of the body. Participants chose one week within the three-week urine collection period to wear the ActiGraph and were free to choose any day within the week to begin wearing the device. The ActiGraph was worn for seven consecutive days from waking until going to bed, unless unfeasible to do so (e.g. swimming, bathing or showering). Participants recorded the times in which the ActiGraph was worn (see appendix W). If participants did not wear the device for a period within the same day; the un-worn times were recorded. If participants missed a whole day; an additional recording day was added directly after the seventh day. The ActiGraph was not worn during sleeping hours. Participants returned the ActiGraph at the end of the recording period either in person or by post.

8.2.6. Blood Treatment

Separate 1.5 µl samples of whole blood were transferred by pipette from the heparinised

vacutainer into separate HbA_{1c} and lipid test cartridges and analysed respectively by an automated point of care blood analyser (see Chapter Four, General Methods, 4.6). The remaining whole blood was centrifuged at 1500 g for 10 minutes at 4˚C. Five 250 µl aliquots of supernatant were pipetted into five individual Eppendorfs and frozen at -80˚C.

8.2.7. Plasma Extraction

Plasma samples were defrosted over night at 4°C. A one micro-spoon of glass beads was added into new pre-labelled 2 ml Eppendorf tube. Raw plasma samples were vortexed for five seconds before 200 µl of the raw plasma sample and 1520 µl pre-chilled solvent mix (methanol/chloroform (4/1, v/v) was pipetted into the new Eppendorf tube containing the glass beads. Sample were then vortexed for five seconds, shaken for 15 minutes at 4°C and then kept at -20 °C for 20 minutes. Samples were then centrifuged at 14 000 rpm at 4°C for 5 minutes and kept at -80 °C until further analysis.

8.2.8. Plasma Analysis

See Chapter Four, General Methods, 4.9.

8.2.9. Urine Treatment

Vacutainer tubes were labelled with participant ID, phase, week and sample number. The first vacutainer tube from each participant was frozen for biobanking at -80°C. The duplicate vacutainer tube was centrifuged at 4000 g for 5 minutes at 4˚C. Three, 1 ml aliquots of supernatant were pipetted evenly into three separate Eppendorfs which represent each collection day (three Eppendorfs were filled to provide adequate sample quantities). From the remaining raw urine, a 200 µL aliquot was used to determine refractive index, measured using a handheld refractometer (see Chapter Four, General Methods, 4.8.).

Following sample preparation, extraction and adjustment (see Chapter Four, General Methods, 4.8.), weekly and phase pools were created on ice. For weekly pools, 200 µL of sample from each of the three extracted spot urines collected per week was pipetted into a new 2 ml Eppendorf and vortexed to create a pooled sample representing weeks one, two and three of each phase. For phase pools (baseline, three- and six-months), 200 µl of sample from each of the three weekly pools was aliquoted into a new 2 ml Eppendorf and vortexed. Next, 20 µl of the sample was pipetted into the micro-insert of a HPLC glass vial and 80 µL 70:30 solution (methanol/water) added before the vial was crimped and vortexed. Pooled samples were stored at -20°C prior to analysis by FIE-HRMS and the remaining volume of weekly pools stored at -80°C. Modelling, classification and feature selection (see Chapter Four, General Methods, 4.9.) was conducted on phase pools to determine differences in the urinary metabolome between baseline, three and six months.

8.2.10. Physical Activity Analysis

Data from ActiGraph were downloaded and analysed using ActiLife software (ActiLife, LLC, Pensacola, FL, USA). Data were filtered according to wear time diaries completed by the participant. Data recorded on the ActiGraph which did not correspond to reported wear time were not included in analysis. Ten second epoch[3] length was selected for valid data. Variables including; kCal expenditure, time spent in light, moderate and vigorous exercise intensities and step counts were downloaded and saved in Excel format prior to statistical analysis.

[3] Accelerometers function by integrating a filtered digitized acceleration signal over a user-specified time interval, referred to as an epoch. The usual accelerometer stored magnitude of accelerations at fixed recording intervals (1, 4, 15, or 60 seconds or longer) is called an "epoch". At the end of each epoch, the some index of physical activity is calculated (Ayabe, Kumahara, Morimura and Tanaka, 2013).

8.2.11. Statistical Analysis

For all physiological, biochemical and questionnaire data a three by two mixed model ANOVA was conducted. Post hoc analysis used Welch's t-test for group differences and Student's t-test (parametric) and Wilcoxon's test (non-parametric) were employed for post hoc time differences, with the Bonferroni correction. Additional analyses, using the same statistical tests as above, were conducted in participants categorised into the 'pre-contemplation' group. Significant findings for this group are reported and discussed where relevant. For correlation analyses, parametric data were analysed using Pearson's correlation and non-parametric data analysed by Spearman's correlation. Parametric data are presented as mean ± standard deviation and non-parametric data presented as median (range). Statistical significance was accepted at $P < 0.05$. All data are presented in tables, where statistically significant findings are observed, the data are also presented in figures.

8.3. Results

8.3.1. Participants

Eighty-eight patients were invited to take part in the study by letter from Church Surgery, 23 of whom (26%) volunteered to take part and completed a baseline visit to the WARU. Twelve participants were randomised into the intervention group. Nineteen participants (83%), 13 females and six males (age 65 ± 9 years) completed all visits and were included in analysis, Figure 8.1. There were no significant differences between groups at baseline except for fat percentage (INT 37.1 ± 33.6%, CON 45.4 ± 45.7%, $P = 0.02$) and hip circumference (INT 106 ± 104 cm, CON 118 ± 122 cm, $P = 0.04$).

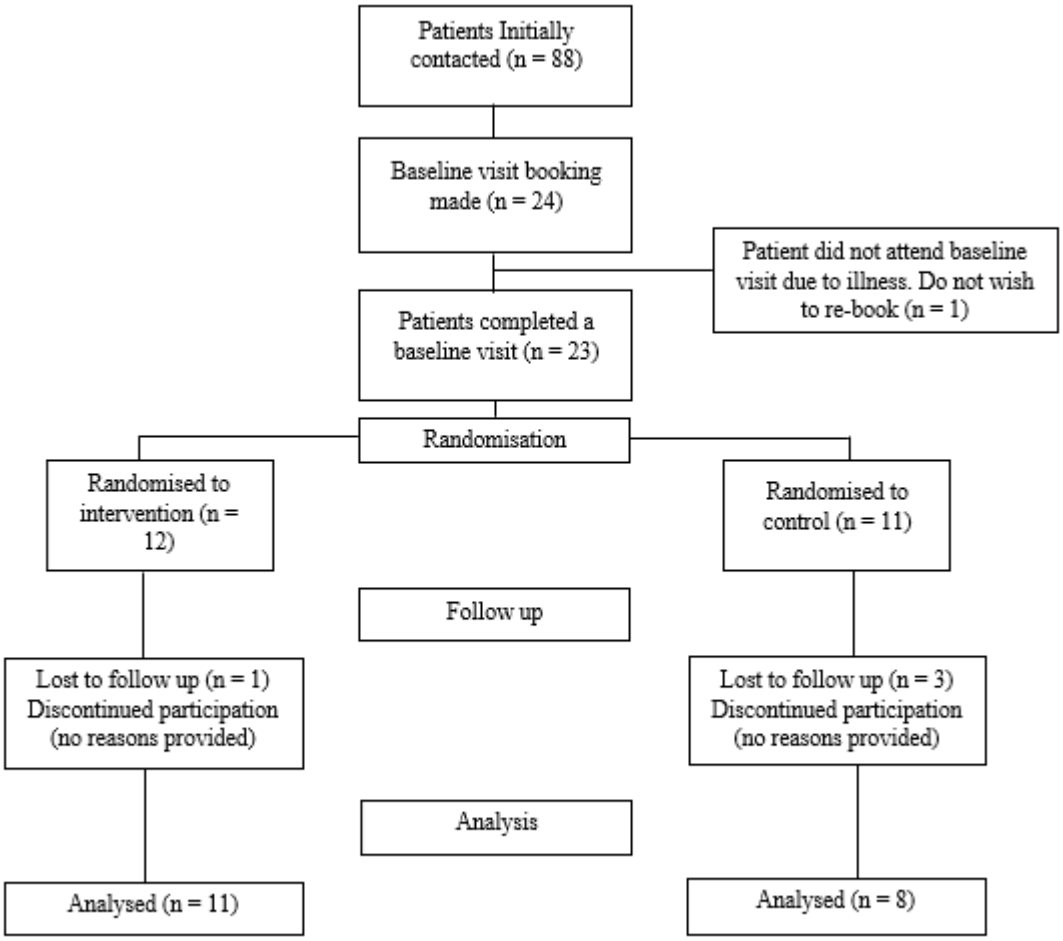


Figure 8.1. Participant recruitment, randomisation, retention and analysis.

8.3.2. Personal Diabetes Questionnaire

Following initial contact regarding T2D risk (and prior to randomisation), 12 participants (six INT / six CON) indicated they had started to act to achieve weight loss, three (two INT / one CON) indicated they were preparing to act, while four (three INT / one CON) were in the pre-contemplation phase and not considering weight loss. Eight (four INT / four CON) participants indicated they had begun to act to change their dietary behaviours, four (two INT / two CON) were preparing to make changes to their dietary behaviours, one (INT) was in the contemplation phase while six (four INT / two CON) were categorised in the pre-contemplation phase. Participants in INT scored lower for barriers to dietary change (12 ± 7 arbitrary units) compared to CON (14 ± 4 arbitrary units) although the difference was not statistically significant ($P = 0.47$). Similarly, INT participants scored lower for barriers to exercise (12 ± 4 arbitrary units) compared to CON participants (13 ± 4 arbitrary units) although the difference was not significant ($P = 0.88$).

8.3.3. HbA_{1c}

HbA_{1c} decreased significantly between baseline and six months ($P = 0.01$) and three months and six months ($P = 0.01$) irrespective of treatment group ($P = 0.89$) despite no initial change between baseline and three months ($P = 0.84$), Figure 8.2. HbA_{1c} did not change in pre-contemplation participants ($P = 0.34$).

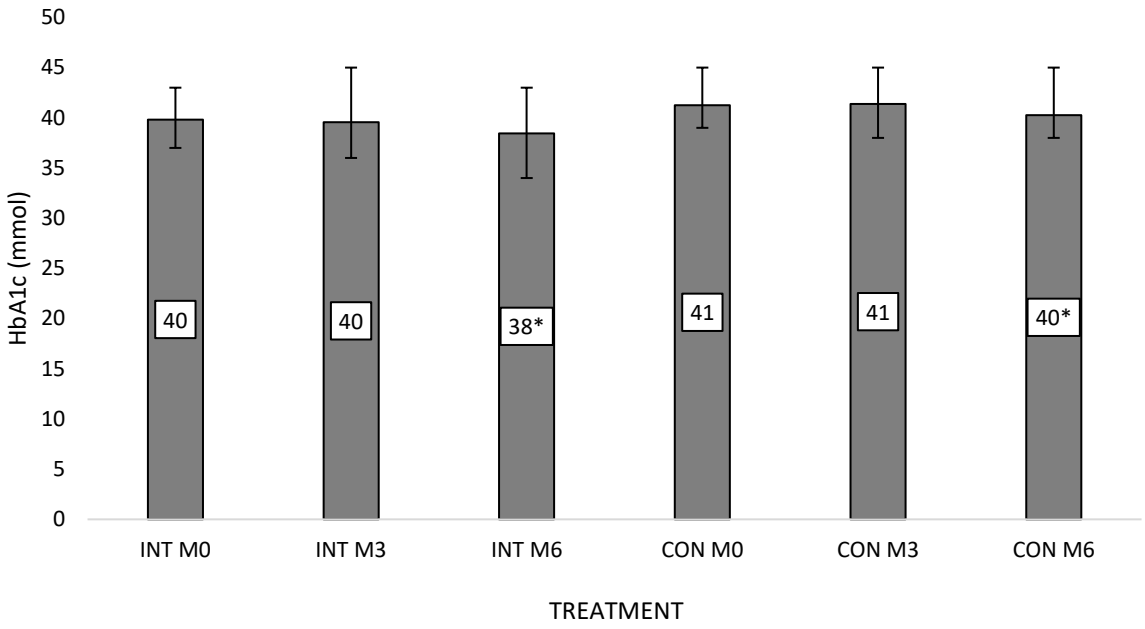


Figure 8.2. HbA_{1c} at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON). * Significantly different ($P < 0.05$) from baseline. # Significant difference between treatments. Data are represented as mean ± standard deviation.

Table 8.1. HbA_{1c}, Total, LDL and HDL Cholesterol and Triglycerides at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON). Parametric data represented as mean ± standard deviation. Non-parametric data represented as median (range). Statistical significance * is accepted at $P < 0.05$.

	HbA_{1c} (mmol/mol)		**Total Cholesterol (mmol/L)**		**LDL Cholesterol (mmol/L)**		**HDL Cholesterol (mmol/L)**		**Triglycerides (mmol/L)**	
Treatment Group	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**
Baseline	40 ± 1.9	41± 2	5.3 ± 0.9	5.1 ± 1.1	2.7 ± 0.9	2.7 ± 0.7	1.5 (1.4)	1.4 (0.8)	1.8 ± 0.8	1.7 ± 0.6
3 Months	40 ± 2.3	41 ± 2	5.1 ± 1.1	5.4 ± 0.8	2.8 ± 1.0	2.9 ± 0.4	1.5 (1.4)	1.7 (1.0)	1.5 ± 0.8	2.0 ± 0.8
6 Months	38 ± 3.0	40 ± 2.3	5.2 ± 1.2	5.4 ± 1.0	2.9 ± 0.9	3.0 ± 0.7	1.4 (1.3)	1.6 (0.9)	1.5 ± 0.7	1.8 ± 0.4
Treatment Effect (*P* value)	0.89		0.03*		0.53		0.19		0.11	
Time Effect (*P* value)	0.02*		0.19		0.03*		0.87		0.68	

Table 8.2. Body Mass, BMI, Hip and Waist Circumference, Fat and Muscle%, Systolic and Diastolic BP at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON). Parametric data represented as mean ± standard deviation. Non-parametric data represented as median (range). Statistical significance * is accepted at $P < 0.05$.

	Body Mass (kg)		**BMI (kg/m^2)**		**Hip Circumference (cm)**		**Waist Circumference (cm)**		**Fat (%)**		**Muscle (%)**		**Systolic BP (mmHg)**		**Diastolic BP (mmHg)**	
Treatment Group	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**
Baseline	79.6 ± 14.3	86.5 ± 10.2	28.9 ± 4.5	31.8 ± 4.5	107 ± 14	119 ± 10	100 (39)	104 (18)	37.1 ± 7.4	45.4 ± 6.9	49.8 ± 10.1	46.8 ± 5.1	136 ± 13	138 ± 23	82 ± 10	83 ± 10
3 Months	78.2 ± 14.4	85.7 ± 10.4	28.3 ± 4.6	31.6 ± 4.7	104 ± 15	116 ± 11	97 (39)	104 (18)	38.4 ± 6.4	46 ± 6.4	47.8 ± 9.1	45.7 ± 5.0	136 ± 18	129 ± 20	83 ± 12	82 ± 6
6 Months	77.6 ± 14.3	85.2 ± 9.1	28.2 ± 4.8	31.4 ± 4.2	104 ± 13	115 ± 10	98 (39)	102 (26)	37.0 ± 8.5	47.4 ± 7.0	48.9 ± 11.4	44.5 ± 4.6	129 ± 13	135 ± 18	81 ± 10	84 ± 6
Treatment Effect (*P* value)	0.71		0.51		0.81		0.82		0.32		0.38		0.09		0.48	
Time Effect (*P* value)	0.06		0.06		> 0.00*		0.03*		0.55		0.09		0.10		0.99	

8.3.4. Biochemical and Anthropometric Outcomes

Total cholesterol increased significantly more in CON compared to INT ($P = 0.03$). In CON, total cholesterol increased from baseline to three months ($P = 0.04$), baseline to six months ($P = 0.02$) and remained similar between three and six months ($P = 0.77$). Total cholesterol remained similar in INT throughout ($P > 0.05$) and in pre-contemplation participants ($P = 0.89$). LDL cholesterol increased significantly between baseline and six months ($P = 0.02$) with no difference in treatment group ($P = 0.53$) although remained unchanged in the pre-contemplation group ($P = 0.76$). HDL cholesterol and triglycerides remained similar over time across both groups ($P > 0.05$; Table 8.1 and Figures 8.4 and 8.5) and in the pre-contemplation group ($P = 0.20$).

Hip circumference decreased similarly in both groups ($P < 0.05$) irrespective of treatment, decreasing from baseline to three months ($P = 0.00$), baseline to six months ($P = 0.00$) and remaining similar between three and six months ($P = 0.40$). A significant decrease was also observed in the pre-contemplation group ($P = 0.01$). Waist circumference changed significantly over time but was unaffected by treatment. Values decreased from baseline to three months ($P = 0.02$), baseline to six months ($P = 0.02$) and unchanged between three and six months ($P = 0.33$). Decreases in waist circumference in the pre-contemplation group did not reach statistical significance ($P = 0.07$) Body mass, BMI, systolic and diastolic BP and fat remained similar across treatment and time ($P > 0.05$; Table 8.2 and Figures 8.6 and 8.7). Muscle percentage remained similar across treatment and time when all participants were analysed together ($P < 0.05$) however a significant decrease was observed in pre-contemplation participants ($P = 0.04$).

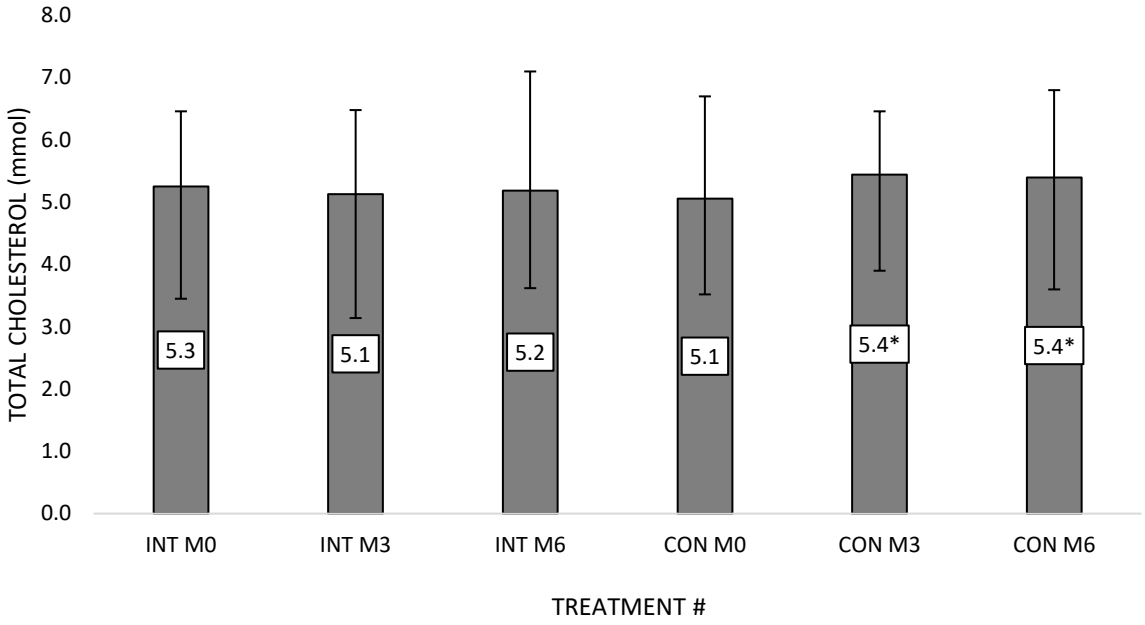


Figure 8.3. Total cholesterol at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON). *Significantly different ($P < 0.05$) from baseline. # Significant difference between treatments. Data are represented as mean ± standard deviation.

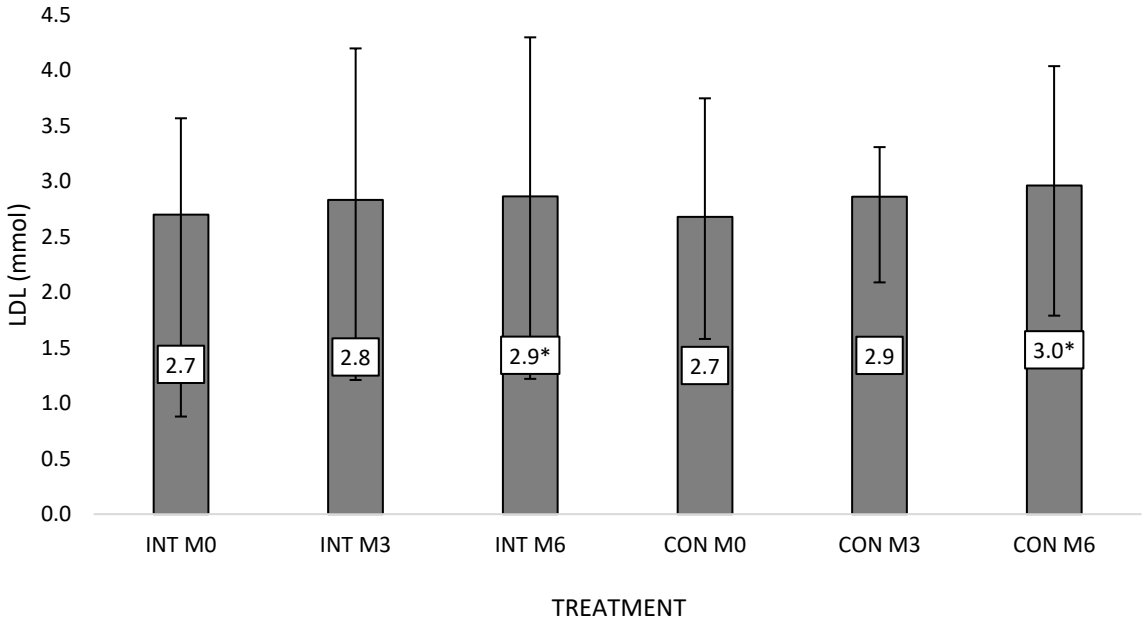


Figure 8.4. LDL cholesterol at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON). * Significantly different ($P < 0.05$) from baseline. # Significant difference between treatments. Data are represented as mean ± standard deviation.

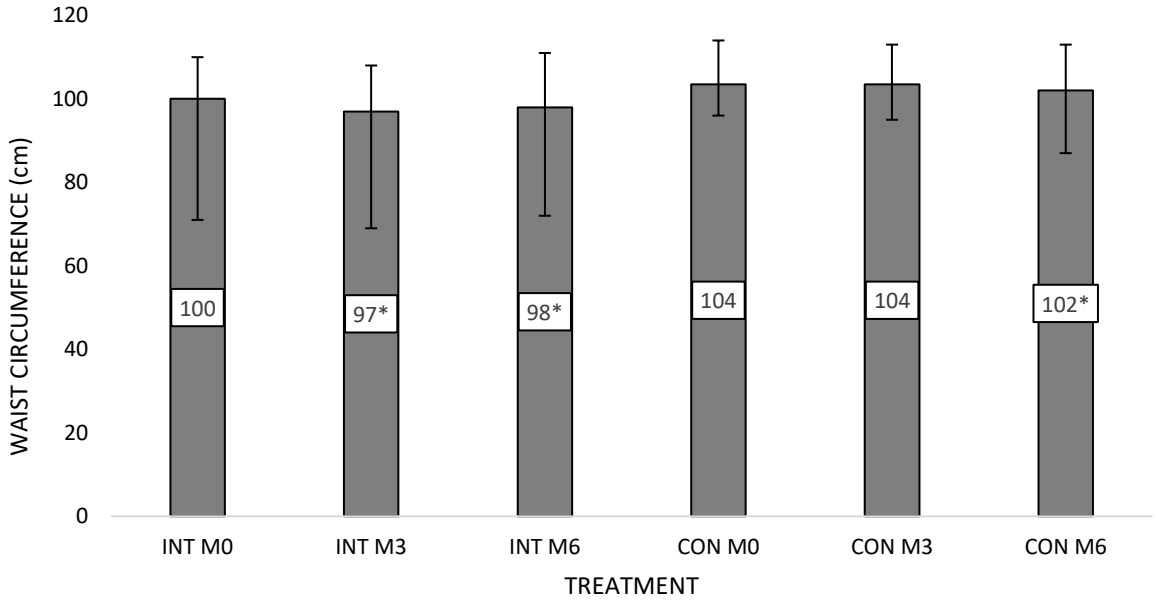


Figure 8.5. Waist circumference at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON). * Significantly different ($P < 0.05$) from baseline. # Significant difference between treatments. Data are represented as median (range).

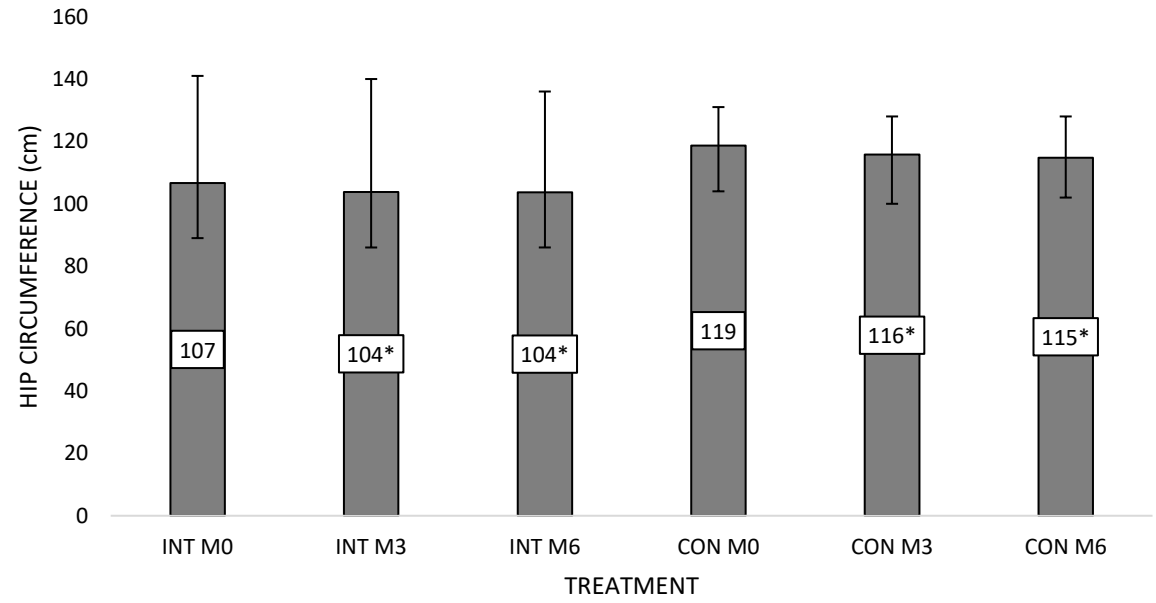


Figure 8.6. Hip circumference at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON). * Significantly different ($P < 0.05$) from baseline. # Significant difference between treatments. Data are represented as mean ± standard deviation.

Table 8.3. Correlation coefficients for change in HbA_{1c} and changes in Body Mass, BMI and Waist Circumference between baseline and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON). [1] Pearson's Correlation Test; [2] Spearman's Correlation Test. Statistical significance* is accepted at $P < 0.05$.

Change in Body Mass / Change in HbA_{1c}	
Correlation Coefficient	0.60 [2]
Significance	0.00*
Change in BMI / Change in HbA_{1c}	
Correlation Coefficient	0.67 [2]
Significance	0.00*
Change in Waist Circumference / Change in HbA_{1c}	
Correlation Coefficient	0.30 [1]
Significance	0.21

8.3.5. Baseline to Six Months Correlation Analysis

Significant correlations were found for changes in HbA_{1c} and changes in body mass ($r = 0.60$, $P = 0.00$) and changes in BMI ($r = 0.67$, $P = 0.00$) between baseline and six months. No significant correlation was identified between change in waist circumference and change in HbA_{1c} ($r = 0.30$, $P = 0.21$), Table 8.3, Figures 8.8 and 8.9.

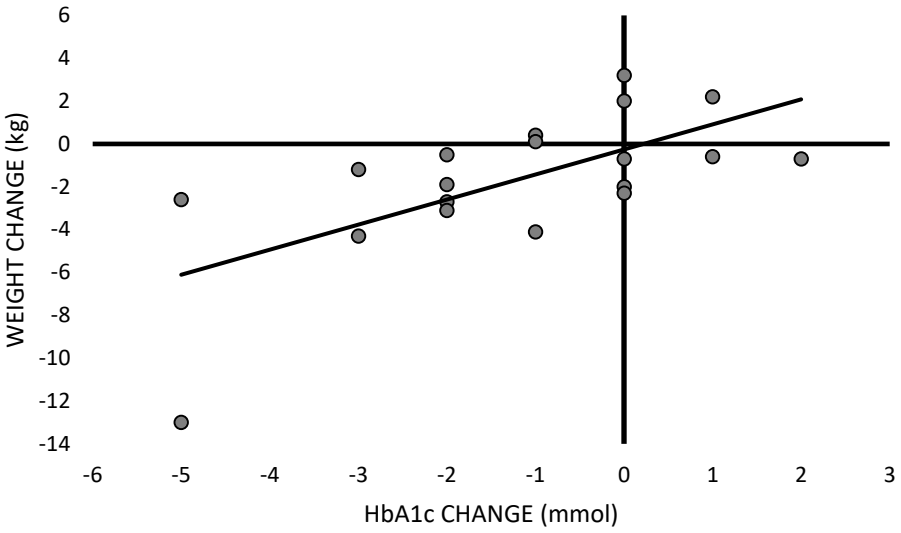


Figure 8.7. Correlation of weight change and HbA_{1c} change between baseline and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON).

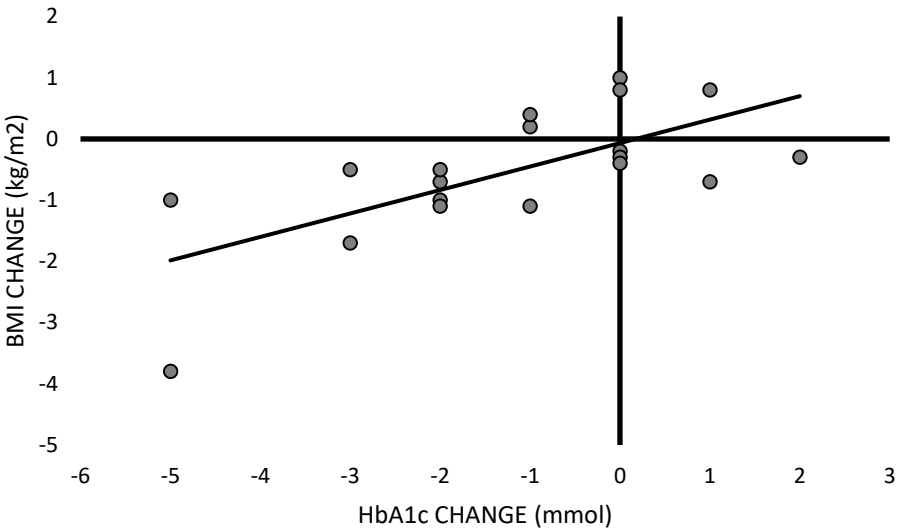


Figure 8.8. Correlation of BMI change and HbA_{1c} change between baseline and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON).

Table 8.4. Effects of treatment and time on urinary biomarkers of dietary intake following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON).

Biomarker	Dietary Component	Treatment Effect (*P*)	Time Effect (*P*)
2-Cyanoethylmercapturic acid	Tar (Smoking)	0.85	0.60
2,3-Dihydroxyphenylpropionic acid	Wholegrain	0.18	0.44
3-Hydroxyhippuric acid	Fruit & Vegetables	0.19	0.36
3-Hydroxypropylmercapturic acid	Tar (Smoking)	0.94	0.25
3-Methyl-xanthine	Cocoa	0.42	0.94
3,4-Dihydroxybenzoic acid	Fruit & Vegetables	0.57	0.33
3,5-Dihydroxybenzoic acid	Fruit & Vegetables	0.12	0.08
3,5-Dihydroxybenzoic acid-3-sulfate	Fruit & Vegetables	0.78	0.78
4-Hydroxyhippuric acid	Fruit & Vegetables	0.51	0.00*
7-Methyl-xanthine	Cocoa	0.33	0.91
Acesulfame-K	Sweetened Drinks	0.15	0.94
Benzoxazolinone	Wholegrain	0.20	0.25
Caffeic acid	Fruit & Vegetables	0.02*	0.02*
Caffeine	Caffeine	0.17	0.68
Chlorogenic acid	Fruit, coffee, green tea, vegetables	0.49	0.71
Cotinine	Nicotine	0.71	0.33
D, L-Sulforaphane-glutathione	Cruciferous Vegetables	1.00	1.00
D, L-Sulforaphane-L-cysteine	Cruciferous Vegetables	0.19	0.12

D, L-Sulforaphane-N-acetyl-L-cysteine	Cruciferous Vegetables	0.07	0.11
Daidzein	Soy	0.08	0.09
Dihydrocaffeic acid	Cocoa	0.13	0.14
Epicatechin	Apple, cocoa, coffee, green/black tea, fruit, vegetables	0.72	0.19
Ferulic acid	Coffee, polyphenol-rich foods	0.84	0.18
Ferulic acid-4-O-b-D-glucuronide	Coffee, polyphenol-rich foods	0.06	0.12
Ferulic acid-4-O-sulfate	Coffee, polyphenol-rich foods	0.49	0.03*
Feruloylglycine	Coffee, polyphenol-rich foods	0.63	0.07
Furaneol	N/A	0.61	0.60
Hippuric acid	Fruit and Vegetables	0.35	0.00*
Isoferulic acid	Coffee, polyphenol-rich foods	0.43	0.94
m-Coumaric acid	Coffee, olives	0.48	0.68
N-(2-Furoyl) glycine	Strongly heated foods	0.32	0.31
Naringen	Citrus	0.15	0.11
Norfuraneol	N/A	0.69	0.66
p-Coumaric acid	Wine/Grapes, Berries, Wholegrains, herbs, peanuts	0.56	0.43
Pyrogallol	Beans, soya, peanuts, almonds	0.45	0.50

Quercetin	Fruits, vegetables, tea	0.48	0.43
Quercetin-3-O-beta-D-glucuronide	Fruits vegetables, tea	0.37	0.36
Resveratrol	Red wine, grapes	0.40	0.35
Vanillic acid	coffee, cocoa, wholegrain, herbs, olive	1.00	0.61
p-Cresol Glucuronide	Protein	0.76	0.35
1-Methyl histidine	Poultry	0.58	0.58
2,3-Dihydroxyphenylpropionic acid-3-sulfate	Wholegrain	0.35	0.54
3-Methyl histidine	Poultry	0.61	0.72
4-Hydroxyproline betaine	Citrus	0.13	0.97
5-Hydroxy-L-tryptophan	Protein	0.20	0.28
5-Methoxyindole-3-acetic acid	Poultry	0.19	0.40
5-Oxo-L-prolyl-L-proline	Processed Cheese	0.72	0.39
B-Alanine	Protein	0.22	0.78
Calystegine A3	Potatoes	0.39	0.47
Calystegine B	Potatoes	0.48	0.96
Creatinine	Meat	0.49	0.82
D-Sucrose	Sugar	0.51	0.16
Dopamine 3-O-Sulfate	Bananas	0.06	0.49
Dopamine-4-O-sulfate	Bananas	0.11	0.19
Ethyl-beta-D-glucuronide	Alcohol	0.59	0.56
Gallic acid	Fruits & vegetables, wine/grapes, tea	0.68	0.40
Indoxyl Sulfate	Protein	0.91	0.29
L-Anserine	Poultry	0.88	0.92

L-Carnitine	Red Meat	0.90	0.63
L-Carnosine	Meat	0.47	0.47
L-Histidine	Protein	0.14	0.79
L-Nicotine	Nicotine	0.30	0.53
L-Tartarate	Grape	0.61	0.40
p-Cresol sulfate	Protein	0.53	0.71
Phenyl-acetyl-L-glutamine	Protein	0.30	0.56
Phenylalanine	Protein	0.95	0.54
Proline betaine	Citrus	0.60	0.58
Rhamnitol	Apple	0.54	0.15
Taurine	Striated muscle meat	0.03*	0.03*
Trans-3'-Hydroxycotinine	Nicotine	0.24	0.15
Trigonelline	Beans, soya, peanuts, almonds, coffee, peas	0.11	0.25
Trimethylamine-N-oxide	Fish	0.97	0.56
Tryptophan	Protein	0.77	0.25

Table 8.5. Dietary biomarker concentrations (mmol/L) of metabolites which exhibited significant differences between groups, changes over time or both (see Table 8.4.) Parametric data presented as mean ± standard deviation. Non-parametric data presented as median (range).

	4-Hydroxyhippuric acid (mmol/L)		Caffeic acid (mmol/L)		Ferulic acid-4-O-sulfate (mmol/L)	
Treatment Group	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**
Baseline	0.01076 (0.01999)	0.01759 (0.01664)	0.01329 ± 0.00522	0.01271 ± 0.00405	0.04761 ± 0.02767	0.03929 ± 0.01782
3 Months	0.01160 (0.01607)	0.01371 (0.01244)	0.01422 ± 0.00693	0.00822 ± 0.00215	0.04233 ± 0.02055	0.03904 ± 0.02060
6 Months	0.00798 (0.01162)	0.00821 (0.01682)	0.00974 ± 0.00322	0.00958 ± 0.00254	0.03730 ± 0.01781	0.03501 ± 0.01718

	Hippuric acid (mmol/L)		Taurine (mmol/L)	
Treatment Group	**INT**	**CON**	**INT**	**CON**
Baseline	3.85798 ± 1.86808	3.76769 ± 0.55655	0.03990 (0.13609)	0.04143 (0.01685)
3 Months	3.80716 ± 1.50185	2.97166 ± 1.03394	0.02536 (0.06943)	0.03054 (0.03728)
6 Months	2.48414 ± 0.88638	2.62659 ± 0.59901	0.02527 (0.07353)	0.01968 (0.03257)

Table 8.6A. Mean fold change in the concentration of 4-Hydroxyhippuric acid, Ferulic acid-4-O-sulfate and Hippuric acid (where treatment had no effect) between baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON).

4-Hydroxyhippuric Acid	**Mean Fold Change**
Baseline to 3 Months	-1.16
Baseline to 6 Months	-1.61
3 Months to 6 Months	-1.32
Ferulic acid-4-O-sulfate	
Baseline to 3 Months	-1.12
Baseline to 6 Months	-1.19
3 Months to 6 Months	-1.01
Hippuric Acid	
Baseline to 3 Months	-1.16
Baseline to 6 Months	-1.53
3 Months to 6 Months	-1.24

Table 8.6B. Mean fold change in the concentration of Caffeic acid and Taurine (where treatment had an effect) between baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON).

Caffeic Acid	**Mean Fold Change**
Intervention	
Baseline to 3 Months	0.99
Baseline to 6 Months	-1.43
3 Months to 6 Months	-1.31
Control	
Baseline to 3 Months	-1.38
Baseline to 6 Months	-1.36
3 Months to 6 Months	1.00
Taurine	
Intervention	
Baseline to 3 Months	-1.50
Baseline to 6 Months	-1.57
3 Months to 6 Months	-1.12
Control	
Baseline to 3 Months	-1.96
Baseline to 6 Months	-1.72
3 Months to 6 Months	-1.03

Table 8.7. Dietary biomarker concentrations (mmol/L) of metabolites which exhibited significant differences in pre-contemplation participants. Data presented as median (range).

	4-Hydroxyhippuric acid (mmol/L)	Dehydroxybenzoic Acid-35 (mmol/L)	Ferulic acid (mmol/L)
Baseline	0.01600 (0.01400)	0.00200 (0.00200)	0.00152 (0.00750)
3 Months	0.01400 (0.01200)	0.00200 (0.00400)	0.00232 (0.00251)
6 Months	0.00900 (0.01100)	0.00100 (0.00200)	0.00087 (0.00179)

	Hippuric acid (mmol/L)	Trans-3-Hydroxycotinine (mmol/L)
Baseline	3.32164 (3.74472)	0.00004 (0.00786)
3 Months	3.88204 (3.22357)	0.00004 (0.00646)
6 Months	2.60405 (1.26709)	0.00001 (0.00595)

Table 8.8. Mean fold change in the concentration of 4-Hydroxyhippuric acid, Dehydroxybenzoic Acid, Ferulic acid, Hippuric acid and Trans-3-Hydroxycotinine in pre-contemplation participants between baseline, three and six months.

4-Hydroxyhippuric Acid	**Mean Fold Change**
Baseline to 3 Months	- 0.00071
Baseline to 6 Months	- 0.00657
3 Months to 6 Months	- 0.00586
Dehydroxybenzoic Acid-35	
Baseline to 3 Months	- 0.00071
Baseline to 6 Months	- 0.00071
3 Months to 6 Months	- 0.00143
Ferulic Acid	
Baseline to 3 Months	- 0.00028
Baseline to 6 Months	- 0.00178
3 Months to 6 Months	- 0.00149
Hippuric Acid	
Baseline to 3 Months	- 0.00616
Baseline to 6 Months	- 1.59526
3 Months to 6 Months	- 1.58910
Trans-3-Hydroxycotinine	
Baseline to 3 Months	- 0.00029
Baseline to 6 Months	- 0.00031
3 Months to 6 Months	- 1.43E-05

Table 8.9. Correlation coefficients for change in HbA_{1c} and changes in the concentration of 4-Hydroxyhippuric acid, Caffeic acid, Ferulic acid-4-O-sulfate, Hippuric acid and Taurine between baseline and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON). 1 Pearson's Correlation Test; 2 Spearman's Correlation Test. Statistical significance* is accepted at $P < 0.05$.

Change in 4-Hydroxyhippuric Acid / Change in HbA_{1c}	
Correlation Coefficient	-0.15[1]
Significance	0.55
Change in Caffeic Acid / Change in HbA_{1c}	
Correlation Coefficient	-0.41[1]
Significance	-0.10
Change in Ferulic acid-4-O-sulfate / Change in HbA_{1c}	
Correlation Coefficient	0.17[2]
Significance	0.49
Change in Hippuric Acid / Change in HbA_{1c}	
Correlation Coefficient	-0.43[1]
Significance	0.07
Change in Taurine / Change in HbA_{1c}	
Correlation Coefficient	-0.01[2]
Significance	0.94

8.3.6. Urine Dietary Biomarkers

Quantified values were available for 73 urinary metabolites at baseline, three months and six months (Tables 8.4 and 8.5) and fold changes displayed in Tables 8.6A/B. Complete urine profiles from all phases were completed by 15 participants and included in analysis (nine INT, six CON). Of the 73 biomarkers identified, five metabolites exhibited significant differences either by treatment, time or both. 4-Hydroxyhippuric acid concentration, a biomarker of fruit and vegetable intake, decreased from baseline to six months ($P = 0.02$) and three months and six months ($P = 0.01$) irrespective of treatment ($P = 0.51$). Concentration of caffeic acid, another biomarker of fruit and vegetable intake, was significantly different between treatments after three months ($P = 0.02$) with both treatments demonstrating decreases in concentration between baseline and six months ($P = 0.01$) with the values similar at six months between both

treatments (P = 0.92). Excretion of ferulic acid-4-O-sulfate, a biomarker representative of polyphenol-rich foods, such as coffee, wine, tomatoes, beer, breakfast cereals, apple wholegrain, cocoa, dried fruit, berries, remained similar between treatments (P = 0.49) but changed significantly over time (P = 0.03) with concentration decreasing overall between baseline and six months (P = 0.01) and three months and six months (P = 0.03). Similarly, hippuric acid, another biomarker of fruit and vegetable consumption, remained similar between treatments (P = 0.35) but decreased significantly overall between baseline and six months (P = 0.00) and three months and six months (P = 0.02). Lastly, but in similar fashion, concentration of taurine, a biomarker for striated muscle meat was significantly different between treatment (P = 0.03) and decreased significantly over time (P = 0.03). Post hoc testing for taurine did not provide conclusive results for pairwise differences. No correlations were found between the change in concentration of 4-Hydroxyhippuric acid, Caffeic acid, Ferulic acid-4-O-sulfate, Hippuric acid and Taurine and change in HbA_{1c} between baseline and six months (Table 8.9). Concentrations of 4-hydroxyhippuric acid and hippuric acid also decreased in pre-contemplation participants ($P < 0.05$). In addition, decreases were also observed dehydroxybenzoic acid (P = 0.01; fruit and vegetable intake), ferulic acid (P = 0.00; polyphenol intake) and trans-3-hydroxycotinine (P = 0.02; nicotine intake).

Table 8.10. RF classification margins for participant's urine in intervention and control groups between baseline and three months, baseline and six months and three months and six months.

Participant	M0-M3	M0-M6	M3-M6
	Intervention		
03	0.195	0.207	0.298
06	0.039	0.169	0.125
07	0.233	0.333	0.094
08	0.201	0.281	0.092
11	0.018	0.094	0.108
12	0.13	0.187	0.273
18	0.24	0.246	0.094
19	0.233	0.234	0.16
22	0.245	0.298	0.137
	Control		
01	0.311	0.23	0.292
02	0.02	0.208	0.157
05	0.1	0.305	0.224
09	0.104	0.113	0.135
16	0.257	0.219	0.457
23	0.223	0.18	0.179

8.3.7. FIE-MS Urine

Metabolome fingerprints were created by non-targeted FIE-HRMS to determine the chemical composition of urines between phases using random forest classification (Table 8.10). Fifteen participants provided urine samples across all phases which were included in analysis. Of the 45 RF margins displayed in Table 8.10, four met the 0.3 RF margin threshold used previously in this thesis and in other previous work (Enot *et al*., 2008), demonstrating acceptable class discrimination in the urinary metabolome which may be suggestive of a change in dietary behaviour between phases. Analysis of RF margins by Kruskal-Wallis test revealed no significant treatment effect between intervention and control at any phase ($P > 0.05$). From the four participants where RF margins suggest a possible change in dietary behaviour, three were part of the control group and one was part of the intervention group, demonstrating the

intervention to have no effect on dietary behaviour in the context of the urinary metabolome, compared to control. To determine if the degree of urine metabolome change by RF margin at each phase was associated with change in HbA_{1c}, correlations were conducted. Pearson's correlation revealed no significant relationship between urine metabolome change and HbA_{1c} change between either baseline and 3 months (r = -0.12, *P* = 0.65) or baseline and six months (r = 0.10, *P* = 0.71). Similarly, Spearman's correlation found no relationship between urine metabolome change and HbA_{1c} change between three and six months (r = 0.12, *P* = 0.65). Given no changes were found in HbA_{1c} values in pre-contemplation participants, no correlation analysis was conducted.

Table 8.11. RF classification margins for plasma in intervention and control groups between baseline and three months, baseline and six months and three months and six months.

M0-M3	M0-M6	M3-M6
	Intervention	
-0.02969	2.35E-04	-0.0697
	Control	
-0.11078	-0.00955	-0.06075

8.3.8. FIE-HRMS Plasma

Following non-targeted FIE-MS, random forest classification margins clearly demonstrate no distinct class separation in plasma metabolome profiles between any phase (Table 8.11). Kruskal-Wallis analysis revealed no difference in RF margins between treatments at any phase ($P > 0.05$) suggesting the intervention had no effect on dietary behaviour in the context of the plasma metabolome, compared to control. Given RF classification margins for plasma between phases were significantly below the 0.3 threshold margin considered satisfactory in discriminating between biological samples, no feature selection of discriminatory signals was conducted.

Table 8.12. AHEI scores from FFQ responses at baseline and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON). 1-vegetables, 2-fruit, 3-wholegrains, 4-sugar-sweetened beverages, 5-nuts, legumes, and vegetable protein, 6-red meat and processed meat, 7-trans-fats, 8-fish: 9-polyunsaturated fats, 10-sodium, 11-alcohol. Data are represented as mean ± SD.

	Baseline												**Six Months**											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Total**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Total**
												Control												
Mean	7.8	5.7	3.4	6.5	2.6	4.5	5.3	3.5	5.5	10.0	9.1	63.8	8.3	6.4	2.1	5.5	4.5	6.3	4.5	3.3	6.2	10.0	8.8	66.0
SD	2.1	2.4	2.4	3.4	3.3	2.6	2.7	1.2	3.2	0.0	1.0	9.4	1.9	2.8	2.1	4.5	3.5	3.3	1.2	2.4	3.2	0.0	1.0	7.5
												Intervention												
Mean	8.3	6.3	2.5	5.0	5.2	4.0	5.4	5.2	3.5	10.0	9.3	64.6	8.2	5.9	1.9	4.9	4.7	4.8	5.7	4.7	5.1	10.0	9.3	65.1
SD	1.7	3.2	1.9	4.2	3.8	2.7	2.5	1.9	2.6	0.0	1.1	11.7	2.2	3.4	1.1	4.8	3.0	2.8	2.7	2.4	2.9	0.0	0.9	12.3

8.3.9. Food Frequency Questionnaire

At baseline, a Kruskal-Wallis test demonstrated intakes of trans-fats to be significantly different between INT and CON ($P = 0.03$). In CON, participants mean score was 3.4 arbitrary units whereas in INT, the mean score was 5.2 arbitrary units, demonstrating participants in INT reported more favourable intakes of trans-fat. After six months, there was no significant difference for trans-fat intake scores between groups. Analysis of the remaining components of the FFQ using the AHEI scoring method demonstrated no differences between groups at baseline or six months ($P > 0.05$). When changes between baseline and six months were examined as a collective group, paired t-tests revealed significant difference in scores for both intake of wholegrains ($P = 0.00$) and red and processed meat ($P = 0.03$). For wholegrain intake, the mean score decreased from 2.8 ± 2.0 to 2.0 ± 1.5 arbitrary units while scores for red and processed meat increased from 4.1 ± 2.5 to 5.3 ± 3.0 arbitrary units. The remaining dietary component scores remained similar between baseline and six months ($P > 0.05$), Table 8.12. Analysis of participants in pre-contemplation found no significant differences ($P > 0.05$).

Table 8.13. kCal expenditure, time spent in light, moderate and vigorous exercise intensities and step counts recorded by ActiGraph at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON). Parametric data represented as mean ± standard deviation. Non-parametric data represented as median (range). Statistical significance* is accepted at $P < 0.05$.

	kCal		Light (mins)		Moderate (mins)		Vigorous (mins)		Step Count	
Treatment Group	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**
Baseline	460 ± 150	323 ± 117	213 ± 46	176 ±37	40 ± 19	20 ± 11	0 (0)	0 (0)	5862 (9693)	4568 (3971)
3 Months	426 ± 128	289 ± 88	201 ± 57	151 ± 49	38 ± 16	21 ± 10	0 (1)	(0)	5527 (8391)	3524 (2771)
6 Months	491 ± 282	305 ± 122	237 ± 68	165 ± 49	43 ± 27	21 ± 14	0 (1)	0 (1)	5911 (7262)	3913 (3133)
Treatment Effect (*P* =)	0.76		0.48		0.78		0.60		0.79	
Time Effect (*P* =)	0.60		0.29		0.79		0.04*		0.71	

8.3.10. Physical Activity Outcomes from ActiGraph

A seven-day average for daily; calorie expenditure, time spent in light, moderate and vigorous physical activity domains and step counts (Table 8.13) were calculated for each participant after baseline, three- month and six-month ActiGraph use. There were no significant differences between kCal expenditure or time spent in light and moderate exercise intensities between INT and CON or between baseline, three and six months ($P > 0.05$). Analysis demonstrated time spent undertaking vigorous exercise increased significantly over time between baseline and six months ($P = 0.04$) although the was no difference between treatments ($P > 0.05$). A significant difference was found in step counts between treatments after three months ($P = 0.04$) although no time differences were found between baseline, three and six months ($P > 0.05$). Correlation analysis between changes in HbA_{1c} and changes in recorded physical activity including kCal expenditure, light, moderate, vigorous and steps counts between time points revealed no significant associations ($P > 0.05$). No significant differences were found in pre-contemplation participants in any variable measured by the ActiGraph ($P > 0.05$).

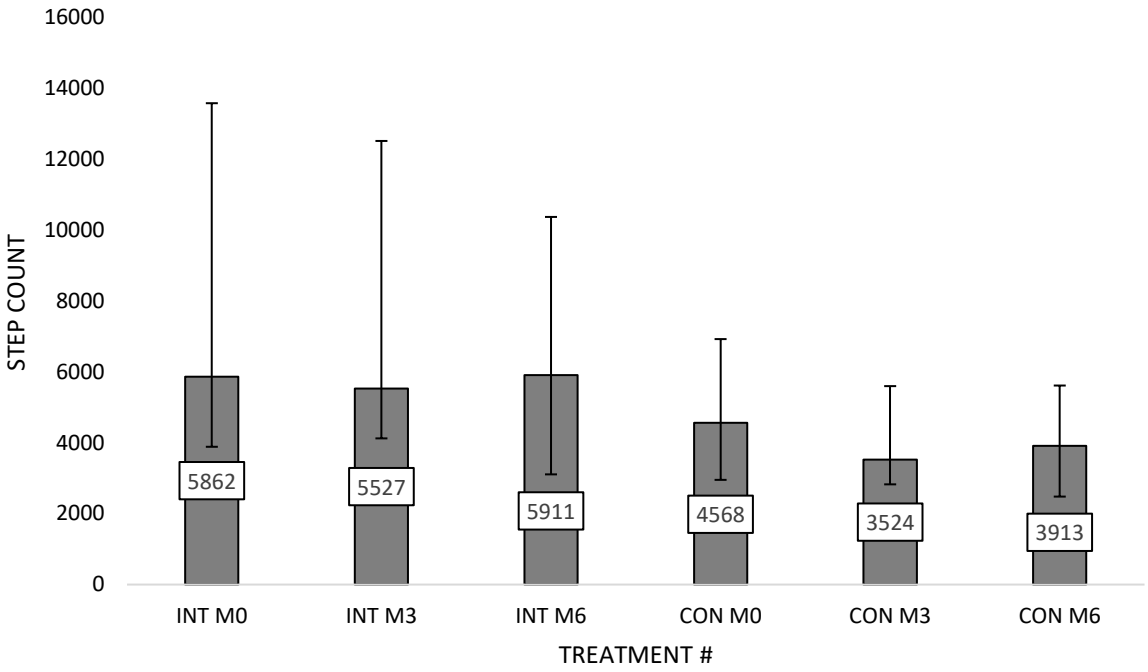


Figure 8.9. Average step counts at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON). * Significantly different ($P < 0.05$) from baseline. # Significant difference between treatments. Data are represented as median (range).

Table 8.14. Weekly walking, moderate, vigorous and total physical activity in met minutes reported in the IPAQ at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON). Data are represented as median (range). Statistical significance* is accepted at $P < 0.05$.

	Walking (met mins)		**Moderate (met mins)**		**Vigorous (met mins)**		**Total (met mins)**	
Treatment Group	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**
Baseline	2772 (5148)	2425 (5148)	2400 (6720)	840 (2880)	0 (1440)	0 (2880)	4638 (11868)	3279 (10428)
3 Months	1386 (5461)	825 (2772)	300 (5760)	1320 (5040)	0 (8640)	0 (1200)	3532 (13861)	3058 (6972)
6 Months	1386 (4686)	1782 (5461)	360 (6720)	1200 (6720)	0 (960)	0 (480)	2826 (11406)	3477 (10878)
Treatment Effect (*P* =)	0.23		0.25		0.35		0.31	
Time Effect (*P* =)	0.03*		0.85		0.40		0.28	

8.3.11. IPAQ-SF Questionnaire

Moderate and vigorous physical activity and total physical activity (Table 8.14) were similar in INT and CON between baseline, three and six months and did not change significantly over time ($P > 0.05$). Reported weekly met minutes for walking decreased significantly over time in both groups ($P = 0.03$), Figure 8.10. Values reported by pre-contemplation participants remained unchanged ($P > 0.05$).

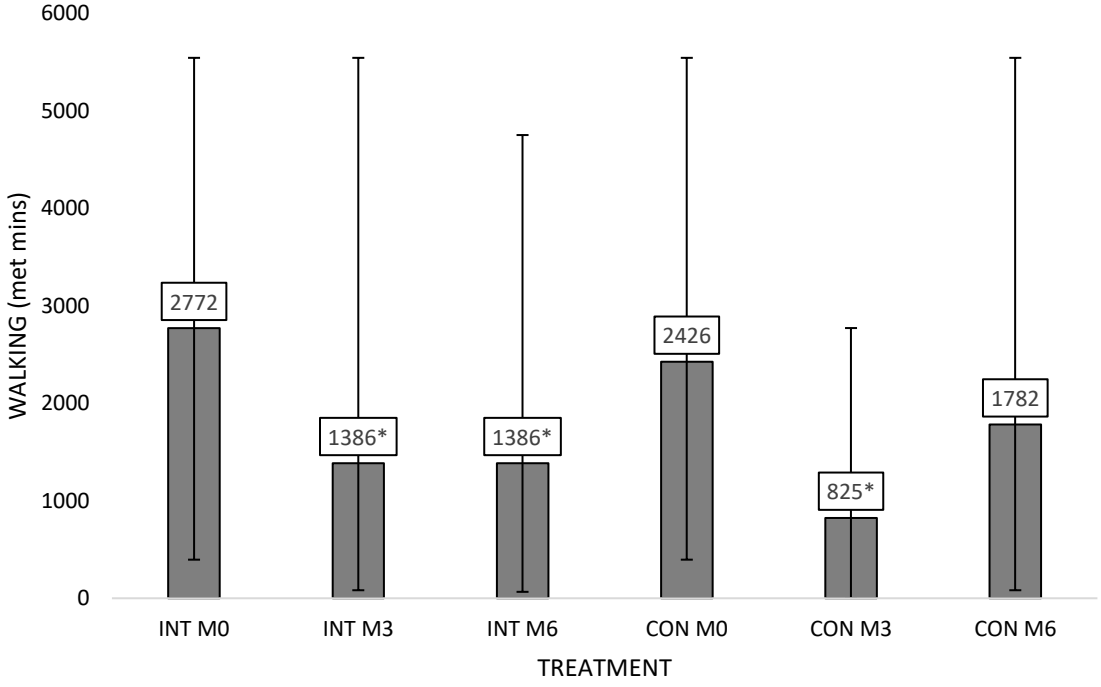


Figure 8.10. Walking met minutes at baseline, three and six months following a 15-minute one-to-one consultation with a health care professional in a primary care setting (INT) or standard care (CON). * Significantly different ($P < 0.05$) from baseline. # Significant difference between treatments. Data are represented as median (range).

Table 8.15A. Physical functioning, role limitations due to physical health, role limitations due to emotional problems and energy/fatigue (a score of 100 represents the highest level of functioning) at baseline, three and six months following a 15-minute one to one consultation with a health care professional in a primary care setting (INT) or standard care (CON). Data are represented as median (range). Statistical significance* is accepted at $P < 0.05$.

	Physical functioning		**Role limitations due to physical health**		**Role limitations due to emotional problems**		**Energy/Fatigue**	
Treatment Group	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**
Baseline	69 (75)	42 (100)	75 (100)	25 (100)	100 (100)	100 (100)	55 (45)	42 (80)
3 Months	75 (80)	62 (95)	87 (100)	37 (100)	100 (67)	100 (67)	55 (45)	50 (60)
6 Months	80 (99)	60 (90)	100 (100)	50 (100)	100 (99)	100 (67)	52 (84)	50 (40)
Treatment Effect (*P* =)	0.20		0.19		0.20		0.20	
Time Effect (*P* =)	0.20		0.19		0.20		0.20	

Table 8.15B. Emotional wellbeing, social functioning, pain and general health (a score of 100 represents the highest level of functioning) at baseline, three and six months following a 15 minute one-to one consultation with a health care professional in a primary care setting (INT) or standard care (CON). Data are represented as median (range). Statistical significance* is accepted at $P < 0.05$.

	Emotional wellbeing		**Social functioning**		**Pain**		**General health**	
Treatment Group	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**	**INT**	**CON**
Baseline	84 (24)	86 (60)	81 (62)	87 (75)	78 (67)	45 (90)	50 (60)	55 (75)
3 Months	84 (36)	88 (56)	88 (37)	100 (75)	72 (77)	45 (77)	57 (60)	62 (60)
6 Months	84 (91)	88 (40)	100 (99)	100 (63)	77 (89)	45 (78)	52 (99)	65 (45)
Treatment Effect (*P* =)	0.20		0.20		0.20		0.20	
Time Effect (*P* =)	0.20		0.20		0.20		0.20	

8.3.12. SF36 Questionnaire

Scores for physical functioning, role limitations due to physical health, role limitations due to emotional problems, energy/fatigue, emotional wellbeing, social functioning, pain and general health (Table 8.15A and 8.15B) were similar in INT, CON and pre-contemplation participants between baseline, three and six months and did not change between time points ($P > 0.05$)

Table 8.16A. Study feedback responses (%) for recruitment and data collection from 19 participants.

Recruitment	**Strongly Disagree**	**Disagree**	**Neither agree or disagree**	**Agree**	**Strongly Agree**
I was happy to receive information about my diabetes risk in the post	0%	0%	0%	53%	47%
The information I received in the post about pre-diabetes was useful and informative	0%	0%	5%	74%	21%
The information I received in the post about the study was clear, useful and understandable	0%	0%	0%	63%	37%
Contacting the research team and becoming a participant was simple and straight forward	0%	0%	0%	47%	53%
The research team explained the study clearly and I understood my role as a participant from the outset	0%	0%	0%	21%	79%
Data Collection					
The research team organised visit times for data collection that were convenient for me	0%	0%	0%	21%	79%
I was happy for the research team to record my physical data	0%	0%	0%	26%	74%
I was happy to provide a blood sample as part of the data collection process	0%	0%	0%	32%	68%
I was happy to complete the questionnaires at the end of each visit	0%	0%	0%	32%	68%

Table 8.16B. Study feedback responses (%) for urine collection from 19 participants.

Urine Collection	Strongly Disagree	Disagree	Neither agree or disagree	Agree	Strongly Agree
I was successful in collecting urine using the urine collection kit	0%	0%	0%	58%	42%
It was easy to collect urine in the pot	0%	5%	0%	58%	37%
I was confident collecting urine in the pot	0%	5%	0%	53%	42%
It was easy to transfer urine from the pot into the sample tube using the transfer straw	0%	5%	5%	48%	42%
I felt confident transferring urine from the pot into the sample tube using the transfer straw	0%	0%	5%	53%	42%
I was happy to write the collection date and time on the sample tube	0%	5%	5%	37%	53%
In general, I think collecting a urine sample is difficult	32%	53%	5%	5%	0%
I think collecting a urine sample in a home environment is embarrassing	53%	42%	5%	0%	0%
I was happy to store several urine samples collected over a week in a small box in my fridge	0%	5%	5%	53%	37%
Returning my urine samples to the research team was easy and arranged at a time convenient for me	0%	5%	0%	58%	37%

Table 8.16C. Study feedback responses (%) for the 15-minute consultation from 11 participants and physical activity recording from 16 participants.

15 Minute Consultation	Strongly Disagree	Disagree	Neither agree or disagree	Agree	Strongly Agree
I was pleased to be told I would be receiving a 15-minute consultation from my GP	0%	0%	9%	27%	64%
Booking a 15-minute consultation with my surgery was simple and straightforward	0%	0%	0%	64%	36%
The consultation provided me with useful information about my diabetes risk and ways I can improve my lifestyle	0%	0%	9%	36%	55%
Since attending the 15-minute consultation I have improved my health through increased exercise and better dietary habits	0%	9%	45%	9%	36%
Physical Activity Recording					
I was happy to be asked to record my levels of physical activity using the ActiGraph	0%	0%	6%	56%	38%
Recording my levels of physical activity using the ActiGraph was easy	0%	6%	6%	69%	19%
Wearing the ActiGraph did not interfere with my daily tasks and routines	0%	6%	6%	56%	31%
Recording the times I wore the ActiGraph was easy	0%	0%	19%	50%	31%
I was more physically active in the weeks I wore the ActiGraph compared to the weeks I did not	0%	31%	63%	6%	0%

8.3.13 Feedback Questionnaire

Feedback responses from 19 participants are detailed in Table 8.16A/B/C. Responses for recruitment and data collection procedures (Table 8.16A) produced no negative responses. Participants either agreed or strongly agreed to each question asked demonstrating acceptability from 100% of respondents. For questions relating to urine collection procedures (Table 8.16B), only 5% of participants provided negative responses while 5% neither agreed or disagreed with the questions asked. From the participants who attended a consultation (Table 8.16C), no negative responses were given to three out of four questions, although 9% of participants indicated they hadn't made positive changes to their levels of physical activity or dietary habits while a further 45% of participants neither agreed or disagreed in their response. Only 6% of participants provided negative responses in relation to ActiGraph use (Table 8.16C) while another 6% neither agreed nor disagreed to ActiGraph related questions. 31% of participants indicated they were not more physically active while wearing the ActiGraph while 63% neither agreed or disagreed, demonstrating the use of wearable technology to monitor exercise did not affect habitual behaviour.

8.4. Discussion

The primary aim of this study was to determine the feasibility of conducting a randomised control trial in primary care targeting lifestyle modification in patients at increased risk of T2D. Feasibility was based upon participant uptake, retention and acceptability. Secondary aims of the work were to determine the impact of the GP-led one-to-one lifestyle consultation on participant biochemistry and anthropometry, dietary intake and physical activity, compared to current standard care.

In Chapter two, a GP-led 30-minute one-to-one consultation targeting lifestyle modification in patients at risk of T2D resulted in statistically significant decreases in HbA_{1c} and other health outcomes associated with the condition. However, the design of the intervention did not include a control group, and as such, direct comparisons with usual care were not possible. Patients in the initial study received written information regarding their T2D risk and ways to improve lifestyle behaviours. Additionally, all patients had the opportunity to attend a 30-minute one-to-one consultation with a healthcare professional to discuss T2D risks and preventative healthy lifestyle behaviours. It is possible the initial literature given to patients provided the appropriate stimulus to initiate behaviour change and subsequently improve HbA_{1c} values, as well as other T2D related outcomes and suggests a one to one consultation may not provide additional benefit. Therefore, a randomised control trial was implemented to examine the effects of the GP-led one-to-one lifestyle consultation in comparison to standard practice in Wales, which is currently written advice only. Due to the complexity and logistical challenges associated with conducting an RCT within primary care, the feasibility of initiating such a design within a smaller cohort of patients was thought prudent prior to a larger study being conducted. Therefore, only one surgery was used for patient recruitment and hosting consultations.

Eighty-eight patients were invited to take part in the study in a letter sent by Church Surgery. The patients invited to take part were newly screened for HbA_{1c} and were not involved in the previous study detailed in Chapter two. Of the patients initially invited, 23 volunteered to take part and successfully completed a baseline visit, this represented 26% of the initially invited cohort. 19 participants, 83% of those who completed an initial visit completed all remaining visits and suggests once participants were enrolled in the research, retention was high. Uptake and retention figures for this study compare similarly with other published work examining T2D prevention. In a similar primary care-based study targeting pre-diabetes, work by Dawes *et al*. (2014) demonstrated a 33% study uptake and 95% retention rate over six months in a group of 180 eligible patients, of which 59 made an appointment for a baseline visit. In other work, a randomised clinical trial examining the effects of resistance exercise in adults with pre-diabetes demonstrated an overall retention rate of 76% across a 15-month period (Davy *et al*., 2017).

From the four participants lost to follow up in this study, one was randomized into the intervention group and three randomized to control. Despite polite attempts made to establish reasons for withdrawal for the purposes of acceptability, it was not possible to ascertain reasons why participants were lost to follow up. While it is possible participants withdrew due to their allocation into the control group, participants randomized to control provided positive feedback regarding their involvement in the research. In one case, a participant commented in their feedback their initial concern regarding their allocation to control, fearing an adverse effect to their health due to perceived loss of treatment benefit from non-allocation into the intervention group. However, they felt they had benefited from their participation in the study and suggested the three-monthly visits to the research facility had a beneficial impact on their overall health and healthy lifestyle behaviors. This may suggest, albeit speculatively, that regular health monitoring, either

within academic or clinical environments, may prove beneficial for some patients in improving healthy behaviors and health outcomes without the need for additional meetings with trained health professionals who provide consultations specific to T2D prevention. The data provides some support for this notion which demonstrate values for HbA_{1c} (Table 8.1), hip and waist circumference decreased significantly over time, regardless of treatment allocation, while decreases in body mass in both groups also approached statistical significance (Table 8.2). These significant decreases may also be considered clinically relevant given the extent of change within the six month data collection period, the acceptability of the intervention, and ease of the intervention implementation. In many other cases, there were no significant differences in T2D related variables across treatment or time suggesting the effect of the consultation was relatively minor when compared to standard written advice. Similarly, results from the SF-36 questionnaire, which provides an indication of perceived health, indicated values to be similar between treatments and over time for all health categories. Of all biochemical, anthropometric, dietary and physical activity variables measured, only total cholesterol and intakes of caffeic acid and taurine (urinary biomarkers of fruit and vegetables and striated muscle meat, respectively) varied significantly between treatments. Total cholesterol decreased in intervention participants by 0.1 mmol/L and increased in control participants by 0.3 mmol/L. Caffeic acid and taurine concentrations decreased in both treatments however the decreases were more pronounced in control subjects. In participants categorized into the 'pre-contemplation' category of behavior change, fewer changes were identified. Values for hip circumference demonstrated a significant decrease similarly to the cohort as a whole. Interestingly, percentage muscle also decreased in this group suggesting that for those whose behaviours and lifestyle remain unchanged following initial intervention, adverse health consequences may follow. This is in contrast to the cohort as a whole where muscle percentage remained similar throughout the study. Specific focus therefore needs to be given to those categorized into the pre-contemplation group as this study's data shows fewer

positive health changes are achieved in these individuals.

Feedback from participants at the end of the study demonstrated the RCT to be an acceptable method for determining the effectiveness of the consultation in combatting T2D. Questions relating to participant recruitment and data collection procedures produced no negative responses, suggesting these elements of the RCT were wholly acceptable to those taking part. Responses to questions relating to the urine collection procedures were positive overall with only 5% of participants reporting they found the procedures for collecting, storing and returning urine samples to be difficult and suggests this procedure for dietary monitoring can acceptably be tested in a larger sample. Similarly, use of the ActiGraph for physical activity monitoring was reported as being both easy to use without causing inconvenience to daily life by an overwhelming majority of respondents, with only 6% providing disagreeable responses. Again, this suggests this would be an acceptable method of physical activity tracking if a larger sample size was used in a future RCT. Significantly, when participants were questioned about the 15-minute consultation, all but two participants indicated they were pleased to be receiving the consultation while 100% either agreed or strongly agreed that booking their consultation was simple and straightforward. Of interest, while 91% of participants agreed the consultation provided them with useful information about their diabetes risk and ways they can improve their lifestyle, only 45% of participants suggested they had improved their lifestyle as a result of attending the consultation, while 55% either disagreed or neither agreed or disagreed. This provides some support for the notion that the consultation had no additional benefit in combating T2D and suggests in this group of participants, the initial written information may have been enough of a stimulus to instigate lifestyle changes. Responses provided in the Personal Diabetes Questionnaire which was completed after receipt of the written information but prior to the consultations supports this. Twelve participants indicated they had actively started to try and achieve weight loss while three were preparing to act. Similarly,

eight participants indicated they had taken steps to change their dietary behaviour while another four were preparing. This suggests the initial literature detailing T2D and its associated risks was largely effective at initiating a change in behaviour associated to the disease prior to contact with a health professional. In participants categorised as 'pre-contemplation', fewer health enhancing changes in the measured variables were found.

Small but statistically significant decreases in outcomes associated to T2D across both intervention and control groups suggests, in this cohort, that written advice outlining T2D risk and preventative healthy behaviors, is as effective as a GP-led one-to-one consultation delivered by a trained healthcare professional. As highlighted above, it may be possible that written advice accompanied by regular health monitoring is beneficial in limiting T2D risk and therefore, the need for an additional consultation is possibly unwarranted, however, the sample size was small and testing on a larger cohort is warranted for greater statistical power. Based on the data, providing patients who are identified as at increased risk of T2D with written information on T2D risk and strategies for improving health, coupled with three or six monthly health monitoring protocols may prove successful in managing HbA_{1c} and other T2D related outcomes as participants may feel motivated to maintain their health by the prospect of being followed up in a healthcare environment, either clinical or academic. This suggestion is also proposed in a previous study by Vermunt *et al*. (2012) which found intervention participants who received eleven 20-minute consultations focussing on behaviour change, five group meetings addressing diet and physical activity and an additional one-hour consultation with a dietician, demonstrated no significant changes in body weight, blood glucose concentration, physical activity and dietary intake in comparison to control participants who received only written T2D advice. The authors suggested a possible explanation may be due to control participants becoming motivated to change behaviour by the prospect of check-up visits with a health care professional. Currently

in Wales, patients identified as at increased risk of T2D are followed up in primary care annually to monitor changes in blood glucose status. As opposed to current practice, bi-annual or quarterly appointments at primary care level may provide the necessary contact time to initiate and maintain decreases in factors relating to T2D risk. While increased patient contact comes with increased financial requirements, the costs of preventing manifest T2D is outweighed by the financial burden of treating diagnosed T2D and its well-established complications, where current estimates suggest annual treatment costs in Wales are currently £500 million (Diabetes UK, State of the Nation, 2015). Previous work suggests lifestyle intervention is cost effective in comparison to pharmacological-only approaches, such as metformin, and can delay and/or prevent the onset of T2D (Herman *et al*., 2009). Future work therefore may examine the effects of written T2D advice with a form of additional health monitoring or follow up compared to written advice only with annual primary care HbA_{1c} measurement (current practice).

A limitation identified in Chapter two was the lack of effective physical activity and dietary monitoring following lifestyle intervention in patients at risk of T2D. In Chapter two participants, many decreases in HbA_{1c} were associated with decreases in body mass, BMI and waist circumference in individual surgeries as well as combined surgery data. Similarly, in the present study, reduction in HbA_{1c} correlated positively with decreases in both body mass and BMI. This has also been demonstrated in recent work by Lean *et al*. (2017) which found significant weight loss was strongly associated with rates of T2D remission. However, in Chapter two, HbA_{1c} decreases also occurred independently of changes in anthropometric measures. A possible explanation for this finding was attributed to increased levels of physical activity undertaken by the patient. As part of the 30-minute one-to-one consultation, patients were encouraged to increase their level of weekly physical activity in line with UK recommendations. In many cases, patients were also referred to classes which specialized in supervised exercise provision, such as NERS.

Previous work demonstrates significant improvements in insulin sensitivity following exercise, independent of significant changes in body mass (Duncan *et al*., 2003, Nassis *et al*., 2005) with the effects of increased exercise induced GLUT4 translocation through increased skeletal muscle contraction supporting this notion (Lund *et al*., 1995). Additionally, Duncan, Perri, Theriaque, Hutson, Eckel and Stacpoole (2003) found insulin sensitivity improved significantly following six months of walking training in previously sedentary individuals with the improvement in insulin sensitivity occurring in the absence of weight loss. While the GP cluster surgeries aimed to significantly reduce anthropometric values, the data attained from that work potentially demonstrates the importance of increasing levels of physical activity in patients with pre-diabetes, even in the absence of weight loss. The data also suggested patients may have engaged more in physical activity compared to improving dietary habits given HbA_{1c} values improved despite lack of body weight decreases in some surgeries. While indications of patient's physical activity and habitual dietary intake were sought prior to the initial consultation visit in Chapter two, this information was not followed up at 12 and 24 months by surgeries, nor were the initial questionnaire responses provided for analysis. Therefore, to gain a better understanding as to the reasons why HbA_{1c} values may improve in relation to physical activity and diet, it was suggested greater evidence regarding physical activity and dietary behaviours should be sought within a similar cohort at risk of T2D.

In the present work, physical activity was recorded by both self-report questionnaire and an electronic activity tracker while dietary monitoring was achieved by self-report questionnaire and by metabolomic analysis of urine and plasma. Previous work has demonstrated the validity of the ActiGraph activity tracker in monitoring physical activity (Powell, Dowd, Carson and Donnelly, 2017) and has been used in similar work examining physical activity in adults at risk of T2D following structured education (Biddle *et al*., 2015). In addition, work demonstrating the utility of

urine sampling as a method of dietary monitoring was reviewed in depth in Chapter three, while work in Chapters five and six demonstrate both a successful, feasible and acceptable protocol for urine collection in the community as well as demonstrating the stability of the urinary metabolome in the presence of nationally recommended moderate intensity exercise.

Although not significant, ActiGraph data demonstrates participants in the intervention group modestly increased kCal expenditure as well as time spent engaging in light and moderate intensity activity as well as increasing step counts between baseline and six months. Conversely, control group participants demonstrated reduced kCal expenditure as well as reduced time spent engaging in light intensity activity and reduced step counts between baseline and six months, although similarly to intervention, the differences did not reach statistical significance. Of interest, trends in ActiGraph data did not match responses given in the self-reported IPAQ-SF. Respondents in the intervention group reported significantly less time walking. Reported time spent engaging in moderate activity and total met minutes also decreased after six months, although neither reached significance. In control participants, reported walking decreased after six months which corresponds with ActiGraph data, while trends between ActiGraph data and self-reported moderate and vigorous were also similar. Such a disparity of findings represents an established limitation in self-reporting questionnaires where misreporting of physical activity has been demonstrated (Shephard, 2003; Finger *et al*., 2015). Participants were instructed to wear the ActiGraph during waking hours and wear time diaries indicate good compliance with this instruction. Given the reported validity of the ActiGraph as a reliable measure of physical activity compared to the limitations of self-reported physical activity, data acquired from the ActiGraph were used to assess the effects of physical activity on HbA_{1c} changes, a key measure of T2D risk. Given the association between exercise and HbA_{1c}, participants exhibiting higher levels of exercise may have had an associated decrease in HbA_{1c} concentration. Conversely, reduced levels

of exercise may have correlated with increased HbA_{1c} values. However, analysis revealed changes in physical activity including; kCal expenditure, time spent in light, moderate and vigorous exercise intensities and step counts between all visits did not correlate with changes in $HbA_{1c.}$ Given the data available, it is reasonable to suggest neither the consultation, nor the written literature, was effective in increasing rates of physical activity in either group. Accepting the documented influence of physical activity in managing blood glucose concentration, future work of similar design may need to place greater emphasis on the importance of engaging in regular exercise as per national guidelines for physical activity. Furthermore, work by Biddle *et al*. (2015) suggests a single three-hour structured education workshop targeting reduced sedentary time in young adults at risk of T2D may not be enough to achieve positive changes in sedentary time. Therefore, it is rational to claim either more extensive literature is provided to at risk patients regarding physical activity or more one-to-one consultation time is spent emphasizing its importance.

Alongside investigating the efficacy of a GP-led one-to-one lifestyle intervention in pre-diabetes, a focus of this thesis has been the use of urine sampling as a means of dietary monitoring to support more established recording methods, such as the FFQ. Evidence outlined in Chapter three demonstrates several urinary biomarkers have been established for foods associated to high health importance as well biomarkers linked to smoking and alcohol consumption. Evidence also demonstrates different dietary patterns can be distinguished in urine. In addition, work in Chapter five demonstrated a feasible and acceptable method of community urine sampling which identified the appropriate time and frequency of urine sampling necessary to represent habitual diet without causing excessive disruption to daily life. Furthermore, given participants in the present study were advised to undertake regular moderate intensity exercise as part of UK national guidelines for physical activity, work in Chapter six aimed to determine the effects this exercise

intensity on the urinary metabolome to establish metabolite presence in urines would be significantly disrupted. Following 30 minutes of moderate intensity exercise, it was found this level of exercise had a negligible impact on the urine metabolome and it was therefore concluded that urine samples provided by individuals undergoing lifestyle modification would be unaffected by exercise at this intensity. In the current study, participants in both the intervention and control groups collected three urine samples a week for three weeks following each visit to the research facility for analysis of diet.

To the investigator's knowledge, this is the first study to use urine sampling for dietary monitoring purposes in an NHS approved study involving patients at risk of T2D. Analysis of samples took both a targeted metabolomic approach to identify quantified metabolites of specific foods and food groups in addition to an untargeted approach which has the ability to identify and discriminate between varying diets. From 19 participants completing all visits, 15 successfully provided adequate samples for all time phases which could successfully be analyzed for dietary content, suggesting urine collection in participants at risk of T2D is feasible. 73 metabolites of known chemical composition reflective of intake of commonly consumed foods/beverages and food types were successfully quantified for each phase demonstrating it possible to monitor consumption of such items in a community setting. In all but five cases, there were no significant changes in the urinary concentration of these metabolites suggesting dietary behaviours remained mostly similar across time points and between treatments. The concentration of five metabolites; 4-hydroxyhippuric acid, caffeic acid, ferulic acid-4-O-sulfate, hippuric acid and taurine decreased significantly over time. 4-hydroxyhippuric acid, caffeic acid, ferulic acid-4-O-sulfate, hippuric acid have been identified as markers of fruit and vegetable and polyphenol intake and as such, an increase in urinary concentration of these metabolites as a result of increased intake may have been expected following dietary advice. Similar decreases in fruit, vegetable (dehydroxybenzoic

acid) and polyphenol (hippuric acid) intake were observed in pre-contemplation participants as well as a promising decrease in trans-3-hydroxycotinine, a biomarker of nicotine and regularly detected in individuals who smoke cigarettes. A possible explanation for reduced presence of these food related metabolites in three- and six-months urines may reflect reduced dietary intake in an attempt at improved calorie control, although this is speculative. Recent work by Lloyd *et al*. (2019) has shown absolute concentrations of selected urinary dietary biomarkers in FMV samples the day after consumption increases with increased portion size of the associated foods and beverages. A lack of change observed in most quantified dietary metabolites over time and between treatments is further reflected in FIE-HRMS data which show overall poor class discrimination in participant urines and plasma between phases, inferring individual dietary patterns remained largely unchanged between baseline, three and six months and between treatments. This is supported by FFQ data which show consumption of most of the food and beverage categories (9 of 11 categories) remained similar between baseline and six months. One possible explanation may be attributed to the timing of the baseline urine collection period. Responses to the Personal Diabetes Questionnaire demonstrate eight participants had begun to act to change their dietary behaviours while four were preparing to make changes to their dietary behaviours. This shows a large proportion of participants had made efforts to change their diet before they visited the research facility for a baseline visit and the provision of baseline urine samples which acted as a comparator for dietary behaviour at three and six months. Therefore, given diets had likely changed before baseline sampling, it is perhaps unsurprising that no clear discrimination was achieved in urine samples across phases as dietary change had already likely occurred prior to urine sampling procedures being introduced.

Future work needs to give this issue consideration in order to detect observable changes in the urine metabolome following dietary advice. Determining how to collect samples at the earliest

possible opportunity following a pre-diabetes diagnosis is therefore a potential focus for further research. Examination of FFQ data suggests dietary behaviour, overall, remained relatively unchanged over six months. However, significant changes were found for intakes of wholegrain and red and processed meat consumption. For wholegrain, while no treatment difference was evident, a significant change over time was found, with the mean AHEI score decreasing from 2.8 to 2.0 arbitrary units. In contrast, the mean AHEI score for red and processed meat consumption increased from 4.1 arbitrary units at baseline to 5.3 arbitrary units at six months, although again no treatment differences were present. Higher AHEI scores are strongly associated with lower risk of major chronic disease suggesting dietary behaviour relating to red and processed meat consumption improved while consumption of wholegrains was less optimal after six months. A benefit of urine sampling is the ability to validate self-report dietary questionnaires using metabolomics. As evidenced in Table 8.4, the urinary concentration of biomarkers for wholegrain intake; 2,3-dihydroxyphenylpropionic acid, benzoxazolinone and 2,3-Dihydroxyphenylpropionic acid-3-sulfate remained unchanged over time. This is also similar for the biomarker of red meat consumption, L-carnitine and suggests the FFQ is either more sensitive to changes in dietary behaviours or participants were wrongly estimating their intake of wholegrain and red meat, a limitation heavily evidenced previously in works in chapter three. Based on the general trend from urine metabolome analysis and self-report FFQ, dietary behaviour remained relatively unchanged over time and suggests using both forms of dietary monitoring in conjunction to assess dietary adherence following intervention is, at least, moderately successful, despite a small number of different dietary changes detected by each method. Future work needs to continue use of such methods to provide further evidence for the utility of urine sampling in free-living community settings for the purposes of dietary monitoring. In relation to the effectiveness of the intervention in promoting dietary change, it is interesting to note that 12 participants indicated they had or

were planning to change their dietary behaviour simply because they had been informed of their heightened risk of T2D and had been provided with some brief information on healthy dietary behaviours. These responses were provided prior to any participant attending a consultation and suggests written advice regarding T2D risk and the importance of a healthy diet is an adequate stimulus to instigate a change in dietary behaviour in some patients.

While care was taken to ensure the RCT was conducted within a robust study design, some limitations arose which warrant discussion, one of which concerned statistical analysis of secondary outcomes. In cases where data was non normally distributed, it was not possible (to the investigator's knowledge) to identify a non-parametric test, equivalent to a three by two mixed model ANOVA, which could provide information on treatment and time interactions of two independent unequal groups measured at multiple time points. While an examination of resources suggested a Kruskal-Wallis test is the equivalent non parametric analysis to an ANOVA, it does not provide the group and time interactions the research team required and therefore this option was not utilised where interaction analyses was sought. According to Blanca, Alarcon, Arnau, Bono and Bendayan (2017) there are also disadvantages to using non-parametric procedures, such as the Kruskal-Wallis test, which converts quantitative continuous data into rank-ordered data, which result in a loss of information. Attempts to log transform the data to a normal distribution were also unsuccessful. Following discussions with academics with expertise in statistical analysis, it was decided a three by two mixed model ANOVA was a robust alternative which would provide the necessary information on treatment and time interactions. This decision is supported in work by Blanca, Alarcon, Arnau, Bono and Bendayan (2017) who suggest ANOVA is robust to violations of normality and can be utilised under a wide variety of conditions when variables including; equal and unequal sample sizes; group sample size and total sample size; shape of the distribution and equal or unequal

distributions were examined. While such work has shown ANOVA to be robust to violations of normality, the use of such a test on non-normal data divides opinion among academics and therefore the results from the present work should be treated with caution.

In conclusion, this NHS approved randomised control trial has successfully shown it feasible to implement an RCT targeting pre-diabetes in primary care. Rates of recruitment and retention as well as feedback relating to acceptability have shown such a design has the potential to be applied ethically and practically to a larger sample at risk of T2D. Given the present work was designed to assess feasibility and was statistically underpowered, development of a similar design and applied to a larger cohort is a prudent next step to determine the true impact of the one-to-one consultation on factors associated to T2D, compared to current practice. Furthermore, this work successfully employed the use of a home-based urine collection kit for the purposes of dietary monitoring which was informed by previous work detailed within this thesis. Use of such urine sampling protocols in a future RCT has the potential to monitor dietary behaviours on a larger scale and demonstrate the utility of such a method in monitoring community wide, free-living dietary behaviours.

Chapter Nine:

9.1. General Discussion and Future Directions

The overall aim of this thesis was to evaluate the efficacy of a one to one lifestyle consultation delivered through primary care in reducing progression to T2D in a north Ceredigion pre-diabetic population. Specific objectives included; 1. The completion of a service evaluation of the North Ceredigion GP cluster pre-diabetes intervention; 2. The development and validation of methods to quantify dietary changes over time; and 3. Establishing the feasibility of conducting a randomised control trial to determine the efficacy of a low-cost diabetes prevention programme delivered through primary care. The work presented here addresses important gaps in the literature. To the investigator's knowledge, this is the first time a T2D prevention programme of this design has been examined both alone and in conjunction with a novel method for dietary monitoring and physical activity tracking. In order to achieve the aims and objectives of this thesis, several studies were conducted. The discussion below will chart the progressive pathway between studies, discuss the limitations of the research and suggest the potential future direction of T2D prevention and lifestyle monitoring within Wales.

9.2. Realisation of Aims

According to a Public Health England report published in 2016, approximately four million people in the UK were suffering with diabetes, 90% with T2D, while a statistic report by the NHS in 2014 demonstrated one in three UK adults were considered to have pre-diabetes, a condition which classifies an individual as being at increased risk of developing T2D. In Wales, the incidence of T2D is increasing unsustainably. In 2015 it was estimated that 182,600 people had T2D and by 2025 that number will have increased to 300,000, the highest percentages by

population in the UK (Diabetes UK, State of the Nation, 2015). The financial implications for Wales' NHS are extremely worrying, with approximately 10% of the annual budget spent on treating a preventable condition and its complications, equating to approximately £500 million (Diabetes UK, State of the Nation, 2015). Of great concern are the estimated 540,000 individuals in Wales at risk of developing T2D, as approximately 10% of unaddressed cases will progress to manifest T2D (Tabak *et al*., 2012). In the absence of effective treatments and interventions these estimations are projected to increase (Diabetes UK, State of the Nation, 2015). Given the increasing rate of T2D cases in Wales and projected future cases, an urgent need for an effective intervention programme which can prevent the onset of T2D was identified as a key objective.

In 2015, a north Ceredigion GP cluster comprised of seven surgeries initiated a pre-diabetes intervention which targeted patient education and lifestyle modification. Lifestyle intervention has been shown to be at least as effective as pharmacological approaches (such as metformin) in preventing progression to T2D (Gillies *et al*., 2007) and more cost effective (Herman *et al*., 2009). The intervention comprised of a 30-minute one-to-one consultation for patients identified as having an HbA_{1c} concentration of between 42 and 47 mmol/mol, the concentration range considered to increase the risk of developing T2D. The 30-minute consultation included the collection of baseline data (including; HbA_{1c}, weight, BMI, waist circumference and blood pressure) and information on diet, exercise and the importance of avoiding manifest T2D. In partnership with the cluster, work outlined within chapter two of this thesis undertook an evaluation of the intervention. Analysis of data at 12 and 24 months following the 30-minute consultation demonstrated a significant overall decrease in HbA_{1c}. In many cases, participation in the intervention led to HbA_{1c} decreasing to values below the range considered to increase the risk of T2D development. The data for HbA_{1c} recorded post intervention reached values

regarded as healthy by NHS Wales, Public Health Wales and Diabetes UK in 37% of cases after 12 months (217 of 592 participants) and 41% of cases after 24 months (119 of 292 participants). Conversely, only 3% (17 of 592 participants) and 6.5% (19 of 292 participants) had HbA_{1c} values which fell in the diabetes range after 12 and 24 months, respectively. Statistically significant overall decreases were also observed for body mass, BMI and waist circumference after 12 months and BMI and waist circumference after 24 months, although changes in BMI were small and unlikely to be clinically relevant. These findings are similar to those observed in an initial analysis of NHS England's DPP which observed a significant decrease in weight after 12 months (NHS DPP, 2018) and a 1.2 mmol/mol decrease in HbA_{1c} (Valabhji *et al*., 2019). In many cases, decreases in HbA_{1c} were associated with decreases in body mass, BMI and waist circumference in individual surgeries and pooled data. In other cases, however, decreases in HbA_{1c} occurred independently of changes in anthropometric measures. A possible explanation for this finding was attributed to increased levels of physical activity undertaken by the patient, independent of weight loss. The benefits of physical activity in improving health outcomes were discussed as part of the 30-minute one-to-one consultation. Patients were encouraged to increase their level of weekly physical activity to 150 minutes of moderate intensity exercise, 75 minutes of vigorous intensity exercise or a combination of both intensities plus additional resistance exercise. In many cases, patients were also referred to classes which specialised in supervised exercise provision, such as NERS. Patients may have engaged more in physical activity compared to improving dietary habits, given HbA_{1c} values improved despite lack of body weight decreases in some surgeries. While patients were asked to provide an indication as to their physical activity and dietary habits during the initial consultation visit, this information was not followed up at 12 and 24 months by surgeries. Furthermore, limitations associated with self-report questionnaires, as discussed in chapter three, suggest information provided by participants at baseline GP visits regarding diet and

physical activity may have been unreliable. To gain a better understanding as to the specific reasons why HbA_{1c} values improved following lifestyle intervention, it was suggested future work within a similar cohort should generate better evidence regarding physical activity and dietary behaviours. Therefore, work was undertaken to determine alternative ways of monitoring healthy behaviours associated to T2D risk which don't rely on self-report. It was subsequently suggested that developing a greater understanding of physical activity and dietary practices following intervention would identify what elements of the intervention were successful and what could be improved upon in future T2D prevention initiatives.

In chapter three, a review of the literature examining traditional dietary monitoring methods highlighted several limitations. As an alternative approach, a significant body of research demonstrated urine metabolome profiling to be another method for measuring dietary exposure as well as providing the opportunity to validate existing dietary recording methods (Favé *et al*., 2011). The establishment of biomarkers of foods of high health importance including fruit, vegetables, meat, fish, wholegrain, tea, coffee, sugar, as well as the ability to discriminate between metabolic profiles derived from different diets means it is possible to both improve and validate traditional dietary monitoring methods with urinary metabolomics. Many studies examining urinary metabolomics for habitual dietary monitoring have involved participants collecting 24-hour urine samples or participating in intensive laboratory-based control studies, which while robust and accurate, place a significant burden on participants due to its adverse impact on normal daily life (John *et al*., 2016). This limitation means it unlikely the use of urine sampling could be applied to a 'real world' setting using current practices. Therefore, work in chapter five aimed to determine whether habitual dietary behaviour could be monitored within a free-living environment without intrusive or disruptive protocols being administered. This was attempted through the collection of a series of spot samples collected over a six-week

period with the ultimate aim to establish a less burdensome collection protocol for participants in future research. Data regarding sample collection compliance, sample collection time and frequency and protocol acceptability were collected to inform the aims of the study. Collection compliance for FMV samples was higher (93% - 100%) compared to BT samples (86% - 100%) while weekday collection compliance (87% - 100%) was greater than weekend compliance (75% - 100%). It was argued that while a weekend sample resulted in lower compliance, a sample should be included in any future protocol as dietary behaviour will likely change on a weekend compared to a week day (An, 2016; Jahns *et al.*, 2016). Therefore, it was proposed a weekend urine sample should be collected to capture that dietary information in future work utilising urinary metabolomics. As compliance for FMV samples was highest, work was undertaken to assess its utility in capturing dietary information compared to BT samples. FMV samples were as effective independently compared to FMV and BT samples combined in capturing dietary behaviour. Given a key aim of the study was to develop a non-disruptive protocol for dietary monitoring, analysis was undertaken to determine how many samples each week were required to provide an accurate representation of dietary behaviour, compared to weekly and global pools, which contained all FMV samples collected throughout the week and entire sampling phase respectively. It was demonstrated that three samples collected each week, two on weekdays and one on a weekend, was effective in capturing similar dietary information compared to the global pool, which comprised of an FMV urine sample collected every day for the six-week sampling period. Data regarding the acceptability of the protocol, which was gathered from feedback questionnaires, suggested an overwhelming majority of participants found the urine collection kit and the collection procedures to be easily utilised and was a feasible method for use in future work. This sample collection protocol structure was therefore used in the RCT detailed in chapter eight.

Following the development of the urine collection protocol for dietary monitoring, further work was conducted to examine the effects of acute moderate exercise on the urine metabolome. The aim of the study detailed in chapter six was to examine the extent of urinary metabolome change following 30-minutes of moderate intensity exercise on a cycle ergometer. Previous work demonstrated the effects of both high intensity exercise and resistance exercise on urinary metabolites. However, the effects of moderate and submaximal exercise were less established. This exercise modality was of interest in this thesis as UK national activity guidelines state all adults should undertake 30 minutes of moderate intensity exercise, five days per week. These recommendations are especially encouraged in patients undergoing lifestyle modification for the prevention of non-communicable diseases, such as those who participated in the initial pre-diabetes intervention, as well as the participants in the later RCT. Prior to testing the urine collection protocol in a population at risk of T2D, the work in chapter six aimed to determine metabolome change in urine following both 30 minutes of standardised moderate exercise on a cycle ergometer, an intensity based on national exercise guidelines, compared to rest. Determining such change provided useful data regarding the extent of urine metabolome disturbance in individuals who engage in moderate physical activity as per advice from lifestyle intervention. The study demonstrated 30 minutes of standardised moderate intensity exercise to have no significant impact on the urinary metabolome. With reference to the RCT, it was concluded that nationally recommended moderate exercise will not result in significant metabolome disturbance in urines which are collected as part of a dietary monitoring protocol.

Prior to conducting the RCT, a short study detailed in chapter seven compared the Alere Afinion AS100 Point of Care Analyser (used in the RCT) and HPLC in the determination of $HbA_{1c.}$ While HPLC is considered the gold standard for clinical monitoring and diagnosis of diabetes status, the Alere Afinion AS100 POC analyser was to be used in the proceeding RCT

for monitoring HbA_{1c}. It was therefore of interest to determine how closely the results from both analytical methods agreed. The data demonstrated the Alere Afinion AS100 POC analyser to underestimate HbA_{1c} concentration by a median of two mmol/mol and in some cases misclassify an individual's glycaemic state. Based on the results, it was concluded the POC analyser was not suitable for clinically diagnosing T2D. However, the significant positive correlations between the POC analyser and HPLC analyses suggested the former can be used, with caution, to assess intervention effectiveness by monitoring longitudinal HbA_{1c} changes without the waiting time, additional costs and expertise associated with HPLC analysis. A comparable study by Grant, Dunseath, Churm and Luzio (2017) also found a median two mmol/mol difference in HbA_{1c} values between HPLC and POC analysis. Similarly, the authors concluded that the POC analyser could be used for HbA_{1c} monitoring, although HPLC analysis should be undertaken for diagnostic purposes.

In relation to the objectives of this thesis, the work detailed in chapter two successfully evaluated the north Ceredigion GP cluster pre-diabetes intervention. Limitations from that work included the lack of accurate and reliable dietary monitoring. Therefore, work detailed in chapters five and six developed methods to quantify dietary change and confirm moderate intensity exercise to have no significant impact on the urine metabolome. Additional work outlined in chapter seven compared two different analytical methods for the determination of HbA_{1c}. The findings from chapters two, five, six and seven informed the development of an RCT, detailed in chapter eight. This study examined the feasibility of conducting a randomised control trial to evaluate the effectiveness of a one-to-one consultation to improve blood glucose control in pre-diabetes. While results from chapter two demonstrated promise in reducing T2D prevalence in at risk groups, the design of the project as a service evaluation was regarded as a limitation, as it was difficult to identify the primary reasons for reduction in HbA_{1c} and

associated risk factors. Potential drivers of behaviour change included; the initial letter confirming T2D risk and accompanying information and/or the information received during the one to one consultation. Therefore, a randomised control trial was proposed to assess the effectiveness of the consultation in comparison to current practice in pre-diabetes care in Wales (written information only).

In both intervention and control participants, the previously tested urine sampling protocol was used for the purposes of dietary monitoring while ActiGraph physical activity trackers were utilised to monitor physical activity levels more closely than in the initial GP cluster intervention. Due to the complexity and logistical challenges of conducting an RCT within primary care, it was thought necessary to first assess the feasibility of initiating such a protocol within a smaller group of patients prior to a future larger study being conducted. Based upon rates of recruitment and retention as well as feedback relating to acceptability, the study demonstrated such a design has the potential to be applied both ethically and practically to a larger sample of patients at risk of T2D. Furthermore, the work successfully employed the use of the urine collection kit for the purposes of dietary monitoring which demonstrated its utility in successfully monitoring a range of dietary biomarkers as well as demonstrating changes in dietary patterns in free-living individuals. Measures of physical activity were also monitored more closely using the ActiGraph activity tracker although few significant changes were found. Neither the diet nor exercise monitoring protocols were administered in the initial GP cluster intervention. The use of such in the RCT represented an alternative and potentially more accurate measure of lifestyle behaviour compared to self-report which have previously been shown to be prone to bias. The results demonstrated values for HbA_{1c}, hip and waist circumference decreased significantly over time irrespective of treatment group. Most other biochemical, anthropometric, dietary and physical activity variables measured remained similar

across treatment and time. While this suggests the intervention had little impact on T2D risk, the study was limited by its sample size and therefore a suitably powered study with a larger sample is the logical next phase.

9.3. Future Directions

The sample size for the RCT was decided based upon potential numbers of eligible patients provided to the research team by the participating general practice. This was based upon the number of registered patients with a BMI > 25 kg/m^2, an existing HbA_{1c} < 12 months between 42 and 47 mmol/mol or patients otherwise deemed at clinical risk of T2D by their GP. As this was a feasibility study designed to inform a future large-scale randomised control trial, no formal sample size calculation was employed. Given this limitation, the development of a similar design applied to an appropriately powered sample is a prudent next step to determine the true impact of the one-to-one consultation on factors associated to T2D, compared to current practice. The initial GP cluster intervention has demonstrated the ability of GP surgeries across north Ceredigion to work in collaboration with a university research group to design, deliver and analyse a T2D prevention programme. With this pathway in place, a future multi-centre randomised control trial utilising a similar method to examine the effectiveness of the one to one consultation is a realistic and feasible next step. The results also demonstrated the minimal impact of the consultation on dietary and physical activity behaviours. Future work may aim to place greater emphasis on the importance improving diet and increasing physical activity in improving blood glucose control. Work detailed in chapter eight has shown an RCT can successfully be conducted alongside primary care in an ethically acceptable and scientifically robust manner. Utilising the services of the initial seven general practices of Borth, Church, Llanilar, Padarn, Tanyfron, Tregaron and Ystwyth and accessing their pre-diabetic populations will facilitate this aim. Additionally to this, a formal financial assessment

should be undertaken to determine the cost effectiveness of the intervention in reducing prevalence of T2D in Wales, as up to now only approximate costs are known.

Given the health complications associated with T2D as well as financial implications involved in treating the condition, it is of extreme importance that an effective pathway is implemented within NHS Wales' healthcare structure which has been thoroughly evaluated and proven to be effective in reducing population T2D risk. Despite significant support being provided for T2D prevention in England, a similar model for Wales has yet to be implemented. This is despite the prevalence of the disease being highest in Wales compared to the remaining areas of the UK (Diabetes UK, State of the Nation, 2015) and there being a considerable portion of the Welsh population considered at increased risk of T2D. In August 2019 it was announced that in addition to the NHS England's Diabetes Prevention Programme, up to 8000 people in England at increased risk of T2D will be offered digital wearable technology to help track physical activity to manage T2D risk. Given the appropriate resources are made available, similar pilot work in Wales should be conducted. One possible line of research may be to offer such technologies to patients who attend a one to one consultation to determine if additional digital support following the consultation has further health benefits compared to a consultation and associated information alone.

9.4. Conclusion

The work presented in this thesis has successfully evaluated a GP led pre-diabetes intervention and informed the development of a future multi-centre randomised control trial which will demonstrate the effectiveness of the intervention in preventing the onset of T2D in patients considered at increased risk of the condition. Furthermore, an effective and acceptable protocol for monitoring habitual diet using urinary metabolomics has been developed and implemented

in a group of participants who have been subjected to dietary advice from a health professional. It is crucial future work continues to develop evidence regarding the most effective approach for T2D prevention in Wales, where prevalence of the disease is increasing unsustainably. This work has shown promise in contributing to that future approach.

References

Abe, H. (1983). Distribution of free L-Histidine and related dipeptides in the muscle of fresh water fishes. *Comparative Biochemistry*, 76, 35-39.

Abe, H., Okuma, E., Sekine, H., Maeda, A. and Yoshiue, S. (1993). Human urinary excretion of L-histidine related compounds after ingestion of several meats and fish muscle. *International Journal of Biochemistry*, 25, 1245-1249.

Absetz, P., Valve, R., Oldenburg, B., Heinonin, H., Nissinen, A., Fogelholm, M., Ilvesmaki, V., Talja, M. and Uutela, A. (2007). One-year results of the GOAL Implementation Trial. *Diabetes Care*, 30, 2465-2470.

Achten, J., Gleeson, M. and Jeukendrup, A.E. (2001). Determination of the exercise intensity that elicits maximal fat oxidation. *Medicine and Science in Sport and Exercise*, 34, 92-97.

Ackerman, R.T., Finch, E.A., Brizendine, E., Zhou, H. and Marrero, D.G. (2008). Translating the Diabetes Prevention Program into the Community; The DEPLOY Pilot Study. *American Journal of Preventive Medicine*, 35, 357-363.

Ahn-Jarvis, J., Clinton, S.K., Riedl, K.M., Vodovotz, Y. and Schwartz, S.J. (2012). Impact of Food Matrix on Isoflavone Metabolism and Cardiovascular Biomarkers in Adults with Hypercholesterolemia. *Food and Function*, 3, 1051-1058.

Alberti, K.G. and Zimmet, P.Z. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabetic Medicine: *A Journal of the British Diabetic Association*, 15, 539-553.

Ali, A.M., Burleigh, M., Daskalaki, E., Zhang, T., Easton, C. and Watson, D.G. (2016). Metabolomic Profiling of Submaximal Exercise at a Standardised Relative Intensity in Healthy Adults. *Metabolites*, 6.

Almeida, F.A., Shetterly, S., Smith-Ray, R.L., Estabrooks, P.A. (2010). Reach and Effectiveness of a Weight Loss Intervention in Patients with Prediabetes in Colorado. *Centers for Disease Control and Prevention*, 7.

An, R. (2016). Weekend-weekday differences in diet among U.S. adults, 2003-2012. *Annals of Epidimiology*, 26, 57-65.

Ayabe, M., Kumahara, H., Morimura, K. and Tanaka, H. (2013). Epoch length and the physical activity bout analysis: An accelerometry research issue. *BMC Research Notes*, 6.

Beaver, W.L., Wasserman, K. and Whipp, B.J. (1985). A new method for detecting anaerobic threshold by gas exchange. *Journal of Applied Physiology*, 60, 2020-2027.

Beckmann, M., Joosen, A.M., Clarke, M.M., Mugridge, O., Frost, G., Engel, B., Taillart, K, Lloyd, A.J., Draper, J. and Lodge, J.K. (2016). Changes in the human plasma and urinary metabolome associated with acute dietary exposure to sucrose and the identification of potential biomarkers of sucrose intake. *Molecular Nutrition & Food Research*, 60, 440-457.

Beckmann, M., Lloyd, A.J., Haldar, S., Fave, G., Seal, C.J., Brandt, K., Mathers, J.C. and Draper, J. (2013). Dietary exposure biomarker-lead discovery based on metabolomics analysis of urine samples. *Proceedings of the Nutrition Society*, 72, 352-361.

Bell, K., Shaw, J.E., Maple-Brown, L., Ferris, W., Gray, S., Murfet, G., Flavel, R., Maynard, B., Ryrie, H., Pritchard, B., Freeman, R. and Gordon, B.A. (2020). A position statement on screening and management of prediabetes in adults in primary care in Australia. *Diabetes Research and Clinical Practice*, 164.

Bhopal, R.S., Douglas, A., Wallia, S., Forbes, J.F., Lean, M.E.J., Gill, J.M.R., McKnight, J.A., Sattar, N., Sheikh, A., Wild, S.H., Tuomilehto, J., Sharma, A., Bhopal, R., Smith, J.B.E., Butcher, I and Murray, G.D. (2014). Effect of a lifestyle intervention on weight change in south Asian individuals in the UK at high risk of type 2 diabetes: a family-cluster randomised controlled trial. *Diabetes and Endocrinology*, 3, 218-227.

Biddle, S.J.H., Edwardson, C.L., Wilmot, E.G., Yates, T., Gorely, T., Bodicoat, D.H., Ashra, N., Khunti, K., Nimmo, M.A. and Davies, M.J. (2015). A Randomised Controlled Trial to Reduce Sedentary Time in Young Adults at Risk of Type 2 Diabetes Mellitus: Project STAND (Sedentary Time ANd Diabetes). *PLOS One*, 10.

Bingham, S.A., Welch, A.A., McTaggart, A., Mulligan, A.A., Runswick, S.A., Luben, R., Oakes, S., Khaw, K.T., Wareham, N. and Day, N.E. (2001). Nutritional methods in the European Prospective Investigation of Cancer in Norfolk. *Public Health Nutrition,* 4, 847-858.

Bingham, S.A. (2002). Biomarkers in nutritional epidemiology. *Public Health Nutrition*, 5, 821-827.

Black, A.E., Bingham, S.A., Johansson, G. and Coward, W.A. (1997). Validation of dietary intakes of protein and energy against 24-hour urinary N and DLW energy expenditure in middle-aged women, retired men and post-obese subjects: comparisons with validation against presumed energy requirements. *European Journal of Clinical Nutrition*, 51, 405-413.

Blanca, M.J., Alarcon, R., Arnau, J., Bono, R. and Bendayan, R. (2017). Non-normal data: Is ANOVA still a valid option? *Psicothema*, 29, 552-557.

Bland, J.M. and Altman, D.G. (1986). Statistical methods for assessing agreement between two methods of clinical measurements. *The Lancet*, i, 307-310.

Borelli, B. (2011). The Assessment, Monitoring, and Enhancement of Treatment Fidelity in Public Health Clinical Trials. *Journal of Public Health Dentistry,* 71, 52-63.

Borg, G.A. (1982). Psychophysical bases of perceived exertion. *Medicine and Science in Sport and Exercise*, 14, 377–381.

Bouatra, S., Aziat, F., Mandal,R., Guo, A.C., Wilson, M.R., Knox, C., Bjorndahl, T.C., Krishnamurthy, R., Saleem, F., Liu, P., Dame, Z.T., Poelzer, J., Huynh, J., Yallou, F.S., Psychogios, N., Dong, E., Bogumil, R., Roehring, C. and Wishart, D.S. (2013). The Human Urine Metabolome. PLOS ONE, 4.

Buhl, K.M., Gallagher, D., Hoy, K., Matthews, D.E. and Heymsfielf, S.B. (1995). Unexplained disturbance in body weight regulation: diagnostic outcome assessed by doubly labelled water and body composition analyses in obese patients reporting low energy intakes. *Journal of the American Diabetic Association*, 95, 1393-1400.

Butler, A.E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R.A. and Butler, P.C. (2003). Beta-Cell Deficit and Increased Beta-Cell Apoptosis in Humans with Type 2 Diabetes. *Diabetes*, 52, 102-110.

Campbell, L., Pepper, T. and Shipman, K. (2019). HbA_{1c}: a review of non-glycaemic variables. *Journal of Clinical Pathology*, 72, 12-19.

Camps, S. G.J.A., Verhoef, S.P.M. and Westerterp, K.R. (2016). Physical Activity and Weight Loss are Independent Predictors of Improved Insulin Sensitivity Following Energy Restriction. *Obesity*, 24, 291-296.

Capita, R. and Alonso-Calleja, C. (2009). Differences in reported winter and summer dietary intakes in young adults in Spain. *International Journal of Food Sciences and Nutrition*, 56, 431-443.

Castell, G. S., Serra-Majem, L. and Ribas-Barba, L. (2015). What and how much do we eat? 24-hour dietary recall method. *Nutricion Hospitilaria*, 31, 46-48.

Cerf, M.E. (2013). Beta cell dysfunction and insulin resistance. *Frontiers in Endocrinology*, 4, 1-12.

Cernea, S. and Dobreanu, M. (2013). Diabetes and beta cell function: from mechanisms to evaluation and clinical implications. *Biochemia Medica*, 23, 266-280.

Champagne, C.M., Bray, G.A., Kurtz, A.A., Monteiro, J.B.R., Tucker, E., Valaufova, J. and Delaney J.P. (2002). Energy intake and energy expenditure: A controlled study comparing dieticians and non-dieticians. *Journal of the American Diabetic Association*, 102, 1428-1432.

Cheung, W., Keski-Rahkonen, P., Assi, N., Ferrari, P., Freisling, H., Rinaldi, S., Slimani, N., Zamora-Ros, R., Rundle, M., Frost, G., Gibbons, H., Carr, E., Brennan, L., Cross, A.J., Pala, V., Panico, S., Sacerdote, C., Palli, D., Tumino, R., Ku¨hn, T., Kaaks, R., Boeing, H., Floegel, A.,Mancini, F., Boutron-Ruault., M., Baglietto, L., Trichopoulou, A., Naska, A., Orfanos, P. and Scalbert., A. (2017). A metabolomic study of biomarkers of meat and fish intake. *American Journal of Clinical Nutrition*, 105, 600-608.

Chiuve, S.E., Fung, T.T., Rimm, E.B., Hu, F.B., McCullough, M.L., Wang, M., Stampfer, M.J. and Willet, W.C. (2012). Alternative dietary indices both strongly predict risk of chronic disease. *The Journal of Nutrition*, 142, 1009-1018.

Chorrel, E., Svensson, M.B., Moritz, T. and Antti, H. (2012). Physical fitness level is reflected by alterations in the human plasma metabolome. *Molecular Biosystems*, 8, 1187-1196.

Colberg, S.R., Sigal, R.J., Fernhall, B., Regensteiner, J.G., Blismer, B.J., Rubin, R.R., Chasan-Taber, L., Albright, A.L. and Braun, B. (2010). Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement. *Diabetes Care*, 33, 147-167.

Conway, J.M., Ingwerson, L.A. and Moshfegh, A.J. (2004). Accuracy of Dietary Recall Using the USDA Five-Step Multiple-Pass Method in Men: An Observational Validation Study. *Journal of the American Dietetic Association*, 104, 595-603.

Costa, B., Barrio, F., Cabre, J.J., Pinol, J.L., Cos, X., Sole, C., Bolibar, B., Basora, J., Castell, C., Sola-Morales, O., Salas-Salvado, J., Lindstrom, J. and Tuomilehto, J. (2012). Delaying progression to type 2 diabetes among high-risk Spanish individuals is feasible in real-life primary healthcare settings using intensive lifestyle intervention. *Diabetologia*, 55, 1319-1328.

Craig, C.L., Marshall, A.L., Sjostrom, M., Bauman, A.E., Booth, M.L., Ainsworth, B.E., Pratt, M., Ekelund, U., Yngve, A., Sallis, J.F. and Oja, P. (2003). International Physical Activity Questionnaire: 12-Country Reliability and Validity. *Medicine and Science in Sport and Exercise*, 35, 1381–1395.

Crews, H.M., Oliver, L. and Wilson, L.A. (2001). Urinary biomarkers for assessing dietary exposure to caffeine. *Food, Additives and Contaminants*, 18, 1075-87.

Cross, A.J., Major, J.M. and Sinha, R. (2011). Urinary Biomarkers of Meat Consumption. *American Association for Cancer Research*, 20, 1107-1111.

Das, S.K. and Elbein, S.C. (2006). The Genetic Basis of Type 2 Diabetes. *Cellscience*, 2, 100-131.

Davies, K., Kingston, A., Robinson, L., Hughes, J., Hunt, J.M., Barker, S.A.H., Edwards, J., Collerton, J., Jagger, C. and Kirkwood, T.B.L. (2014). Improving Retention of Very Old Participants in Longitudinal Research: Experiences from the Newcastle 85+ Study. *PLOS ONE*, 9.

Davy, B.M., Winett, R.A., Savla, J., Marinik, E.L., Baugh, M.E., Flack, K.D., Halliday, T.M., Kelleher, S.A., Winnett, S.G., Williams, D.M and Boshra, S. (2017). Resist diabetes: A randomized clinical trial for resistance training maintenance in adults with prediabetes. *PLOS One*, 12.

Dawes, D., Ashe, M., Campbell, K., Cave, D., Elley, C.R., Kaczorowski, J., Sohal, P., Ur, E. and Dawes, M. (2015). Preventing Diabetes in Primary Care: A Feasibility Cluster Randomized Trial. *Canadian Journal of Diabetes*, 39, 111-116.

Department of Health. (2014). Living Well for Longer: National Support for Local Action to Reduce Premature Avoidable Mortality.

Diabetes State/Territory Organisations. (2012). Talking Diabetes; pre-diabetes (IFG and IGT).

Diabetes UK. (2015). STATE OF THE NATION; Challenges for 2015 and beyond.

Diabetes UK. (2019). Facts & Figures. Available here: https://www.diabetes.org.uk/professionals/position-statements-reports/statistics. Accessed on: 09.12.2019

Dragsted, L.O. (2010). Biomarkers of meat intake and the application of nutrigenomics. *Meat Science*, 84, 301-307.

Draper, J., Enot, D.P., Parker, D., Beckmann, M., Snowdon, S., Wanchang, L. and Zubair, H. (2009). Metabolite signal identification in accurate mass metabolomics data with MZedDB, an interactive m/z annotation tool utilising predicted ionisation behaviour 'rules'. *Bioinformatics*, 10.

Dresner, A., Laurent, D., Marcucci, M., Griffin, M.E., Dufour, S., Cline, G.W., Slezak, L.A., Andersen, D.K., Hundal, R.S., Rothman, D.L., Petersen, K.F. and Shulman, G.I. (1999). Effects of free fatty acids on glucose transport and IRS-1–associated phosphatidylinositol 3-kinase activity. *The Journal of Clinical Investigation*, 103, 253-259.

Duncan, G.E., Perri, M.G., Theriaque, D.W., Hutson, A.D., Eckel, R.H. and Stacpoole, P.W. (2003). Exercise Training, Without Weight Loss, Increases Insulin Sensitivity and Postheparin Plasma Lipase Activity in Previously Sedentary Adults. *Diabetes Care*, 26, 557-562.

Dunkley, A.J., Bodicoat, J.H., Greaves, C.J., Russell, C., Yates, T., Davies, M.J. and Khunti, K. (2014). Diabetes Prevention in the Real World: Effectiveness of Pragmatic Lifestyle Interventions for the Prevention of Type 2 Diabetes and of the Impact of Adherence to Guideline Recommendations; A Systematic Review and Meta-analysis. *Diabetes Care*, 37, 922-933.

Edmands, W.M.B., Ferrari, P., Rothwell J.A., Rinaldi, S., Slimani, N., Barupal, D.K., Biessy, C., Jenab, M., Clavel-Chapelon, F., Fagherazzi, G., Boutron-Ruault, M.C., Katzke, V.A., Kuhn, T., Boeing, H., Trichopoulou, A., Lagiou, P., Trichopoulos, D., Palli, D., Grioni, S., Tumino, R., Vineis, P., Mattiello, A., Romieu, I and Scalbert, A. (2015). Polyphenol metabolome in human urine and its association with intake of polyphenol-rich foods across European countries. *American Journal of Clinical Nutrition*, 102, 905-913.

Eizirik, D.L., Cardozo, A.K. and Cnop, M. (2008). The Role for Endoplasmic Reticulum Stress in Diabetes Mellitus. *Endocrine Reviews*, 29, 42-61.

El-Sohemy, A., Baylin, A., Kabagambe, E., Ascherio, A., Spiegelman, D and Campos, H. (2002). Individual carotenoid concentrations in adipose tissue and plasma as biomarkers of dietary intake. *American Journal of Clinical Nutrition*, 76, 172-179.

Enea, C., Seguin, F., Petitpas-Mulliez, J., Boildieu, N., Boisseau, N., Delpech, N., Diaz, V., Eugene, M and Degue, B. (2010). 1H NMR-based metabolomics approach for exploring urinary metabolome modifications after acute and chronic physical exercise. *Analytical and Bioanalytical Chemistry*, 396, 1167-1176.

Enot, D.P., Wanchang, L., Beckmann, M., Parker, D., Overy, D.P. and Draper, J. (2008). Pre-processing, classification modelling and feature selection using flow injection electrospray mass spectrometry metabolite fingerprint data. *Nature Protocols*, 3, 446-470.

Esko, T., Hirschhorn, J.N., Feldman, H.A., Hsu, Y.H.H., Deik, A.A., Clish, C.B., Ebbeling, C.B. and Ludwig, D.S. (2017). Metabolomic profiles as reliable biomarkers of dietary composition. *American Journal of Clinical Nutrition*, 105, 547-554.

Evans, S., Royston, P. and Day, S. (2017). Minim: allocation by minimisation in clinical trials.

Falade, J. (2012). Prediabetes: help before it's too late. *The Pharmaceutical Journal*, 288, 643-646.

Fave, G., Beckmann, M., Lloyd, A.J., Zhou, S., Harold, G., Lin, W., Tailliart, K., Xie, L., Draper, J. and Mathers, J.C. (2011). Development and validation of a standardized protocol to monitor human dietary exposure by metabolite fingerprinting of urine samples. *Metabolomics*, 7, 469–484.

Fave, G., Beckmann, M.E., Draper, J.H. and Mathers, J.C. (2009). Measurement of dietary exposure: a challenging problem which may be overcome thanks to metabolomics? *Genes and Nutrition*, 4, 135-141.

Floegal, A., Stefan, N., Yu, Z., Muhlenbruch, K., Drogan, D., Joost, H.G., Fritsche, A., Haring, H.U., de Angelis, M.H., Peters, A., Roden, M., Prehm, C., Wang-Sattler, R., Illig, T., Schulze, M.B., Adamski, J., Boeing, H. and Pischon, T. (2013). Identification of Serum Metabolites Associated with Risk of Type 2 Diabetes Using a Targeted Metabolomic Approach. *Diabetes*, 62, 639-648.

Franz, M.J., Bantle, J.P., Beebe, C.A., Brunzell, J.D. Chiasson, J.L. Garg, A., Holzmeister, L.A., Hoogwerf, B., Mayer-Davies, E., Mooradian, A.D., Purnell, J.Q. and Wheeler, M. (2003). Evidence-Based Nutrition Principles and Recommendations for the Treatment and Prevention of Diabetes and Related Complications. *American Diabetes Association*, 26, 51-61.

Fujimoto, W.Y., Abbate, S.L., Kahn, S.E., Hokanson, J.E. and Brunzell, J.D. (1994). The Visceral Adiposity Syndrome in Japanese-American Men. *Obesity Research*, 2, 364-371.

Garcia-Perez, I., Posma, J.M., Chambers, E.S., Nicholson, J.K., Mathers, J.C., Beckmann, M., Draper, J., Holmes, E. and Frost, G. (2016). An Analytical Pipeline for Quantitative Characterization of Dietary Intake: Application to Assess Grape Intake. *Journal of Agriculture and Food Chemistry*, 64, 2423-2431.

Garcia-Perez, I., Posma, J.M., Gibson, R., Chambers, E.S., Hansen, T.H., Vestergaard, H., Hansen, T., Beckmann, M., Pedersen, O., Elliot, P., Stamler, J., Nicholson, J.K., Draper, J., Mathers, J.C., Holmes, E. and Frost, G. (2017). Objective assessment of dietary patterns by use of metabolic phenotyping: a randomised, controlled, crossover trial. *Lancet Diabetes Endocrinology*.

Garvey, W.T., Maianu, L., Zhu, J.H., Brechtel-Hook, G., Wallace, P. and Baron, A.D. (1998). Evidence for Defects in the Trafficking and Translocation of GLUT4 Glucose Transporters in Skeletal Muscle as a Cause of Human Insulin Resistance. *The Journal of Clinical Investigation*, 101, 2377–2386.

Gersovitz, M., Madden, J.P. and Smiciklas-Wright, H. (1978). Validity of the 24hr dietary recall and seven-day record for group comparisons. *Journal of the American Diabetes Association*, 73, 48-55.

Gibbons, H., Carr, E., McNulty, A.B., Walton, J., Flynn, A., Gibney, M.J. and Brennan, L. (2017). Metabolomic-based identification of clusters that reflect dietary patterns. *Molecular Nutrition and Food Research*, 61.

Gibney, M.J., Walsh, M., Brennan, L., Roche, H.M., German, B. and van Ommen, B. (2005). Metabolomics in human nutrition: opportunities and challenges. *American Journal of Clinical Nutrition*, 82, 497-503.

Gillies, C.L., Abrams, K.R., Lambert, P.C., Cooper, N.J., Sutton, A.J., Hsu, R.T. and Khunti, K. (2007). Pharmacological and lifestyle interventions to prevent or delay type 2 diabetes in people with impaired glucose tolerance: systematic review and meta-analysis. *The British Medical Journal*, 334.

Goodpaster, B.H., Kelley, D.E., Wing, R.R., Meler, A. and Thaete, F.L. (1999). Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. *Diabetes*, 48, 839-847.

Goran, M.I. and Poehlman, E.T. (1992). Total energy expenditure and energy requirements in healthy elderly persons. *Metabolism*, 41, 744-753.

Goris, A.H.C., Westerterp-Plantenga, M.S. and Westerterp, K.R. (2000). Undereating and under recording of habitual food intake in obese men: selective underreporting of fat intake. *American Journal of Clinical Nutrition*, 71, 130-134.

Grant, D.A., Dunseath, G.J., Churm, R. and Luzio, S.D. (2017). Comparison of a point-of-care analyser for the determination of HbA1c with HPLC method. *Practical Laboratory Medicine*, 8, 26-29.

Gray, L.J., Khunti, K., Williams, S., Goldby, S., Troughton, J., Yates, T., Gray, A. and Davies, M.J. (2012). Let's prevent diabetes: study protocol for a cluster randomised controlled trial of an educational intervention in a multi-ethnic UK population with screen detected impaired glucose regulation. *Cardiovascular Diabetology*, 11.

Gregg, E.O., Minet, E., McEwan, M. (2013). Urinary biomarkers of smokers' exposure to tobacco smoke constituents in tobacco products assessment: a fit for purpose approach. *Biomarkers*, 18, 467-486.

Grosso, G., Stepaniak, U., Micek, A., Topor-Madry, R., Pikhart, H., Szafraniec, K. and Pajak, A. (2015). Association of daily coffee and tea consumption and metabolic syndrome: results from the Polish arm of the HAPIEE study. *European Journal of Nutrition*, 54, 1129-1137.

Grundy, S.M., Benjamin, I.J., Burke, G.L., Chait, A., Eckel, R.H., Howard, B.V., Mitch, W., Smith, S.C. and Sowers, J.R. (1999). Diabetes and Cardiovascular Disease. A Statement for Healthcare Professionals from the American Heart Association. *Circulation*, 100, 1134-1146.

Guasch-Ferré, M., Bhupathiraju, S.N. and Hu, F.B. (2018). Use of Metabolomics in Improving Assessment of Dietary Intake. *Clinical Chemistry*, 64, 82-98.

Gummesson, A., Nyman, E., Knutsson., M and Karpefors, M. (2017). Effect of weight reduction on glycated haemoglobin in weight loss trials in patients with type 2 diabetes. *Diabetes, Obesity and Metabolism*, 19, 1295–1305.

Hand, D.J. and Till, R.J. (2001). A simple generalisation of the area under the ROC curve for multiclass classification problems. *Machine Learning*, 45, 171-186.

Hamer, M., McNaughton, S.A., Bates, C.J. and Mishra, G.D. (2010). Dietary patterns, assessed from a weighed food record, and survival among elderly participants from the United Kingdom. *European Journal of Clinical Nutrition*, 64, 853–861.

Hardin, D.S., Azzarelli, B., Edwards, J., Wigglesworth, J., Maianu, L., Brechtel, G., Johnson, A., Baron, A. and Garvey, W.T. (1995). Mechanisms of enhanced insulin sensitivity in endurance-trained athletes: effects on blood flow and differential expression of GLUT 4 in skeletal muscles. *The Journal of Endocrinology and Metabolism*, 80, 2437-2446.

Hardin, D.S., Grilley, K., Baron, B. and Hale, K.A. (1999). Accelerated Red Blood Cell Turnover Can Invalidate the Use of Hemoglobin A1c as a Diagnostic Test for Cystic Fibrosis Related Diabetes. *Pediatric Research*, 45, 90.

Hays, R.D., Sherbourne, C.D. and Mazel, R.M. (1993). The RAND 36-Item Health Survey 1.0. *Health Economics*, 2, 217-227.

Heinrich, J., Holscher, B., Seiwert, M., Carty, C.L., Merkel, G. and Shulz, C. (2005). Nicotine and cotinine in adults' urine: The German Environmental Survey 1998. *Journal of Exposure Analysis and Environmental Epidemiology*, 15, 74–78.

Heinzmann, S.S., Brown, I.J., Chan, Q., Bictash, M., Dumas, M.E., Kochhar, S., Stamler, J., Holmes, E., Elliot, P. and Nicholson, J.K. (2010). Metabolic profiling strategy for discovery of nutritional biomarkers: proline betaine as a marker of citrus consumption. *American Journal of Clinical Nutrition*, 92, 436-443.

Herman, W.H., Hoerger, T.J., Brandle, M., Hicks, K., Sorenson, S., Zhang, P., Hamman, R.F., Ackermann, R.T., Engelgau, M.M. and Ratner, R.E. (2009). The Cost-Effectiveness of Lifestyle Modification or Metformin in Preventing Type 2 Diabetes in Adults with Impaired Glucose Tolerance. Annals of Internal Medicine, 142, 323-332.

Hill, R.J. and Davies, P.S.W. (2002). Energy intake and energy expenditure in elite lightweight female rowers. *Medicine and Science in Sport and Exercise*, 34, 1823–1829.

Hodgson, J.M., Chan, S.Y., Puddey, I.B., Devine, A., Wattanapenpaiboon, N., Wahlqvist, M.L., Lukito, W., Burke, V., Ward, N.C., Prince, R.L. and Croft, K.D. (2004). Phenolic acid metabolites as biomarkers for tea- and coffee-derived polyphenol exposure in human subjects. *British Journal of Nutrition*, 91, 301–305.

Horowitz, J.F. and Klein, S. (2000). Lipid metabolism during endurance exercise. *American Journal of Clinical Nutrition*, 72, 558-563.

Hosomi, R., Yoshida, M. and Fukunaga, K. (2012). Seafood Consumption and Components for Health. *Global Journal of Health Science*, 4, 72-86.

Houmard, J.A., Tanner, C.J., Slentz, C.A., Duscha, B.D., McCartney, J.S. and Kraus, W.E. (2004). Effect of the volume and intensity of exercise training on insulin sensitivity. *Journal of Applied Physiology*, 96, 101-106.

Huang, S. and Czech, M.P. (2007). The GLUT4 Glucose Transporter. *Cell Metabolism*, 5, 237-252.

Jahns, L., Conrad, Z., Johnson, L.K., Scheet, A.J., Stote, K.S. and Raatz, S.K. (2017). Diet Quality Is Lower and Energy Intake Is Higher on Weekends Compared with Weekdays in Midlife Women: A 1-Year Cohort Study. *Journal of the Academy of Nutrition and Dietetics*, 117, 1080-1086.

Jelleyman, C., Yates, T., O'Donovan, G., Gray, L.J., King, J.A. Khunti, K. and Davies, M.J. (2015). The effects of high-intensity interval training on glucose regulation and insulin resistance: a meta-analysis. *Obesity Reviews*, 16, 942-961.

Jensen, M.D., Ryan, D.H., Apovian, C.M., Ard, J.D., Comuzzie, A.G., Donato, K.A., Hu, F.B., Hubbard, V.S., Jakicic, J.M., Kushner, R.F., Loria, C., Millen, B, E., Nonas, C.A., Pi-Sunyer, F,X., Stevens, J., Stevens, V.J., Wadden, T.A., Wolfe, B.M. and Yanovski, S,Z. (2013). A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *Circulation*, 129, 102-138.

John, A.K., Cogswell, M.E., Campbell, N.R., Nowson, C.A., Legetic, B., Hennis, A.J.M. and Patel, S.M. (2016). Accuracy and Usefulness of Select Methods for Assessing Complete Collection of 24-Hour Urine: A Systematic Review. *The Journal of Clinical Hypertension*, 18, 456-467.

Johnson, R.K. (2002). Dietary Intake—How Do We Measure What People Are Really Eating? *Obesity Research*, 10, 63-68.

Kahn, H.A. (1987). A step towards using urinary nitrogen as a tool for validating 24-hour dietary recall interviews. *European Journal of Epidemiology*, 3, 308-311.

Kahn, H.A., Whelton, P.K., Appel, L.J., Kumanyika, S.K., Meneses, J.L., Hebert, P.R. and Woods, M. (1995). Validity of 24-hour dietary recall interviews conducted among volunteers in an adult working community. *Association of Educational Psychologists*, 5, 484-489.

Kahn, N. and Mukhtar, H. (2013). Tea and health: studies in humans. *Current Pharmaceutical Design*, 19, 6141-6147.

Kahn, S.E. (2003). The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia*, 46, 3-19.

Karami, A. and Baradaran, A. (2014). Comparative evaluation of three different methods for HbA1c measurement with High-performance liquid chromatography in diabetic patients. *Advanced Biomedical Research*, 25.

Katula, J.A., Vitolins, M.Z., Rosenberger, E.L., Blackwell, C.S., Morgan, T.M., Lawlor, M.S. and Goff, D.C. (2011). One-Year Results of a Community-Based Translation of the Diabetes Prevention Program. *Diabetes Care*, 34, 1451–1457.

Khalesi, S., Doshi, D., Burys, N. and Sun, J. (2017). Validation of a short food frequency questionnaire in Australian adults. *International Journal of Food Sciences and Nutrition*, 68, 349-357.

King, D.S., Dalksy, G.P., Staten, M.A., Clutter, W.E., Van Houten, D.R. and Holloszy, J.O. (1985). Insulin action and secretion in endurance-trained and untrained humans. *Journal of Applied Physiology*, 63, 2247-2252.

Knowler, W.C., Barrett-Connor, E., Fowler, S.E., Hamman, R.F., Lachin, J.M., Walker, E.A. and Nathan, D.M. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *The New England Journal of Medicine*, 346, 393-403.

Koistinen, V.M., Mattila, O., Katina, K., Poutanen, K., Aura, A.M. and Hanhineva, K. (2018). Metabolic profiling of sourdough fermented wheat and rye bread. *Scientific Reports*, 8.

Kowalkowska, J., Slowinska, M. A., Slowinski, D., Dlugosz, A., Niedzwiedzka, E. and Wadolowska, L. (2013). Comparison of a Full Food-Frequency Questionnaire with the Three-Day Unweighted Food Records in Young Polish Adult Women: Implications for Dietary Assessment. *Nutrients*, 5, 2747-2776.

Kulzer, B., Hermanns, M., Gorges, D., Schwarz, P. and Haak, T. (2009). Prevention of Diabetes Self-Management Program (PREDIAS): Effects on Weight, Metabolic Risk Factors, and Behavioral Outcomes. *Diabetes Care*, 32, 1143-1146.

Laatikainen, T., Dunbar, J.A., Chapman, A., Kilkkinen, A., Vartiainan, E., Heistaro, S., Philpot, B., Absetz, P., Bunker, S., O'Neil, A., Reddy, P., Best, J.D. and Janus, E.D. (2007). Prevention of Type 2 Diabetes by lifestyle intervention in an Australian primary health care setting: Greater Green Triangle (GGT) Diabetes Prevention Project. *Biomed Central Public Health*, 7.

Lafay, L., Mennen, L., Basdevant, A., Charles, M.A., Borys, J.M., Eschwege, E. and Romon, M. (2000). Does energy intake underreporting involve all kinds of food or only specific food items? Results from the Fleurbaix Laventie Ville Santé (FLVS) study. *International Journal of Obesity*. 24, 1500–1506.

Landgraf, R. (2006). HbA1c-the Gold Standard in the Assessment of Diabetes Treatment? *Deutsche Medizinische Wochenschrift*, 131, 243-246.

Larsson, S.C. and Orsini, N. (2013). Red Meat and Processed Meat Consumption and All-Cause Mortality: A Meta-Analysis. *American Journal of Epidemiology*, 179, 282-289.

Lean, M.E.J., Leslie, W.S., Barnes, A.C., Brosnahan, M., Thom, G., McCombie, L., Peters, C., Zhyzhneuskaya, S., Al-Mrabeh, A., Hollingsworth, K.G., Rodrigues, A.M., Rehackova, L., Adamson, A.J., Sniehotta, F.F., Mathers, J.C., Ross, H.M., Mcllvenna, Y., Stefanetti, R., Trennell, M., Welsh, P., Kean, S., Ford, I., McConnachie, A., Sattar, N. and Taylor, R. (2018). Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. *The Lancet*, 391, 541-551.

Lee, P.H., Yu, Y.Y., McDowell, I., Leung, G.M., Lam, T.H. and Stewart, S.M. (2011). Performance of the international physical activity questionnaire (short form) in subgroups of the Hong Kong Chinese population. *International Journal of Behavioural Nutrition and Physical Activity*, 8, 81.

Lewis, G.D., Farrell, L., Wood, M.J., Martinovic, M., Arany, Z., Rowe, G.C., Souza, A., Cheng, S., McCabe, E.L., Yang, E., Shi, X., Deo, R., Roth, F.P., Asnani, A., Rhee, E.P., Systrom, D.M., Semigran, M.J., Vasan, R.S., Carr, S.A., Wang, T.J., Sabatine, M.S., Clish, C.B. and Gerszten, R.E. (2010). Metabolic Signatures of Exercise in Human Plasma. *Science Translational Medicine*, 26.

Lichtman, S.W., Pisarska, K., Berman, E.R., Pestone, M., Dowling, H., Offenbacher, E., Weisel, H., Heshka, S., Mathews, D.E and Heymsfield, S.B. (1992). Discrepancy between self-reported and actual caloric intake and exercise in obese subjects. *The New England Journal of Medicine*, 327, 1893-1898.

Liu, L., Wang, P.P., Roebothan, B., Ryan, A., Tucker, C.S., Colbourne, J., Baker, N., Cotterchio, M., Yi, Y. and Sun, G. (2013). Assessing the validity of a self-administered food-frequency questionnaire (FFQ) in the adult population of Newfoundland and Labrador, Canada. *Nutrition Journal*, 12.

Lloyd, A.J., Beckmann, M., Fave, G., Mathers, J.C. and Draper, J. (2011). Proline betaine and its biotransformation products in fasting urine samples are potential biomarkers of habitual citrus fruit consumption. *British Journal of Nutrition*, 106, 812–824.

Lloyd, A.J., Beckmann, M., Haldar, S., Seal, C., Brandt, K. and Draper, J. (2013). Data-driven strategy for the discovery of potential urinary biomarkers of habitual dietary exposure. *American Journal of Clinical Nutrition*, 97, 377-389.

Lloyd, A.J., Fave, G., Beckmann, M., Lin, W., Tailliart, K., Xie, L., Mathers, J.C. and Draper, J. (2011). Use of mass spectrometry fingerprinting to identify urinary metabolites after consumption of specific foods. *American Journal of Clinical Nutrition*, 94, 981-991.

Lloyd, A.J., Willis, N.D., Wilson, T., Zubair, H., Chambers, E., Garcia-Perez, I., Xie, L., Tailliart, K., Beckmann, M., Mathers, J.C. and Draper, J. (2019). Addressing the pitfalls when designing intervention studies to discover and validate biomarkers of habitual dietary intake. *Metabolomics*, 15.

Lloyd, A.J., Zubair, H., Willis, N.D., Wilson, T., Xie, L., Tailliart, K., Chambers, E.S., Garcia-Perez, I., Holmes, E., Frost, G., Mathers, J.C. Beckmann, M. and Draper, J. (2016). Quantification of dietary biomarkers in spot urine samples reflects the intake of foods of UK high public health importance. *Proceedings of the Nutrition Society*, 75.

Lohmann, D., Liebold, F., Heilmann, W., Senger, H. and Pohl, A. (1978). Diminished insulin response in highly trained athletes. *Metabolism*, 27, 521-524.

Lund, S., Holman, G.D., Schmitz, O. and Pedersen, O. (1995). Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through a mechanism distinct from that of insulin. *Proceedings of the National Academy of Sciences of the United States of America*, 92, 5817–5821.

Ma, Y., Olendzki, B.C., Hafner, A.R., Chiriboga, D., Hebert, J.R., Campbell, M., Sarnie, M. and Ockene, I.S. (2006). Seasonal variation in food intake, physical activity, and body weight in a predominantly overweight population. *European Journal of Clinical Nutrition*, 60, 519-528.

Mackus, M., Van de Loo, A., Raasveld, S.J., Hogewoning, A., Torano, S., Flesch, F.M., Korte-Bouws, G.A.H., Van Neer, R.H.P., Wang, X., Nguyen, T.T., Brookhuis, K.A., Kraneveld, A.D., Garssen, J. and Verster, J.C. (2017). Biomarkers of the alcohol hangover state: Ethyl glucuronide (EtG) and ethyl sulfate (EtS). *Human Psychopharmacology*, 32.

Mailankot, M., Thomas, T., Praveena, P., Jacob, J., Benjamin, J.B. and Vasudevan, D.M. (2012). Various Anticoagulants and Fluoride do not Affect HbA1C Level. *Indian Journal of Clinical Biochemistry*, 27.

Mainous III, A.G., Tanner, R.J., Baker, R., Zayas, C.E. and Harle, C.A. (2014). Prevalence of prediabetes in England from 2003 to 2011: population-based, cross-sectional study. *British Medical Journal*, 4, 1-8.

Malin, S.K., Finnegin, S., Fealy, C.E., Filion, J., Rocco, M.B. and Kirwan, J.P. (2014). b-Cell Dysfunction Is Associated with Metabolic Syndrome Severity in Adults. *Metabolic Syndrome and Related Disorders*, 12, 79-85.

Mason, J.B. and Mitchell, J.T. (1983). Nutritional surveillance. *Bulletin of the World Health Organization*, 61, 745-755.

Mendonca, N., Hill, T.R., Granic, A., Davies, K., Collerton, J., Mathers, J.C., Siervo, M., Wrieden, W.L., Seal, C.J., Kirkwood, T.B.L., Jagger, C. and Adamson, A.J. (2016). Macronutrient intake and food sources in the very old: analysis of the Newcastle 85+ Study. *British Journal of Nutrition*, 115, 2170–2180.

Meng, X., Maliakal, P., Lu, H., Lee, M.J. and Yang, C.S. (2004). Urinary and Plasma Levels of Resveratrol and Quercetin in Humans, Mice, and Rats after Ingestion of Pure Compounds and Grape Juice. *Journal of Agriculture and Food Chemistry*, 52, 935-942.

Mennen, L.I., Sapinho, D., Ito, H., Bertrais, S., Galan, P., Hercberg, S. and Scalbert, A. (2006). Urinary flavonoids and phenolic acids as biomarkers of intake for polyphenol-rich foods. *British Journal of Nutrition*, 96, 191–198.

Michielsen, C.C.J.R., Almanza-Aguilera, E., Brouwer-Brolsma, E.M., Urpi-Sarda, M. and Afman, L.A. (2018). Biomarkers of food intake for cocoa and liquorice (products): a systematic review. *Genes and Nutrition*, 13.

Mikines, K.J., Farrell, P.A., Sonne, B., Tronier, B. and Galbo, H. (1985). Postexercise dose-response relationship between plasma glucose and insulin secretion. *Journal of Applied Physiology*, 64, 988-999.

Montague, C.T. and O'Rahilly, S. (2000). Perspectives in Diabetes. The Perils of Portliness Causes and Consequences of Visceral Adiposity. *Diabetes*, 49, 883-888.

Morimoto, Y., Beckford, F., Franke, A.A. and Maskarinec, G. (2014). Urinary isoflavonoid excretion as a biomarker of dietary soy intake during two randomized soy trials. *Asia Pacific Journal of Clinical Nutrition*, 23, 205-209.

Myint, T., Frase, G.E., Lindsted, K.D., Knutsen, S.F., Hubbard, R.W. and Bennett, H.W. (2000). Urinary 1-Methylhistidine Is a Marker of Meat Consumption in Black and in White California Seventh-day Adventists. *American Journal of Epidemiology*, 152, 752-755.

Nassis, G.P., Papatakou, K., Skenderi, K., Triandafillopoulou, M., Kavouras, S.A., Yannakoulia, M., Chrousos, G.P. and Sidossis, L.S. (2005). Aerobic exercise training improves insulin sensitivity without changes in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. *Metabolism Clinical and Experimental*, 54, 1472-1479.

NHS England. (2018). Healthier You: NHS Diabetes Prevention Programme.

Nilsen, V., Bakke, P.S. and Gallefoss, F. (2011). Effects of lifestyle intervention in persons at risk for type 2 diabetes mellitus - results from a randomised, controlled trial. *Biomed Central Public Health*, 11.

Novotny, J.A., Rumpler, W.V., Riddick, H., Hebert, J.R., Rhodes, D., Judd, J.T., Baer, D.J., McDowell, M., Briefel, R. (2003). Personality characteristics as predictors of underreporting of energy intake on 24-hour dietary recall interviews. *Journal of the American Diabetic Association*, 103, 1146-1151.

O'Gorman, A., Gibbons, H. and Brennan, L. (2013). Metabolomics in the identification of biomarkers of dietary intake. *Computational and Structural Biotechnology Journal*, 4.

O'Loughlin, G., Cullen, S.J., McGoldrick, A., O'Connor, S., Blain, R., O'Malley, S and Warrington, G.D. (2013). Using a Wearable Camera to Increase the Accuracy of Dietary Analysis. *American Journal of Preventative Medicine*, 44, 297-301.

O'Sullivan, A., Gibney, M.J. and Brennan, L. (2011). Dietary intake patterns are reflected in metabolomic profiles: potential role in dietary assessment studies. *American Journal of Clinical Nutrition*, 93, 314-321.

Ockene, I.S., Tellez, T.L., Rosal, M.C., Reed, G.W., Mordes, J., Merriam, P.A., Olendzki, B.C., Handelman, G., Nicolosi, R. and Ma, Y. (2012). Outcomes of a Latino Community-Based Intervention for the Prevention of Diabetes: The Lawrence Latino Diabetes Prevention Project. *Research and Practice*, 102, 336-342.

Olokoba, A, B., Abateru, O.A. and Olokoba, L.B. (2012). Type 2 Diabetes Mellitus: A Review of Current Trends. *Oman Medical Journal*, 27, 269-273.

Paalanen, L., Mannisto, S., Virtanen, M.J., Knekt, P., Rasanen, L., Montonen, J. and Peitinen, P. (2006). Validity of a food frequency questionnaire varied by age and body mass index. *Journal of Clinical Epidemiology*, 59, 994-1001.

Pandis, N. (2011). Randomization. Part 2: Minimization. *American Journal of Orthodontics and Dentofacial Orthopaedics*, 140, 902-904.

Patel, K.P., Luo, F.J.G., Plummer, N.S., Hostetter, T.H. and Meyer, T.W. (2012). The Production of p-Cresol Sulfate and Indoxyl Sulfate in Vegetarians Versus Omnivores. *Clinical Journal of the American Society of Nephrology*, 7, 982-988.

Paxton, A.E., Strycker, L.A., Toobert, D.J., Ammerman, A.S., and Glasgow, R.E. (2011). Starting the conversation performance of a brief dietary assessment and intervention tool for health professionals. *American Journal of Preventative Medicine*, 40, 67-71.

Payne, W.R., Walsh, K.J., Harvey, J.T., Livy, M.F., McKenzie, K.J., Donaldson, A., Atkinson, M.G., Keogh, J.B., Moss, R.S., Dunstan, D.W. and Hubbard, W.A. (2008). Effect of a Low–Resource-Intensive Lifestyle Modification Program Incorporating Gymnasium-Based and Home-Based Resistance Training on Type 2 Diabetes Risk in Australian Adults. *Diabetes Care*, 31, 2244–2250.

Pechlivanis, A., Kostidis, S., Saraslanidis, P., Petridou, A., Tsalis, G., Mougios, V., Gika, H.G., Mikros, E. and Theodoridis, G.A. (2010). 1H NMR-Based Metabonomic Investigation of the Effect of Two Different Exercise Sessions on the Metabolic Fingerprint of Human Urine. *Journal of Proteome Research*, 9, 6405–6416.

Pechlivanis, A., Papaioannou, K.G., Tsalis, G., Saraslanidis, P., Mougios, V. and Theodoridis, G.A. (2015). Monitoring the Response of the Human Urinary Metabolome to Brief Maximal Exercise by a Combination of RP-UPLC-MS and 1H NMR Spectroscopy. *Journal of Proteome Research*, 14, 4610-4622.

Penn, L., White, M., Oldroyd, J., Walker, M., Alberti, K.G.M.M. and Mathers, J.C. (2009). Prevention of type 2 diabetes in adults with impaired glucose tolerance: the European Diabetes Prevention RCT in Newcastle upon Tyne, UK. *Biomed Central Public Health*, 9.

Petersen, G.O., Leite, C.E., Chatkin, J.M. and Thiesen, F.V. (2010). Cotinine as a biomarker of tobacco exposure: Development of a HPLC method and comparison of matrices. *Journal of Separation Science*, 33, 516-521.

Peterson, K.F., Dufour, S., Morino, K., Yoo, P.S., Cline, G.W. and Shulman, G.I. (2012). Reversal of muscle insulin resistance by weight reduction in young, lean, insulin-resistant offspring of parents with type 2 diabetes. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 8236-8240.

Petta, S. and Marchesini, G. (2017). Coffee and tea breaks for liver health. *Journal of Hepatology*, 67, 221-223.

Piper, C., Marossy, A., Griffiths, Z. and Adegboye, A. (2017). Evaluation of a type 2 diabetes prevention program using a commercial weight management provider for nondiabetic hyperglycemic patients referred by primary care in the UK. *Diabetes Research and Care*, 5.

Playdon, M.C., Sampson, J.N., Cross, A.J., Sinha, R., Guertin, K., Moy, K.A., Rothman, N., Irwin, M.L., Mayne, S.T., Stolzenberg-Soloman, R. and Moore, S.C. (2016). Comparing metabolite profiles of habitual diet in serum and urine. *American Journal of Clinical Nutrition.*

Poesen, R., Mutsaers, H.A.M., Windey, K., van den Broek, P.H., Verweij, V., Augustijns, P., Kuypers, D., Jansen, J., Evenepoel, P., Verbeke, K., Meijers, B. and Masereeuw, R. (2015). The Influence of Dietary Protein Intake on Mammalian Tryptophan and Phenolic Metabolites. *PLOS ONE*, 10.

Pohjanen, E., Thysell, E., Jonsson, P., Eklund, C., Silfver, A., Carlsson, I.B., Lundgren, K., Moritz, T., Svensson, M.B. and Antti, H. (2007). A Multivariate Screening Strategy for Investigating Metabolic Effects of Strenuous Physical Exercise in Human Serum. *Journal of Proteome Research*, 6, 2113-2120.

Poitout, V. and Robertson, R.P. (2008). Glucolipotoxicity: fuel excess and beta-cell dysfunction. *Endocrine Reviews*, 29, 351-366.

Pollard, J., Wild, C.P., White, K.L.M., Greenwood, D.C., Cade, J.E, and Kirk, S.F.L. (2003). Comparison of plasma biomarkers with dietary assessment methods for fruit and vegetable intake. *European Journal of Clinical Nutrition*, 57, 988-998.

Poslusna, K., Ruprich, J., de Vries, J.H.M., Jakubikova, M. and van't Veer, P. (2009). Misreporting of energy and micronutrient intake estimated by food records and 24-hour recalls, control and adjustment methods in practice. *British Journal of Nutrition*, 101, 73-85.

Powell, C., Carson, B.P., Dowd, K.P. and Donnelly, A.E. (2016). Simultaneous validation of five activity monitors for use in adult populations. *Scandinavian Journal of Medicine and Science in Sports*, 27, 1881-1892.

Prentice, A.M., Black, A.E., Coward, W.A., Davies, H.L., Goldberg, G.R., Murgatroyd, P.R., Ashford, J., Sawyer, M and Whiteheard, R.G. (1986). High levels of energy expenditure in obese women. *British Medical Journal*, 292, 983-987.

Prentki, M. and Nolan, C.J. (2006). Islet b cell failure in type 2 diabetes. *The Journal of Clinical Investigation*, 116, 1802-1812.

Radin, M.S. (2014). Pitfalls in Hemoglobin A1c Measurement: When Results may be Misleading. *Journal of General Internal Medicine*, 29, 388-394.

Raina, S.K. (2013). What and how much do we eat? 24-hour dietary recall method. *North American Journal of Medical Sciences*, 5, 498.

Ralib, A, M., Pickering, J.W., Shaw, G.M., Devarajan, P., Edelstein, C.L., Bonventre, J.V. and Endre, Z.H. (2012). Test Characteristics of Urinary Biomarkers Depend on Quantitation Method in Acute Kidney Injury. *Journal of the American Society of Nephrology*, 23, 322-333.

Randle, P.J., Garland, P.B., Hales, C.N. and Newsholme, E.A. (1963). The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet*, 13, 785-789.

Ranganathan, P., Pramesh, C.S. and Buyse, M. (2015). Common pitfalls in statistical analysis: Clinical versus statistical significance. *Perspectives in Clinical Nutrition*, 6, 169-170.

Rechner, A.R., Spencer, J.P.E., Kuhnle, G., Hahn, U. and Rice-Evans, C. (2001). Novel biomarkers of the metabolism of caffeic acid derivatives in vivo. *Free Radical Biology & Medicine*, 30, 1213-1222.

Reilly, J.J., Lord, A., Bunker, V.W., Prentice, A.M., Coward, W.A., Thomas, A.J. and Briggs, R.S. (1993). Energy balance in healthy elderly women. *British Journal of Nutrition*, 69, 21-27.

Reilly, J.J., Montgomery, C., Jackson, D., MacRitchie, J. and Armstrong, J. (2001). Energy intake by multiple pass 24 h recall and total energy expenditure: a comparison in a representative sample of 3–4-year-olds. *British Journal of Nutrition*, 86, 601–605.

Roden, M., Price, T.B., Perseghin, G., Petersen, K.F., Rothman, D.L., Cline, G.W. and Shulman, G.I. (1996). Mechanism of Free Fatty Acid–induced Insulin Resistance in Humans. *The American Society for Clinical Investigation*, 97, 2859-2865.

Ross, R. (2003). Does Exercise Without Weight Loss Improve Insulin Sensitivity? *Diabetes Care*, 26, 944-945.

Rothberg, A.E., McEwen, L.N., Kraftson, A.T., Ajluni, N., Fowler, C.E., Nay, C.K., Miller, N.M., Burant, C.F. and Herman, W.H. (2017). Impact of weight loss on waist circumference and the components of the metabolic syndrome. *Diabetes and Research Care*, 5.

Roumen, C., Corpeleijn, E., Feskens, E.J.M., Mensink, M., Saris, W.H.M. and Blaak, E.E. (2008). Impact of 3-year lifestyle intervention on postprandial glucose metabolism: the SLIM study. *Diabetic Medicine*, 25, 597-605.

Ruxton, C. (2009). Health aspects of caffeine: benefits and risks. *Nursing Standard*, 24.

Saaristo, T., Moilanen, L., Korpi-Hyovalti, E., Vanhala, M., Saltevo, J., Niskanen, L., Jokelainen, J., Peltonen, M., Oksa, H., Tuomilehto, J., Uusitupa, M. and Keinanen-Kiukaanneimi, S. (2010). One-year follow-up of the Finnish National Diabetes Prevention Program (FIN-D2D). *Diabetes Care*, 33, 2146–2151.

Sakane, N., Sato, J., Tsushita, K., Tsujii, S., Kotani, K., Tsuzaki, K., Tominaga, M., Kawazu, S., Sato, Y., Usui, T., Kamae, I., Yoshida, T., Kiyohara, Y., Sato, S. and Kuzuya, H. (2011). Prevention of type 2 diabetes in a primary healthcare setting: Three-year results of lifestyle intervention in Japanese subjects with impaired glucose tolerance. *Biomed Central Public Health*, 11.

Samaras, K., Kelly, P.J. and Campbell, L.V. (1999). Dietary underreporting is prevalent in middle-aged women and is not related to adiposity. *International Journal of Obesity*, 23, 881-888.

Sang, S., Lee, M.J., Yang, I., Bucley, B. and Yang, C.S. (2008). Human urinary metabolite profile of tea polyphenols analyzed by liquid chromatography/electrospray ionization tandem mass spectrometry with data-dependent acquisition. *Rapid communications in mass spectrometry*, 22, 1567-1578.

Savage, D.B., Petersen, K.F. and Shulman, G.I. (2005). Mechanisms of Insulin Resistance in Humans and Possible Links with Inflammation. *Hypertension*, 45, 828-833.

Scarborough, P., Bhatnagar, P., Wickramasinghe, K.K., Allender, S., Foster, C. and Rayner, M. (2011). The economic burden of ill health due to diet, physical inactivity, smoking, alcohol and obesity in the UK: an update to 2006–07 NHS costs. *Journal of Public Health*, 33, 527-535.

Schaefer, E.J., Augustin, J.L., Schaefer, M.M., Rasmussen, H., Ordovas, J.M., Dallal, G.E and Dwyer, J.T. (2000). Lack of efficacy of a food-frequency questionnaire in assessing dietary macronutrient intakes in subjects consuming diets of known composition. *American Journal of Clinical Nutrition*, 71, 746-751.

Schatzkin, A., Kipnis, V., Carroll, R.J., Midthune, D., Subar, A.F., Bingham, S., Schoeller, D.A., Troiano, R.P and Freedman, L.S. (2003). A comparison of a food frequency questionnaire with a 24-hour recall for use in an epidemiological cohort study: results from the biomarker-based Observing Protein and Energy Nutrition (OPEN) study. *International Journal of Epidemiology*, 32, 1054–1062.

Scheen, A.J. (2003). Pathophysiology of type 2 diabetes. *Acta Clinica Belgica*, 58, 335-41.

Scherer, G., Urban, M., Hagedorn, H.W., Serafin, R., Feng, S., Kapur, S., Muhammad, R., Jin, Y., Sarkar, M. and Roethig, H.J. (2010). Determination of methyl-, 2-hydroxyethyl- and 2-cyanoethylmercapturic acids as biomarkers of exposure to alkylating agents in cigarette smoke. *Journal of Chromatography*, 870, 2520-2528.

Schlienger, J.L. (2013). Complications du diabète de type 2. *La Presse Medicale*, 42, 839-848.

Schmider, E., Ziegler, M., Danay, E., Beyer, L. and Buhner, M. (2010). Reinvestigating the Robustness of ANOVA Against Violations of the Normal Distribution Assumption. *Methodology*, 6, 147-151.

Schmidt, J.A., Rinaldi, S., Ferrari, P., Carayol, M., Achaintre, D., Scalbert, A., Cross, A.J., Gunter, M.J., Fensom, G.F., Appleby, P.N., Key, T.J. and Travis, R.C. (2015). Metabolic profiles of male meat eaters, fish eaters, vegetarians, and vegans from the EPIC-Oxford cohort. *American Journal of Clinical Nutrition*, 102, 1518-1526.

Searle, J.L., Klein, G., Friedmann, J., Jensen, G.L., Mitchell, D.C. and Smiciklas-Wright, H. (2002). Energy Expenditure Measured by Doubly Labelled Water, Activity Recall, and Diet Records in the Rural Elderly. *Nutrition*, 18, 568 –573.

Sesti, G. (2006). Pathophysiology of insulin resistance. *Best Practice & Research Clinical Endocrinology & Metabolism*, 20, 665-679.

Sheng, Z., Cao, J-Y., Pang, Y-C., Xu, H-C., Chen, J-W., Yuan, J-H., Wang, R., Zhang, C-S., Wang, L-X. and Dong, J. (2019). Effects of Lifestyle Modification and Anti-diabetic Medicine on Prediabetes Progress: A Systematic Review and Meta-Analysis. *Frontiers in Endocrinology*, 455.

Sharma, B., Sarmah, D. and Sonker, P. (2013). Effect of different anticoagulants on HBA1C estimation and its stability. *Journal of Laboratory Physicians*, 5, 143-144.

Shephard, R.J. (2003). Limits to the measurement of habitual physical activity by questionnaires. *British Journal of Sports Medicine*, 37, 197-206.

Shim, J.S., Oh, K. and Kim, H.C. (2014). Dietary assessment methods in epidemiologic studies. *Epidemiology and Health*, 36.

Sigal, R.J., Kenny, G.P., Wassermann, D.H., Castaneda-Sceppa, C. and White, R.D. (2006). Physical activity/exercise and type 2 diabetes: a consensus statement from the American Diabetes Association. *Diabetes Care*, 29, 1433-1438.

Simundic, A.M. (2016). Statistical analysis in method comparison studies – Part one. Downloaded from acutecaretesting.org.

Siopi, A., Deda, O., Manou, V., Kellis, S., Kosmidis, I., Komninou, D., Raikos, N., Christoulas,K., Theodoridis, G.A. and Mougios, V. (2017). Effects of Different Exercise Modes on the Urinary Metabolic Fingerprint of Men with and without Metabolic Syndrome. *Metabolites*, 7.

Slavin, J.L. and Lloyd, B. (2012). Health benefits of fruits and vegetables. *Advances in Nutrition*, 3, 506-516.

Slow, S., Donaggio, M., Cressey, P.J., Lever, M., George, P.M. and Chambers, S.T. (2005). The betaine content of New Zealand foods and estimated intake in the New Zealand diet. *Journal of Food Composition and Analysis*, 18, 473–485.

Slupsky, C.M., Rankin, K.N., Wagner, J., Fu, H., Chang, D., Weljie, A.M., Saude, E.J., Lix, B., Adamko, D.J., Shah, S., Greiner, R., Sykes, D. and Marrie, T.J. (2007). Investigations of the Effects of Gender, Diurnal Variation, and Age in Human Urinary Metabolomic Profiles. *Analytical Chemistry*, 79, 6995-7004.

Sodorholm, P.P., Koskela, A.H., Lundin, J.E., Tikkanen, M.J. and Aldercruetz, H.C. (2009). Plasma pharmacokinetics of alkylresorcinol metabolites: new candidate biomarkers for whole-grain rye and wheat intake. American Journal of Clinical Nutrition, 90, 1167-1171.

Stella, C., Beckwith-Hall, B., Cloarec, O., Holmes, E., Lindon, J.C., Powell, J., van der Ouderaa, F., Bingham, S., Cross, A.J. and Nicholson, J.K. (2006). Susceptibility of Human Metabolic Phenotypes to Dietary Modulation. *Journal of Proteome Research*, 5, 2780-2788.

Stetson, B., Schlundt, D., Rothschild, C., Floyd, J.E., Rogers, W. and Moksshagundam, S.P. (2011). Development and validation of The Personal Diabetes Questionnaire (PDQ): A measure of diabetes self-care behaviours, perceptions and barriers. *Diabetes Research and Clinical Practice*, 91, 321-332.

Sun, Q. (2017). Re: The use of food frequency questionnaires is both pseudo-scientific and illogical. Response to author.

Tabak, A.G., Herder, C., Rathmann, W., Brunner, E.J. and Kivimaki M. (2012). Prediabetes: a high-risk state for diabetes development. *The Lancet*, 379, 2279-2290.

Tanti, J.F., Ceppo, F., Jager, J. and Berthou, F. (2013). Implication of inflammatory signalling pathways in obesity-induced insulin resistance. *Frontiers in Endocrinology*, 3, 1-15.

Thatcher, R. and Gregory, N. (2017). Reduction in prevalence of pre-diabetes following one to one consultation delivered through general practice. Poster session at the Welsh Endocrine and Diabetes Society conference, Wales, UK.

Tomita, S., Nemoto, T., Matsuo, Y., Shoji, T., Tanaka, F., Nakagawa, H., Ono, H., Kikuchi, J., Ohnishi-Kameyama, M. and Sekiyama, Y. (2015). An NMR-based, non-targeted multistep metabolic profiling revealed L-rhamnitol as a metabolite that characterised apples from different geographic origins. *Food Chemistry*, 174, 163-172.

Tomoyasu, M.J., Toth, M.J. and Poehlman, E.T. (1999). Misreporting of total energy intake in older men and women. *Journal of the American Geriatrics Society*, 47, 710-715.

Trabulsi, J. and Schoeller, D.A. (2001). Evaluation of dietary assessment instruments against doubly labelled water, a biomarker of habitual energy intake. *American Journal of Physiology, Endocrinology and Metabolism*, 281, 891-899.

UK Government. 2019. UK Chief Medical Officers' Physical Activity Guidelines.

Valabhji, J., Barron, E., Bradley, D., Bakhai, C., Fagg, J., O'Neill, S., Young, B., Wareham, N., Khunti, K., Jebb, S. and Smith, J. (2019). Early Outcomes from the English National Health Service Diabetes Prevention Program. *Diabetes Care,* (Ahead of print).

Van den Brandt, P. (2018). Coffee or Tea? A prospective cohort study on the associations of coffee and tea intake with overall and cause-specific mortality in men versus women. *European Journal of Epidemiology*, 33, 183–200.

Van Der Ploeg, H.P., Tudor-Locke, C., Marshall, A.L., Craig, C., Hagstromer, M., Siostrom, M., Bauman, A. (2010). Reliability and validity of the international physical activity questionnaire for assessing walking. *Research Quarterly for Exercise and Sport*, 81, 97-101.

Varraso, R., Chiuve, S.E., Fung, T.T., Barr, R.G., Hu, F.B., Willet, W.C. and Camargo, C.A. (2015). Alternate Healthy Eating Index 2010 and risk of chronic obstructive pulmonary disease among US women and men: prospective study. *British Medical Journal*, 3.

Vasquez-Fresno, R., Llorach, R., Urpi-Sarda, M., Lupianez-Barbero, A., Estruch, R., Corella, D., Fito, M., Aros, F., Ruiz-Canela, M., Salas-Salvado, J. and Andres-Lacueva, C. (2014). Metabolomic Pattern Analysis after Mediterranean Diet Intervention in a Nondiabetic Population: A 1- and 3-Year Follow-up in the PREDIMED Study. *Journal of Proteome Research*, 14, 531-540.

Vermunt, P.W.A., Milder, I.E.J., Wielaad, F., de Vries, J.H.M., Baan, C.A., van Oers, J.A.M. and Westert, G.P. (2012). A lifestyle intervention to reduce Type 2 diabetes risk in Dutch primary care: 2.5-year results of a randomized controlled trial. *Diabetic Medicine*, 29, 223–231.

Vermunt, P.W.A., Milder, I.E.J., Wielaard, F., de Vries, J.H.M., Baan, C.A., van Oers, J.A.M. and Westert, G.P. (2012). A lifestyle intervention to reduce Type 2 diabetes risk in Dutch primary care: 2.5-year results of a randomized controlled trial. *Diabetic Medicine*, 29, 223-231.

Vetrani, C., Rivellese, A.A., Annuzzi, G., Mattila, I., Meudec, E., Hyötyläinen, T., Orešič, M. and Aura, A.M. (2014). Phenolic metabolites as compliance biomarker for polyphenol intake in a randomized controlled human intervention. *Food Research International*, 63, 233-238.

Wang, C., Guan, Y. and Yang, J. (2010). Cytokines in the Progression of Pancreatic β-Cell Dysfunction. *International Journal of Endocrinology*, 2010, 1-10.

Wang, Y., Gapstur, S.M., Carter, B.D., Hartman, T.J., Stevens, V.L., Gaudet, M.M. and McCullough, M.L. (2018). Untargeted Metabolomics Identifies Novel Potential Biomarkers of Habitual Food Intake in a Cross-Sectional Study of Postmenopausal Women. *The Journal of Nutrition*, 148, 932-943.

Weber, J.L., Reid, P.M., Greaves, K.A., DeLany, J.P., Stanford, V.A., Going, S.B., Howell, W.H. and Houtkooper, L.B. (2001). Validity of self-reported energy intake in lean and obese young women, using two nutrient databases, compared with total energy expenditure assessed by doubly labelled water. *European Journal of Clinical Nutrition*, 55, 940–950.

Whitton, C., Ho, J.C.Y., Tay, Z., Rebello, S.A., Lu, Y., Ong, C.N. and van Dam, R.M. (2017). Relative Validity and Reproducibility of a Food Frequency Questionnaire for Assessing Dietary Intakes in a Multi-Ethnic Asian Population Using 24-h Dietary Recalls and Biomarkers. *Nutrients*, 9.

Williams, N. (2017). The Borg Rating of Perceived Exertion (RPE) scale. *Occupational Medicine*, 67:404–405.

Wilson, L.A., Wilkinson, K., Crews, H.M., Davies, A.M., Dick, C.S. and Dumsday, V.L. (1999). Urinary monitoring of saccharin and acesulfame-K as biomarkers of exposure to these additives. *Food Additives and Contaminants*, 16, 227-238.

Wilson, T., Garcia-Perez, I., Posma, J.M., Lloyd, A.J., Chambers, S.E., Tailliart, K., Zubiar, H., Beckmann, M., Mathers, J.C., Holmes, E., Frost, G. and Draper, J. (2019). Spot and Cumulative Urine Samples Are Suitable Replacements for 24-Hour Urine Collections for Objective Measures of Dietary Exposure in Adults Using Metabolite Biomarkers. *The Journal of Nutrition.*

Wing, R.R., Blair, E.H., Bononi, P., Marcus, M.D., Watanabe, R. and Bergman, R.N. (1994). Calorie restriction per se is a significant factor in improvements in glycaemic control and insulin sensitivity during weight loss in obese NIDDM patients. *Diabetes Care*, 17, 30-36.

Wiseman, H., Casey, K., Bowey, E.A., Duffy, R., Davies, M., Rowland, I.R., Lloyd, A.S., Murray, A., Thompson, R. and Clarke, D.B. (2004). Influence of 10 wk of soy consumption on plasma concentrations and excretion of isoflavonoids and on gut microflora metabolism in healthy adults. *American Journal of Clinical Nutrition*, 80, 692-696.

World Health Organisation. 2016. Global Report on Diabetes.

Wood, J.R., Kaminski, B.M., Kollman, C., Beck, R.W., Hall, C.A., Yun, J.P., Cengiz, E., Haller, M.J., Hassan, K., Klingensmith, J. and Tamborlane, W.V. (2012). Accuracy and Precision of the Axis-Shield Afinion Hemoglobin A1c Measurement Device. *Journal of Diabetes Science and Technology*, 6, 380-386.

Wrieden, W., Peace, H., Armstrong, J. and Barton, K. (2003). A short review of dietary assessment methods used in National and Scottish Research Studies. Working Group on Monitoring Scottish Dietary Targets Workshop.

Yan, B., Jiye, A., Wang, G., Lu, H., Huang, X., Liu, Y., Zha, W., Hao, H., Zhang, Y., Liu, L., Gu, S., Huang, Q., Zheng, Y. and Sun, J. (2008). Metabolomic investigation into variation of endogenous metabolites in professional athletes subject to strength-endurance training. *Journal of Applied Physiology*, 106, 531-538.

Yates, T., Davies, M.J., Sehmi, S., Gorely, T. and Khunti, K. (2011). The Pre-diabetes Risk Education and Physical Activity Recommendation and Encouragement (PREPARE) programme study: are improvements in glucose regulation sustained at 2 years? *Diabetic Medicine*, 28, 1268–1271.

Young, J.F., Nielsen, S.E., Haraldsdóttir, J., Daneshvar, V., Lauridsen, S.T., Knuthsen, P., Crozier, A., Sandstrom, B. and Dragsted, L. O. (1999). Effect of fruit juice intake on urinary quercetin excretion and biomarkers of antioxidative status. *American Journal of Clinical Nutrition*, 69, 87-94.

Yungsheng, M.A., Olendzki, B.C., Pagoto, S.L., Hurley, T.G., Magner, R.P., Ockene, I.S., Schneider, K.L., Merriam, P.A. and Hebert, J.R. (2009). Number of 24-Hour Diet Recalls Needed to Estimate Energy Intake. *Annals of Epidemiology*, 19, 553-559.

Zhu, Y., Wang, P., Sha, W. and Sang, S. (2016). Urinary Biomarkers of Whole Grain Wheat Intake Identified by Non-targeted and Targeted Metabolomics Approaches. *Scientific Reports*, 6.

Zierath, J.R., He, L., Guma, A., Wahlstrom, O., Klip, A. and Wallberg-Henriksson, H. (1996). Insulin action on glucose transport and plasma membrane GLUT4 content in skeletal muscle from patients with NIDDM. *Diabetologia*, 39, 1180-1189.

Appendix A: Patient Invitation Letter

Practice details

Dear

We are writing to you because a blood test you have had in the last year or so, suggests you *may* be at risk of becoming diabetic. Unfortunately, being at risk of diabetes (called pre-diabetes) also increases your risk of developing heart disease or a stroke. However, recently it has become very clear that improving diet and increasing physical activity can massively reduce the risk of getting diabetes (even if your weight stays the same). This is good news! The enclosed leaflet has more information.

GP surgeries in North Ceredigion are offering a 30-minute consultation with a Lifestyle Advisor; in our surgery this will be provided by _____. Please will you fill in the short questionnaires on diet and physical activity, enclosed with this letter and bring these along to your appointment. During the consultation there is the option of giving some measurements such as blood pressure, weight, and having a blood test if not done recently. _____ will also have useful information to share with you and be able to signpost resources and make referrals to exercise classes or food education classes if needed.

If you would like to take part, then please make your 30-minute appointment by telephoning _____. If you wish to discuss anything *before* booking your appointment please contact _____ at the surgery. You are encouraged to bring someone with you.

GP surgeries in North Ceredigion with Hywel Dda Health Board have joined with health scientists at Aberystwyth University to evaluate this project; this will not affect your confidentiality or the service we provide. There will be the opportunity to find out more about this and future opportunities for research when you attend.

We are excited to be involved in this project and we hope you take this opportunity to make positive lifestyle changes which will benefit you in many ways.

Best wishes,

Your Team at ---------- Surgery

Appendix B: Diabetes Information Leaflet

Will I receive check-ups?

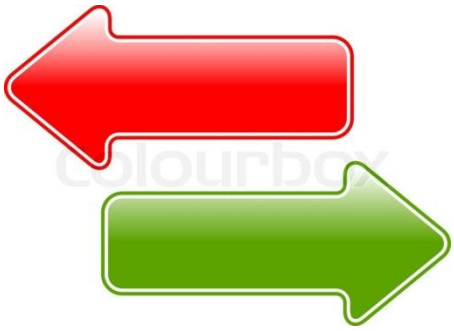
Yes, at least once a year around the time of your birthday, your GP will invite you for a check-up. Your blood will be tested and any changes to your blood sugar and what it means for your health will be discussed.

In most cases you only need to have your blood tested once a year as it takes time for significant changes to happen. Testing too often can lead to misleading results.

Feeling anxious?

Being told you have pre-diabetes can be difficult to hear, it does need to be taken seriously, but keep in mind that at this early stage, simple changes to your lifestyle can quite quickly help to prevent you developing diabetes.

Early diagnosis, treatment and good control of diabetes is vital to reduce the chances of developing serious diabetes



Diabetes symptoms

Some people may go on to develop diabetes. If you experience any of the symptoms below, please visit your GP: -

- Feeling very thirsty.
- Urinating frequently, particularly at night.
- Feeling very tired.
- Weight loss and loss of muscle.

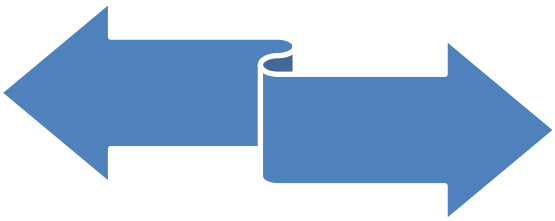
For general support and advice about lifestyle changes, call .us at Church Surgery on 01970 624855 and make an appointment with Mandy Glennie (Lifestyle advocate)

https://www.diabetes.org.uk for more information

Understanding

Pre-Diabetes.

People who have pre-diabetes have a blood sugar level that is higher than normal but not quite high enough to be classed as diabetic. Having pre-diabetes puts people at a high risk of developing Type 2 diabetes (which is controlled by diet or tablets). Unfortunately, people with pre-diabetes are also at almost double the risk of suffering from a heart attack and/or stroke compared to someone who has a normal blood sugar level.



The good news is something can be done to reduce the risk of pre-diabetes progressing to diabetes.

Find out more inside.....

What causes Pre- Diabetes?

There are three main things that contribute to Pre-Diabetes and the progression to Type 2 Diabetes: -

1. **What you eat and drink:** eating healthy food and drinking water can help you keep well. Irregular and unhealthy eating habits cause blood sugars to rise and fall.
2. **Being overweight:** this affects the body's ability to process sugar in your blood.
3. **What you do:** long periods of being inactive reduce the ability of your body to deal with sugar in your blood.

For some people, **the genes you inherit** from your family can also contribute to the development of borderline diabetes, your risk is increased if a close relative has Type 2 Diabetes.

Research suggests that up to 60% of Type 2 diabetes cases could be prevented by making healthy lifestyle changes.

What do I need to do?

If you are overweight the most important thing you can do is to stop high risk diabetes progressing is to lose weight. Any weight loss will make a difference, but if you are able to lose 5% of your body weight in the next few months you could reduce your chance of developing Type 2 diabetes by almost 60%.

Your starting weight	Amount to lose (5% of body weight).
8st 8 (55kg)	6lb (2.8kg)
9st 12 (57kg)	7lb (3kg)
11st (69kg)	8lb (3.5kg)
12 9 (81kg)	9lb (4kg)
14st 5 (92kg)	10lb (4.6kg)
15st 10 (95kg)	11lb (4.8kg)
17st 5 (111kg)	12lb (5.6kg)
19st 2(121kg)	13lb (6kg)
20st 6 (130kg)	14lb (6.5kg)
22st (139kg)	15.5lb (7kg

Eating a balance of different food groups and portion control will help control your blood sugar levels which reduced your risk of getting diabetes and heart disease.

Being more physically active increases how well your body can deal with and manage sugar which also helps you to control your blood sugar levels.

A combination of healthy eating and being more physically active will have the greatest impact. The more you do the more the risk of developing diabetes reduces.

What support is available?

When you are diagnosed with Pre-Diabetes you benefit from the free support of a Lifestyle Advisor.

- Lifestyle Advisors are in your GP Surgery
- They offer a range of times and places to meet
- They will work with you to assess your current lifestyle and help you to set some goals
- They can book you on to weight management and exercise programmes.

Appendix C: Starting the Conversation Questionnaire

Starting The Conversation: Diet

(Scale developed by: the Center for Health Promotion and Disease Prevention, University of North Carolina at Chapel Hill, and North Carolina Prevention Partners)

Over the past few months:

1.	How many times a week did you eat fast food meals or snacks?	Less than 1 time ☐ 0	1-3 times ☐ 1	4 or more times ☐ 2
2.	How many servings of fruit did you eat each day?	5 or more ☐ 0	3-4 ☐ 1	2 or less ☐ 2
3.	How many servings of vegetables did you eat each day?	5 or more ☐ 0	3-4 ☐ 1	2 or less ☐ 2
4.	How many regular fizzy pops or glasses of sweet tea did you drink each day?	Less than 1 ☐ 0	1-2 ☐ 1	3 or more ☐ 2
5.	How many times a week did you eat beans (like pinto or black beans) chicken or fish?	3 or more times ☐ 0	1-2 times ☐ 1	Less than 1 time ☐ 2
6.	How many times a week did you eat regular snack crisps or crackers (not low-fat)?	1 time or less ☐ 0	2-3 times ☐ 1	4 or more times ☐ 2
7.	How many times a week did you eat desserts and other sweets (not the low-fat kind)?	1 time or less ☐ 0	2-3 times ☐ 1	4 or more times ☐ 2
8.	How much margarine, butter, or meat fat do you use to season vegetables or put on potatoes, bread, or corn?	Very little ☐ 0	Some ☐ 1	A lot ☐ 2

SUMMARY SCORE (sum of all items): _____

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

(August 2002)

SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is supported to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation

methods available on the IPAQ website. If possible, please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

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.

6. 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.

6. 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***

6. 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.

6. 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

Appendix E: Participant Information Sheet

ABERYSTWYTH UNIVERSITY

IBERS

PARTICIPANT INFORMATION

Viability of urine collection and analysis for identifying nutrient content of diet in a free living environment

Introduction

Analysis of human urine is able to provide us with comprehensive information on the diet you consume up to 24 hours before providing a urine sample. While a single urine sample does not provide us with the information necessary to inform us on your long term dietary exposure, a number of urine samples over a prolonged period of time can give us a robust overview of your long term dietary exposure. Our aim is to determine how many urine samples are needed to give us a strong overview of an individual's habitual dietary exposure without causing unnecessary disturbances to their everyday lives. This information will help us to better understand the dietary habits of different individuals and how that relates to their long term health.

What is required of me if I agree to take part in the study?

If you agree to participate you will be provided with all the information necessary to successfully complete the study. Before beginning the study, you will be asked to complete a simple physical activity questionnaire and a diet questionnaire which will provide us with information on your current physical activity and exercise habits. You will then be provided with a urine sampling kit. You will be given verbal and written information on how to use the kit and at what times of day you must provide a urine sample.

Participation in this study requires you to provide two urine samples a day for a total of six weeks. You must provide us these urine samples every day, when you first wake up in the morning and before you go to bed in the evening. You will be provided with all of the equipment and instruction necessary to successfully collect and store your urine samples. You will be required to store your urine samples in a refrigerator for a maximum of 5 days before returning the samples to our laboratory in the envelopes provided to you. The kits you will use to store your urine samples are fully sealed and safe to store in the refrigerator. You do not need to alter your diet at any point during the 6 week period; you're free to consume any foods or drink you normally would. You will be required to complete an online electronic daily diet diary during the 6 weeks which records all of the food and drink you have consumed during the day. Upon completion of the 6 weeks, you will be asked to complete a questionnaire where you will have the opportunity to provide feedback on the ease of using the urine

analysis kit.

All the urine samples you have provided to us will be stored safely and securely for the duration of the study. Urine samples may be stored safely and securely until the host institute (IBERS) deems that they are no longer required. Urine samples will only be used for analysis which directly pertains to this study.

During analysis of urine, only biomarkers which relate to either acute or habitual dietary exposure will be measured. Urine samples will never be used to test for anything which is not a dietary biomarker.

Can I change my mind if I decide I no longer want to take part in the study?

You are free to leave the study at any time, without giving prior notification or a reason for doing so.

Other Information

Should you have any further questions regarding participation in the study, we will be pleased to answer them, and may be contacted on:

Dr Thomas Wilson
Email: tpw2@aber.ac.uk

Nick Gregory (PhD Candidate)
Email: nig5@aber.ac.uk

Prof John Draper
Email: jhd@aber.ac.uk

Dr Rhys Thatcher
Email: ryt@aber.ac.uk

If you have any complaints about any aspect of this study you can contact:

Dr Rhys Thatcher (Academic Supervisor to Nick Gregory)
Email: ryt@aber.ac.uk
Telephone: 01970628425

Institute Director of Research: Dr Alison Kingston-Smith
Email: ahk@aber.ac.uk
Telephone: 01970 823062

ABERYSTWYTH UNIVERSITY
IBERS

INFORMED CONSENT

Viability of urine collection and analysis for identifying nutrient content of diet in a free-living environment

NAME OF VOLUNTEER: …….....

PRINCIPAL INVESTIGATOR: Dr Thomas Wilson**,** Nick Gregory

ADDITIONAL INVESTIGATORS: Dr Rhys Thatcher, Prof John Draper

I have read the volunteer information sheet on the above study and have had the opportunity to discuss the details and ask questions. The investigator has explained to me the nature and purpose of the study to be undertaken. I understand fully what is proposed to be done. ☐

I have agreed to take part in the study as it has been outlined to me, but I understand that I am completely free to withdraw from the study at any time I wish. ☐

I understand that this study is part of a research project designed to promote scientific knowledge, which has been approved by Aberystwyth University Ethics Committee, and may be of no benefit to me personally. ☐

I understand that participation in the research study involves providing multiple urine samples ☐

I understand any data collected will be kept confidential, and only be seen by the principal investigators ☐

I hereby fully and freely consent to participate in the study which has been fully explained to me. ☐

SIGNATURE OF VOLUNTEER: ….……..….

Date: ….....

I confirm that I have explained to the volunteer named above, the nature and purpose of the tests to be undertaken.

SIGNATURE OF INVESTIGATOR: ….....

Date: ….....

Instructions for Urine Collection

A part of this study each week you will be given a urine collection kit, which will enable you to collect two urine samples a day, for 7 days.

The contents of the urine collection kit are;

1 x Sealed plastic box; containing 7 urine transfer straws and 14 white ended 4mL vacutainter tubes

1 x Empty sealed plastic box

1 x Small plastic bag

1 x 125mL urine collection up.

1. To collect a urine sample; take a clean collection straw and empty vacutainer tube.
2. Fill the collection cup with a mid-stream urine sample.
3. Submerge the tip of the transfer straw into the collection cup.
4. Insert the vacutainer tube into the transfer straw and keep pressure applied until tube is filled to capacity.
5. Label the tube according to the information on the sample log sheet
6. Place the labelled vacutainer tube in the empty plastic box, and store in your refrigerator.
7. There is one transfer straw for each day. After providing your morning

sample, simply rinse the straw with cold water and allow to dry at room temperature. After using the straw again in the evening, rinse the straw with cold water and place in the plastic bag provided. You can return the bag of used straws each week when you drop off your urine samples, so they can be disposed safely.

8. Empty the remaining contents of the urine collection cup into the toilet and rinse the collection cup with cold water and allow to dry. The collection cup will need to be re-used throughout the week.

Appendix H: Urine Collection Feedback Questionnaire

The following questions relate to the use of the urine sampling kit you have been using over the past six weeks. Please indicate your answer in each case by circling the appropriate response and when further detail is requested please use the space provided.

1. Did the time of day you were required to provide a urine sample cause disruption to your usual daily routine?

   YES NO

   If you answered **YES** could you please indicate why this was in one or two sentences

2. Was the urine sampling kit easy to use?

   YES NO

   If you answered **NO** could you please indicate why this was in one or two sentences

3. Did you use the urine sampling kit successfully every time?

   YES NO

   If you answered **NO** could you please indicate why this was in one or two sentences

4. Was it easy to store your urine samples once they had been collected?

   YES NO

   If you answered **NO** could you please indicate why this was in one or two sentences

5. Having completed this process and knowing what it involves, would you be prepared to use the urine sampling kit twice a day over a 6 week period for research purposes in the future?

YES NO

If you answered **NO** could you please indicate why this was in one or two sentences

6. Please provide any further comments and feedback here

Thank you for your time

Appendix I: Participant Information Sheet

Participant Information

Monitoring the metabolic response of human urine following a bout of prolonged moderate intensity exercise

Introduction

It is now established that analysis of human urine is able to provide us with comprehensive information on the diet you consume up to 24 hours before providing a urine sample. Previous work in the Institute of Biological, Environmental and Rural Sciences (IBERS) has investigated the feasibility of urine collection in a free living environment and the subsequent analysis for identifying nutrient content of diet. This work has demonstrated that urine can be collected in the home successfully and analysis of the samples provides robust information on habitual diet. Some evidence suggests that exercise can cause changes to human urine which may also influence the dietary information present in the urine. Given the general recommendations that individuals should complete at least 150 minutes of exercise each week in order to maintain a healthy lifestyle, it is our aim to investigate the effects of exercise on human urine and how that may influence our ability to monitor diet effectively.

What is required of me if I agree to take part in the study?

If you agree to participate, you will be provided with all the information necessary to successfully complete the study. You will need to sign a consent form and complete health and physical activity questionnaires. The study requires you to visit the laboratory on three separate occasions. We will ask you to drink at least a pint (or 500 ml) of ordinary tap water before visiting the laboratory to ensure that you are properly hydrated. You must arrive at the laboratory for visit 2 and 3 following an overnight fast of at least 8 hours.

During the first visit (1), we will assess your body composition using a DEXA scanner. We will then determine your maximal oxygen uptake value ($\dot{V}O_2max$), which we will use to calculate the appropriate exercise intensity for subsequent visits. This is a maximal test where you will be asked to cycle at a fixed rev rate until you fatigue, while the workload gets progressively harder, and normally lasts between 10 and 15 minutes. You will breathe into a mouthpiece which is connected to a computer, while wearing a nose clip throughout the exercise, to allow us to measure your oxygen uptake.

At least 48 hours after the first visit; you will begin the main experiment. You are required to visit the laboratory in the morning to complete two experimental trials following an overnight fast of at least 8 hours. You must record your diet on the evening prior to each visit using a simple dietary record provided to you. The two trials will be separated by at least 7 days. You must provide a urine sample when you wake up prior to each trial. We will give you all the equipment you need to collect the sample at home. When you arrive at the laboratory, you will be asked to provide another urine sample in a private room. A heart rate monitor will be

positioned on your chest. You will then be asked to either (1) exercise at a moderate intensity for 30 minutes on a cycle ergometer or (2) sit quietly for 30 minutes (you are free to bring reading materials or electronic devices for this trial). You are free to drink water throughout each trial, if you wish. We will measure your heart rate in both trials every 5 minutes .We will ask you how hard you think you're working (usual a simple visual scale) every 5 minutes in the exercise trial. The order in which you complete the trials will be randomised by the study investigators. Once complete, you will provide a second urine sample in private. You will then be free to use the shower and changing facilities in the building before you leave, if you wish. Please allow approximately 90 minutes for these visits.

It is important to maintain similar conditions during each trial. We will therefore ask you to refrain from unaccustomed strenuous activity, and avoid drinking alcohol for 24 hours prior to each visit. You should consume foods of similar quantity and nature in the evening before each trial.

Can I change my mind if I decide I no longer want to take part in the study?

You are free to leave the study at any time, without giving prior notification or a reason for doing so.

Other information

Should you have any further questions regarding participation in the study, we will be pleased to answer them, and may be contacted on:

Nick Gregory (PhD Candidate)
Email: nig5@aber.ac.uk

Dr Rhys Thatcher
Email: ryt@aber.ac.uk

If you have any complaints about any aspect of this study you can contact:

Dr Rhys Thatcher (Academic Supervisor to Nick Gregory)
Email: ryt@aber.ac.uk
Telephone: 01970628425

Institute Director of Research: Dr Alison Kingston-Smith
Email: ahk@aber.ac.uk

Appendix J: Informed Consent

ABERYSTWYTH UNIVERSITY
IBERS

INFORMED CONSENT

Monitoring the metabolic response of human urine following a bout of prolonged exercise

NAME OF VOLUNTEER: .....

PRINCIPAL INVESTIGATOR: Nick Gregory (PhD Candidate)

ADDITIONAL INVESTIGATORS: Dr Rhys Thatcher, Dr Thomas Wilson

I have read the volunteer information sheet on the above study and have had the opportunity to discuss the details and ask questions. The investigator has explained to me the nature and purpose of the study to be undertaken. I understand fully what is proposed to be done. ☐

I have agreed to take part in the study as it has been outlined to me, but I understand that I am completely free to withdraw from the study at any time I wish. ☐

I understand that this study is part of a research project designed to promote scientific knowledge, which has been approved by Aberystwyth University Ethics Committee, and may be of no benefit to me personally. ☐

I understand this study involves a whole body scan using dual energy x-ray absorptiometry (DXA) which equates to 1 days' worth of natural radiation exposure or 1/10th of a chest x-ray ☐

I understand that participation in the research study involves a maximal exercise test and two further trials involving submaximal exercise, both of which require attendance following an overnight fast ☐

I understand any data collected will be kept confidential, and only be seen by the principal investigators ☐

I hereby fully and freely consent to participate in the study which has been fully explained to me. ☐

SIGNATURE OF VOLUNTEER: .....

Date: .....

I confirm that I have explained to the volunteer named above, the nature and purpose of the tests to be undertaken.

SIGNATURE OF INVESTIGATOR: .....

Date: .....

Appendix K: 24 Hour Food Diary

For the day BEFORE urine collections please note everything you eat, without specifying the amounts. Thank you

Example	**24 hr before test : breakfast, lunch, snacks dinner**
Date	/ /2017
Breakfast	**Breakfast**
Wholegrain toast butter jam Coffee Skimmed milk …	
Lunch	**Lunch**
White bread Bacon Lettuce Tomato Mayonnaise Fruit salad shortcake	
snacks	**snacks**
Coca Cola Apple Tea with milk …	
Dinner	**Dinner**
Steak pie Chips Peas Beer	

Vanilla ice cream Chocolate fondant	

Appendix L: Participant Information Sheet

Participant Information Sheet

A comparison of blood sugar values between a commercial point of care analyser and a hospital pathology analyser

You are being invited to take part in a research study. Before you make a decision to take part, it is important to explain why the research is being done and what it will involve. Please take your time to read the following information carefully. Please do not hesitate to contact the research team if you would like any additional information about the study. It is important that you fully understand what the study will involve, so take your time when deciding if you would like to take part.

What is the purpose of the study?

Pre-diabetes is defined as a higher than normal blood sugar level and is associated with an increased risk of developing diabetes. A number of research projects currently taking place at Aberystwyth University are examining the effects of lifestyle interventions on blood sugar control in participants at risk of diabetes and other chronic diseases. Blood sugar is regularly monitored in these studies using a commercial point of care analyser located within the Wellbeing and Health Assessment Research Unit (WARU) at Aberystwyth University.

The aim of this study is to compare blood sugar results between the point of care analyser used at the WARU laboratory and results provided by a local NHS Pathology Laboratory. Comparison of the results from both laboratories will help us to understand if there are any differences in blood sugar values and if so, to what extent they different.

Do you have to participate in the study?

It is up to you to decide if you would like to participate or not. You will be asked to sign a consent form at the start of the study. If you decide to take part but then for any reason wish to withdraw your participation, you can do so at any point without giving reason or prior notice, this will not stop you from participating in any future research studies.

What will happen if you agree to take part in the study?

You will be asked to visit the Carwyn James Building at Aberystwyth University on one occasion at a time of your choice. When you arrive, the researcher will explain the purpose of the study, provide you with the opportunity to ask any questions and then ask you to sign the consent form. A blood sample will be obtained from your arm and collected into two 4 ml tubes and one 6 ml tube. The

blood sample will be collected by a trained member of the research team. Another blood sample will then be collected from your index finger.

The first tube we collect will be sent to Bronglais Pathology Laboratory, Aberystwyth, for them to analyse. The second and third tubes and the blood sample collected from your finger will be analysed at the WARU laboratory.

What will you have to do?
You are free to eat and drink what you wish prior to your visit to the WARU. However, please refrain from alcohol consumption and any vigorous exercise for 24 hours prior to your visit.

Will there be any negative side effects if you participate?
There will be no long term side effects if you participate in the study. Blood tests can cause some mild discomfort when being collected however all members of the research team who are responsible for blood collection are highly trained and experienced. Attendance to your WARU visit will alter your daily routine however the research team will do their best to organise a date and time most convenient for you.

Will you benefit from taking part in the study?
By participating in the study, you will know your current blood sugar value which will provide an indication as to your current risk of diabetes.

What happens if something goes wrong?
If you feel that you have a reason to complain about any aspect of the study or the way you have been treated, you can do so by instigating Aberystwyth University complaints procedures. Contact information is at the end of this document.

Will my participation in the study be kept confidential?
All the information about you will be kept strictly confidential throughout the study.

What will happen with the results generated by the research?
The findings from our study may be published in scientific journals and presented at conferences. However all the results will be anonymous and will not identify you as a participant in any way. We will provide you with your results either in person or by telephone at your request. If your results suggest an increased risk of diabetes, we will recommend you visit your GP for further investigation.

Who is organising and funding the research?
Aberystwyth University have organised and are funding the research study.

Who has reviewed the study?
Our research study has been reviewed by academic scientific researchers and approved by Aberystwyth University Ethics Procedures.

Contact Information:
Investigator
Nicholas Gregory BSc (Hons), MPhil
PhD Researcher
Email: nig5@aber.ac.uk
Telephone: 01970 622075

Academic Supervisor
Dr Rhys Thatcher BSc (Hons), MSc, SFHEA, FBASES
Reader in Exercise Physiology
Email: ryt@aber.ac.uk
Telephone: 01970 628630

If you have a complaint about the research, you can contact:

Dr Alison Kingston Smith
IBERS Director of Research
Email: ahk@aber.ac.uk
Telephone: 01970 823062

Research Site Address:

Wellbeing Assessment Research Unit
Carwyn James Building
Aberystwyth University
Aberystwyth
SY23 3FD

Appendix M: Informed Consent

ABERYSTWYTH UNIVERSITY
-Institute of biological, Environmental and Rural Sciences-
-Wellbeing Assessment Research Unit-

INFORMED CONSENT

A comparison of blood sugar values between a commercial point of care analyser and an NHS hospital pathology analyser

NAME OF PARTICIPANT: .....

INVESTIGATOR: Mr Nick Gregory

ADDITIONAL INVESTIGATORS: Dr Rhys Thatcher

I have read the participant information sheet about the above study and have had the opportunity to discuss the details and ask questions. The investigator has explained to me the nature and purpose of the study to be undertaken. I understand fully what is expected of me as a participant. ☐

I have agreed to take part in the study as it has been outlined to me, but I understand that I am free to withdraw from the study at any time I wish. ☐

I understand that this study is part of a research project designed to promote scientific knowledge and may be of no benefit to me personally. ☐

I understand that my anonymised personal information will be stored confidentially for the entire duration of the study and will be retained by the research team after completion of the study ☐

☐

I hereby fully and freely consent to participate in the study which has been fully explained to me.

SIGNATURE OF PARTICIPANT: .....

Date: .....

I confirm that I have explained to the volunteer named above, the nature and purpose of the tests to be undertaken.

SIGNATURE OF INVESTIGATOR: .....

Appendix N: Blood Test Invitation Letter

Examining the feasibility of conducting a randomised control trial to evaluate the effectiveness of a focussed 15-minute one-to-one consultation to improve blood glucose control in pre-diabetes

Dear (Insert Patient Name),

We are writing to invite you to the surgery to take part in our diabetes screening programme. Our patient records suggest that you *may* be at risk of developing diabetes. A simple blood test can help us to identify if you are at risk or not.

Being at risk of diabetes (called pre-diabetes) can increase your risk of developing heart disease and/or stroke risk. However, recently it has become very clear that improving your lifestyle, such as eating more healthily and exercising more can massively reduce the chances of getting diabetes (even if your weight stays the same). This is good news! It is important that we test you so we can monitor your diabetes risk in the future.

We have an exciting partnership with Aberystwyth University who are interested in preventing diabetes in people who live in and around Ceredigion. Based on your results, you may be able to take part in their work.

If you would like to book an appointment at the surgery to be tested for diabetes, please call us on 01970 624 855 or visit the surgery in person.

Appendix O: Study Invitation Letter

Examining the feasibility of conducting a randomised control trial to evaluate the effectiveness of a focussed 15-minute one-to-one consultation to improve blood glucose control in pre-diabetes

Dear (Insert Patient Name)

We are writing to you because a blood test you have had recently suggests you *may* be at risk of becoming diabetic. Being at risk of diabetes (called pre-diabetes) can increase your risk of developing heart disease and/or stroke risk. However, recently it has become very clear that improving diet and increasing physical activity can massively reduce the risk of getting diabetes (even if your weight stays the same). This is good news! The enclosed leaflet has more information.

Scientists at Aberystwyth University are conducting a research study which, in part, is examining the effectiveness of a 15 minute one to one consultation which focuses on preventing diabetes in people who are at risk. You have been sent this letter because you may be eligible to participate in their research. You can read more about the study in the accompanying Participant Information Sheet.

You can contact the research team at Aberystwyth University if you would like to participate in the research study using the contact information on the participant information sheet. It is important to highlight that at this stage, Aberystwyth University have no information relating to you and will only be made aware of your diabetes risk if you choose to participate in their research. If you wish to discuss anything *before* participating, you can contact the research team who will be happy to answer any questions. Choosing not to take part will not affect you rights as a patient in any way and will not stop you from participating in future research studies should you wish to.

Church Surgery and Aberystwyth University are working together to help improve diabetes care in Ceredigion. We are excited to be involved in this project and we hope you take this opportunity to make positive lifestyle changes which will benefit you in many ways.

Appendix P: Participant Information Sheet

Participant Information Sheet

Examining the feasibility of conducting a randomised control trial to evaluate the effectiveness of a 15-minute one-to-one consultation to improve blood glucose control in pre-diabetes

You are being invited to take part in a research study. Before you make a decision to take part, it is important to explain why the research is being done and what it will involve. Please take your time to read the following information carefully and discuss it with friends and family if you wish. Please do not hesitate to contact the research team if you would like any additional information about the study. It is important that you fully understand what the study will involve, so take your time when deciding if you would like to take part.

What is the purpose of the study?

Pre-diabetes is defined as a higher than normal blood sugar level and is associated with an increased risk of developing diabetes. As the complications of diabetes, such as heart problems blindness and risk of amputation, develop over time it is beneficial to delay the onset of the condition for as long as possible. Researchers at Aberystwyth University aim to assess the feasibility of running a large scale scientific trial to evaluate the effectiveness of a 15-minute one-to-one consultation to improve blood sugar control in people with pre-diabetes and delay the development of diabetes.

Why have you been chosen to participate?

You have been chosen as a potential participant because your GP surgery has identified you as someone who is at increased risk of developing diabetes based on a recent blood test you have provided.

Do you have to participate in the study?

It is up to you to decide if you would like to participate or not. Deciding not to participate will not affect your rights as a patient in any way. It is important to highlight that the research team will only be made aware of your diabetes risk if you decide to take part. At this stage, only your GP surgery knows of your risk. If you do decide to take part, you will need to contact the research team to book your first appointment. You will be asked to sign a consent form at the start of the study. If you decide to take part but then for any reason wish to withdraw your participation, you can do so at any point without giving reason or prior notice, this will not affect your rights as a patient or stop you from participating in any future research studies.

What will happen if you agree to take part in the study?
If you would like to participate, you can arrange your first visit by contacting the research team. You can find contact details at the end of this information sheet.

You will attend the Wellbeing Assessment Research Unit (WARU) at the Carwyn James Building, Aberystwyth University on 3 separate occasions. After your first visit, you will come back to the WARU after 3 months and 6 months. During these visits, we will record some physical data including your weight, height, waist circumference, blood pressure and body composition. We will take a blood sample from your arm to test your blood sugar level and analyse it further to understand what kinds of food you consume. We will ask you to fill out some questionnaires which relate to your diet, level of physical activity and general wellbeing. After your first visit to the WARU, the research team may inform you that you have been selected to visit your GP surgery to attend a pre-diabetes consultation with a practice nurse. Participants who attend a pre-diabetes consultation will be randomly selected by an electronic system.

What will you have to do?
You will also be asked to collect some urine samples when you are at home using a simple urine collection kit so we can analyse your diet. You will need to store the samples in your fridge before returning them by free post or in person. Full instructions will be provided to you on how to use the collection kit.

You might also be asked to record your levels of physical activity for one week after each visit your make to the WARU. We will provide you with a simple activity tracker which sits around your waist like a belt. You only need to wear this during the hours you are awake. You do not need to wear the tracker when it is not feasible to do so (e.g. when you are washing). Participants who are asked to track their physical activity levels will be picked randomly.

Will there be any negative side effects if you participate?
There will be no long term side effects if you participate in the study. Blood tests can cause some mild discomfort when being collected however all members of the research team who are responsible for blood sampling are highly trained and experienced. Attendance to each WARU visit will alter your daily routine however the research team will do their best to organise a date and time most convenient for you.

Will you benefit from taking part in the study?
By participating in the study, you will gain a better understanding and increase your knowledge of the factors that need to be controlled in managing your blood sugar levels.

What happens if something goes wrong?
If you feel that you have a reason to complain about any aspect of the study or the way you have

been treated, you can do so by instigating NHS complaints procedures or Aberystwyth University complaints procedures. Contact information is at the end of this document.

Will my participation in the study be kept confidential?
All the information about you will be kept strictly confidential throughout the study. Your GP will be made aware of your participation if you are randomly selected to receive a consultation. Similarly, we will inform your GP if we suspect you have developed diabetes, with your permission.

What will happen with the results generated by the research?
The findings from our study may be published in scientific journals and presented at conferences. However all the results will be anonymous and will not identify you as a participant in any way. You are welcome to request a summary of our findings once the study is complete.

Who is organising and funding the research?
Aberystwyth University and Church Surgery (Aberystwyth) have organised the research study. Aberystwyth University is funding the research.

Who has reviewed the study?
Our research study has been reviewed by academic scientific researchers and medical professionals. A NHS Research Ethics Committee has reviewed and accepted the study's proposals.

Contact Information:

Chief Investigator
Nicholas Gregory BSc (Hons), MPhil
PhD Researcher
Email: nig5@aber.ac.uk
Telephone: 01970 622075

Academic Supervisor
Dr Rhys Thatcher BSc (Hons), MSc, SFHEA, FBASES
Reader in Exercise Physiology
Email: ryt@aber.ac.uk
Telephone: 01970 628630

If you have a complaint about the research, you can contact:

Dr Alison Kingston Smith
IBERS Director of Research
Email: ahk@aber.ac.uk
Telephone: 01970 823062

Research Site Addresses:

Wellbeing Assessment Research Unit
Carwyn James Building
Aberystwyth University
Aberystwyth
SY23 3FD

Church Surgery
Portland Street
Aberystwyth
SY23 2DX

Appendix Q: Informed Consent

ABERYSTWYTH UNIVERSITY
-Institute of biological, Environmental and Rural Sciences-
-Wellbeing Assessment Research Unit-

INFORMED CONSENT

Examining the feasibility of conducting a randomised control trial to evaluate the effectiveness of a 15-minute one-to-one consultation to improve blood glucose control in pre-diabetes

NAME OF PARTICIPANT: .....

CHIEF INVESTIGATOR: Mr Nick Gregory

ADDITIONAL INVESTIGATORS: Dr Rhys Thatcher, Dr Simon Payne, Dr Thomas Wilson, Mr Sam Chapman, Dr Heather Cox

I have read the participant information sheet (Version 1, 22/1/18) about the above study and have had the opportunity to discuss the details and ask questions. The investigator has explained to me the nature and purpose of the study to be undertaken. I understand fully what is expected of me as a participant. ☐

I have agreed to take part in the study as it has been outlined to me, but I understand that I am completely free to withdraw from the study at any time I wish. ☐

I understand that this study is part of a research project designed to promote scientific knowledge, which has been approved by an NHS Research Ethics Committee, and may be of no benefit to me personally. ☐

I understand that my anonymised personal information will be stored confidentially for the entire duration of the study and may be retained by the research team even if I decide to withdraw my participation. ☐

I understand that my GP will be made aware of my participation in the study if I am to receive a consultation. ☐

I understand that my GP will be informed if the research team suspect I have become diabetic during my participation in the study. The research team will inform my GP with my permission. ☐

I hereby fully and freely consent to participate in the study which has been fully explained to me. ☐

SIGNATURE OF PARTICIPANT: .....

Date: .....

I confirm that I have explained to the volunteer named above, the nature and purpose of the tests to be undertaken.

SIGNATURE OF INVESTIGATOR: .....

Date: .....

Appendix R: Readiness to Change Questionnaire (Extracts from)

A. WEIGHT CHANGE READINESS.

1. Are you currently trying to lose weight?

   - Yes, I am trying to lose weight ☐
   - No, but I am trying to keep from gaining weight ☐
   - No, I am not making any attempts to control my weight now ☐

2. If you are NOT currently trying to lose weight or avoid gaining weight, is this something you plan to do in the future?

   - Yes, I plan to start within the next month ☐
   - Yes, I plan to start within the next six months ☐
   - No, I have no plans right now for starting a weight control plan ☐
   - I am already following a weight control plan ☐

B. DIET CHANGE READINESS.

Please answer the following questions about the things you may or may not do to control your blood glucose using food.

1. Are you currently trying to follow a diet plan in order to better control your blood glucose?

   - Yes, I have a plan I am trying to follow ☐
   - No, I am not following a plan but I am conscious of how food affects my blood sugar ☐
   - No, I really do not pay attention to how food affects my blood sugar ☐

2. If you are following a plan, what kind of plan are you using?

   - I do not use any kind of diet plan ☐
   - Carbohydrate counting ☐
   - The food exchange system ☐
   - Total available glucose (TAG) ☐
   - Healthy foods ☐
   - The food guide pyramid ☐
   - Fat gram counting ☐
   - Other ☐

3. If you are NOT currently following a diet plan or meal plan to better control your blood glucose, is this something you plan to do in the future?

- I am already following a diet or meal plan ☐
- Yes, I plan to start within the next month ☐
- Yes, I plan to start within the next six months ☐
- No, I have no plans right now for starting to follow a diet or meal plan ☐

Rate questions C and D with the following scale:

(1) Never,
(2) 1 time per month or less,
(3) 2-3 times per month,
(4) 1-2 times per week,
(5) 4-6 times per week,
(6) 1 or more times per day

C. DIET BARRIERS.

The next set of questions has to do with when and where overeating, unplanned snacks and poor food choices occur. Each question asks about a particular kind of situation. Think about these behaviors and how much of a problem each situation is for you in trying to control these behaviors.

During the past 3 months, how often have you had a problem with each of the following?

1. Eating problems when feeling stressed, anxious, depressed, angry, or bored ☐
2. Eating problems because of hunger or food cravings ☐
3. Eating problems because family or friends tempt you or are not very supportive of your efforts to eat right ☐
4. Eating problems when eating away from home (e.g. fast food, restaurants) ☐
5. Eating problems because you feel deprived due to trying to follow a diet ☐
6. Eating problems because you feel discouraged due to lack of results (e.g. no weight loss, high blood sugars) ☐
7. Eating problems because you are too busy with family, work, or other responsibilities ☐

D. EXERCISE BARRIERS. **The next set of questions has to do with why you find it hard to start exercising or hard to stick with an exercise plan.**

During the past 3 months, how often have you had trouble exercising because of each of the following?

1. Feeling stressed, anxious, depressed, angry, or bored ☐
2. Exercise and physical activity cause pain and discomfort for me ☐
3. Family or friends are not very supportive ☐
4. When away from home (e.g. on vacation, business trips, at relatives) ☐
5. My daily schedule (waking, go to bed, eat, work, etc) is different from one day to the next ☐
6. Feel discouraged due to lack of results (e.g. no weight loss, high blood sugars) ☐
7. Being too busy with family, work, or other responsibilities ☐

Subscale Description and scoring:

A (Weight Change Readiness): readiness for change for attempting weight loss; “Pre-contemplation” if A2=3 or A1=3; “contemplation” if A2=2; “preparation” if A2=1; “action” if A1>1 or A2=4

B (Diet Change Readiness): Readiness for change for attempting dietary self-management; “Pre-contemplation” if B3=4; “contemplation” if B3=3; “preparation” if B3=2; “action” if B1=1 B2≥1 or B3=1

C (Diet Barriers): Environmental, social, and emotional factors interfering with attempts to adhere to regimen; Sum C1-C7 (Higher score indicates more frequent barriers)

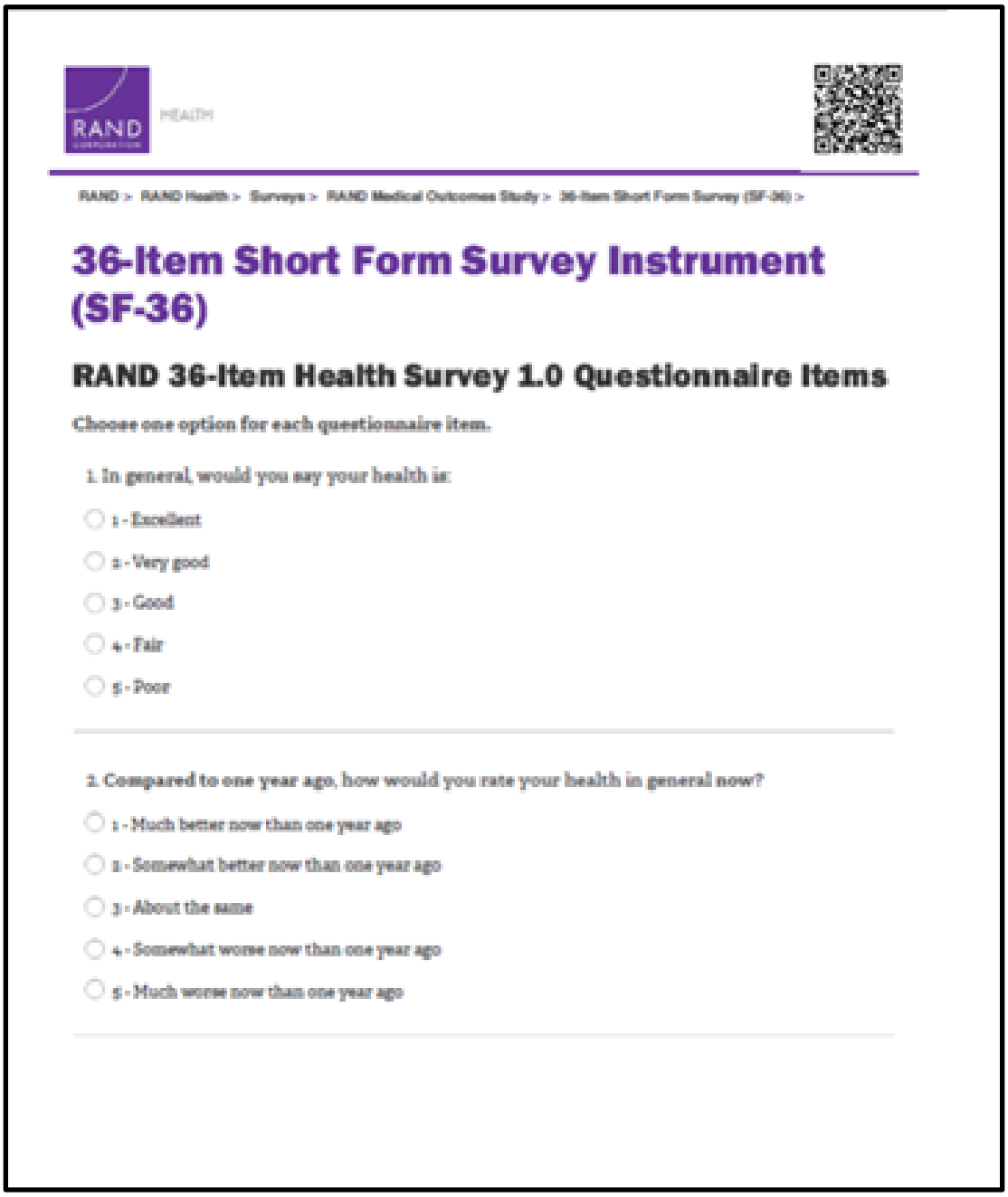
D (Exercise Barriers): Environmental, social and emotional factors interfering with attempts to adhere to regimen; Sum D1-D7 (Higher score indicates more frequent barriers)

The PDQ survey and scoring protocol may both be obtained from the authors by request or may be downloaded from the internet (PDQ survey: http://healthbehavior.psy.vanderbilt.edu/PDQ.pdf A PASW syntax file is available upon request.

The questions in the above questionnaire have been drawn from the validated Personal Diabetes Questionnaire and have not been changed or altered in any way.

Stetson, B., Schlundt, D., Rothschild, C., Floyd, J.E., Rogers, W. and Mokshagundam, S.P. (2011). Development and validation of The Personal Diabetes Questionnaire (PDQ): A measure of diabetes self-care behaviors, perceptions and barriers. Diabetes Research and Clinical Practice, 321-322.

Appendix S: SF-36 Questionnaire



RAND HEALTH

RAND > RAND Health > Surveys > RAND Medical Outcomes Study > 36-Item Short Form Survey (SF-36) >

36-Item Short Form Survey Instrument (SF-36)

RAND 36-Item Health Survey 1.0 Questionnaire Items

Choose one option for each questionnaire item.

1. In general, would you say your health is:

- 1 - Excellent
- 2 - Very good
- 3 - Good
- 4 - Fair
- 5 - Poor

2. Compared to one year ago, how would you rate your health in general now?

- 1 - Much better now than one year ago
- 2 - Somewhat better now than one year ago
- 3 - About the same
- 4 - Somewhat worse now than one year ago
- 5 - Much worse now than one year ago

The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
3. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	○ 1	○ 2	○ 3
4. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	○ 1	○ 2	○ 3
5. Lifting or carrying groceries	○ 1	○ 2	○ 3
6. Climbing **several** flights of stairs	○ 1	○ 2	○ 3
7. Climbing **one** flight of stairs	○ 1	○ 2	○ 3
8. Bending, kneeling, or stooping	○ 1	○ 2	○ 3
9. Walking **more than a mile**	○ 1	○ 2	○ 3
10. Walking **several blocks**	○ 1	○ 2	○ 3
11. Walking **one block**	○ 1	○ 2	○ 3
12. Bathing or dressing yourself	○ 1	○ 2	○ 3

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

	Yes	No
13. Cut down the **amount of time** you spent on work or other activities	○ 1	○ 2
14. **Accomplished less** than you would like	○ 1	○ 2
15. Were limited in the **kind** of work or other activities	○ 1	○ 2
16. Had **difficulty** performing the work or other activities (for example, it took extra effort)	○ 1	○ 2

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

	Yes	No
17. Cut down the **amount of time** you spent on work or other activities	○ 1	○ 2
18. **Accomplished less** than you would like	○ 1	○ 2
19. Didn't do work or other activities as **carefully** as usual	○ 1	○ 2

20. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

- ○ 1 - Not at all
- ○ 2 - Slightly
- ○ 3 - Moderately
- ○ 4 - Quite a bit
- ○ 5 - Extremely

21. How much **bodily** pain have you had during the **past 4 weeks**?

- 1 - None
- 2 - Very mild
- 3 - Mild
- 4 - Moderate
- 5 - Severe
- 6 - Very severe

22. During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

- 1 - Not at all
- 2 - A little bit
- 3 - Moderately
- 4 - Quite a bit
- 5 - Extremely

These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the **past 4 weeks**...

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
23. Did you feel full of pep?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
24. Have you been a very nervous person?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
25. Have you felt so down in the dumps that nothing could cheer you up?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
26. Have you felt calm and peaceful?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
27. Did you have a lot of energy?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
28. Have you felt downhearted and blue?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
29. Did you feel worn out?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
30. Have you been a happy person?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
31. Did you feel tired?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6

32. During the **past 4 weeks**, how much of the time has **your physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

○ 1 - All of the time

○ 2 - Most of the time

○ 3 - Some of the time

○ 4 - A little of the time

○ 5 - None of the time

How TRUE or FALSE is each of the following statements for you.

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
33. I seem to get sick a little easier than other people	○ 1	○ 2	○ 3	○ 4	○ 5
34. I am as healthy as anybody I know	○ 1	○ 2	○ 3	○ 4	○ 5
35. I expect my health to get worse	○ 1	○ 2	○ 3	○ 4	○ 5
36. My health is excellent	○ 1	○ 2	○ 3	○ 4	○ 5

Appendix T: Food Frequency Questionnaire

FFQ Questionnaire

ID:_____

Phase:_____

Thank you for agreeing to take part in this study.

This is a simple questionnaire designed to help us understand what foods you normally eat. It is not a test, so there are no right or wrong answers. It is your usual diet we are interested in.

All information will be treated in the strictest confidence.

Please turn over and read the instructions for answering questions before completing the questionnaire. Thank you for your time.

ID	M	E	D	E	s	v	V
	Date	d	d	m	m	y	y

For office use only

About the food you eat

The following questions are about the food you usually eat and how often you eat certain foods. Please read the following instructions before answering the questions.

For each food there is an amount shown, either a "medium serving" or a common household unit such as a slice or teaspoon. Please put a tick (√) in the box to indicate how often, **on average**, you have eaten the specified amount of each food **during the past 6 months**.

EXAMPLE:

For white bread the amount is one slice, so if you ate 4 or 5 slices a day, you should put a tick in the column headed "4-5 per day".

FOODS & AMOUNTS	AVERAGE USE LAST 6 months								
BREAD & SAVOURY BISCUITS (one slice or biscuit)	Never or less than once / month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
White bread and rolls								✔	

EXAMPLE:

For chips, the amount is a "medium serving", so if you had a helping of chips twice a week you should put a tick in the column headed "2-4 per week".

FOODS & AMOUNTS	AVERAGE USE LAST 6 months								
POTATOES, RICE & PASTA (medium serving)	Never or less than once / month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Chips				✓					

It is your turn to answer now!

Please put a tick (√) in each box to indicate how often, **on average**, you have eaten each food **during the past 6 months.**

Please estimate your average food use as best you can, and please answer every question - do not leave **ANY** lines blank. **Please put a tick (√) on every line.**

FOODS & AMOUNTS	**AVERAGE USE LAST 6 months**								
19. MEAT & FISH ***(medium serving)***	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Beef: roast, steak, mince, stew casserole, curry or bolognese	1	2	3	4	5	6	7	8	9
Beefburgers	1	2	3	4	5	6	7	8	9
Pork: roast, chops, stew, slice or curry	1	2	3	4	5	6	7	8	9
Lamb: roast, chops, stew or curry	1	2	3	4	5	6	7	8	9
Chicken, turkey or other poultry: including fried, casseroles or curry									

	1	2	3	4	5	6	7	8	9
Bacon	1	2	3	4	5	6	7	8	9
Ham	1	2	3	4	5	6	7	8	9
Corned beef, Spam, luncheon meats	1	2	3	4	5	6	7	8	9
Sausages	1	2	3	4	5	6	7	8	9
Savoury pies, e.g. meat pie, pork pie, pasties, steak & kidney pie, sausage rolls, scotch egg	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (√) on EVERY line

PLEASE PUT A TICK (√) ON EVERY LINE.

FOODS & AMOUNTS	**AVERAGE USE LAST 6 months**								
19. MEAT & FISH, (continued) ***(medium serving)***	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Liver, liver pate, liver sausage	1	2	3	4	5	6	7	8	9
Fried fish in batter, as in fish and chips	1	2	3	4	5	6	7	8	9
Fish fingers, fish cakes	1	2	3	4	5	6	7	8	9
Other white fish, fresh or frozen, e.g. cod, haddock, plaice, sole, halibut	1	2	3	4	5	6	7	8	9
Oily fish, fresh or canned, e.g. mackerel, kippers, tuna, salmon, sardines, herring	1	2	3	4	5	6	7	8	9
Shellfish, e.g. crab, prawns, mussels	1	2	3	4	5	6	7	8	9

20. BREAD & SAVOURY BISCUITS ***(one slice or biscuit)***									
White bread and rolls	1	2	3	4	5	6	7	8	9
Scones, teacakes, crumpets, muffins or croissants	1	2	3	4	5	6	7	8	9
Brown bread and rolls	1	2	3	4	5	6	7	8	9
Wholemeal bread and rolls	1	2	3	4	5	6	7	8	9
Cream crackers, cheese biscuits	1	2	3	4	5	6	7	8	9
Pitta bread, naan bread, chapati	1	2	3	4	5	6	7	8	9
Garlic bread	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (√) on EVERY line

PLEASE PUT A TICK (√) ON EVERY LINE.

FOODS & AMOUNTS	**AVERAGE USE LAST 6 months**								
21. CEREALS ***(one bowl)***	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Porridge, Readybrek	1	2	3	4	5	6	7	8	9
Sugar coated cereals e.g. Sugar Puffs, Cocoa Pops, Frosties	1	2	3	4	5	6	7	8	9
Non-sugar coated cereals e.g. Cornflakes, Rice Crispies	1	2	3	4	5	6	7	8	9
All Bran, Bran Flakes, Muesli	1	2	3	4	5	6	7	8	9
Wholegrain cereals e.g. Cheerios, Weetabix, Shredded Wheat	1	2	3	4	5	6	7	8	9
22. POTATOES, RICE & PASTA ***(medium serving)***									
Boiled, mashed, instant or jacket potatoes	1	2	3	4	5	6	7	8	9
Chips, potato waffles									

	1	2	3	4	5	6	7	8	9
Roast potatoes	1	2	3	4	5	6	7	8	9
Yorkshire pudding, pancakes, dumpling	1	2	3	4	5	6	7	8	9
Potato salad	1	2	3	4	5	6	7	8	9
White rice	1	2	3	4	5	6	7	8	9
Brown rice	1	2	3	4	5	6	7	8	9
White or green pasta, e.g. spaghetti, macaroni, noodles	1	2	3	4	5	6	7	8	9
Tinned pasta, e.g. spaghetti, ravioli, macaroni	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

PLEASE PUT A TICK (√) ON EVERY LINE.

FOODS & AMOUNTS	**AVERAGE USE LAST 6 months**								
22. POTATOES, RICE & PASTA (continued) ***(medium serving)***	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Super noodles, pot noodles, pot savouries	1	2	3	4	5	6	7	8	9
Wholemeal pasta	1	2	3	4	5	6	7	8	9
Lasagne, moussaka, cannelloni	1	2	3	4	5	6	7	8	9
Pizza	1	2	3	4	5	6	7	8	9
23. DAIRY PRODUCTS & FATS									
Single or sour cream *(tablespoon)*	1	2	3	4	5	6	7	8	9
Double or clotted cream *(tablespoon)*									

	1	2	3	4	5	6	7	8	9
Low fat yoghurt, fromage frais *(125g carton)*	1	2	3	4	5	6	7	8	9
Full fat or Greek yoghurt *(125g carton)*	1	2	3	4	5	6	7	8	9
Dairy desserts *(125g carton)*, e.g. mousse	1	2	3	4	5	6	7	8	9
Cheese, e.g. Cheddar, Brie, Edam *(medium serving)*	1	2	3	4	5	6	7	8	9
Cottage cheese, low fat soft cheese *(medium serving)*	1	2	3	4	5	6	7	8	9
Eggs as boiled, fried, scrambled, omelette etc. *(one)*	1	2	3	4	5	6	7	8	9
Quiche *(medium serving)*	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (√) on EVERY line

PLEASE PUT A TICK (√) ON EVERY LINE.

FOODS & AMOUNTS	AVERAGE USE LAST 6 months								
23.(b) The following on bread or vegetables *(teaspoon)*	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Butter	1	2	3	4	5	6	7	8	9
Block margarine, e.g. Stork, Krona	1	2	3	4	5	6	7	8	9
Polyunsaturated margarine, e.g. Flora sunflower	1	2	3	4	5	6	7	8	9
Other soft margarine, dairy spreads, e.g. Blue Band, Clover	1	2	3	4	5	6	7	8	9
Low fat spread, e.g. Gold	1	2	3	4	5	6	7	8	9
Vegetable oil	1	2	3	4	5	6	7	8	9
Olive oil	1	2	3	4	5	6	7	8	9

24. SWEETS & SNACKS									
Sweet biscuits, chocolate, e.g. digestive *(one)*	1	2	3	4	5	6	7	8	9
Sweet biscuits, plain, e.g. Nice, ginger *(one)*	1	2	3	4	5	6	7	8	9
Cakes e.g. fruit, sponge, sponge pudding *(medium serving)*	1	2	3	4	5	6	7	8	9
Sweet buns & pastries e.g. flapjacks, doughnuts, Danish pastries, cream cakes *(medium serving)*	1	2	3	4	5	6	7	8	9
Fruit pies, tarts, crumbles *(medium serving)*	1	2	3	4	5	6	7	8	9
Milk puddings, e.g. rice, custard, trifle *(medium serving)*	1	2	3	4	5	6	7	8	9
Ice cream, choc ices *(one)*	1	2	3	4	5	6	7	8	9
Chocolates *(small bar or $\frac{1}{4}$ pound of chocolates)*	1	2	3	4	5	6	7	8	9

Please check that you have a tick (√) on EVERY line

PLEASE PUT A TICK (√) ON EVERY LINE.

FOODS & AMOUNTS	AVERAGE USE LAST 6 months								
24. SWEETS & SNACKS (continued)	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Sweets, toffees, mints *(one packet)*	1	2	3	4	5	6	7	8	9
Sugar added to tea, coffee, cereal *(teaspoon)*	1	2	3	4	5	6	7	8	9
Crisps or other packet snacks e.g. Wotsits *(one packet)*	1	2	3	4	5	6	7	8	9
Peanuts or other nuts *(one packet)*	1	2	3	4	5	6	7	8	9
25. SOUPS, SAUCES AND SPREADS									
Vegetable soups *(bowl)*	1	2	3	4	5	6	7	8	9
Meat soups *(bowl)*	1	2	3	4	5	6	7	8	9

Sauces, e.g. white sauce, cheese sauce, gravy *(medium serving)*	1	2	3	4	5	6	7	8	9
Tomato based sauces e.g. pasta sauces *(medium serving)*	1	2	3	4	5	6	7	8	9
Tomato ketchup, brown sauce *(tablespoon)*	1	2	3	4	5	6	7	8	9
Relishes e. g. pickles, chutney, mustard *(tablespoon)*	1	2	3	4	5	6	7	8	9
Low calorie, low fat salad cream or mayonnaise *(tablespoon)*	1	2	3	4	5	6	7	8	9
Salad cream, mayonnaise *(tablespoon)*	1	2	3	4	5	6	7	8	9
French dressing *(tablespoon)*	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (√) on EVERY line

PLEASE PUT A TICK (√) ON EVERY LINE.

FOODS & AMOUNTS	AVERAGE USE LAST 6 months								
25. SOUPS, SAUCES AND SPREADS (continued)	Never or less than once/ month	1-3 per month	Once A Week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Other salad dressing *(tablespoon)*	1	2	3	4	5	6	7	8	9
Marmite, Bovril *(teaspoon)*	1	2	3	4	5	6	7	8	9
Jam, marmalade, honey, syrup *(teaspoon)*	1	2	3	4	5	6	7	8	9
Peanut butter *(teaspoon)*	1	2	3	4	5	6	7	8	9
Chocolate spread, chocolate nut spread *(teaspoon)*	1	2	3	4	5	6	7	8	9
Dips e.g. houmous, cheese and chive *(tablespoon)*	1	2	3	4	5	6	7	8	9
26. DRINKS									
Tea *(cup)*									

	1	2	3	4	5	6	7	8	9
Coffee, instant or ground *(cup)*	1	2	3	4	5	6	7	8	9
Coffee whitener, e.g. Coffee-mate *(teaspoon)*	1	2	3	4	5	6	7	8	9
Cocoa, hot chocolate *(cup)*	1	2	3	4	5	6	7	8	9
Horlicks, Ovaltine *(cup)*	1	2	3	4	5	6	7	8	9
Wine *(glass)*	1	2	3	4	5	6	7	8	9
Beer, lager or cider *(half pint)*	1	2	3	4	5	6	7	8	9
Port, sherry, vermouth, liqueurs *(glass)*	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (√) on EVERY line

PLEASE PUT A TICK (√) ON EVERY LINE.

FOODS & AMOUNTS	AVERAGE USE LAST 6 months								
26. DRINKS (continued)	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Spirits, e.g. gin, brandy, whisky, vodka *(single)*	1	2	3	4	5	6	7	8	9
Low calorie or diet fizzy soft drinks *(glass)*	1	2	3	4	5	6	7	8	9
Fizzy soft drinks, e.g. Coca cola, lemonade *(glass)*	1	2	3	4	5	6	7	8	9
Pure fruit juice (100%) e.g. orange, apple juice *(glass)*	1	2	3	4	5	6	7	8	9
Fruit squash or cordial *(glass)*	1	2	3	4	5	6	7	8	9
27. FRUIT *(1 fruit or medium serving)* *For very seasonal fruits such as strawberries, please estimate your average use when the fruit is in season									
Apples	1	2	3	4	5	6	7	8	9
Pears									

	1	2	3	4	5	6	7	8	9
Oranges, satsumas, mandarins, tangerines, clementines	1	2	3	4	5	6	7	8	9
Grapefruit	1	2	3	4	5	6	7	8	9
Bananas	1	2	3	4	5	6	7	8	9
Grapes	1	2	3	4	5	6	7	8	9
Melon	1	2	3	4	5	6	7	8	9
*Peaches, plums, apricots, nectarines	1	2	3	4	5	6	7	8	9
*Strawberries, raspberries, kiwi fruit	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (√) on EVERY line

PLEASE PUT A TICK (√) ON EVERY LINE.

FOODS & AMOUNTS	**AVERAGE USE LAST 6 months**								
27. FRUIT (continued) ***(1 fruit or medium serving)***	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Tinned fruit	1	2	3	4	5	6	7	8	9
Dried fruit, e.g. raisins, prunes, figs	1	2	3	4	5	6	7	8	9
28. VEGETABLES Fresh, frozen or tinned ***(medium serving)***									
Carrots	1	2	3	4	5	6	7	8	9
Spinach	1	2	3	4	5	6	7	8	9
Broccoli	1	2	3	4	5	6	7	8	9
Brussels sprouts	1	2	3	4	5	6	7	8	9
Cabbage									

	1	2	3	4	5	6	7	8	9
Peas	1	2	3	4	5	6	7	8	9
Green beans, broad beans, runner beans	1	2	3	4	5	6	7	8	9
Marrow, courgettes	1	2	3	4	5	6	7	8	9
Cauliflower	1	2	3	4	5	6	7	8	9
Parsnips, turnips, swedes	1	2	3	4	5	6	7	8	9
Leeks	1	2	3	4	5	6	7	8	9
Onions	1	2	3	4	5	6	7	8	9
Garlic	1	2	3	4	5	6	7	8	9
	Never or less than	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

	once/ month								

Please check that you have a tick (√) on EVERY line

PLEASE PUT A TICK (√) ON EVERY LINE.

FOODS & AMOUNTS	**AVERAGE USE LAST 6 months**								
28. VEGETABLES Fresh, frozen or tinned (continued) ***(medium serving)***	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Mushrooms	1	2	3	4	5	6	7	8	9
Sweet peppers	1	2	3	4	5	6	7	8	9
Beansprouts	1	2	3	4	5	6	7	8	9
Green salad, lettuce, cucumber, celery	1	2	3	4	5	6	7	8	9
Mixed vegetables (frozen or tinned)	1	2	3	4	5	6	7	8	9
Watercress	1	2	3	4	5	6	7	8	9
Tomatoes	1	2	3	4	5	6	7	8	9

Sweetcorn	1	2	3	4	5	6	7	8	9
Beetroot, radishes	1	2	3	4	5	6	7	8	9
Coleslaw	1	2	3	4	5	6	7	8	9
Avocado	1	2	3	4	5	6	7	8	9
Baked Beans	1	2	3	4	5	6	7	8	9
Dried lentils, beans, peas	1	2	3	4	5	6	7	8	9
Tofu, soya meat, TVP, Vegeburger	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (√) on EVERY line

Appendix U: Urine Sample Collection Log Sheet

Urine Sample Collection Log Sheet

Participant ID.....

Week	Sample ID	Date	Time
1	01		
	02		
	03		
2	04		
	05		
	06		
3	07		
	08		
	09		

- Please collect 3 urine samples a week for 3 weeks

- 2 urine samples should be collected on non-consecutive days in the week (e.g. Tuesday and Thursday) and the 3rd sample should be collected during the weekend

- All urine samples should be collected during the first time you go to the toilet after waking up in the morning

- Keep your collected samples in the fridge within the absorbent pouches provided

- Post the samples back to us using the addressed box provided or bring them back in person to the Carwyn James Building

Appendix V: ActiGraph Instructions

Instructions for wearing the Actigraph activity monitor

For a period of 7 days:

- Wear the device around your waist during the day, with the device on your **RIGHT** side.
- Record when you wear the device on the time sheets provided

More details:

- The device consists of a red small box (the ActiGraph), attached to a strap. The strap should go around your waist and fit close to your body. One option would be to wear it as a normal belt; that it is the best position to track your movements. Alternatively, over your underwear but under clothing is also fine.

    - Other clothing such as shirts and coats could be placed over the device, which will also help protect it from getting wet, and getting caught.
    - Adjust the belt so that the Actigraph is positioned just above the right hipbone (see photo). The Actigraph must fit tightly but comfortably against your body. Adjust the strap to make a snug and comfortable fit.

- You should start to wear the Actigraph within 7 days during one week of the 3 week urine collection period, for the next 7 days.
    - Your week can start mid-week, but you should wear it for the 7 days that follow.
    - Before and after this week, it is recommended to keep the device in one place and not move it.
    - You can return the device in person, via the postal bag we can provide, or let us know when you're done and we could be able to pick it up.

- Wear the device for 7 days, every day, throughout the complete time, including sleeping. Write down when you start wearing the device on the sheet. Remember to put it on your **RIGHT** side on your waist when getting out of bed.

- It might not be feasible to wear the device at certain times. In that case, record the time you taken it off on the accompanying timesheet.
    - An example of non-wear would be when you go swimming, have a bath or shower, or some sporting activities where you're not allowed to wear jewellery. The device is not waterproof. Please remember to put it on again afterwards. Record on the time sheet the periods when the Actigraph was

stopped being worn, including the type of activities you did, and for how long (see the timesheet).
    - If you simply forget to wear the device, please note that also on the timesheet. If you miss a whole day; record for an extra day directly after the 7th day

- Every evening, record the time you go to bed, on the log sheet.
- Every morning, record the time you get up, on the log sheet.
- At the end of the measurement period, please return the Actigraph and the time sheets

- When you receive the Actigraph, a light might be flashing; this is normal and shows the device is set up, but not recording yet. Once it starts actually recording, the light will disappear. So, don't wear the device as long as the light is flashing (possibly for one or two days at the start).

PLEASE RETURN THE ACTIGRAPH WHEN YOU'RE DONE

Illustration of where to place the ActiGraph during the day, at this height, with the red device on the side. It can be placed under your clothing.

The Actigraph is NOT waterproof, so please do not shower with it.

Any questions, or not sure about anything, or experiencing problems? Contact Nick Gregory (nig5@aber.ac.uk/01970 622075) or Sam Chapman (sac37@aber.ac.uk)

Appendix W: ActiGraph Log Sheet

Day	Date	Start of day wear (time of getting out of bed)	End of day wear (time of going to bed)	Non wear from (time)	Non-wear until (time)	Reason of non-wear	Any Additional Comments

Appendix X: Feedback Questionnaire

Study Feedback Questionnaire

The following questions relate to the pre-diabetes study you have been involved in.

Recruitment

The following questions relate to the recruitment process:

1. I was happy to receive information about my diabetes risk in the post

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

2. The information I received in the post about pre-diabetes was useful and informative

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

3. The information I received in the post about the study was clear, useful and understandable

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

4. Contacting the research team and becoming a participant was simple and straightforward

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

5. The research team explained the study clearly and I understood my role as a participant from the outset

○	○	○	○	○
Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree

6. Further Comments *(if required)*

Data collection

The following questions relate to your baseline, 3 month and 6 month visit to the Carwyn James Building:

1. The research team organised visit times for data collection that were convenient for me

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

2. I was happy for the research team to record my physical data (e.g. weight/height/waist circumference/blood pressure etc.)

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

3. I was happy to provide a blood sample as part of the data collection process

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

4. I was happy to complete the questionnaires at the end of each visit

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

6. Further comments (*if required*)

Urine Collection

The following questions relate to the urine collection procedures in the home:

1. I was successful in collecting urine using the urine collection kit

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

2. It was easy to collect urine in the pot

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

3. I was confident collecting urine in the pot

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

4. It was easy to transfer urine from the pot into the sample tube using the transfer straw

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

5. I felt confident transferring urine from the pot into the sample tube using the transfer straw

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

6. I was happy to write the collection date and time on the sample tube

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

7. In general, I think collecting a urine sample is difficult

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

8. I think collecting a urine sample in a home environment is embarrassing

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

9. I was happy to store several urine samples collected over a week in a small box in my fridge

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

10. Returning my urine samples to the research team was easy and arranged at a convenient time for me

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

11. Further comments (if required)

15 Minute Consultation

The following questions relate to the 15-minute consultation received in your GP surgery. These questions only need to be answered if you attended a consultation:

1. I was pleased to be told I would be receiving a 15 minute consultation from my GP

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

2. Booking a 15 minute consultation with my surgery was simple and straightforward

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

3. The consultation provided me with useful information about my diabetes risk and ways I can improve my lifestyle

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

4. Since attending the 15-minute consultation I have improved my health through increased exercise and better dietary habits

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

5. The 15-minute consultation has had a beneficial impact on my health and I would recommend it to other people who are at risk of developing diabetes

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

Further comments (*if required*)

Physical Activity recording

The following questions relate to the methods for recording physical activity. These questions only need to be answered if the research team asked you to record your physical activity:

1. I was happy to be asked to record my levels of physical activity using the Actigraph

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

2. Recording my levels of physical activity using the ActiGraph was easy

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

3. Wearing the ActiGraph did not interfere with my daily tasks and routines

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

4. Recording the times I wore the ActiGraph was easy

Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
○	○	○	○	○

5. I was more physically active in the weeks I wore the ActiGraph compared to the weeks I did not

○	○	○	○	○
Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree

6. Further comments (*if required*)