Dietary prevention of type 2 diabetes: the role of fruit and vegetable intake

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Abstract

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This thesis begins with a background chapter which explores the current diabetes epidemic and examines the role of obesity and oxidative stress as causative factors. Current dietary recommendations for prevention of type 2 diabetes are critically evaluated.

A systematic review and meta-analysis was conducted to determine the independent role of fruit and vegetables in preventing diabetes. Convincing benefit for greater consumption of green leafy vegetables was demonstrated. An insignificant trend towards benefit was observed for fruit and vegetables.

The Fruit and Vegetable Intake and Glucose Control Study (FIVE) is a sub study of the Let's Prevent Diabetes Study. FIVE includes cross sectional analysis of baseline plasma vitamin C, (a biomarker for fruit and vegetable intake) from 2101 participants. FIVE further includes 12 months analysis of individuals with impaired glucose regulation, randomised to receive group education or usual care.

Results demonstrate 29% of the population consumed at least 5 portions of fruit and vegetables a day. Fewer South Asian individuals met the recommendation compared to White Europeans (21% *vs*. 30% p = 0.003).

Each additional piece of fruit or vegetable consumed (21.8μmol/l plasma vitamin C) was associated with a reduction of 0.04% in HbA1c, 0.05mmol/l in fasting and 0.22mol/l in 2 hour blood glucose. Participants who consumed 5 portions a day compared to those who did not, had a 24% associated reduced risk of being diagnosed with impaired glucose regulation (OR = 0.76, 95% CI: 0.59 to 0.98).

At 12 months follow up those receiving lifestyle education had greater levels of plasma vitamin C compared to those in the usual care arm (36.1μmol/l (SD 20.7) *vs.*29.9μmol/l (SD 20.3)). No statistical difference in mean change between intervention arms was seen.

The thesis provides novel, robust nutritional biomarker data from a large at risk, multi ethnic population. Results support recommendations to promote fruit and vegetables in the diet to prevent diabetes. The potential for tailored advice on increasing green leafy vegetables among those at risk of diabetes should be investigated further.

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Table of Abbreviations

Abbreviation	Full Terminology
2 hour glucose	2 hour post challenge glucose
ADA	American Diabetes Association
AgRP	Agouti related protein
BMI	Body mass index
CAD	Coronary artery disease
CAT	Catalase
CCK	Cholecyostokinin
CART	Cocaine amphetamine related transcript
CP	Coefficient of precision
CRP	C-reactive protein
CV	Coefficient of variance
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DESMOND	Diabetes Education and Self-Management for On-going and Newly Diagnosed
DINE	Dietary Instrument for Nutrition Education
DPP	Diabetes Prevention Programme
DUK	Diabetes UK
E%	Percentage of total energy intake
FDPS	Finnish Diabetes Prevention Study
FFA	Free fatty acids
FFQ	Food frequency questionnaire
GLUT-4	Glucose-4 transporters
GLP-1	Glucagon-like peptide 1
GPx	Glutathione peroxidise
HbA1c	Haemoglobin A1c
HDL	High density lipoprotein
HR	Hazard ratio
HSE	Health survey for England
IDPP	Indian Diabetes Prevention Programme
IFG	Impaired fasting glucose
IGR	Impaired glucose regulation
IGT	Impaired glucose tolerance
IKK-β	Inhibitor of nuclear factor kappa beta kinase
IL-6	Interleukin 6
IMCL	Intracellular muscle lipids
ISoPs	Isoprostanes
JNK	c-Jun amino terminal kinase
JT	Jacqui Troughton
KANWU Study	Kupoio, Aarhus, Naples, Wollongong, Uppsala (centres in the study)
LDL	Low density lipoprotein
LSM	Lifestyle modification
LSMP	Lifestyle modification programme

MI	Myocardial infarction
MOOSE	Meta-analysis of observational studies in epidemiology
MUFA	Monounsaturated fatty acids
NEFA	Non-esterified fatty acids
NF-Kβ	Nuclear factor kappa beta
NGT	Normal glucose tolerance
NHS	National Health Service
NIHR	National Institute of Health Research
NO	Nitric oxide
NPY	Neuropeptide Y
OGTT	Oral glucose tolerance test
OR	Odds ratio
PC	Patrice Carter
PDM	Pre-diabetes mellitus
POMC	Pro-opiomelanocortin
PREPARE	The Pre-diabetes Risk Education and Physical Activity Recommendation and Encouragement study
PUFA	Polyunsaturated fatty acids
PYY	Peptide YY
QATSO	Quality assessment tool for systematic reviews of observational studies
RCT	Randomised controlled trial
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RR	Relative risk
SA	South Asian
SAA	Serum amyloid A
SBP	Systolic blood pressure
SD	Standard deviation
SCFA	Short chain fatty acid
SFA	Saturated fatty acid
SOD	Superoxide dismutase
STROBE	The strengthening of the reporting of observational studies in epidemiology
T2DM	Type 2 diabetes mellitus
TG	Triglycerides
TNK-α	Tumor necrosis factor alpha
USFA	Unsaturated fatty acids
WC	Waist circumference
WE	White European
WHO	World Health Organisation
WHR	Waist hip ratio

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Thesis overview

Lifestyle modification programmes have shown convincing evidence for the prevention of type 2 diabetes. However the independent effects of individual dietary factors remain to be elucidated. The majority of these programmes recommend that participants should increase their consumption of fruit and vegetables. Furthermore current prevention guidelines from Diabetes UK and the American diabetes Association recommend a high intake of fruit and vegetables; yet the evidence for these recommendations is lacking.

This thesis aimed to explore the independent role that fruit and vegetables play in preventing or reducing the risk of developing type 2 diabetes. Current evidence was evaluated via a systematic review on the independent effects of fruit and vegetable consumption and risk of type 2 diabetes. A nutritional sub-study, utilising a large community screening and intervention study was then conducted. This study aimed to examine glycaemic parameters in relation to current fruit and vegetable intake using plasma vitamin C as a validated biomarker. Together this work was designed to investigate the independent effects that fruit and vegetable intake play in the prevention of type 2 diabetes and to determine whether current recommendations regarding fruit and vegetable intake are appropriate.

Note: Work conducted by others

The statistical analysis for the meta-analysis in chapter 3 was conducted by Laura J Gray; Lecturer of Population and Public Health Sciences, The University of Leicester. The laboratory measurement of stabilised plasma vitamin C was conducted by Sarah Gilchrist and Jayne Woodside within the Nutrition and Metabolism Group at Queens University Belfast.

Chapter 1

Obesity and Diabetes

1.0 Chapter summary

This section is the first of two introductory chapters. Chapter 1 will describe obesity, its prevalence, causes, and the risk that obesity poses to the development of type 2 diabetes (T2DM). The chapter includes definitions of T2DM and impaired glucose regulation (IGR), also known as pre-diabetes (PDM). In addition the prevalence and complications of both T2DM and IGR will be discussed. Finally the chapter explores the relationship between obesity and diabetes and the role that oxidative stress plays in these interlinked diseases.

1.1 Obesity

Body weight is controlled by interacting regulatory systems which influence food intake, body composition and energy expenditure.[1] In healthy individuals these systems work to keep an equal balance between energy input and energy output; if disturbed, either overweight, obesity or malnutrition can result.

1.1.1 Defining weight status

Overweight and obesity are defined as a condition where abnormal or excessive body fat accumulates to an extent that presents a risk to the individual's health.[2] In adults one of the easiest and most commonly used anthropometric measures of body fatness is the Body Mass Index (BMI). BMI, developed by Keys in 1972 is a measure of weight for height, defined as weight in kg divided by the square of height in metres.[1] BMI is commonly used at the population level as it can be used in adults of either sex at any age. BMI does not however distinguish between lean mass, fat mass or water content, nor does it determine where fat is accumulated. Skin-fold thickness, underwater weighing or bioelectric impedance can separate

body mass into fat and fat free mass, however these techniques are expensive and time consuming therefore BMI remains the most routinely used method for classification of weight status in a health care setting.[3] In addition waist circumference (WC) and waist to hip ratio (WHR) are increasingly used as simple measures of abdominal obesity.

A healthy BMI, defined as 18.5kg/m^2 to 24.9kg/m^2, overweight (≥25kg/m^2) and obesity (≥30kg/m^2) (figure 1-1, page 5) were originally calculated using North American mortality data by insurance companies to differentiate death rates.[4] There is now a plethora of data which demonstrates that being overweight or obese is detrimental to health. The Whitehall Study included data from over 17 000 men and showed that in those free from coronary heart disease (CHD), being overweight or obese increased an individual's risk of all-cause, cardiovascular disease (CVD), CHD and stroke mortality.[5] Furthermore studies using different measures of body composition, BMI, WC or WHR all show strong associations with increasing weight and risk of mortality and morbidity.[6-8] Both overall and abdominal obesity are also strong and independent predictors of T2DM risk.[9] However all measures of obesity are highly correlated[6] and regardless of measurement technique it is now generally accepted that being overweight or obese carries a profound health burden.[5, 7, 10]

1.1.2 South Asian cut points

Cut points for both BMI and WC are derived from European and North American Caucasian populations[4,11] however there is on-going debate about whether these classification indices are relevant for all ethnicities. It has been suggested that the

current cut points for the normal range, overweight and obesity may be misleading in non-European populations. Indeed, differences in body composition have been observed in different ethnic groups. For any given BMI various ethnic groups will have a different percentage of body fat.[12] Asian populations have less skeletal muscle, lower bone mineral content and excess body fat for a given BMI as compared to White Caucasians.[13] Evidence indicates that Asian groups suffer from elevated risk of T2DM, hypertension and dsylipidemia even at BMIs below 25kg/m^2.[14-16] South Asians (SA) make up the largest ethnic minority group in the UK therefore this debate is important in a UK clinical setting. Although cut points are arbitrary they are important in clinical interventions aimed at reducing disease risk, therefore it is important to be aware of differences in risk between ethnic groups.[17]

The World Health Organisation (WHO) convened an expert consultation on BMI in Asian populations and although they concluded that the percentage of Asians with a high risk of CVD and T2DM is substantial at BMIs lower than the existing WHO cut off point for overweight, the cut off varies in different Asian populations and therefore the current WHO BMI cut-offs remain.[18] However another recent report has showed that SA display equivalent levels of dysglycaemia and dyslipidemia as White Europeans (WE) at lower cut points;[15] again highlighting that the burden of obesity in the SA population may be underestimated if traditional BMI cut offs are used.[14] Indeed a consensus statement headed by the director for the centre of diabetes and lifestyle disease research in New Delhi states that due to Asian Indians having an increased risk of developing obesity related co-morbidities at lower levels of BMI and WC, then the cut points should be changed.[19] In addition the South

Asian Health Foundation in the UK has recommended that health initiatives targeting the SA population should use lower thresholds for BMI.[16]

Figure 1-1: World Health Organization weight classification guide[20]

BMI

	kg/m^2	
Underweight	< 18.5	
Normal	18.5 - < 25	
Overweight	25 - < 30	(23 in S.E. Asians)
Obese	30 - < 40	(27.5 in S.E. Asians)
Obese I	30 - < 35	
Obese II	35 - < 40	
Morbidly Obese	≥ 40	

Waist Circumference

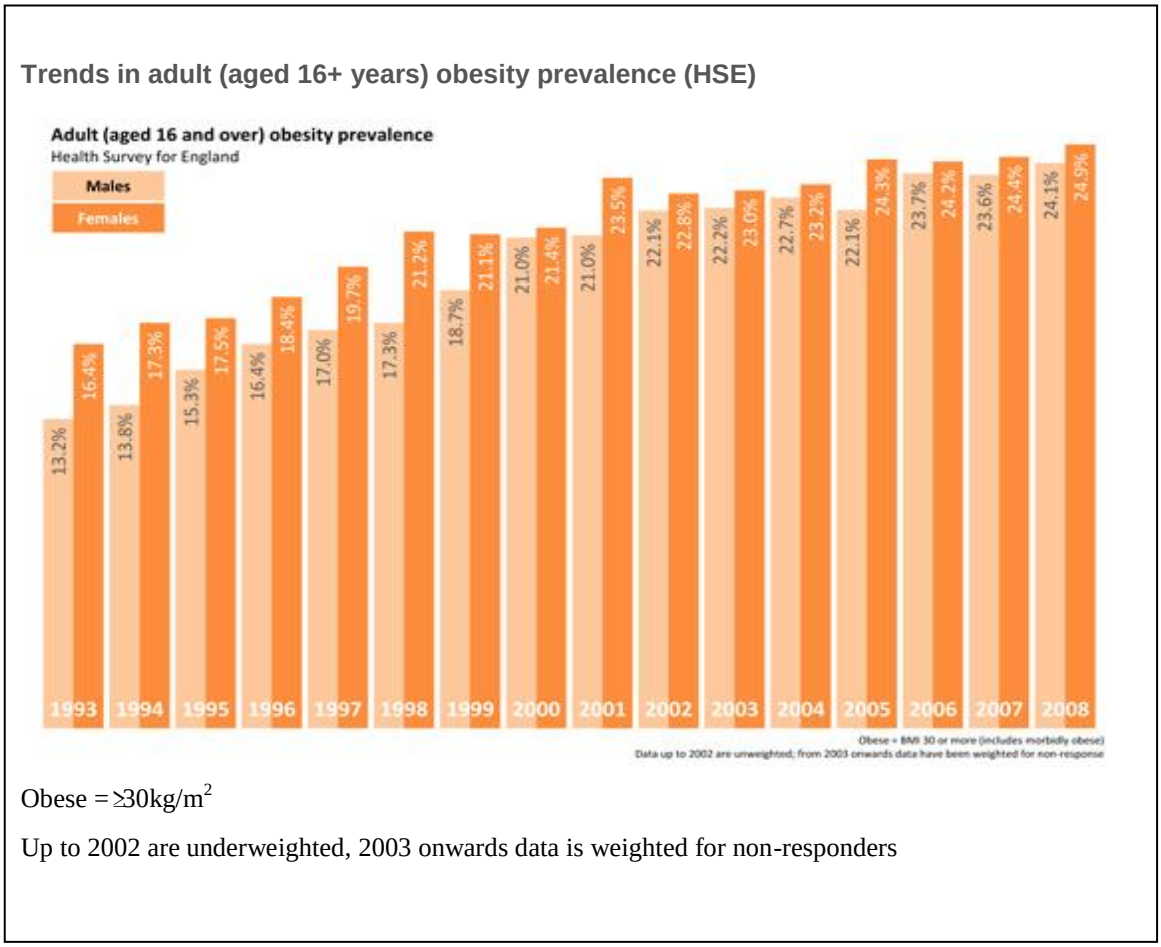
Risk	**Men**	**Women**
Low	<94cm	<80cm
High	94 - 102cm (>94 in SAs)	80 – 88cm (>80 in SAs)
Very High	≥102cm	≥88cm

1.1.3 Prevalence of obesity

The prevalence of obesity is increasing throughout the world in all age groups.[3] The prevalence of obesity in England has more than doubled in the last 25 years, with only 32.5% of men and 41.1% of women having a healthy BMI in 2008.[21] Data from 2009 show that in England 22% of adult men and 24% of adult women were obese.[22] Morbid obesity has risen in both men and women between the years 1993/1995 and 2006/2008, from 0.3% to 1.5% and 1.3 % to 2.6% in men and

women respectively.[21] In addition 38% of all adults have a waist circumference above the recommended measures (32% of men and 44% of women).[22] Predictions calculate that by 2050, 60% of adult men, 50% of adult women and 25% of children in the UK will be obese.[21]

Figure 1-2: Trends in adult obesity[21]



Obese = ≥30kg/m^2

Up to 2002 are underweighted, 2003 onwards data is weighted for non-responders

1.1.4 Causes of obesity

The law of thermodynamics states that energy cannot be created or destroyed so any imbalance between energy intake and energy output requires the ability to store energy.[23] Storage capacity of protein and carbohydrates is limited; however fat

stores can easily expand to accommodate any excess energy intake.[24] Thus imbalance between energy input and output leads to accumulation of body fat. Genetic factors can influence variability in body size and body composition,[25] as may maternal nutrition.[26] However the potential to develop obesity is not a new concept and the human species has not changed genetically for millions of years, yet the environment in which we live has changed dramatically.[27, 28]

Following the end of World War II there has been a gradual increase in obesity prevalence which coincided with an increase in meat and fat intake,[3] greater consumption of food eaten outside the home, larger portions sizes, increased energy intake from salty snacks and a plentiful supply of sugary drinks.[23, 24] It has been estimated that over the past 20 years there has been an increase in calorie intake of about 300kcal/day per person, more than enough to produce an extra 1kg increase in body weight per person per year.[24] In addition a decrease in daily physical activity has also occurred. Regulation of a stable body weight requires tight control of energy homeostasis; however, the current environment favours poor dietary choices and reduced physical activity leading to obesity.[27]

1.1.5 Regulation of energy intake

Regulation of food intake is controlled by several neuronal systems which include both hedonic and homeostatic mechanisms.[29] Peripheral signals include leptin, and ghrelin. Leptin is an adipokine involved in long term energy regulation, increasing with greater levels of adiposity. Ghrelin is synthesised in the gut and concentrations increase during periods of food deprivation; release is thought to stimulate food intake.[24] Both leptin and ghrelin have receptors located in the hypothalamus which

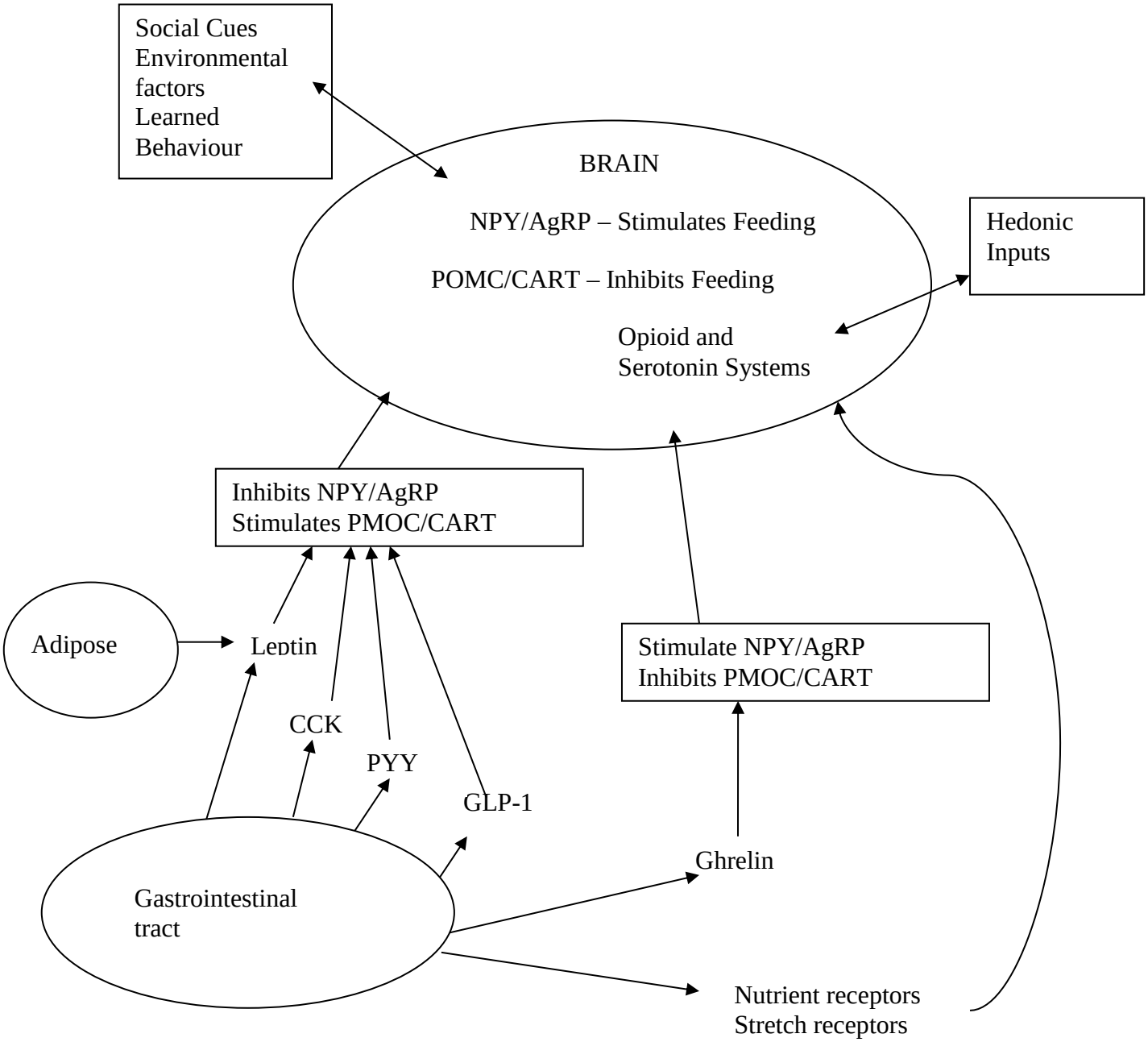
can stimulate neuropeptide Y (NPY) and Agouti related protein (AgRP). NPY/AgRP neurons are believed to constitute a potent feeding system and NPY is thought to be the strongest stimulator of food intake.[24] The NPY/AgRP system is opposed by the satiety system which includes pro-opiomelanocortin (POMC) and cocaine and amphetamine related transcript (CART) factors also located in the hypothalamus.[29]

Gastrointestinal and pancreatic peptides are also thought to influence food intake. Satiety signals include cholecystokinin (CCK), which has been shown to decrease food intake.[24] Peptide YY (PYY) is released from the small intestine and appears to inhibit NPY/AgRP neurons, thus food intake.[24, 29] Glucagon-like peptide 1 (GLP-1) is a circulatory hormone produced by the gut which may decrease food intake and appears to induce a sensation of nausea at raised concentrations.[24] Central pathways involved in the regulation of food intake include the melanocortin system, which acts to inhibit feeding and the orexin melanin concentrating hormone system that acts to drive feeding behaviour.[29]

Hedonistic factors are also important for the regulation of energy intake and centres of food rewards within the brain share common neuronal pathways as drug rewards. Both the endogenous opioid systems and serotonin systems can influence the hedonic value of food independently from the individual's metabolic needs.[29] Serotonin and opioid receptors may modulate feeding, quantity of food intake and macronutrient selection.[24] In addition learned behaviour and social and emotional factors can influence food intake.[30] Differences in susceptibility to obesity may be linked to variation in the efficiency of the central control mechanisms which

influence eating behaviour.[31] Theoretically obesity is a simple energy equation, however a range of factors influence energy intake not just essential metabolic requirements (Figure 1-3, page 9).

Figure 1-3: Regulation of food intake through the gut brain axis (Adapted from (Bray[24], Badman[30], Saper[29])



1.2 Diabetes

Diabetes Mellitus is the term given to a group of metabolic disorders which are characterised by hyperglycaemia, an elevated glucose concentration in the plasma. The most prevalent form is T2DM, accounting for about 90% of all diabetes cases.[32] T2DM was previously known as Non-insulin dependent Diabetes Mellitus or maturity onset diabetes as historically it occurred in later life. However T2DM incidence is now increasing in younger generations.[33] T2DM can be caused by impaired insulin secretion, insulin resistance or a combination of both.[34]

1.2.1 Normal glucose homeostasis

Glucose levels are maintained by the anabolic hormone insulin. Insulin is synthesised and secreted from the β-cells within the islets of Langehans in the pancreas. Throughout the day insulin is secreted at low basal levels, the remainder is secreted in association with a rise in portal plasma glucose following ingestion of a meal.[35] As food is consumed glucose levels in the blood rise and the pancreas responds by releasing insulin. Insulin acts to suppress hepatic glucose production by stimulating glycogen synthesis and inhibiting glucogenolysis (the breakdown of glycogen to glucose) and gluconeogenesis (the synthesis of glucose from non-carbohydrate precursors, glycerol, lactate and amino acids). Insulin promotes glucose uptake via stimulation of tissue receptors and the translocation of glucose transporters within cells.[36, 37] Hepatocytes, adipocytes and skeletal myocytes are the main targets for glucose uptake and are characterised by having numerous glucose-4 transporters (GLUT-4).

1.2.2 Impaired glucose regulation (IGR)

The development of T2DM is a gradual process, thus an intermediate stage between normoglycaemia and diabetes occurs, known as impaired glucose regulation (IGR) or PDM. Often before the onset of T2DM insulin resistance occurs, the normal response to insulin becomes impaired. As insulin is released cells do not respond adequately therefore glucose does not enter the cells and blood levels remain elevated. The body's initial response is to release more insulin, resulting in hyperinsuliniaemia. In some subjects this response continues without T2DM developing, however if the increase in insulin is not sufficient or the pancreas stops being able to produce enough insulin (beta cell dysfunction) to compensate for the insulin resistance then T2DM will present. Two forms of IGR exist, Impaired Fasting Glucose (IFG) and Impaired Glucose Tolerance (IGT), they represent two metabolically distinct abnormalities and are not interchangeable.[38] Individuals can suffer from IFG, IGT or both.

IFG and IGT are not classed as clinical entities in themselves, but as risk factors for future diabetes and or adverse outcomes.[39] Studies have demonstrated that raised fasting glucose levels carry a statistically significant risk for both all cause and coronary mortality.[40, 41] A recent study demonstrated that individuals with a fasting blood glucose greater than 7mmol/l had an 89% associated increased risk of vascular death (Hazard ratio (HR) = 1.89 95% confidence intervals (CI): 1.69 to 2.10) compared to those with fasting blood glucose between 3.9mmol/l and 5.6mmol/l.[42] Furthermore previous data has demonstrated that for each 1mmol/l incremental rise in fasting blood glucose there was an associated 6% rise in risk of

total mortality.[41] The authors of this study concluded that fasting plasma glucose is an independent risk factor for CV events even in levels below the diabetic range.[41]

1.2.3 Impaired glucose tolerance (IGT)

The prevalence of IGT is around 10% of the global population and is defined by WHO as fasting glucose less than 7.0mmol/l, if measured and a 2 hour post glucose load between 7.8-11.0mmol/l.[39] In 1979, the category IGT was proposed explicitly to define those at increased risk of large vessel disease but at a low level risk of small vessel disease.[43] The WHO states that IGT is a state of increased risk of progressing to diabetes, however many subjects will revert back to normal glucose tolerance.[39] It has been demonstrated that subjects with IGT have reduced insulin mediated glucose disposal and that individuals are characterised by moderate to severe muscle insulin resistance and diminished early phase insulin secretion.[44] Subjects with IGT manifest dual defects characteristic of T2DM, having a combination of deficient insulin secretion and muscle insulin resistance resulting in a less than efficient disposal of glucose during a glucose load.[44]

1.2.4 Impaired fasting glucose (IFG)

IFG occurs in around 5% of the population and is defined by WHO as fasting glucose of 6.1-6.9mmol/l and a 2 hour post glucose load of less than 7.8 mmol/l.[39] IFG has different pathological mechanisms to IGT, subjects with IFG demonstrate total glucose disposal similar to that of normal glucose tolerant subjects however they are characterised by hepatic insulin resistance.[44] Fasting glucose levels are raised due to hepatic glucose output being poorly controlled.

1.2.5 Diagnosis of diabetes

Glucose concentrations of a healthy individual lie between 4-7mmol/l, this represents a balance between the rate of glucose delivery into the blood and the rate of removal from circulation.[36] Diagnosis is based on the ability to predict and deter microvascular complications associated with high blood glucose concentrations. A fasting blood glucose of ≥7mmol/l is thought to increase the risk of microvascular complications.[39]

Historically considered the gold standard for the diagnosis of diabetes, an oral glucose tolerance test requires the subject to give a fasted blood sample, take a 75g glucose load, then after two hours give a repeat blood sample. The procedure demonstrates how adequately the individual responds to a glucose challenge. Diabetes is diagnosed if the two hour blood level is ≥11.1mmol/l. A fasting blood concentration of equal or greater to 7mmol/l, on more than one occasion can also be used to diagnose diabetes. Diabetes is often an asymptomatic condition, thus diagnosis should not be made on a single blood result. If no symptoms are present at least two abnormal blood results must be obtained. If symptoms such as excessive thirst, high urine volume, recurrent infections or unexplained weight loss exist then a single abnormal blood result may suffice.[39] However HbA1c can now also be considered for diagnosis.

1.2.6 HbA1c

HbA1c reflects average plasma glucose over the previous 8 to 12 weeks and is regularly used in diabetes management. However HbA1c is now considered as a diagnostic tool. HbA1c has several advantages over other methods; it can be taken

at any time of the day and does not require a fasted sample. The American Diabetes Association (ADA) recommends HbA1c as the preferred choice for diagnosis.[45] The WHO state that HbA1c of 6.5% is recommended as the cut point for diagnosis of diabetes, but a value less than 6.5% does not exclude diabetes diagnosed using glucose tests. Furthermore HbA1c cannot be used to diagnose IGR. In addition WHO state that the test can only be recommended if stringent quality assurance tests are in place and the assays are standardised.[46]

Table 1-1: 2006 WHO recommendations for diagnosis of diabetes and intermediate hyperglycaemia[39]

Diabetes	Fasting Plasma Glucose 2hr Glucose	≥ 7.0mmol/l Or ≥11.1mmol/l
Impaired Glucose Tolerance (IGT)	Fasting Plasma Glucose 2hr Glucose	<7.0mmol/l & ≥7.8mmol/l but <11.1mmol/l
Impaired Fasting Glucose (IGF)	Fasting Plasma Glucose 2hr Glucose	6.1mmol/l to 6.9mmol/l & if measured <7.8mmol/l

1.2.7 Prevalence of diabetes

The prevalence of T2DM is currently estimated to be 6.4% worldwide.[47] In the UK alone there are around 2.8 million people with diabetes[48] and T2DM accounts for approximately 90% of cases. In the past two decades there has been a dramatic increase in the diagnosis of T2DM worldwide.[47] T2DM is increasing in all populations and all age groups throughout the world. This rapid increase in prevalence over the last two decades suggests that environmental factors must be important. In addition populations undergoing rapid lifestyle changes and rapid nutrition transition have seen the greatest increase in prevalence.[49]

The prevalence of diabetes varies in different ethnic groups. The Southall Study in 1985 demonstrated that prevalence of diabetes for Asians living in that area was 3.8 times greater than those of European descent.[50] Today prevalence in SAs is estimated to be between 2 to 6 times that of WEs and many more maybe undetected.[51] ADDITION Leicester found 4.7% of SAs had screen detected T2DM as compared to 2.8% of WEs.[52] In addition diabetes occurs 5 to 10 years earlier in the SA population compared to WEs.[50, 52, 53]

1.2.8 Complications of diabetes

Microvascular injury; damage to small blood vessels, causes common complications associated with diabetes, and includes retinopathy, nephropathy and neuropathy. Diabetic subjects are also at an increased risk of macrovascular injury; damage to large blood vessels. Responsible for an excess of CHD, stroke and peripheral vascular disease. 70% of those with diabetes will die from cardiovascular disease.[33, 34, 35, 41] Life expectancy is reduced by 5 to 10 years as compared to those without diabetes.[54] Recent estimates showed that compared to those free from diabetes, individuals with diabetes had an 80% age adjusted risk for all-cause mortality (HR = 1.80 95% CI: 1.71 to 1.90) and a 132% age adjusted risk for vascular disease (HR = 2.32 95% CI: 2.11 to 2.56).[42]

1.3 Obesity prevalence and diabetes

It is widely accepted that obesity is the single most important risk factor for T2DM. Being overweight, having abdominal fat distribution and obesity are associated with around 90% of all cases of T2DM.[55]

Evidence from large prospective studies in both men and women have demonstrated strong associations between BMI and risk of T2DM.[56-58] The Male Health Professionals study observed that the risk of T2DM increased continuously with increasing levels of BMI. Individuals with a BMI between 25kg/m^2 and 26.9kg/m^2 had a 2.2 times greater risk of developing T2DM compared to those with a BMI of <23kg/m^2 (95% CI: 1.3-3.8).[56] A further study in both men and women demonstrated that for each 1 unit increase in BMI at age 25 there was an associated increase in risk of T2DM independently of any subsequent weight change (male Relative Risk (RR) = 1.15, 95% CI: 1.11 – 1.19 and female RR = 1.11, 95%CI: 1.07 – 1.15).[59] It has also been demonstrated that an increase in risk of developing T2DM occurs with increasing BMI levels not considered as overweight.[58]

Furthermore, prospective studies show that baseline BMI can influence subsequent development of T2DM. Both weight gain and weight loss over time have been associated with a change in risk of incidence of T2DM.[60] In the Male Health Professional study long term weight gain was strongly associated with risk of T2DM.[61] Those who gained just 3-6kg throughout adulthood had a 1.8 times greater risk of T2DM (95% CI: 1.0 – 3.2) compared to those whose weight remained stable.[61] Ford and colleagues estimated that 27% of T2DM cases could have been avoided in a US cohort if weight gain had been avoided.[62] In addition weight loss has been shown to be beneficial in reducing the risk of T2DM. A US study in overweight men and women observed that intentional weight loss over 13 years reduced the rate of developing T2DM by 25% compared to those individuals who did not report intentional weight loss.[63] Studies aimed at the prevention of T2DM have shown that 5% loss of initial body weight is important for the

prevention of T2DM.[64] Furthermore a recent report estimated that a 1% reduction in BMI across the USA and UK could reduce incident cases of diabetes from 2.4 to 2.1 million and 202 to 179 thousand respectively.[65]

1.4 Central obesity and diabetes

It has been hypothesised that central obesity is associated with a greater risk of developing T2DM than general obesity as visceral fat secretes hormones directly into the portal system.[66] Thus WC measurement may be more predictive of T2DM than other obesity measurements. A study investigating the risk of CVD in subjects with T2DM showed that WC and WHR were positively and continuously correlated to cardiovascular and coronary events yet BMI showed no significant relation.[67] However a recent meta-analysis demonstrated that the pooled relative risk for WC was only modestly stronger than BMI at predicting incident T2DM and no significant differences were observed between the three indices of weight (WC, WHR, or BMI).[68] The review confirmed that all measures of obesity are strongly predictive of T2DM.[68]

1.4.1 Pathogenesis of diabetes due to obesity

The close relationship observed between obesity and T2DM suggests the conditions share common pathophysiology. Indeed obesity and T2DM share a number of characteristics including raised free fatty acids (FFA), raised levels of oxidative stress and chronic inflammation. These abnormalities have a complicated interrelated relationship with each other and act in a positive feedback loop to exacerbate one another.

1.5 Oxidative stress

Oxidative metabolism is an essential part of normal cell physiology.[69] A delicate balance exists between beneficial and harmful levels of reactive oxygen species (ROS).[70, 71] Oxidative stress occurs when an imbalance between pro-oxidants and antioxidants occur in a biological system and it is widely accepted that oxidative stress acts as a participant in the development and progression of T2DM.[72] It is proposed that the accelerated complications and increased risk of coronary heart disease seen in T2DM are due to the presence of oxidative stress. Levels of ROS can be increased under a state of chronic inflammation; in addition excess substrate availability can result in an overproduction of ROS.[70]

1.5.1 Reactive oxygen species

ROS are highly reactive, short lived derivates of oxidative metabolism.[71] ROS include O_2^-, OH^- and H_2O_2. Reactive nitrogen species (RNS), such as NO^- have similar properties and also cause cellular damage. Free radicals are molecules containing one or more unpaired electrons and are highly reactive as they need to obtain an extra electron for their outer orbit, to do this it is necessary to break up a pair of electrons which results in a chain reaction of free radical production.[69] O_2^- is often considered the primary oxidant as it can arise from metabolic processes and then go on to generate secondary ROS.[70] ROS can cause lipid peroxidation, DNA damage and alter protein structure resulting in metabolic dsyregulation and alterations in cellular signalling and cellular functions.[71] Under normal metabolic conditions ROS are produced at low levels, the main source being the mitochondrial electron transport chain. It is estimated that 1-3% of electrons in the electron transport chain leak prematurely during energy transduction.[70] Over nutrition

results in an increase in substrate load which increases the citric acid cycle activity producing an excess of ROS. In an attempt to reduce the presence of harmful ROS insulin stimulated uptake of substrate is inhibited reducing substrate load on the mitochondria. Thus insulin resistance is a compensatory mechanism, protecting cells from further damage.[73]

1.5.2 Antioxidant systems

An antioxidant is any substrate when present at low concentrations in comparison to an associated oxidisable substrate, significantly delays or inhibits oxidation of that substrate.[74] A number of antioxidant enzyme and non-enzymatic antioxidant systems exist which contain ROS and RNS within normal limits. Primary antioxidant enzymes include catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). The superoxide dismutase family of antioxidants act to eliminate O_2^- whereas catalase and glutathione peroxidase families eliminate H_2O_2.[69] Non-enzymatic antioxidants include vitamin C, vitamin E, beta-carotene, flavonoids and phytochemicals. These dietary antioxidants are found in foods, including fruit and vegetables and can significantly reduce the adverse effects of ROS. Antioxidants work in synergy with one another and are capable of adapting to changing needs.[72, 74]

1.5.3 Oxidative stress and insulin resistance

When an imbalance between ROS and antioxidants occurs in the favour of ROS, oxidative stress results. Raised levels of ROS result in the oxidation and cellular damage of DNA, proteins and lipids. Membrane phospholipids can become inexplicably modified by lipid oxidation resulting in disturbed physiochemical

functions, including insulin sensitivity of cells.[75-77] In a state of obesity high concentrations of lipoproteins are available for lipid peroxidation; once lipids are modified they themselves can induce a pro-inflammatory and a pro-atherogenic response.

1.5.4 Lipotoxicity

Elevated FFA are present in both overweight and obese individuals prior to the onset of T2DM.[78] The Paris Prospective Study observed higher levels of FFA in subjects who developed glucose intolerance over 5 years and concluded that FFA significantly contributed to an increased risk of developing IGT and T2DM. For each 0.12mmol/l increase in fasting non-esterified fatty acids (NEFA) there was a 30% increase risk of glucose deterioration.[79]

In 1963 Randle first proposed that raised FFA would result in competition between glucose and FFA as energy substrates within skeletal muscle cells. It was proposed that FFA would be preferentially oxidised when present in high amounts resulting in raised blood glucose concentrations.[80, 81] This theory helps us to understand the development of insulin resistance seen prior to T2DM. However T2DM is not only characterised by raised FFA but by high intracellular muscle lipids (IMCL).[82] IMCL have been positively correlated to BMI and negatively correlated to insulin sensitivity in Europeans.[83] A further study demonstrated that IMCL were correlated to whole body glucose uptake independently of BMI, fasting plasma glucose and age.[84] In addition lipid fusion studies have demonstrated that raised FFA result in a decrease in insulin stimulated glucose metabolism; 90% of which was associated with reduced insulin stimulated phosphorylation, indicating a reduced function of

the insulin stimulated signalling cascade.[85] Raised FFA due to obesity may directly impact on insulin sensitivity of cells and indirectly by causing an increase in ROS due to excess substrate load within the mitochondria.

1.5.5 Inflammation

Historically adipose tissue was considered a simple storage organ; however discovery of leptin in the early 1990s changed this perception. It is now accepted that adipose is an endocrine organ involved in the homeostasis of a number of metabolic processes including energy expenditure, appetite regulation, insulin sensitivity, reproductive functions, bone metabolism, inflammation and immunity.[86] Increased adiposity can result in the dsyregulation of these processes resulting in pathogenic consequences. A number of bioactive compounds known as adipokines have now been identified which are integrated into a communications network to maintain metabolic homeostasis.[87] Adiponectin is one such adipokine, and concentrations are inversely related to body weight.[88] However adiponectin appears to have an effect on the glycaemic state independently of adipose levels and has been shown to be low in insulin resistant states.[89] In a population of Pima Indians, individuals matched by age, sex and BMI, those who subsequently went on to develop T2DM had lower baseline adiponectin concentrations compared to those who did not develop T2DM.[90]

Adiponectin may also have anti-inflammatory properties with an ability to suppress tumor necrosis factor (TNF-α) and interleukin 6 (IL-6). Indeed both obesity and T2DM are characterised by a state of chronic inflammation. Obesity results in chronic activation of the innate immune response;[91] leading to the expression of

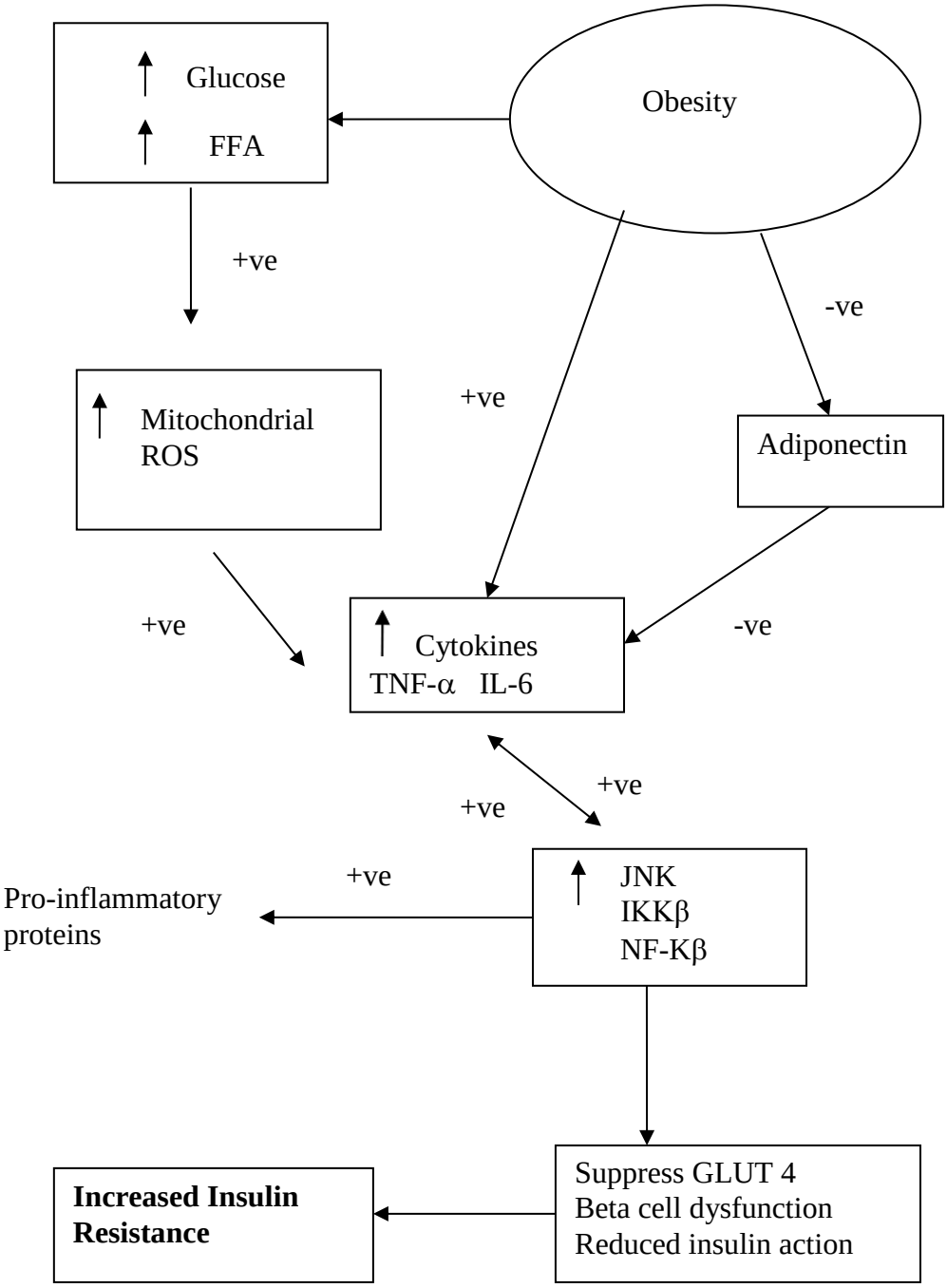
cytokines such as TNF-α and IL-6 which have both local and systemic pro-inflammatory effects. TNF-α is the first step in the inflammatory cascade[92] and stimulates the c-Jun amino terminal kinase (JNK) and the IKK-β/NF-Kβ pathways. These pathways up regulate the expression of additional mediators of inflammation.[93] In addition increased levels of NF-Kβ have been correlated to insulin sensitivity.[94] (Figure 1-4, page 24).

Concentrations of IL-6 and TNF-α are positively associated with obesity markers, including percentage body fat, BMI, WC, body weight and fat mass.[95, 96] In addition it has been demonstrated that weight loss can result in subsequent reduction of both TNF-α and IL-6 levels.[97, 98] Increased levels of walking activity have also been associated with lower levels of circulatory pro-inflammatory markers.[99] However TNF-α and IL-6 are also associated with glycaemic state independently of adiposity levels. Subjects matched for BMI and age with insulin resistance showed greater IL-6 plasma levels and had significantly higher secretion of TNF-α compared to those without insulin resistance.[95] In a German population median IL-6 levels differed across individuals with normal glucose tolerance (NGT), IGT and T2DM. Both those with IGT and T2DM were observed to have higher IL-6 levels than those with NGT.[100] Expression of cytokines may initiate the development of insulin resistance via inhibition of insulin signalling pathways.[91] TNF-α is believed to inhibit glucose uptake via suppression of GLUT-4 receptors thus disrupting insulin stimulated glucose uptake.[93]

IL-6 and TNF-α mediate the production of acute phase proteins such C-Reactive Protein (CRP) and Serum Amyloid A (SAA).[89] CRP is considered one of the most

important inflammatory markers in humans and can stimulate the synthesis of other cytokines such as cell adhesion molecules.[92] Baseline IL-6 and CRP levels have been observed to be significantly higher among women who develop T2DM compared to those who do not develop T2DM.[101] The Insulin Resistance Atherosclerosis study also observed a significant linear increase in T2DM incidence across increasing CRP quintiles.[102] A recent meta-analysis showed SAA to be positively associated with BMI, and weight loss was significantly associated with lowering SAA levels.[103] SAA has also been implicated in down regulating the genes involved in insulin sensitivity.[89] A raised inflammatory response may also be implicated in the increased prevalence of T2DM in SA individuals. Higher levels of CRP have been observed in SAs as compared to WEs.[104, 105] In addition lower concentrations of adiponectin have been reported in SAs as compared to WEs.[106]

Figure 1-4: Inflammation and insulin resistance (adapted from Evans 2003[107] and Wright 2006[108])

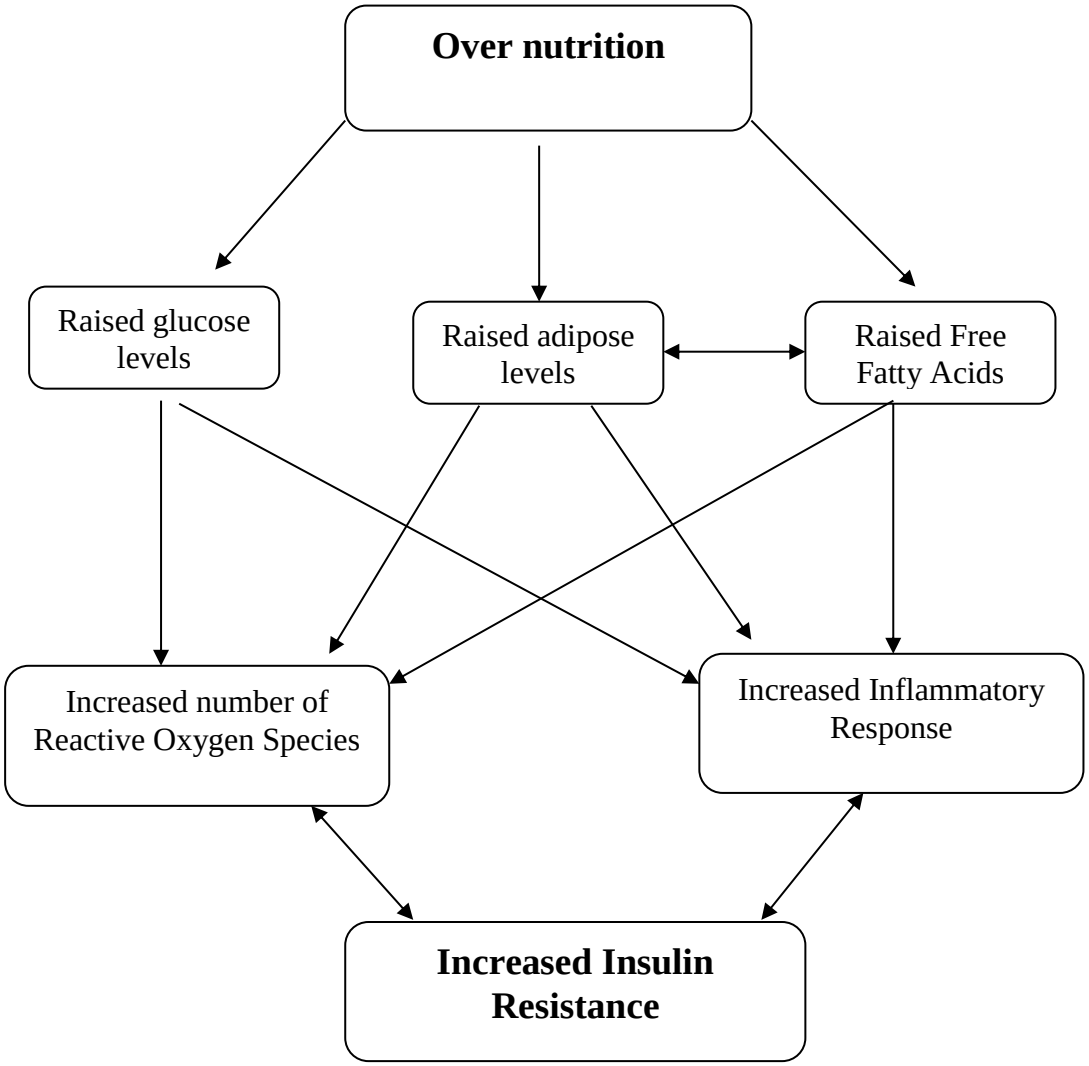


1.5.6 Interaction between inflammation, oxidative stress and free fatty acids

The exact mechanistic link between obesity and T2DM is still uncertain and developing research shows a complex relationship.[93] Increased FFA, raised levels of oxidative stress and chronic inflammation may all act independently to increase risk of insulin resistance and T2DM however they also interact and regulate one another to create a complicated pathology.

Oxidative stress can occur due to both systemic inflammation and by accumulation of fat. It has been demonstrated that adipose tissue is a major source of ROS[109] and that cytokines enhance ROS production by stimulating macrophage and mitochondrial release of ROS.[110] In addition the inflammatory response is up regulated by both modified lipoproteins caused by ROS and by the presence of ROS themselves, which up regulate the NK-Kβ pathway, demonstrating a positive feedback mechanism between inflammation and oxidative stress. The exact relationships are still poorly understood and further research is warranted.

Figure 1-5: Interaction between inflammation, oxidative stress and FFA



1.6 Chapter summary

Chapter 1 has presented the current state of obesity and diabetes within the UK. The chapter demonstrated how the causes of obesity and diabetes are closely linked and that both conditions are increasing in prevalence across the UK and throughout the world. Chapter 1 has introduced oxidative stress as a potential mechanism which may link obesity and diabetes.

Chapter 2

Prevention of Diabetes

2.0 Chapter overview

Following on from the first chapter where diabetes was introduced, chapter 2 will discuss the importance of prevention of T2DM. Studies which have been conducted across the world to determine whether T2DM is indeed a preventable disease will be examined. The chapter will then attempt to elicit the role that dietary manipulation plays in the prevention of T2DM. Whether current dietary recommendations are appropriate will also be explored.

2.1 Prevention of diabetes

Diabetes and the serious co-morbidities associated with it are both difficult to treat and expensive to manage.[111] It is estimated that approximately 5% of NHS resources and 10% of hospital in-care resources are devoted to the care and treatment of T2DM.[112] Prevention of T2DM is a global priority as preventing a small proportion of cases would not only save thousands of lives[113] but also substantially reduce costs to the health care system.

T2DM is increasing in all populations and age groups throughout the world. This rapid increase in prevalence over the last two decades suggests that environmental factors are important.[93] In addition populations undergoing rapid lifestyle changes and rapid nutrition transition have seen the greatest increase in prevalence.[49] Primary prevention is the prevention of a disease by targeting or controlling modifiable risk factors in a population[114] and is key to improving public health. In 1921 Elliot Joslin was the first to propose that the primary prevention of T2DM was important[115] yet nearly ninety years later this is still not successfully implemented within the UK. However awareness of the need for successful prevention

programmes has increased in recent years[114] and a number of trials have provided evidence to support the theory that T2DM is a preventable and indeed potentially reversible disease.[64, 116-118]

2.2 Lifestyle modification programmes for the prevention of type 2 diabetes

2.2.1 The Malmo feasibility study

The Malmo study[119] was one of the first lifestyle modification (LSM) intervention trials, carried out in Sweden and reported in 1991. 415 men with a mean age of 48 years were enrolled into four different treatment groups. There were two intervention groups, a newly diagnosed T2DM patient group and another with IGT; both groups received advice on LSM. LSM consisted of 6 months dietary and physical activity advice either as a group or individually after initial instruction. The two control groups were a non-randomised group of IGT subjects and a group of normal controls. The two control groups did not receive specific diabetes prevention treatment but were referred to their own physician. After 5 years follow up significant weight loss was seen in subjects with IGT and T2DM who had received LSM as compared to both control groups. The treatment group's initial weight loss was greatest at the end of the first year, with weight increasing subsequently. However mean weight stabilised at lower levels compared to both control groups where there was an overall increase in weight. Weight reduction was maintained over 5 years by 82% and 71% of participants in the T2DM and IGT groups. Physical activity was also improved in the two treatment groups, demonstrated by an increase in oxygen uptake. In the T2DM group 53.8% of participants had improved glucose levels. In the IGT group glucose tolerance

improved in 75.8% of cases. Yet in the IGT non-randomised control group, glucose tolerance had deteriorated in 67.1% of cases. An additional, important outcome of this early trial was the demonstration that a large number of sedentary and overweight, middle aged, glucose intolerant and diabetic subjects could successfully participate in a 5 year intervention programme. The study demonstrated that LSM could be implemented within a target group for effective prevention of T2DM.

2.2.2 The Da Qing study

The Da Qing randomised controlled trial (RCT) was carried out in Chinese men and women with IGT and reported in 1997.[120] The LSM intervention was carried out in 33 local health clinics with a 6 year follow up; 530 study participants completed the trial. The clinics were randomised to give diet, exercise, diet and exercise advice or as control groups. Subjects in the dietary treatment groups were set individual goals for calorie consumption and received individual counselling and group counselling sessions weekly for one month, monthly for three months and then once every three months for the entire study period. Participants in each clinic were categorized into two groups according to BMI ($<25 kg/m^2$ and $\geq 25 kg/m^2$); only those in the overweight category were encouraged to lose weight. Those receiving exercise advice were educated on how to increase activity levels, again at weekly then monthly sessions. Those in the control arm were given general information on diet and physical activity. Incidence of T2DM was reduced by 31% in the diet arm, 46% in the exercise arm and 42% in the exercise and diet arm as compared to control. In addition, incidence of T2DM in both BMI categories was significantly lower in the intervention arms as compared to the corresponding control groups

(except for BMI<25kg/m^2 diet group). The study added to the supporting evidence for benefits of LSM in different ethnic groups.

2.2.3 The Finnish Diabetes Prevention Study

Following on from the Malmo feasibility study the Finnish Diabetes Prevention Study was developed.[64] The study was a multi-centre RCT, enrolling males and females with IGT between the ages of 40 and 65 years. Participants were randomised to either control or treatment groups and followed for a 6 year period. The groups were given five main goals to achieve: weight loss of ≥5%, moderate exercise of ≥30minutes per day, intake of dietary fat to be less than 30% of total energy intake, saturated fat to be less than 10% of total energy intake and fibre intake ≥15g per 1000kcal ingested. Subjects in the treatment group were given face to face consultations of between 30 minutes to 1 hour and had a total of seven individualised sessions which were based on a completed three day diet diary. Subjects could also attend voluntary cookery lessons and were offered free supervised individually tailored exercise sessions. The control groups were given verbal and written behaviour modification information. The study reported a 58% reduced incidence of T2DM in the treatment group compared to the control group. Weight loss was modest over the three years (3.2kg ±4.5) but was still significantly different between the two groups. In addition a strong correlation was demonstrated between success of subjects meeting the five goals and the incidence of diabetes, demonstrating the combined benefits of diet and exercise modification.

2.2.4 Diabetes Prevention Programme (DPP)

In 2002 a large trial from America, The Diabetes Prevention Programme (DPP) was reported.[116] The trial included 27 health centres with 3234 subjects randomly assigned to three intervention groups, placebo tablets plus standard lifestyle advice, metformin (an antihyperglycemic agent) plus standard lifestyle advice and an intensive lifestyle modification programme (LSMP). Subjects were followed up for an average of 2.8 years. The LSM group were advised to achieve and maintain a weight loss of at least 7% of their initial body weight through a healthy low fat diet and to engage in moderate intensity exercise for 150 minutes per week. Subjects attended 16 sessions with a curriculum based on diet, exercise and behaviour modification which was taught on a one to one basis. This was the first trial to include a range of ethnic communities, with 54.7% White American, 19.9% African American, 15.7% Hispanic, 5.3% American Indian and 4.4% Asian populations. Weight loss between the three treatment groups was statistically different with an average loss of 0.1kg, 2.1kg and 5.6kg in the placebo, metformin and LSM groups respectively. The incidence of T2DM was 58% lower in the LSM group compared to placebo. The Metformin group achieved 31% lower incidence compared to the placebo group, demonstrating that LSM was more effective than placebo or metformin.

2.2.5 Indian Diabetes Prevention Programme (IDPP)

Within the UK and worldwide Asian Indians have a higher incidence of T2DM as compared to other ethnic groups.[50, 121] The Indian Diabetes Prevention Programme (IDPP) provided important information on LSM for the prevention of T2DM in this susceptible group.[122] The IDPP was a RCT enrolling native urban Asian Indian

individuals with IGT. Over three years 502 subjects were randomised between four groups. The control group were given standard health care advice, the second group given LSM, the third group given metformin and the final group metformin plus LSM. Dietary modification in the LSM arms was individualised for each subject and personal sessions were conducted at six monthly intervals. Results showed that the cumulative incidence of T2DM was significantly reduced in all three treatment groups. Over 3 years cumulative incidence of T2DM was 28.5% for LSM alone, 26.4% for metformin and 28.2% with LSM plus metformin as compared to the 55% in the control arm. An important observation of the study was that without weight loss T2DM was still preventable in Asian Indian subjects with IGT. The study also added to data showing Asian Indians to be a high risk group, progression rates to T2DM were on average 18.3% per year as compared to 6% per year in the Finnish DPS study.

2.2.6 The SLIM Study

A smaller trial, the Study on Lifestyle intervention and impaired glucose tolerance Maastricht (SLIM) was carried out in the Netherlands.[123] This RCT combined dietary and physical activity advice and was designed to improve glucose tolerance in IGT subjects. 147 individuals were randomised to either control or intervention groups. Those in the intervention arm were given dietary advice based on a three day food record every three months in a one hour counselling session with the objective of losing 5-7% of their initial body weight. They were also given advice on how to increase activity levels to at least 30 minutes per day for five days a week. Over the three years, improvements in 2 hour glucose were seen in the intervention arm despite the fact that initial weight loss did not persist. In

concurrence with the IDPP, results suggested that weight loss is not the only important factor in decreasing the risk of T2DM. In this study there was a high dropout rate (28%) which had not been seen in earlier studies, prompting questions on the adherence to LSM. A possible reason for the high dropout rate suggested by the investigators was the fact that no weight loss programme was offered to those who did not lose weight, so subjects not seeing success may have lost motivation.

2.2.7 The Pre-diabetes Risk Education and Physical Activity Recommendation and Encouragement study (PREPARE)

PREPARE was carried out in the UK and was designed specifically to increase physical activity.[124] Participants were invited to enrol into the RCT after diagnosis with IGT at an on-going screening study in the Leicester area. Participants were randomly assigned to one of three arms, usual care, PREPARE or PREPARE plus pedometer. The PREPARE programme consisted of a single three hour group education session. The programme discussed causes, complications, and timelines of IGT. It also addressed the perceived effectiveness of exercise for treatment of IGT, self-belief about walking ability and barriers to increasing activity. Participants were encouraged to carry out 30 minutes of moderate activity a day. Those in the education plus pedometer arm were provided with a pedometer and an activity diary. They were advised to increase their step count by 3000 steps per day. After 2 years those in the PREPARE plus pedometer group had significantly reduced 2 hour glucose compared to those in the usual care arm, (-1.6mmol/l (95% CI: -0.4 to -2.7)). No significant differences were observed in the education only arm compared to usual care after 2 years.[125]

Table 2-1: Lifestyle intervention programmes

Study	Population	Length of Follow up	Treatment Arms	Intervention	Results	
					Weight Change Mean change (kg)	**T2DM Outcomes**
Malmo Feasibility Study[119] 1991	370 men Individuals with NGT,IGT and T2DM Mean age = 48 years Mean BMI = 26kg/m^2	5 years	Intervention arms: G1: T2DM (n=39) G2: IGT (n=161) Control Arms G3: IGT (n=56) G4: NGT (n=114)	Intervention: 18 months dietary and physical activity advice in a group or individually Control: Referred to own physician for routine care	G1: -2.0 to 3.3 kg G2: -2.0 to 3.3kg G3: +0.2 to 2kg G4: + 0.2 to 2kg	G1: 53.8% improved glucose G2: 75.8% improved glucose G3: 67.1% had deteriorated glucose tolerance G4: no cases diabetes
The Da Qing Study[120] 1997	530 men and women All individuals had IGT Mean age = 45 years Mean BMI = 26kg/m^2	6 years	Intervention arms: Diet: (n=130) Exercise: (n=141) Diet & Exercise: (n=126) Control arm: (n=133)	Intervention: Individuals received individual and group counselling on either diet, exercise or diet and exercise depending on treatment arm Counselling conducted weekly for 1 month, monthly for 3 months then every 3 months up to 24 months. Control: Individuals given brochures on healthy diet and exercise	Diet: -2.43kg Exercise: -1.93 kg Diet & Exercise: -3.33kg Control: -1.55kg	As compared to control: Diet: 33% reduced cases Exercise: 47% reduced cases Diet & Exercise: 38% reduced cases

Study	Population	Length of Follow up	Treatment Arms	Intervention	Results	
					Weight Change Mean change (kg)	**T2DM Outcomes**
The Finnish Diabetes Prevention Study (FDPS)[64] 2001	523 men and women All individuals had IGT Mean age = 55 years Mean BMI = 31kg/m^2	3.2 years	Intervention arm (n= 265) Control arm (n=257)	Intervention: Individuals given tailored diet and exercise advice. 5 main goals: Reduce weight by 5%, fat intake <30%, SFA intake <10%, consume 15g fibre per 1000kcal, complete 30minutes moderate exercise per day Control: Received general oral and written advice on diet and exercise	After 2 years: Intervention: -3.5kg Control: -0.8kg	Cumulative incidence T2DM: Intervention: 11% Control = 23% 58% reduced incidence in intervention arm
Diabetes Prevention Programme (DPP)[116] 2002	3234 men and women All individuals had IGT Mean age = 51years Mean BMI = 34kg/m^2	4 years	Lifestyle & Placebo (n=1082) Lifestyle & Metformin (n=1073) Intensive Lifestyle (n=1079)	Intensive Lifestyle: Received individual and group sessions on diet, exercise and behaviour change. Aimed to reduce body weight by 7%, consume low fat, low calorie diet and complete 150minutes of moderate exercise a week. Lifestyle & Placebo or Metformin: Given written information and an initial individual session	Lifestyle & Placebo: -0.1kg Lifestyle & Metformin: -2.1kg Intensive Lifestyle: -5.6kg	Incidence diabetes: Lifestyle & Placebo = 7.8% Lifestyle & Metformin = 11% Intensive Lifestyle = 4.8% 58% reduced cumulative incidence in intensive lifestyle arm

Study	Population	Follow up	Treatment Arms	Intervention	Results	
					Mean weight change (kg)	**T2DM Outcomes**
Indian Diabetes Prevention Programme (IDPP)[122] 2006	502 men and women All individuals had IGT Mean age = 46years Mean BMI =26kg/m^2	3 years	Control (n=133) Lifestyle (n=120) Metformin (n=128) Lifestyle & Metformin (n=121)	Lifestyle intervention arms: Given personalised diet and exercise advice every 6 months, and monthly phone calls. Advised to reduce calories, fat, refined carbs, avoid sugar and increase fibre rich foods Control: Given standard health care advice	No significant changes in weight reported	Compared to control group, risk of diabetes was reduced by: Lifestyle: 15.7% Metformin: 14.5% Lifestyle & Metformin: 15.5%
SLIM study[123] 2008	96 men and women All individuals had IGT Mean age = 53 years Mean BMI =29kg/m^2	3 years	Control (n=49) Intervention (n=47)	Intervention: Given personalised diet and activity advice. Aim to meet Dutch healthy diet guidelines, to reduce body weight by 5 to 7% and complete 30 minutes per day moderate activity Control: Briefly informed about the benefits of a healthy diet and physical activity	Intervention: -1.08kg Control: +0.16kg	Cumulative incidence diabetes: Intervention: 18% Control: 38%

Study	Population	Follow up	Treatment Arms	Intervention	Results	
					Mean weight change (kg)	**T2DM Outcomes**
PREPARE study[125] 2011	73 men and women All individuals had IGT Mean age = 65years Mean BMI =29 kg/m^2	2 years	Education (n=22) Education & pedometer (n=22) Usual care (n=29)	Education: Single 3 hour group education session aimed at increasing physical activity Education & pedometer: In addition to the group education, participants were given a pedometer and an activity diary, and advised to increase step counts by 3000steps per day Usual care: Sent a brief information sheet in the post	Compared to control arm: Education: +0.2kg Education & pedometer: +1.6kg	Compared to control arm: Education: -0.4mmol/l in 2 hour glucose Education & pedometer: -1.6mmol/l in 2 hour glucose

2.3 Sustained effects of lifestyle modification

The evidence from these LSM studies supports the need and success of LSM in high risk individuals. Indeed a meta-analysis examining the effectiveness of LSM in prevention of T2DM concluded that there was overwhelming evidence to support the benefit of LSM intervention to prevent or delay T2DM.[126] In further support of the success of LSM is the demonstration of sustained beneficial effects in those enrolled to treatment arms. The Finnish DPS reported that those enrolled to the intervention arm had a relative risk reduction of 43% after 7 years of follow up.[127] Beneficial lifestyle changes achieved by those in the active intervention arm were sustained even after discontinuation of active counselling. The Da Qing 20 year follow up study observed a cumulative incidence T2DM of 80% in the intervention group as compared to 93% in the control group.[128] In addition participants in the active intervention had 3.6 fewer years with T2DM.

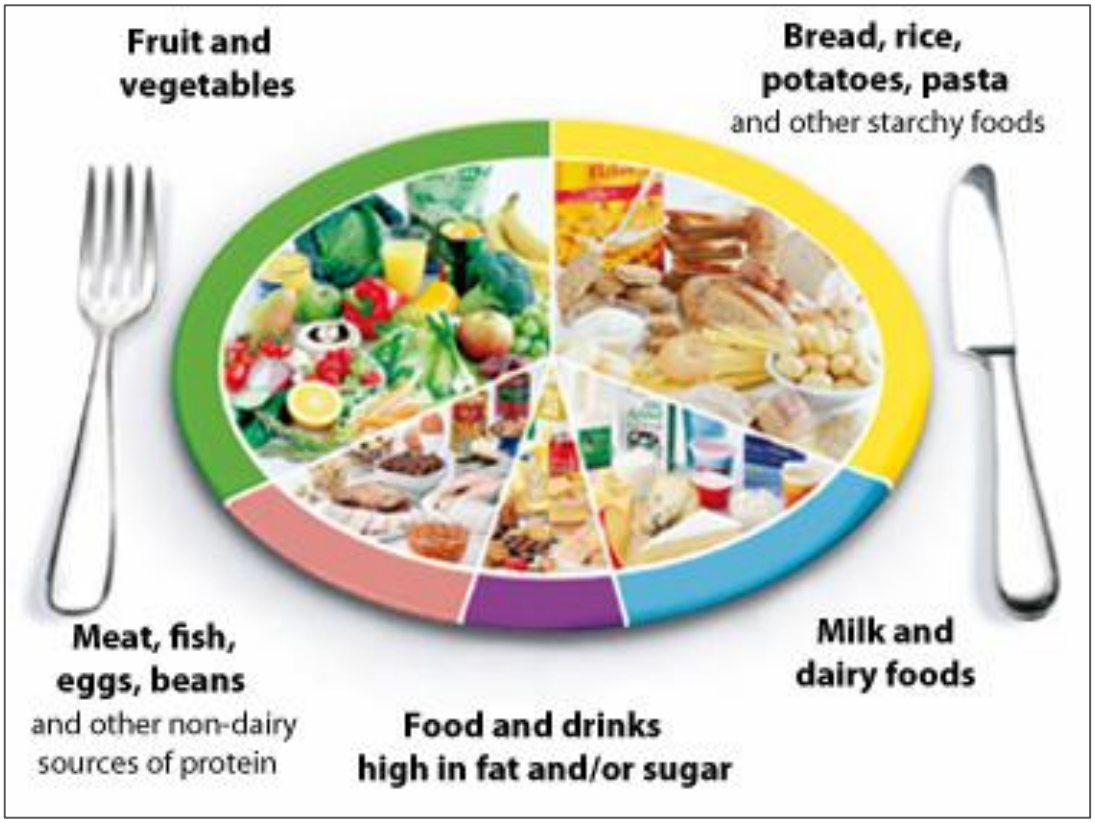
2.4 Dietary modification for the prevention of type 2 diabetes

2.4.1 Dietary change as part of lifestyle programmes

It has been demonstrated that dietary interventions alone can significantly reduce the risk of T2DM.[126] A meta-analysis calculated a pooled HR of 0.67 (95% CI: 0.49 to 0.92). However the role of individual aspects of diet for the prevention of T2DM remains to be fully elucidated. The WHO state that data from LSMPs does not allow for the disentanglement of dietary aspects to determine which nutrients are important for the prevention of T2DM.[129] The role of dietary intake separate from weight loss also remains to be fully explored.

Both the FDPS study and the DPP reported a decrease in average fat intake of individuals enrolled into treatment arms.[116, 130] The FDPS also found an increase in percentage energy from carbohydrates and fibre; these changes were significant after 3 years. Adjustment for weight change did not significantly alter the results[131] suggesting that a change in diet without a reduction of calories may also be important. The LSM studies discussed all recommended similar dietary advice; encouraging a high carbohydrate (55-65% of energy intake), high fibre intake (≥15g/1000kcal) and restricted fat (≤30% or ≤35% in FDPS) and saturated fat (<10%) intake. The advice given was similar to that given to the general population for healthy eating.[132] (Figure 2-1)

Figure 2-1: The Eatwell Plate[132]



2.4.2 Dietary recommendations for the prevention of type 2 diabetes

Although it is still unclear which aspects of diet are important for the prevention of T2DM, the WHO, ADA and Diabetes UK have produced dietary guidelines for the prevention of T2DM.[129, 133, 134] It has been suggested that obesity is the single most important risk factor for T2DM, being overweight, having abdominal fat distribution and obesity are associated with around 90% of all T2DM cases.[49, 55] Guidelines emphasise the need to maintain a healthy weight (<25kg/m^2), or for those who are overweight to reduce weight.[129, 133, 134] The ADA suggests that those developing lifestyle modification programmes for those at risk of T2DM should recommend reducing dietary fat and increasing physical activity to reduce weight. The only direct recommendation for dietary changes, independent of weight loss is that those at risk of T2DM should achieve 14g per 1000kcal of fibre intake and that whole grains should make up half of total grain intake.[133] However the WHO state that there is only probable evidence that intake of non-starch polysaccharides reduces risk of T2DM. The WHO claims that the only convincing evidence for the dietary prevention of T2DM is weight loss.[129] However a meta-analysis of the effectiveness of LSMP showed that dietary modification alone compared to usual care was associated with a 33% reduced risk of developing T2DM (HR = 0.67 95% CI: 0.49 to 0.92).[126] Furthermore the Network to Prevent Diabetes team have developed a toolkit which provides advice to those trying to develop prevention programmes (Figure 2-2, page 42).[135]

There is a clear need for further research into the effects of different dietary components and the relationship to the prevention of T2DM. Although these guidelines have been produced; is there any evidence that changes in dietary intake

to delay or prevent the onset of developing T2DM should be any different from that given for a general healthy diet?

Figure 2-2: Nutrition and dietary guidance to prevent diabetes[135]

Goals for Food Intake	Goals for Long-Term Nutrient Intake
• Consuming fruit, vegetables and legumes in abundance (>500g or 5 portions per day) • Choosing wholegrain in all cereal products • Limiting sugar to <50g/day, including sugar in food and beverages • Consuming vegetable oil and/or soft margarines and/or nuts as the primary source of fat • Limiting butter, other saturated fat and partially hydrogenated fats • Choosing low fat milk and meat products • Consuming fish regularly (>2per week) • Consuming alcoholic beverages in moderation (<2drink/day for men and <1 drink/day for women) if at all	• Energy intake balanced with physical activity levels to achieve or maintain healthy body weight • Total fat 25-35E% (60-80g/day with 2000kcal daily intake), of which saturated or trans-fat <10E% • Dietary fibre 25-35g/day • Salt (NaCl) <6g/day • Alcohol <5E% E% = proportion of total energy

2.4.3 Dietary fibre

Dietary fibre is the term given to the components of food derived from non-digestible plant cell walls; that are not absorbed in the small intestine. Fibre is generally split into two groups, soluble and insoluble. Soluble fibre includes pectins, gums, storage polysaccharides and some hemi-celluloses. Insoluble fibres include cellulose, lignin and many hemi-celluloses.[136] However it can be argued that simply splitting fibre into either of these two groups over simplifies the

complex nature of dietary fibre; extent of fermentation, viscosity and binding capacity are all important aspects of fibre and its effect on physiological response.[137, 138]

Fibre can impact on intestinal tract time, absorption of macronutrients and contains a number of potential beneficial compounds such as vitamin E, B vitamins and minerals. Fibre can also affect the action of digestive enzymes and the secretion of gastrointestinal and pancreatic hormones.[136] Insoluble fibre reduces intestinal tract time, potentially reducing time for carbohydrates to be absorbed in the jejunum.[139] Soluble fibre delays gastric emptying and absorption, therefore slowing the absorption and digestion of carbohydrates,[139] potentially delaying the insulin response. Non digestible fibre passes through to the large intestine and becomes available for fermentation by the microflora of the colon, resulting in the production of short chain fatty acids (SCFA). SCFA include acetate, propionate and butyrate. These compounds are important not only for local energy supplies of the colon mucosa but they may also potentially impact on carbohydrate metabolism.[140]

Dietary fibre and wholegrain intake are the only components strongly recommended for the prevention of T2DM by the ADA,[133] and the evidence from epidemiological studies is fairly compelling. The Nurses Health Study[141] a large prospective study, was carried out in the USA and enrolled over 75,000 females. The study compared the highest consumption of whole grains to the lowest and found a 38% reduced risk of T2DM (RR = 0.62, 95% CI: 0.53-0.71, p=<0.0001). The Iowa Women's Health study also demonstrated wholegrain intake to be strongly inversely associated with

a reduced risk of T2DM (RR = 0.79, 95% CI: 0.65-0.96, p=0.009).[142] An inverse relationship between total dietary fibre and T2DM was also reported.

A further prospective study from America, The Male Health Professionals follow up study also supports the importance of cereal fibre in the diet.[143] The authors reported a strong inverse relationship between wholegrain intake and T2DM incidence, (RR 0.58, 95% CI: 0.47-0.70, p=<0.0001). The relationship was largely explained by cereal fibre, which has also been shown to explain the observed relationship between whole grains and T2DM in other studies.[139] A recent report examined both the Nurses Health Study I and II plus the Male Health Professionals follow up and specifically investigated the potential role that white and brown rice may have on the development of T2DM.[144] The data showed that greater consumption of white rice was associated with an increased risk of T2DM while a greater intake of brown rice was associated with a reduced risk of developing T2DM. Substituting brown rice for white rice decreased risk of T2DM independently of other lifestyle factors, supporting the role of whole grains for the prevention of T2DM.[144] A meta-analysis of prospective studies examining whole grains and incidence T2DM also reported that studies consistently found wholegrain intake to reduce the incidence of T2DM. Pooled summary estimates found for each two serving increase per day there was a 21% reduction in risk of T2DM (RR 0.79, 95% CI: 0.72 – 0.87).[145]

The relationship between wholegrain consumption and diabetes prevention appears strongly associated with BMI. When determining relative risks between consumption of whole grains and T2DM adjustment for BMI must be considered.

The meta-analysis carried out by de Munter et al found that adjustment for BMI substantially weakened observed associations between wholegrain intake and T2DM.[145] In addition the Health Professionals Follow up study reported that a greater intake of whole grains protected from long term weight gain.[146] Dietary fibre and cereal fibre were also inversely related to weight gain, independently of whole grains. It has been claimed that BMI is the strongest confounding factor when determining relationships between wholegrain intake and risk of T2DM.[141] Fung et al reported differences in the effects of wholegrain consumption with risk of T2DM depending on an individual's weight status.[143] In obese men wholegrain intake was only weakly associated with T2DM incidence but in those with a BMI <30kgm^2, a higher intake resulted in a 50% reduced incidence of T2DM when comparing the highest to lowest consumption levels. Thus BMI may potentially modify the association between wholegrain intake and T2DM risk. Data suggests that once BMI is greater than 30kgm^2 the influence which wholegrains may play on glucose regulation is reduced.

Studies also exist which demonstrate that a change in wholegrain consumption can influence glucose and insulin levels. A cross over study provided obese subjects with two six week feeding periods and reported significantly lower fasting insulin levels during the wholegrain feeding period compared to the refined grain feeding period. There was also a tendency for fasting glucose to be lower in the wholegrain period but the results were not significant.[147] Aller et al suggest that modest increases in soluble fibre can affect glucose levels; subjects without T2DM increased fibre in the diet which resulted in a 12.3% reduction in glucose levels.[148] However fibre intake was increased via an increase in breakfast cereal and by

consuming two apples per day; the study claims fibre was the influencing factor, yet it cannot exclude other potential benefits of increased apple consumption. It is well documented that fruits are a rich source of antioxidants which may play a role in glucose control.[149] A more robustly designed study enriched different breakfast meals with oat powder, rye bran or sugar beet fibre; the fat and carbohydrate content of the meals was balanced by rapeseed oil and dextrose powder. All test meals reduced incremental postprandial glucose compared to the control meal, however, only the rye bran meal showed significant improvements.[150]

Although the data on wholegrain intake and risk of T2DM appears consistent a Cochrane review concluded that as most evidence is from observational studies where confounding factors cannot be ruled out, then more research is needed before whole grains can truly be termed preventative.[151] In addition the Scientific Advisory Committee on Nutrition states that studies are inconsistent and inconclusive so firm recommendations cannot be made regarding fibre, whole grains and T2DM.[152] Therefore it seems that more research into wholegrain consumption is required before their role in the prevention of T2DM will be accepted by governing bodies. RCTs need to be developed which investigate the potential glycaemic benefits of independently increasing wholegrain intake in those with habitually low wholegrain consumption.

2.4.4 Dietary fat

Dietary fat can be derived from both plant and animal sources. Animal fats have a high percentage of saturated fatty acids (SFA) and are therefore solid at room temperature. Coconut oil and palm oil also contain high levels of SFA so are also

solid at room temperature; however fats from most plant sources are liquid at room temperature due to a high percent of the unsaturated fatty acids (USFA).[153] USFA acids contain double bonds and the degree of un-saturation determines whether a fat is mono or poly unsaturated.

Reduction of SFA intake is generally included in any dietary advice to prevent T2DM; however, evidence is contradictory and inconclusive. Plasma membranes are composed of lipids and the incorporation of different fat types into cell membranes can alter the properties of the membrane as its composition is not fixed, but adaptive and highly varied.[154] Chain length and number of double bonds present in the membrane can influence physical properties and thus the function of the membrane. Altering phospholipid fatty acid composition can affect membrane fluidity, cellular functions, carrier mediated receptors and properties of membrane bound enzymes.[150, 155] Insulin receptors are embedded in the lipid bilayer of plasma membranes of cells including skeletal and adipose cells;[156] thus the composition of membranes may be implicated in insulin sensitivity.

Early rodent studies consistently showed that rats fed high fat diets went on to develop insulin resistance in both skeletal and adipose cells.[157, 158] The effect on insulin action appeared to coincide with an accumulation of body fat.[157] Human studies also support this theory; over 20 years ago a study carried out in healthy men showed a positive correlation between arachidonic acid and two hour glucose while SFAs were negatively correlated to insulin secretion.[150] A study in older men showed that the fatty acid composition of both serum and skeletal muscle was affected by the level of SFA present.[159] In particular, a strong relationship between

levels of palmitic acid content in skeletal muscle lipids and insulin sensitivity was observed. The authors estimated that the proportion of palmitic acid in the muscle lipids independently explained 23% of the variation in observed insulin sensitivity.[159] The San Luis study demonstrated that those with impaired glucose tolerance who consumed greater amounts of total fat had an increased risk of going on to develop T2DM and results remained in subjects with similar levels of obesity, insulin and glucose levels at baseline.[160] Further supporting the role of fat type in the diet is the KANWU study, a RCT where participants were randomized to receive diets high in SFA or Monounsaturated fatty acids (MUFA).[161] Changes in serum phospholipids reflected the two test diets and insulin sensitivity was significantly decreased in the group consuming high SFA diet. However insulin sensitivity was unchanged in the MUFA group. The study also randomly allocated placebo tablets of n-3 fish oils to participants; however, addition of n-3 to the diet had no effect on insulin sensitivity.[161]

These studies support the biological plausibility that dietary fat can influence insulin sensitivity, however a number of large epidemiological studies have been less conclusive. The Nurses Health Study found only weak associations between total fat, SFA, MUFA and risk of developing T2DM. However poly unsaturated fatty acids (PUFA) were inversely related to risk of T2DM, (RR = 0.75, 95%CI: 0.65-0.88, p=0.0002).[162] Similar results were observed in the Iowa Women's Health study.[163] The strongest relationship observed in the Nurses Health study was between trans fatty acid consumption and incidence T2DM.[162] The authors concluded that by replacing trans fatty acids with PUFA, T2DM risk could be reduced by 40%. In addition the Male Health Professional follow up study found a

positive significant association between both total fat and SFA and incidence T2DM,[164] however relative risks were attenuated with adjustments for cereal fibre and lost once BMI was incorporated into the model.

The EPIC-Norfolk study included both male and female participants and investigated the relationship between PUFA and SFA intake ratio (P:S).[165] Adjusting for total energy intake the P:S ratio significantly reduced risk of T2DM (OR=0.84, 95%CI: 0.75-0.94). Yet again, adjustment for BMI and WHR resulted in the loss of a significant association (OR=0.91, 95%CI: 0.81-1.03). The study appears to suggest that levels of body fat rather than dietary fat are more important for the development of T2DM. However changes in the phospholipid membrane in comparison to changes made in the diet are only small,[154] therefore the use of food frequency questionnaires (FFQ) in epidemiological research may not be sensitive enough to pick out differences in fat intake. The close association between fat intake and obesity and between obesity and T2DM may make it difficult to disentangle small differences. In addition self report of dietary fat intake may be open to criticism as it is well documented that overweight individuals underreport intake.[166]

2.4.5 Dietary patterns

Determination of interactions between individual foods and disease are difficult to detect; in addition foods are rarely eaten in isolation but in combination with other food groups.[167] The combination of food groups may be interactive, creating an additive effect on health.[168] Analysis of dietary patterns rather than of individual food groups takes this food synergy into account.[167] Logically a number of studies

have examined dietary patterns and the incidence of T2DM and results are largely consistent. Factor analysis was used to determine dietary patterns in the Male Health Professionals follow up study.[169] Two patterns were identified, the prudent pattern based on high intakes of vegetables, legumes, fruit, whole grains, fish and poultry and a Western pattern, characterized by red meat, processed meat, refined grains, French fries, high fat dairy products, sweets, desserts, high sugar drinks and eggs. The prudent diet was only moderately associated with a reduced risk of T2DM but the Western pattern strongly increased risk of T2DM (RR 1.59, 95%CI: 1.32 – 1.93).[169] The prudent pattern was shown also to predict lower T2DM incidence over 23 years of follow up in the Finnish Mobile Clinic Health Examination Survey.[170] Evidence appears to suggest that diets rich in fruit and vegetables, whole grains yet with a low intake of red meat and SFA reduce risk of T2DM.[169-171] The Mediterranean diet is one such diet to consist of these food types. High adherence to a Mediterranean diet in the Seguimiento Univesidad de Navarra study was associated with an 83% reduced risk of T2DM, and this was despite a high prevalence of risk factors in these individuals.[172] The value of examining dietary patterns rather than individual food items is supported by the analysis of dietary intake in the Multi Ethnic Study of Atherosclerosis.[173] A dietary pattern characterized by high intake of whole grains, fruit, nuts and seeds, green leafy vegetables and low fat dairy products was found to reduce diabetes risk by 15%; however no individual food group was independently associated with T2DM.[173]

2.5 South Asian diets

Guidelines for the dietary prevention of T2DM set by WHO, ADA and Diabetes UK make no reference to different cuisines consumed by various ethnic groups

across the world.[129, 133, 134] In addition the advice given to individuals enrolled into the IDPP was similar to the recommendations given in other lifestyle modification programmes.[122] SAs are the largest ethnic minority group in the UK yet studies regarding both the dietary profiles and the relationship between dietary intake and insulin resistance and T2DM are scarce.[174, 175]

Individuals of SA origin are at high risk of developing T2DM and the prevalence of T2DM is two to four times greater in SAs than in their WE counterparts.[176] Furthermore diabetes occurs five to ten years earlier in SAs compared to WEs.[176,53, 177] Evidence suggests that migrant SA populations and those in urban areas of India are at greater risk of T2DM than those in rural areas, and lifestyle factors, including dietary habits are likely to have greater impact on insulin resistance than genetic factors.[174] The work within this thesis was conducted in Leicestershire, UK; an area with a large population of SA individuals. Therefore the study is ideally located to specifically investigate potential differences in dietary intake between SA and WE individuals.

Investigation of dietary intake is made difficult by the heterogeneous nature of SA diets; there are not only differences between, but also within subgroups. Hindu, Punjab, Guajarati, Bangladeshi and Pakistani populations make up the large SA community of the UK.[178] A number of factors such as religion, food beliefs and acculturation and generation will affect dietary intake;[178] therefore a variety of research is needed.

The relationship between fat intake and disease incidence among SA groups has produced conflicting data. It has been suggested that SA cuisine is characterised by high SFA intake.[174] Ghee, a trans fatty acid commonly associated with SA diets, was introduced in the 1960s and is proposed as a potential risk factor for CVD. ghee intake has been identified as a significant risk factor for acute myocardial infarction (MI) in SA adults in Pakistan.[179] A further study also found Indian ghee to be higher in urban populations as compared to rural populations in India and demonstrated a relationship between intake and coronary artery disease (CAD).[180] However Ghee intake is not the same throughout different SA groups, intake is high in groups originating from North India and Bangladesh but it is rarely consumed by those coming from South India, however both groups have the same increased risk of diabetes.[181]

SA populations have been seen to consume lower total percentage energy from fat as compared to WEs.[182] Yet another study showed significantly higher fat intakes in SA migrants as compared to the general population in Scotland.[183] More than half of the SA population derived >15% of food from SFA and only 8.6% of migrants achieved fat intake of <35% as compared to 20% of the general population.[183] The assessment of n-3 PUFA in SA groups has been more consistent, SA populations appear to have low levels of n-3 fatty acids in plasma membranes,[175, 182] in concordance with observed low intakes of steamed and boiled fish.[184] However a study investigated the role of n-3 supplementation and found no positive outcome in insulin sensitivity.[185]

Consumption of large carbohydrate meals has also been suggested as a potential cause of hyerinsulineamia in SA populations.[174] It has been demonstrated that SA individuals have higher percentage energy from carbohydrates as compared to European men.[182] However this study made no observation as to whether the carbohydrates consumed were refined or wholegrain. Conflicting data with regard to wholegrain consumption exists in SA populations. In has been reported that 86% of all SAs consume rice weekly and that SA individuals were less likely to consume brown rice as compared to European counterparts.[184] However in apparent contrast it has also been reported that SA individuals in Glasgow were more likely to consume brown rice than the general population.[186]

The relationship between fruit and vegetables appears more consistent, with reported intake of fruit and vegetables being low in both SA individuals living in India and in migrant SA communities. Mean average intake of fruit and vegetables was 265g/day in SAs residing in a South Indian urban community, with no difference between vegetarians and non-vegetarians.[187] The INTERHEART study demonstrated that daily consumption of fruit and vegetables by SAs was lower than individuals from other countries, despite vegetarianism being common in the population examined.[188] Low fruit and vegetable intake in migrant SAs within the UK may also be common as nutrients associated with fruit and vegetables have been observed to be low in SA populations.[183, 189] SA women living in Glasgow had significantly lower potassium dense diets than Italian migrants and White British controls.[183] In addition vitamin C levels were lowest in SA men and women in a cross sectional study in a London population.[189] As well as reflecting short term intake of vitamin C,[190] plasma vitamin C has consistently been shown to be

correlated with habitual, reported intake of fruit and vegetables.[191] Low vitamin D, vitamin C, vitamin E, selenium, beta-carotene and folate have all been observed in SA populations[183, 192, 193]

The data on dietary intake in SA populations is not only scare but conflicting; in addition many of the studies are over 10 years old. Dietary intake of migrant populations will be affected by acculturation therefore more up to date research is needed, indeed greater changes to a more westernised diet are evident in younger populations.[178] Determining both dietary patterns and the association with insulin resistance and T2DM are urgently needed in this high risk population.

2.6 Conclusion

The current dietary recommendations for the prevention of T2DM are similar to those given to the general population for a healthy diet. The recommendations are appropriate to the available evidence. Although data on individual dietary items remains inconclusive the analysis of dietary patterns is compelling. Studies consistently demonstrate that diets rich in fruit and vegetables, whole grains, legumes and low in red and processed meat, SFA and refined grains protect against T2DM development. However the evidence largely originates from observational studies which are open to criticism due to confounding factors that cannot always be controlled for. In addition most observational studies rely on FFQs which are recognized as being insensitive to small yet significant interactions between diet and disease. Despite the plethora of studies further robust RCT with larger sample sizes and studies which determine the mechanistic actions of foods and nutrients are

needed before many recognized organizations will truly accept that the foods we consume can prevent T2DM.

2.7 Chapter summary

Chapter 2 has demonstrated that LSMP can successfully prevent the development of T2DM in high risk individuals. Dietary manipulation has played a key role in these studies, but determination of ideal dietary practice remains unclear. Observational and clinical studies have shown how fat and fibre intake can potentially influence T2DM risk. In addition dietary patterns with common elements have shown the potential to reduce development of T2DM. The exact role of different dietary components remains to be elicited and research is warranted.

Chapter 3

Fruit and Vegetable Intake and Incidence of Type 2 Diabetes Mellitus: Systematic Review and Meta-analysis

3.0 Chapter overview

A number of dietary patterns characterised by high intakes of fruit and vegetables have shown associations with reduced incidence of diabetes.[164, 170, 171] Chapter 3 presents a systematic review and meta-analysis which was conducted to determine the independent effects of fruit and vegetable intake on the incidence of T2DM.

3.1 Introduction

Dietary factors are important and potentially modifiable risk factors for the prevention of T2DM. There has been a focus on the role of carbohydrates, fat and fibre intake.[194, 195,196] However the relationship between fruit and vegetable intake and incidence of T2DM is not fully understood. Chapter 2 discussed the overwhelming support for the benefit of lifestyle interventions to prevent T2DM. It has been shown in a meta-analysis of lifestyle intervention studies that dietary interventions alone are associated with a 33% reduced incidence of T2DM (HR = 0.67 95%CI: 0.49 to 0.92).[126] The majority of these intervention studies included the promotion of fruit and vegetables in the diet. However the relationship between fruit and vegetable intake and incidence T2DM has not been fully elucidated.

Low consumption of fruit and vegetables is common throughout the world.[197] The National Diet and Nutrition Survey[198] in 2002 showed that 86% of all men and women in the UK consumed less than the recommended five portions of fruit and vegetables per day, with 62% consuming less than three portions. It was estimated that inadequate consumption of fruit and vegetables could have accounted for 2.7 million deaths worldwide in the year 2000.[199]

High intakes of fruit and vegetables have proven benefit for reducing the incidence of both cancer and CVD.[200, 201] Diabetes is a strong independent risk factor for CVD[41] and frequently the conditions exist together, sharing common modifiable risk factors.[202] However as yet no firm conclusions have been made as to whether increasing fruit and vegetable intake can decrease the risk of T2DM itself, given the abundance of conflicting evidence within the literature. The exact mechanisms by which fruit and vegetables reduce the risk of CVD and cancer are not precisely known. It is hypothesised that a combination of antioxidants and phytochemicals found in both fruit and vegetables may promote health by combating free radicals which are linked with early phase development of some chronic diseases, [203] (as discussed in section 1.5.1). High intakes of fruit and vegetables have been shown to increase plasma carotenoids and vitamin C[204, 205] both of which have antioxidant properties. It has also been demonstrated that an increase in fruit and vegetables in the diet of people with T2DM can lower markers of oxidative stress.[206]

A previous review in 2007[207] concluded that consumption of three or more daily servings of fruit and vegetables was not associated with a substantial reduction in the risk of T2DM. However, the review was restricted by language and searched only a small number of electronic databases. In addition recent studies have been published which could further contribute to the pooled data and allow further investigation into the relationship between fruit and vegetable consumption and T2DM risk.

3.2 Methods

3.2.1 Search strategy

Using the Cochrane handbook[208] and the CRD's guide to systematic reviews[209] a systematic review protocol was developed in consultation with a clinical librarian. To ensure a broad search, the search strategy included the Medical subject headings: Type 2 Diabetes, Pre-Diabetes, Impaired Glucose Tolerance, Impaired Fasting Glucose, Fruits, Vegetables, Citrus, Follow Up and Prospective Studies. Text word, title word, abstract and subject headings were also searched for the above terms plus a number of non-Medical Subject Headings to cover fruit, vegetables and diabetes.

Five electronic databases were searched; OVID MEDLINE(R) – In process and other non-indexed citations and OVID MEDLINE(R), 1950 to February 2009, EMBASE, 1980 to March 2009,The Cumulative Index to Nursing and Allied Health Literature (CINAHL) and the British Nursing Index (BNI) were searched from inception, (1981 and 1985 respectively) until March 2009, via NLH Search 2.0 and three databases in The Cochrane Library (CDSR, CENTRAL, DARE) were searched from inception to Issue 1, 2009.

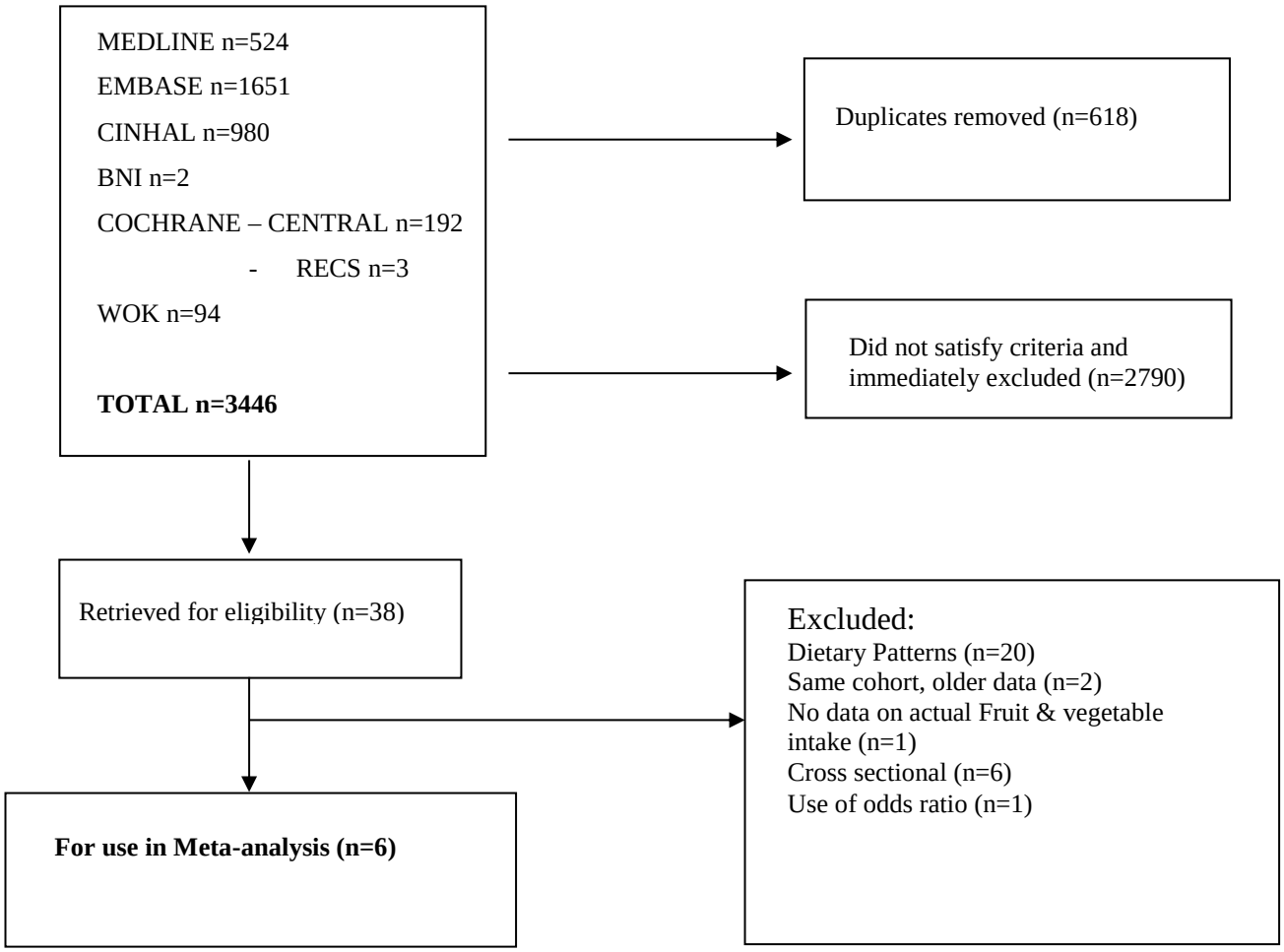
Expert opinion was sought and references checked in any articles which met the inclusion criteria. No language restrictions were applied.

3.2.2 Study selection

Study selection was restricted to prospective cohort studies which included an individual measure of either fruits, vegetables or fruit and vegetable intake along

with an assessment of the development of T2DM. One reviewer (Patrice Carter (PC)) performed the search and reviewed the results. Studies that did not meet the inclusion criteria were discarded during the initial review. Where uncertainty existed the full text article was retrieved and assessed. Two reviewers (PC and Jacqui Troughton (JT)) independently assessed all potentially relevant studies and resolved any uncertainty through discussion. No relevant papers were found in languages other than English.

Figure 3-1: Process of study selection



3.2.3 Validity assessment

Two authors (PC and JT) independently assessed all studies for quality. As no validated tool exists for scoring observational studies a scoring system was created to account for:

(1) Subjects: 1 point if any justification was given for the cohort and 1 point for appropriate inclusion and exclusion criteria.

(2) Outcome: 1 point if diagnosis of type 2 diabetes was confirmed according to accepted clinical criteria[39, 210] and not based on self report.

(3) Intervention: 1 point if participants' usual fruit and vegetable consumption was assessed using a validated tool.[211]

(4) Statistical Analysis: 1 point was given if adjustments were made for age, body mass index and family history of type 2 diabetes, these being proven risk factors for type 2 diabetes. Another point was given for any other adjustments, for example physical activity.

The system was designed with reference to MOOSE,[212] QUATSO[213] and STROBE[214] allowing a total score from 0 to 6 points, with 6 reflecting the highest quality.

3.2.4 Data abstraction and synthesis

Two authors (PC and JT) independently extracted data on the diagnosis of T2DM, intake of fruit and vegetables and the associated risk. HR and RR were used as a measure of the association between intake of fruit and vegetables and risk of T2DM. We assumed relative risks to be a valid approximation of hazard ratio[208] enabling the use of one consistent measure. The studies reported fruit and vegetable intake in

a variety of measurements, for example servings per week, grams per day. We standardised all data into servings per day, using a standard portion of 106g.[215] Any disagreement was resolved through discussion. Where insufficient data were published authors were contacted.

3.2.5 Statistical methods

HR and RR were transformed by taking their natural logarithms, standard error and corresponding confidence intervals were thus calculated.[216] Laura Gray a Medical Statistician carried out the following analysis, HR and their standard errors were pooled using a random effects model to account for statistical heterogeneity between studies to calculate summary HRs and 95% confidence intervals for the highest versus lowest level of consumption.[217] The data was analysed using Stata (version 10). Heterogeneity was assessed using the I^2 statistic. After discussion with PC subgroup analyses was carried out based on the quality of the study (high quality (4-6) versus lower quality (<4)), gender (males and females included versus females only), length of follow up (<10 years versus >10 years), quantiles of intake (comparison of the different quantification of intake, either tertiles, quartiles or quintiles) and location (USA and Europe versus China) as these were thought to be possible sources of heterogeneity. One study was excluded from the analysis as it reported data as odds ratio (OR);[218] the combination of OR and RR can lead to misinterpretation of results.[208] These data were added in the sensitivity analysis to see if they significantly altered the observed associations. Significance was set at $p<0.05$ and 95% confidence intervals are quoted throughout.

3.3 Results

The search identified 3,446 articles (Figure 3-1, page 60). Titles and abstracts were assessed and full articles of potentially relevant studies were obtained. A number of articles examined fruit and vegetable intake within a dietary pattern only or were cross sectional in design and therefore could not be included. Papers reporting the same study data were identified in two cases, with the older papers being excluded.[219, 220] One study did not give enough detail on actual fruit and vegetable intake to warrant inclusion within the meta-analysis.[221]

3.3.1 Study characteristics

Six studies met all the inclusion criteria.[222-227] The study characteristics and main outcomes are given in Table 3-1, (page 64). The combined population resulted in 223,512 study participants; only two studies[224, 226] included male participants. Age of participants ranged from 30 to 74 years. Study length ranged from 4.6 years to 23 years (median of 13.4 years). Three papers provided information on fruit and vegetable intake separately and combined.[223, 225, 227] Two papers[222, 224] only provided information on fruit and vegetable intake separately and another paper only provided the combined data.[226] Four papers also included separate data on the intake of green leafy vegetables (GLV).[222-225] In the majority of papers intake of fruit and vegetables was divided into quintiles, however the paper by Ford[226] analysed the data as tertiles and the paper by Montonen and colleagues[224] examined quartiles.

Table 3-1: Study characteristics

Author	Trial	Number	Number of Cases/non cases	Age	Measure of Fruit & Vegetables	Confounders measured	Follow Up	Assessment of type 2 diabetes	Quantity (highest v lowest intakes as servings/day)	Quality Score
Villegas et al 2008 [222]	Shanghai Women's Health Study-china	64,191 women	896/63,295	40-70	Personal interview FFQ Calculated g/day for fruit & vegetables separately. Defined green leafy vegetables as greens/Chinese greens/spinach Data divided into quintiles Calculated hazard ratio	BMI, WHR, age, level of education, smoking status, alcohol use, hypertension, disease history, hormone use, occupational history physical activity	4.6yrs	Confirmed by ADA criteria	Fruit- 4.56 v 0.82 Vegetables- 4.04 v 1.15 Green leafy vegetables– 1.28 v 0.26	4
Bazzano et al 2008 [223]	Nurses Health Study USA (1984 onwards)	71,346 women	4529/66,817	30-55	Self completed FFQ Calculated servings/day of fruit, vegetables and combined. Defined green leafy vegetables as spinach/kale/lettuce Data divided into quintiles Calculated hazard ratio	BMI, physical activity, smoking status, alcohol use, hormone therapy, family history, hypertension, cholesterol	18yrs	Confirmed if met WHO criteria (before 1997) or ADA criteria (after 1998)	Fruit– 2.5 v 0.5 Vegetables- 5.2 v 1.5 Fruit & Vegetables– 7.5 v 2.1 Green leafy vegetable data not given	4
Montonen et al 2005 [224]	Finnish Mobile Clinic Health Examination Survey	4304 men and women	383/3921	40-69	Dietary History Interview. Calculated g/day for fruit and vegetables separately Gave no definition for green leafy vegetables. Data divided into quartiles Calculated relative risks	Occupation, illness, medication, health status, smoking status, blood pressure	23yr	Confirmed via social insurance institutions register	Fruit– >1.47 v <0.31 Vegetables– >1.23 v <0.4 Green leafy Vegetables– >0.4 v <0.1	3

Author	Trial	Number	Number of Cases/non cases	Age	Measure of Fruit & Vegetables	Confounders measured	Follow Up	Assessment of type 2 diabetes	Quantity (highest v lowest intakes as servings/day)	Quality Score
Liu et al 2004 [225]	Women's Health Study, USA	38,018 women	1614/36,404	≥45	Self completed FFQ Calculated servings/day for fruit, vegetables and combined. Defined green leafy vegetables as spinach/kale/lettuce Data divided into quintiles Calculated relative risks	BMI, smoking status, alcohol use, exercise, family history, menopausal state, vitamin use, blood pressure, cholesterol	8.8yr	Based on self reported	Fruit– 3.91 v 0.62 Vegetables– 6.84 v 1.47 Fruit & Vegetables– 10.16 v 2.54 Green leafy vegetables- 1.42 v 0.14	3
Ford et al 2001 [226]	NHANES, USA	9665 men and women	1018/8647	25-74	Single 24 hour recall Calculated servings per week for fruit and vegetables combined. Data divided into tertiles Calculated hazard ratios	BMI, age, ethnicity smoking, blood pressure, hypertension medication, cholesterol, exercise, alcohol, education	20 yr	Confirmed either by self report or hospital records	Fruit & Vegetables- >5 v 0	1
Meyer et al 2000 [227]	Iowa women's health study, USA	35,988 women	1141/34,847	55-69	Self completed FFQ Calculated servings per day for fruit, vegetables and combined. Data divided into quintiles Calculated hazard ratios	BMI, WHR, age level of education, physical activity, smoking habits, alcohol intake, medication use,	6 yr	Based on self reported	Fruit- 3.36 v 0.57 Vegetables- 5.93 v 1.57 Fruit & Vegetables- 8.86 v 2.57	2

3.3.2 Study quality and publication bias

None of the papers met all of the criteria of the quality assessment tool, with all papers missing out on a point for justification of the cohort. The papers did not state that a power calculation had been undertaken, or any justification given for the numbers of subjects needed to detect an effect of differences in fruit and vegetable intake and the incidence of T2DM. All papers made some adjustments for potential confounding factors, however only three[223-225] adjusted for age, BMI, and family history of T2DM. Only two papers used appropriate inclusion and exclusion criteria, (it was assumed that authors should have excluded subjects with a history of T2DM, cancer, CVD and those with implausibly high or low dietary intake). Two papers[224, 226] did not use what we considered to be a validated tool to assess fruit and vegetable intake.[211]

Publication bias was assessed by visually examining a funnel plot of precision against hazard ratio (not shown) with asymmetry being formally assessed using the Egger test. No significant bias was shown (p=0.27).

3.3.3 Analysis of summary estimates

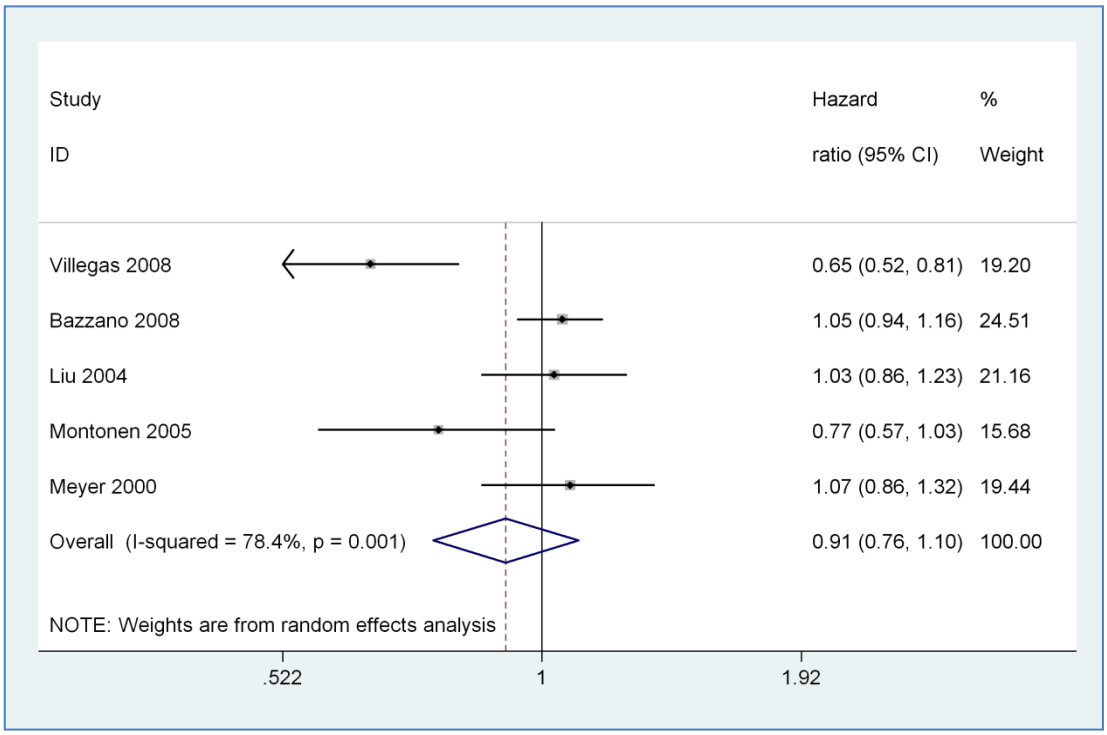
The summary estimates of HR or RR from each publication were pooled to give a total estimate of risk (Table 3-2, page 67). Lowest intake values versus highest intake values were specifically examined. The meta-analysis did not show any significant reductions in risk of T2DM incidence for consumption of fruit, vegetables or vegetables plus fruit combined (Figure 3-2, 3-3, 3-4, page 68 and 69); however the data does suggest a trend towards a benefit of consuming greater quantities (Table 3-2, page 67). All studies that examined GLV intake showed a

benefit of consuming greater quantities (Figure 3-4, page 69). Summary estimates showed that consuming 1.35 servings per day of GLV (highest intake) compared to 0.2 servings (lowest intake) resulted in a 14% reduction in risk (p=0.01) of T2DM incidence (HR= 0.86, 95%CI: 0.77-0.97). Sensitivity analysis was carried out due to the significant heterogeneity observed between studies (Table 3-3, page 70). Separate analysis examined quality of articles, sex, length of follow up and location as these were assumed to be potential sources of bias. Furthermore, investigation as to whether the different ways in which authors had grouped intake (tertiles, quartiles or quintiles), affected the results. However no significant interactions existed between any of these variables to explain the heterogeneity seen. In addition we re-ran the meta-analysis to include the EPIC study,[218] which presented data as OR. Inclusion of this study did not alter the associations previously observed.

Table 3-2: Meta-analysis of highest versus lowest intake

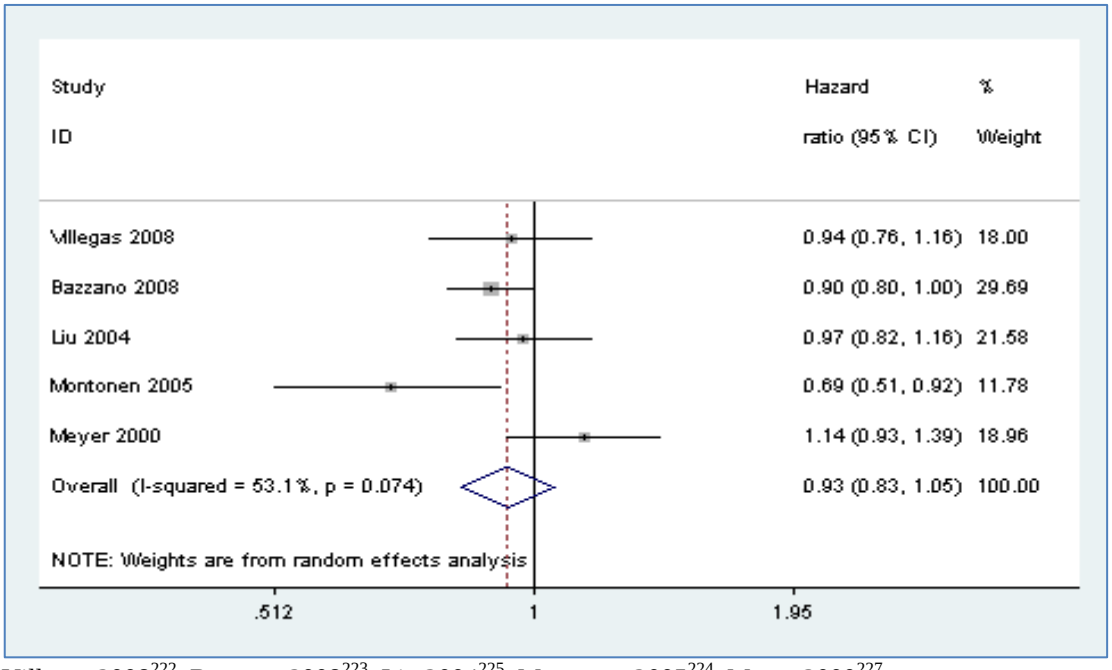
Comparison	Studies	Cases / Non-cases	Pooled HR (95% CI)	P value	Heterogeneity I^2	p value
Vegetables only	5[222-225, 227]	8563/204,654	0.91 (0.76 to 1.09)	0.32	78.1	0.001
Fruit only	5[222-225, 227]	8563/204,654	0.93 (0.83 to 1.01)	0.27	52.6	0.07
Fruit and vegetables	4[223, 225-227]	8302/146,715	1.00 (0.92 to 1.09)	0.97	0	0.40
Green leafy vegetables	4[222-225]	7422/169,807	0.86 (0.77 to 0.97)	0.01	39.6	0.18

Figure 3-2: Forest plot comparing highest versus lowest intake of vegetables



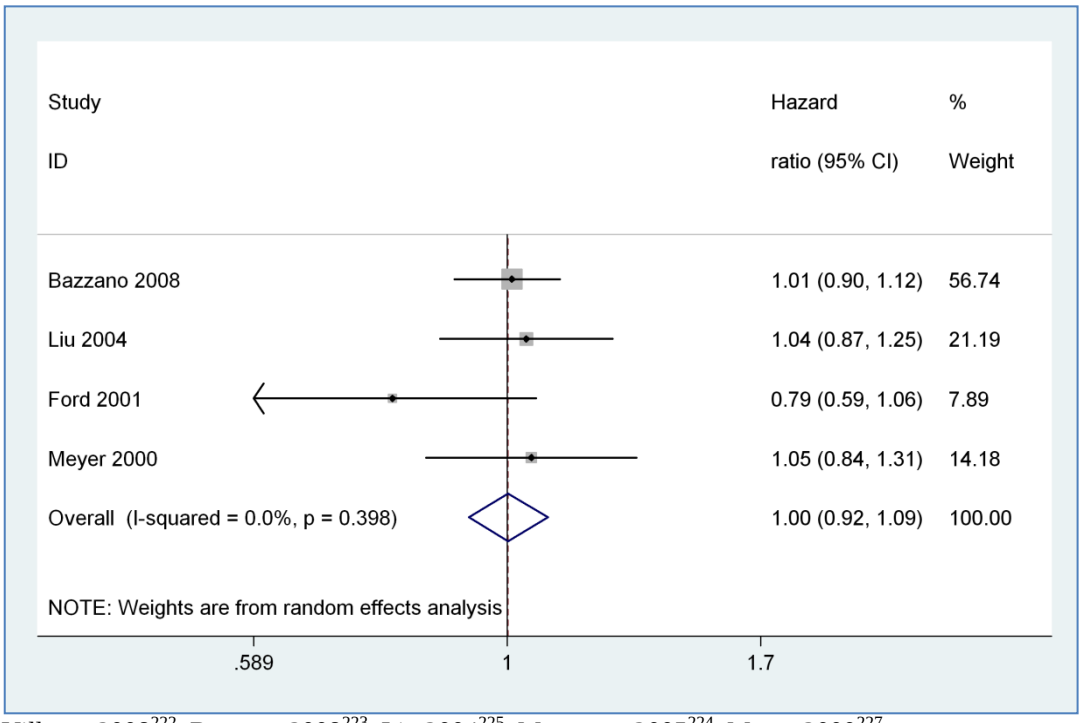
Villegas 2008[222], Bazzano 2008[223], Liu 2004[225], Montonen 2005[224], Meyer 2000[227]

Figure 3-3: Forest plot comparing highest versus lowest intake of fruit



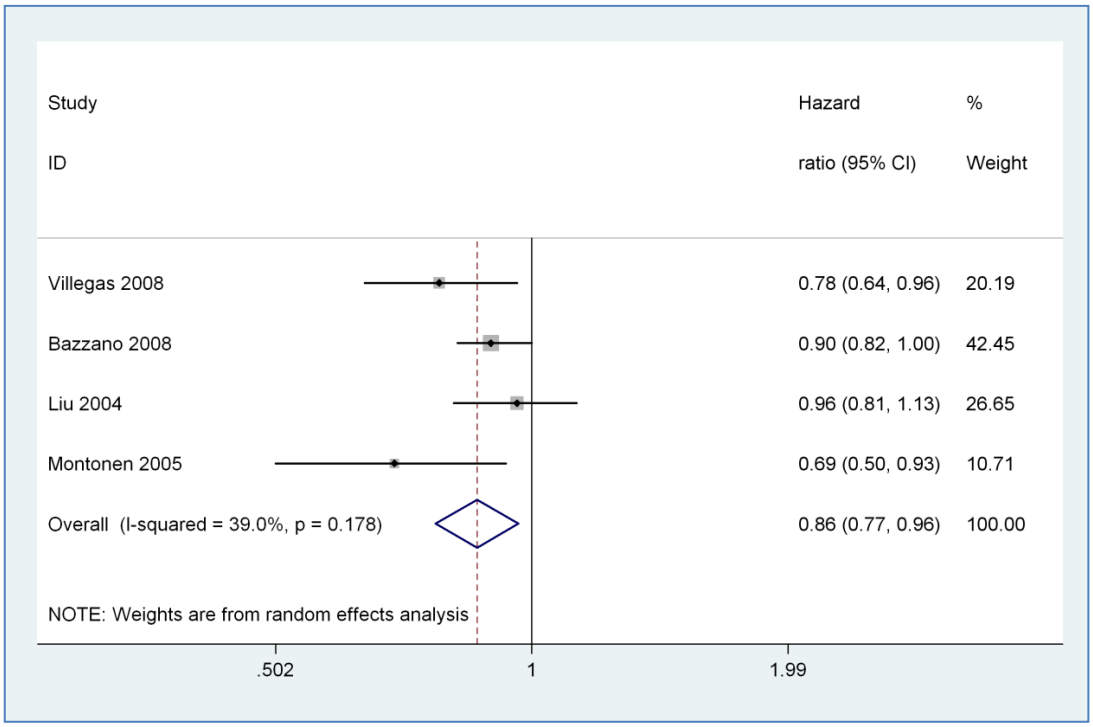
Villegas 2008[222], Bazzano 2008[223], Liu 2004[225], Montonen 2005[224], Meyer 2000[227]

Figure 3-4: Forest plot comparing highest versus lowest intake of fruit and vegetables combined



Villegas 2008[222], Bazzano 2008[223], Liu 2004[225], Montonen 2005[224], Meyer 2000[227]

Figure 3-5: Forest plot comparing highest versus lowest intake of green leafy vegetables



Villegas 2008[222], Bazzano 2008[223], Liu 2004[225], Montonen 2005[224], Meyer 2000[227]

Table 3-3: Sensitivity analysis to investigate differences between studies included in the meta-analysis

	Vegetables only			Fruit only			Fruit and vegetables			Leafy green vegetables	
	S	Pooled HR (95% CI)	P value	s	Pooled HR (95% CI)	P value	s	Pooled HR (95% CI)	P value	s	Pooled HR 95% CI)	P value
Quality												
High (4/5)	2	0.84 (0.52 to 1.34)		2	0.91 (0.82 to 1.00)		1	1.01 (0.91 to 1.13)		2	0.86 (0.76 to 0.98)	
Low (<4)			0.61			0.87			0.87			0.94
	3	0.98 (0.82 to 1.16)		3	0.94 (0.73 to 1.19)		3	0.98 (0.84 to 1.15)		2	0.84 (0.61 to 1.15)	
Sex												
Males and females	1	0.77 (0.57 to 1.04)		1	0.69 (0.51 to 0.93)		1	0.79 (0.59 to 1.06)		1	0.69 (0.51 to 0.94)	
Females only			0.54			0.14			0.23			0.31
	4	0.94 (0.77 to 1.15)		4	0.96 (0.86 to 1.06)		3	1.02 (0.94 to 1.12)		3	0.89 (0.81 to 0.98)	
Length of follow up												
<10 years	3	0.90 (0.67 to 1.21)		3	1.01 (0.90 to 1.13)		2	1.04 (0.91 to 1.20)		2	0.87 (0.71 to 1.07)	
>10 years			0.92			0.19	2		0.54			0.77
	2	0.93 (0.69 to 1.25)		2	0.82 (0.64 to 1.05)			0.93 (0.74 to 1.17)		2	0.82 (0.64 to 1.05)	
Location												
USA and Europe	4	1.02 (0.92 to 1.12)		4	0.93 (0.80 to 1.08)		4	1.00 (0.92 to 1.09)		3	0.89 (0.78 to 1.01)	
China			0.23			0.97	0	-	-			0.73
	1	0.65 (0.52 to 0.81)		1	0.94 (0.76 to 1.16)					1	0.78 (0.64 to 0.96)	
Quantiles												
Tertiles	0	-		0	-		1	0.79 (0.59 to 1.06)		0	-	
Quartiles	1	0.77 (0.57 to 1.04)	0.54	1	0.69 (0.51 to 0.93)	0.14	0	-	0.23	1	0.69 (0.51 to 0.94)	0.31
Quintiles	4	0.94 (0.77 to 1.15)		4	0.96 (0.88 to 1.06)		3	1.02 (0.94 to 1.12)		3	0.89 (0.81 to 0.98)	
Adding EPIC study[218]	6	0.90 (0.76 to 1.05)		6	0.90 (0.79 to 1.02)	-	5	0.96 (0.86 to 1.07)	-	5	0.86 (0.78 to 0.94)	-

3.4 Discussion

3.4.1 Principle finding

The results of this meta-analysis suggest that increasing the amount of GLV in an individual's diet is beneficial in reducing the risk of T2DM. An increase of 1.15 servings per day decreased incidence by 14%. The data did not show any significant relationships between the consumption of fruits, vegetables or fruit and vegetables combined and incidence of T2DM. However there was significant heterogeneity between studies so a sensitivity analysis was carried out. The sensitivity analysis showed no significant interactions between variables examined. Thus differences that exist between the studies included in the meta-analysis were not identified within the sensitivity analysis.

3.4.2 Exploration of heterogeneity

Within the sensitivity analysis examination of location as a possible source of heterogeneity was carried out. Traditional Chinese diets are high in fruit and vegetables,[228] therefore the expectation may be that intake would be greater in China than USA or Europe, however they were in fact quite similar. This may reflect a change from traditional foods of China to a more westernised diet.

Sex was also examined as a possible source of heterogeneity; again this factor did not show any significant interaction between variables. Only two of the studies included male participants and both of these studies showed significant benefits of increasing intake of fruit and vegetables. Therefore the possibility exists that the results may alter if more studies with male subjects were included. There is a need

for further examination of the effects of fruit and vegetable consumption in male participants before firm conclusions can be made.

Although the sensitivity analysis could not explain the level of heterogeneity a number of differences exist between the studies which may explain the observed heterogeneity. Estimations of daily consumption differed between the studies. Ford[226] estimated servings per week, intake from three studies was calculated as servings per day[223, 225, 227] and the remaining two studies calculated grams per day.[222, 224] In order to carry out the meta-analysis it was necessary to standardise all data into servings per day, therefore conclusions drawn should be done so with caution.

All data was standardised into servings per day using a standard portion size of 106g. This was used in agreement with other meta-analysis studies which have analysed fruit and vegetable intake and risk of chronic disease.[207, 215] However the current UK recommendation to consume five portions of fruit and vegetables per day is based on 80g as a serving size. Therefore from the analysis we can calculate that increasing consumption of GLV by one and a half UK portions per day (121.9g) results in 14% reduction in the incidence of T2DM.

In addition to calculating intake in different formats the studies also grouped foods differently. Three papers[223, 225, 227] examined intake of fruit and vegetables separately and combined, one paper[226] examined the combined consumption and two[222, 224] only examined intake separately. These two papers both present significant benefits of greater consumption of fruit, vegetables and GLV. However

there is no explanation as to why they have not reported combined data and this could reflect a bias in the reporting of positive results.

The different studies also split dietary intake into different quantiles, either tertiles,[39] quartiles[37] or quintiles. However the sensitivity analysis showed that this made no significant difference to the results.

Heterogeneity could also be due to differences in food group classification. The articles which investigated GLV did not all use the same criteria, two papers included spinach, kale and lettuce,[223, 225] another included Chinese greens, greens and spinach[222] and the other paper did not provide a definition.[224] This demonstrates a need for one uniformed definition of different fruit and vegetable groups. GLV in actuality include brassicas, such as cabbage, brussel sprouts and cauliflower, compositae, for example lettuce, umbelliferous vegetables, which are plants grown for their leaves and leaf stems, these are often consumed as herbs, for example parsley, dill and fennel. Other leafy vegetables such as spinach are also included.[229] The investigators did not include all of these foods in their GLV categories, if included the observed results may be altered.

Another possible explanation for the differences between the studies may be the method of dietary assessment. Ford[226] collected data via a single 24 hour recall, two studies[222, 224] used dietary assessment interviews and the remaining studies used self-completed FFQ. Assessment of true dietary intake is inherently difficult and the use of FFQ has been criticised in the past.[230, 231] FFQ are subject to a combination of random and systematic errors.[232] These errors in measurement can

underestimate true diet-disease interactions.[233, 234] The possibility exists that such attenuation has obscured a relationship between fruit and vegetable consumption in the meta-analysis. Indeed studies have previously demonstrated that diet-disease interactions have been masked by the use of FFQ but identified by food diaries and nutritional biomarkers.[235, 236, 237] The use of biomarkers avoids problems associated with self report and they can be collected for large numbers of subjects. Thus there is a need to incorporate more biological markers of fruit and vegetable intake, such as plasma vitamin C into prospective nutritional assessment studies.

In the meta-analysis the most fully adjusted HR presented in the articles were included. However not all authors made the same adjustments and this may have impacted on the overall data set. Indeed, only just over half of the papers adjusted for what were considered in our quality assessment tool as essential confounders, (age, BMI and family history of T2DM).

3.4.3 Potential benefits of green leafy vegetables

Although our results for fruit and vegetable consumption were not significant the data do suggest a trend towards a benefit of consuming greater quantities, this supports evidence previously reported in cross sectional studies.[221, 238] In addition a number of studies examining dietary patterns and T2DM incidence have consistently shown that the dietary patterns that are associated with decreased risk of T2DM include fruit and vegetables as important components.[171, 239, 240]

A possible benefit of fruit and vegetables in the diet for the prevention of chronic diseases is their antioxidant content and thus a contribution to reduction of systemic

oxidative stress. The results support this as GLV, such as spinach, have been shown to contain high levels of beta-carotene and vitamin C,[241] both of which have antioxidant properties. GLV also contain polyphenols,[242] which are also known for their antioxidant properties. GLV may also act to reduce T2DM risk due to their magnesium content. A recent meta-analysis[243] found magnesium intake to be inversely associated with incidence of T2DM. GLV are also good sources of α-Linolenic acid,[244] which is an omega-3 polyunsaturated fatty acid. As discussed in chapter 2 (section 2.4.4) evidence exists that the fatty acid profile of the diet is important in determining the fatty acid composition of the phospholipid bilayer. The composition of the phospholipid bilayer is related to insulin sensitivity within skeletal muscle.[245] Thus there are a number of possible mechanisms which may explain the benefit of consuming GLV in the diet.

The results support the evidence that "foods" rather than isolated components such as antioxidants are beneficial for health. Results from a number of supplement trials have produced disappointing results for prevention of disease in contrast to epidemiological evidence.[246, 247] For example, a RCT found no difference in blood pressure, cholesterol or fasting blood glucose between those randomised to antioxidants supplements or placebo.[248] Further investigation is warranted to understand the mechanisms involved in the proposed relationship between GLV and risk of T2DM.

3.4.4 Strengths and limitations

A broad search of both Medical subject headings and keywords which covered diabetes and fruit and vegetable consumption was carried out. The search was

conducted on multiple databases and was carried out by two independent authors. In addition all authors of included articles were contacted in request of any further information.

As with all meta-analysis, a number of limitations must be considered. Publication bias is a potential concern when conducting analysis on published studies; however the statistical tests carried out suggest bias is not present in this meta-analysis. The statistical power of the study may be limited as we only included six studies and only four studies for the examination of GLV. In addition the meta-analysis only includes one study from Europe, highlighting a lack of information from European countries which could potentially add significant evidence to this area of research.

There was significant heterogeneity between the studies examined; therefore overall conclusions must be regarded with caution. However a thorough sensitivity analysis to investigate possible sources of heterogeneity was carried out.

To further examine the association between intake and risk of T2DM we investigated the possibility of carrying out a dose response, however only one paper[222] provided the information required for this analysis. In addition four out of the six studies included[224-227] were given quality assessment scores of less than 4. When these studies were removed from the analysis the results did not significantly alter, however conclusions drawn must be done so with caution. Previous studies have also shown that greater intake of fruit and vegetables are linked to other lifestyle factors such as physical activity.[52] Thus it cannot be excluded that other variables which were not adequately controlled for may have influenced the data

from these studies. The results highlight a call for standardisation of nutritional epidemiology, with emphasis on the additional use of biological markers and uniformity of food groups.

3.5 Conclusions

Results from the meta-analysis support recommendations to promote the consumption of GLV in the diet for reducing the risk of T2DM. The results support the growing body of evidence that lifestyle modification is an important factor in the prevention of T2DM. The potential for tailored advice on increasing intake of GLV to reduce the risk of T2DM should be investigated further.

3.6 Chapter summary

Chapter 3 has demonstrated that greater intake of GLV should be promoted for the prevention of T2DM. The role of fruit and vegetables in general is still hypothetical however a trend towards benefit has been indicated. The reliance on self reported intake and FFQs is a distinct limitation of observational studies. Future work should incorporate nutritional biomarkers where possible.

Chapter 4

Fruit and Vegetable Intake and Glucose Control Study (FIVE) – Study Design and Rationale

4.0 Chapter overview

Chapter 4 will summarise the study design and rationale for the Fruit and Vegetable Intake and Glucose Control (FIVE) study. FIVE examines the relationship between fruit and vegetable intake and the risk of T2DM. Throughout the study vitamin C was used as a biomarker for fruit and vegetable intake. FIVE is a two part study, the first section was a cross sectional study, exploring the relationship between baseline glucose levels and plasma vitamin C concentrations. The study also investigated differences between SA and WE individuals. The second study was a prospective RCT to compare implementation of a structured education programme as compared to standard care in the effectiveness of increasing fruit and vegetable consumption in individuals with IGR.

The chapter will begin by exploring current evidence available on fruit and vegetable intake and risk of T2DM. The chapter will outline the study aims, provide justification for the work and describe the methodology of FIVE. Results and discussion will be presented in chapters 5 and 6 respectively.

4.1 Background

The consumption of five portions of fruit and vegetables per day (400g/day) is widely recommended as part of a healthy balanced diet and for the prevention of chronic disease.[129, 132] This recommendation has been incorporated into health messages following a plethora of epidemiological data on the associations between greater fruit and vegetable intake and reduced risk of CVD and cancer.[201, 249, 250,215, 251,252] Meta-

analyses have been carried out to determine whether consuming the recommended 5 a day as compared to less than three servings per day can significantly improve health. Pooled summary statistics found that the risk of stroke was reduced by 26% and CHD by 17%.[251, 252] Indeed it has been estimated that up to 2.7 million lives around the world could be saved with sufficient fruit and vegetable consumption.[129] Inadequate fruit and vegetable intake is amongst the top ten risk factors for global mortality.[129, 253] However recent data from England found that only 25% of men and 28% of women consume the recommended 400g/day.[22] In addition as discussed in chapter 2, SA individuals both within the UK and in India have low intake of fruit and vegetables[183, 189] which may contribute to their greater risk of developing T2DM than WE individuals. Available evidence suggests that low fruit and vegetable intake is a major modifiable risk factor which could be targeted to improve public health. The systematic review and meta-analysis in Chapter 3 demonstrated a trend towards benefit for greater fruit and vegetable intake in association with reducing risk of T2DM.

Dietary recommendations for the prevention of T2DM also include the message to consume at least 400g fruit and vegetables per day. [129, 133, 134] In addition as discussed in chapter 2, the majority of LSM studies aimed at the prevention of T2DM have included a recommendation to increase fruit and vegetable intake in the diet.[116, 120, 140] The meta-analysis carried out in chapter 3 found no significant benefits for greater consumption of fruit and vegetables for the prevention of T2DM. However, as discussed there are many potential explanations as to why a meta-analysis based on observational studies may not have been able to identify small differences in diet as

assessed by FFQ. Accurate measurement of habitual diet is perhaps the most difficult area of nutritional research.[254] However, errors due to self report can be eliminated by the use of nutritional biomarkers.[255] Vitamin C consistently demonstrates a high correlation with fruit and vegetable intake.[204, 256] A study providing a variety of vegetable supplemented meals, found that the increase in plasma vitamin C was consistent across the different meals yet other carotenoids varied in response.[257] It has been proposed that vitamin C intake is the strongest predictor of plasma vitamin C and around 90% of dietary vitamin C is obtained from fruit and vegetable consumption.[258] The EPIC study observed a strong relationship between fruit and vegetables as assessed by vitamin C and incidence of T2DM (OR = 0.38, 95%CI: 0.28-0.5) yet the relationship when measured by FFQ was much weaker (OR = 0.87, 95%CI: 0.69-1.11).[218]

A potential benefit to greater fruit and vegetable intake is their plentiful supply of antioxidants. As discussed in chapter 1 (section 1.5.2) antioxidants counteract the harmful effects of reactive oxygen species which have been implicated in the development and progression of T2DM.[72] A study in people with T2DM found that an increase in fruit and vegetable consumption resulted in a rise in plasma antioxidants which was negatively correlated to lipid peroxidation.[206] A further study in healthy individuals found that greater fruit and vegetable intake resulted in increased plasma carotenoids. In addition the study reported that markers of oxidative stress were reduced proportionally to a change in plasma carotenoids.[259]

Lipid peroxidation is a central feature of oxidative stress and a variety of lipid by-products are produced as a consequence.[75, 260] F_2-isoprostanes are a complex family of compounds produced from arachidonic acid via free radical catalysed action.[261] Free radicals result in the formation of F_2-isoprostanes in membrane phospholipids; once formed, cellular activation by phospholipase results in their release and circulation in the plasma. F_2-isoprostanes are then excreted in the urine.[260] 8-epi-PGF2 is one type of isoprostane produced in abundance.

Measurement of F_2-isoprostanes in body fluids provides a reliable, non-invasive approach to assess lipid peroxidation and is more accurate than other available methods.[260, 262] Urinary F_2-isoprostanes have been extensively used as a clinical measure of lipid peroxidation and have several advantages, concentration is unchanged following storage at -20^{o}C, measurement of a single morning sample adequately represents daily excretion and they are not modified by lipid content of the diet.[261] In addition urine only contains small quantities of lipid therefore autoxidation is not considered a problem.

4.2 Study design and rationale

It was considered that FIVE would contribute to the growing area of research on fruit and vegetable intake and risk of T2DM. FIVE was carried out as a sub study of a large, community intervention trial for the prevention of T2DM, Let's Prevent Diabetes. FIVE utilised this large study to provide currently unavailable evidence on fruit and vegetable intake in relation to glucose control. Participants enrolled provided a cross

sectional data set of individuals identified with IGR as well as those with NGT and T2DM. In addition a large number of SA individuals were enrolled to provide information on fruit and vegetable intake in this high risk population, information on SA consumption of fruit and vegetable intake in the UK is currently limited. FIVE used plasma vitamin C as a biomarker for fruit and vegetable intake. Nutritional biomarkers can be used effectively across different ethnic groups and do not rely on self report or require any nutritional knowledge from participants.

In an attempt to explore the potential mechanistic actions of fruit and vegetables in the prevention of T2DM FIVE also measured participants' oxidative stress levels. Urine samples were taken from individuals so that urinary F_2-isoprostane analysis could be carried out to determine whether any relationship existed between fruit and vegetable intake and levels of oxidative stress.

The cross sectional study provides evidence of associations but cannot infer cause and effect or determine direction of the association. Thus to establish whether increasing fruit and vegetable intake reduces glycaemic measures over time a prospective study was also conducted. The second part of FIVE was a prospective RCT; utilising participants enrolled to Let's Prevent Diabetes, section 4.6 provides information on the Lets Prevent Study design. FIVE compared initial plasma vitamin C status of individuals identified with IGR to levels post intervention. The intervention part of Let's Prevent Diabetes compared standard care to a structured education programme designed to inform participants about IGR and how to prevent diabetes. The education

includes advice on increasing fruit and vegetable consumption. FIVE specifically measured plasma vitamin C pre and post intervention to assess change in fruit and vegetable consumption between the two groups. Urinary F_2-isoprostanes were again measured to determine any associations in change between fruit and vegetable intake and oxidative stress levels. At the initial screening study and at the annual clinics of Let's Prevent Diabetes, participants provided a fasting blood sample for the assessment of plasma vitamin C and a urine sample for the analysis of F_2-isoprostanes (Table 4-1 page 91). Incorporation of these additional blood and urine samples required a minor amendment to the original study protocol. This was written, submitted and accepted before the first screening sessions of Lets Prevent Diabetes were carried out (appendix 2).

4.3 Study aims

4.3.1 Cross-sectional analysis

- Aim 1: To determine whether fruit and vegetable intake, as assessed by plasma vitamin C is independently associated with the baseline glycaemic state of a population identified as being at high risk of T2DM.
    - 1a: Analysis of plasma vitamin C as a continuous variable
    - 1b: Analysis of plasma vitamin C as a categorical variable
    - 1c: To determine whether plasma vitamin C is associated with diagnosis of IGR

- Aim 2: To determine whether baseline fruit and vegetable intake is associated with levels of urinary F_2-isoprostanes in a population identified as being of high risk of T2DM.
- Aim 3: To determine whether any differences exist in fruit and vegetable intake between SA individuals and WE.

4.3.2 Prospective analysis

- Aim 4: To determine whether structured education for those identified with IGR can significantly improve fruit and vegetable consumption as compared to standard care. Plasma vitamin C was used to determine levels of fruit and vegetable intake pre and post intervention.

4.4 Methodology

4.4.1 Sample size

The first three aims of FIVE are exploratory by design, thus samples were taken from all participants who gave consent. The greater the number in the cohort, the stronger the study power to determine associations.

The fourth aim of FIVE required information from both the initial screening study and follow up clinics. In consultation with a clinical statistician a power calculation was carried out to determine the number of participants needed to detect a clinically

significant change in vitamin C over time and to detect a difference between groups. For 80% power at the 5% significance level with an interclass correlation of 0.05, 52 patients per arm were required to detect a change of 10μmol/l vitamin C, equivalent to about half an orange. Change in F_2-isoprostanes was the secondary outcome of FIVE. Previous studies have shown differences in urinary F_2-isoprostanes in fewer than 52 participants,[263] thus any associations should be detected.

4.4.2 Vitamin C analysis

Participants had a 9ml fasted blood sample collected by a trained health professional in a labelled potassium EDTA monovette syringe. The sample was immediately stored in a dark, sealed box at an average temperature of 4^{o}C. Storage of whole blood at 4^{o}C for 24 hours prior to freezing does not cause significant breakdown or changes in vitamin C levels.[264] Within six hours of blood collection, samples were centrifuged at 3000rpm for 10 minutes at 4^{o}C by PC. Three 100ul plasma aliquots were extracted and placed in labelled 2ml vials. 900ul of 5% Metaphosphoric acid, made up on a weekly basis was added to stabilize the vitamin C. Samples were vortexed briefly then stored at -80^{o}C.

Analysis of plasma vitamin C was carried out by Dr Jayne Woodside and Dr Sarah Gilchrist from Queens University of Belfast, Centre for Public Health, Nutrition and Metabolism Group. PC observed analysis of a sub set of the samples in Belfast. Samples were transported on dry ice to Belfast via courier.

Vitamin C (Ascorbic Acid) was measured on a Cobas FARA centrifugal analyser with a fluorescent attachment. A 2mol/l acetate buffer (pH 6.2) was prepared by adjusting a solution of sodium acetate trihydrate with acetic acid using a pH meter (pH 210 Microprocessor, Hannah Instruments). A stock solution of ascorbate oxidase (250 units) was reconstituted with 0.85mls of 2mol/l acetate buffer and stored at –70^{o}C until required. The oxidising reagent was prepared by diluting 0.1ml of this stock with 9.9mls of the 2mol/l acetate buffer pH 6.2 before use. The coupling reagent was prepared with 20mg of 1,2-Phenylenediamine dissolved in 20mls of deionised water. This was made up fresh for each run and protected from light.

A range of standards was used containing 0, 1, 5, 10, 15, 20μmol/l ascorbic acid diluted with 5% metaphosphoric acid. Frozen preserved plasma samples were thawed and centrifuged. Approximately 200μl of the clear supernatant was transferred into sample cups.

All samples and reagents were placed onto the analyser and the programme for measuring vitamin C initiated. The vitamin C assay has been validated against the National Institute of Standards and Technology Standard Reference Material 970 for ascorbic acid in serum. The vitamin C assay is externally quality assured three times a year using samples supplied by the French Society for Vitamins and Bio-factors. In-house quality control samples are also included in every run. At a mean concentration of 4.2μmol/l, the inter-assay CV was 6.80% (n=9), at 50.6μmol/l the inter-assay CV was 0.72%, while at 151.0μmol/l the inter-assay CV was 1.50%.

4.4.3 Urinary F_2-isoprostane analysis

A spot urine sample was provided by participants into labelled 20ml sterile universals, 2ml aliquots were taken and placed into sterile vials and stored at -80^{o}C. All Urinary F_2-isoprostane and creatinine analysis was carried out by PC under the supervision of Duncan Talbot at Unilever, Colworth Science Park, Bedford. 8-epi-PGF2 were measured using an AutoDelfia (Delfia: Dissociation Enhanced Lanthanide Fluorescent ImmunoAssay) automatic immunoassay system (Perkin Elmer Life Sciences). A time-resolved fluorescence system with a large stokes shift to ensure unique, specific sensitivity. The system uses assay buffer, wash concentrate, enhancement solution, trace stability buffer and solid yellow low fluorescence anti-mouse plates (Perkin Elmer Life Science).

Mouse monoclonal anti-8-IsoProstaglandin $F_2\alpha$ antibody was diluted in assay buffer and filtered through a sterile 0.2µl filter. Europium labelled ovalbumin-8-iso Prostaglandin $F_2\alpha$ tracer was prepared using stability buffer and filtered through a 0.2µl sterile filter. These reagents were made up fresh every 2 weeks or once used.

4.4.4 Assay Protocol for AutoImmuneAutoDelfia System

1. 50µl of standard, quality control or urine sample was dispensed into a dry anti-mouse plate.
2. AutoDelfia further dilutes the stock anti-8-isp Prostaglandin$F_2\alpha$ antibody, 1 in 100 with assay buffer. 100µl of antibody in assay buffer is then added to each well of the plate.

3. AutoDelfia dilutes the Europium labelled ovalbumin-8-isp Prostaglandin $F_2\alpha$ tracer, 1 in 100 with assay buffer. 50µl of diluted tracer is then added to each well of the plate.
4. The plate is incubated with shaking for 60 minutes.
5. The plate is washed with 6 x 400µl wash buffer.
6. 200µl of enhancement solution is added to each well plate and shaken for 5 minutes before counts are read.

   (Figure 4-1, page 91)

Each assay plate contained 6 standards, 3 quality control standards (low, medium and high) plus 39 samples, all tested in duplicate. Muticalc, a data package produces a standard curve and determines the unknown concentrations for each sample from this curve. Precision (CP) is also calculated for each sample, any sample with CP greater than 10 is repeated.

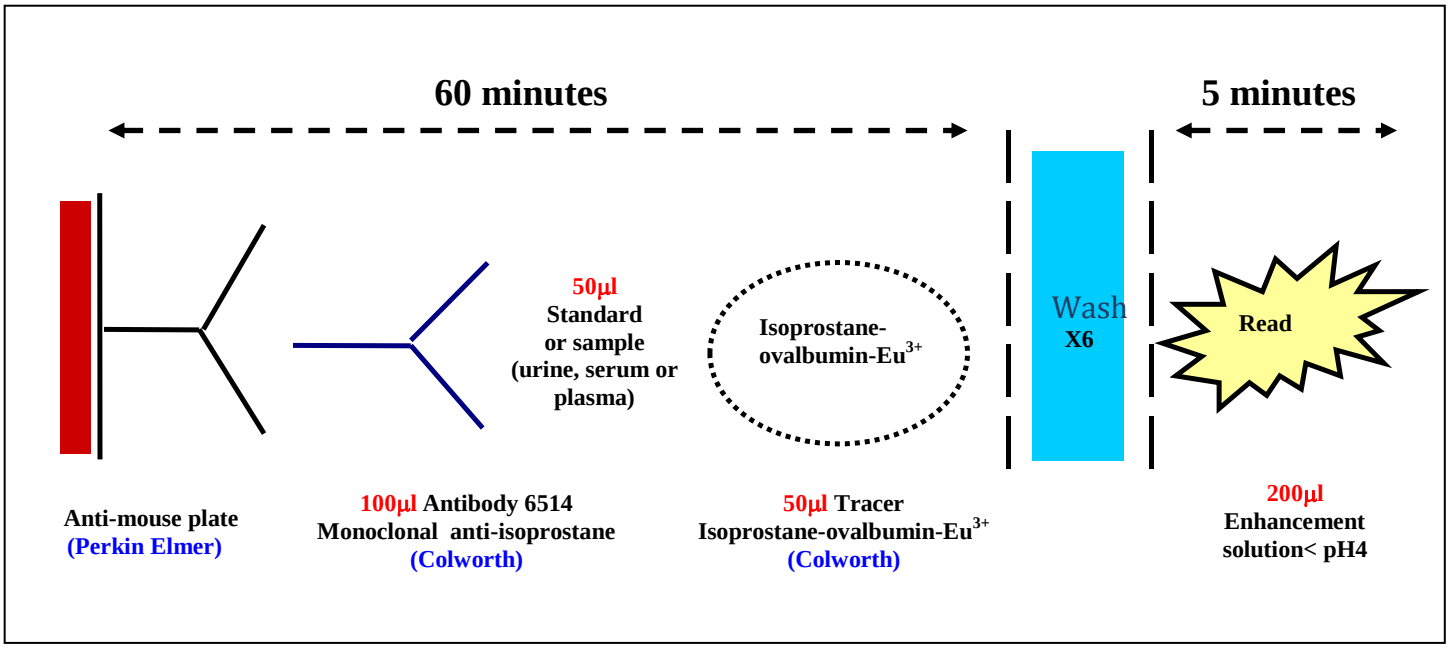
To account for the total urine volume of each participant urinary creatinine was measured to calculate isoprostane per volume creatinine

4.4.5 Determination of urinary creatinine

Urinary creatinine was measured using ABX Pentra 400 (Horiba ABX). Urine samples were centrifuged at 3000rpm for 10 minutes. 120µl volumes were then aliquoted into sample cups, up to 60 samples at a time could be run on the ABX Pentra 400. The APX Pentra 400 was calibrated using ready-to-use ABX Pentra Creatinine 120CP,

ABX Pentra Mulit-cal, positive and negative controls and ABX Pentra urine low and high controls.

Figure 4-1: AutoImmuneAutoDelfia System



4.4.6 Clinical blood sample analysis

Analysis of all blood samples routinely collected within the Let's Prevent Diabetes Study was conducted in the pathology laboratories of Leicester Royal Infirmary. The following quantification systems used Abbot Clinical chemistry assays and were performed on the ARCHITECH c Systems^{TM}/AREOSET systems:

- High density Lipoprotein (HDL) was quantified using the Ultra HDL assay
- Serum Cholesterol was quantified using the cholesterol enzymatic assay

- Serum Triglyceride was measured using the Triglyceride Glycerol Phosphate Oxidase assay

HbA1c was quantified using High Performance Liquid Chromatography (HPLC) on and automated glycohaemoglobin HLC-723G analyser, Tosoh Bioscience Ltd. Plasma glucose was determined using the hexokinase method.

4.4.7 Dietary questionnaire

The Dietary Instrument for Nutrition Education (DINE) questionnaire was administered to participants. DINE is a validated questionnaire for use in a primary health care setting.[265] DINE is a simple scoring tool which ranks individuals into low, medium or high fat and fibre categories. The tool is suitable for self completion.[266, 267] The questionnaire also contains one question on fruit intake and another on vegetable intake (appendix 3).

4.5 Statistical methods

All statistical analysis was carried out using SPSS version 18.0. All variables were imported from the excel database containing baseline questionnaire and blood test results of participants in the Let's Prevent Diabetes screening trial. The DINE and IPAQ questionnaires were scored using guidelines to ascertain fat, fibre and USFA tertiles and activity levels of participants.[265,268] To create a categorical variable for plasma vitamin C tertiles were calculated using the visual binning technique in SPSS.

Baseline characteristics of the cohort were determined using frequency and descriptive techniques for categorical and continuous variables. Differences in the main characteristics between male and female participants, between individuals in tertiles of fat, fibre, USFA and plasma vitamin C were determined using independent T-Tests or ANOVA for continuous variables and Chi-Square tests for categorical variables. Levenes' test of variance was used to determine whether the comparative groups had equal variance.

Normality of continuous dependent variables was determined by visually assessing normality plots. Urinary F_2-isoprostanes were not normally distributed, thus data were log transformed. Geometric means were calculated after analysis.

4.5.1 Linear regression

Linear regression was carried out to determine the relationships between plasma vitamin C, both as a continuous and categorical variable against HbA1c, fasting and 2 hour glucose levels. For categorical variables, the highest category was used as the reference category. HbA1c, fasting glucose and 2 hour glucose were run against all independent variables separately to determine which factors were significantly associated with the glycaemic outcomes and could therefore affect relationships of interest. Variables identified as having a significant association to HbA1c were, BMI, WC, sex, ethnicity, gender, Triglycerides (TG), high density lipoprotein (HDL) and history of CVD disease. Variables identified as having a significant association with fasting glucose were, sex, BMI, WC, HDL, TG and systolic blood pressure (SBP).

Variables associated with 2 hour glucose were age, ethnicity, BMI, WC, history of CVD disease, low density lipoprotein (LDL), HDL, TG, SBP and diastolic blood pressure (DBP). Variables associated with plasma vitamin C were age, sex, vitamin use, BMI, WC, smoking status, HDL, TG, DBP, social deprivation score, fibre intake, fat intake and glycaemic state.

For all linear regression models, the independent variable of interest was firstly run against the dependent variable in an unadjusted model. If a significant relationship was found, model 1 was carried out. This included baseline demographics, age, BMI, gender and ethnicity to determine if the relationship still remained. The second model included baseline demographics plus the independent variables found to have a significant association with the dependent variable of interest. Family history of T2DM was also included in model 2 as this is a known risk factor, however it did not show any independent relationships in this cohort. If a significant relationship still remained a third model was run, this included factors found to be independently associated with the independent variable of interest. Finally a fourth model to include the DINE questionnaire data was included. Thus adjustment was carried out depending on the outcome variable; if associations no longer remained significant at any point further modelling was not carried out.

4.5.2 Logistic regression

Logistic regression was used to determine whether plasma vitamin C levels could predict diagnosis of IGR (defined as IGT, IFG, T2DM or both IGT and IFG) or NGT.

For the categorical variables the lowest category was used as the reference category. All independent variables were entered separately into the logistic regression model to determine which variables were independently significantly associated with prediction of diagnosis. Factors identified were history of CVD, LDL, HDL, TG, SBP and ethnicity.

4.6 Let's Prevent Diabetes Study

FIVE is a specific sub-study of Lets Prevent Diabetes; a clinical cluster randomised controlled trial funded by the National Institute of Health Research (NIHR). The study is being conducted by University Hospitals of Leicester Diabetes Research Team in collaboration with other departments across the University Hospitals NHS Trust and the South East Diabetes Research Network. The project was developed based on evidence from previous prevention studies carried out across USA and Europe, discussed in chapter 2. The trial enrolled clinical practices from a multi ethnic population, thus the intervention has been designed specifically to be sensitive to their needs. The primary outcome of Let's Prevent Diabetes is a reduction in the incidence of diabetes at 3 years. The secondary outcomes include change in HbA1c, fasting and 2 hour glucose.

4.6.1 Screening study

Study participants were identified by a risk factor score carried out on information held on GP surgery computer information. An initial key word search was carried out for terms such as "pre-diabetes, impaired glucose tolerance, impaired fasting glucose or

abnormal glucose". A risk factor scoring method was additionally carried out, an algorithm based on large screening studies from the Leicester area was used.[269] Using stored electronic information on GP databases a risk score was created. Basic information such as age, sex, BMI, family history of T2DM and use of medication was incorporated. Those in the top 10% of risk of T2DM were invited to the initial screening study.

At the screening session an OGTT was carried out. Participants also had anthropometric measurements and fasting blood samples taken, and provided a spot urine sample (Table 4-1, page 100). Participants identified with IGT, IFG or both using the 2006 WHO criteria[270] were invited to enrol into the Lets Prevent Diabetes RCT. Those identified with T2DM or NGT were not eligible to take part. Those with T2DM were referred to their GP for follow up care and those with NGT were advised of their results and thanked for their involvement.

4.6.2 Prevention Study

Participants identified with IGR at the screening session who met the inclusion and exclusion criteria (Figure 4-2, page 97) were invited to take part in the prevention study.

Practices were randomised to avoid contamination between participants. In total 44 clinical practices were enrolled to the study, each practice was randomised to receive either control or intervention. Those in the control arm received standard care, current

guidelines for those identified with IGR is that individuals should receive reading material and general lifestyle advice from their GP or practice nurse. Patients in the intervention arm were given structured group education and follow up care.

Figure 4-2: Inclusion and exclusion criteria for Let's Prevent Diabetes intervention

Inclusion Criteria:

- Diagnosed with IGT or IFG or IGT & IFG at baseline screening visit
- Aged 40-75 years if WE or 25-75 years if SA
- Able to attend group education sessions

Exclusion Criteria:

- Unable to give consent
- Unable to attend group education sessions
- Diagnosis of T2DM at screening session
- Require an interpreter other than for a SA language

4.6.3 Let's Prevent Diabetes intervention

The Let's Prevent programme is a modified version of DESMOND (Diabetes Education and Self-Management for On-going and Newly Diagnosed).[271] This evidence based and cost effective programme is supported by Diabetes UK and the Department of Health and is co-ordinated by University of Hospitals Leicester.[271, 272] DESMOND is a person centred structured education programme based on self management. The education is underpinned by psychological theories, Leventhal's

Common Sense Model,[273] Bandura's concept of self efficacy,[274] Gollwitzer's implementation intention theory[275] and Chiaken's dual processing theory.[276]

Individuals enrolled in to the intervention arm of Let's Prevent attended either one six hour structured education session or two three hour sessions, not more than three weeks apart. The education was delivered by two trained educators who used open questions and encouraged discussion to elicit information and encourage learning. The programme aims to increase self-efficacy, overcome barriers and set personal goals over both the long and short term. In the case of subjects where English was not their first language there were four 3 hour sessions with an educator and interpreter present.

4.6.4 The Lets Prevent Diabetes curriculum

The lets Prevent Diabetes Curriculum was developed by a multi-disciplinary team in partnership with the DESMOND collaborative and The National Activity Centre in Loughborough, PREPARE Study.[124, 271] The written curriculum is suitable for a broad range of participants and has the potential to be integrated into routine care. The curriculum is split into eleven sessions aimed at informing participants about IGR, their risk of T2DM and how they can make tailored lifestyle changes to reduce their risk. (Figure 4-3, page 99).

Participants in the intervention arm received phone calls every three months for the entire study. The phone calls are semi-structured to discuss the changes participants

may have made and to discuss any concerns they may have. The phone calls are delivered with the same philosophy and behaviours as the initial education sessions

Participants return for follow up education sessions at 12 and 24 months; these sessions last 3 hours and review the key messages from the initial education session. They aim to re-enforce the importance of behaviour change.

Figure 4-3: The Let's Prevent education curriculum

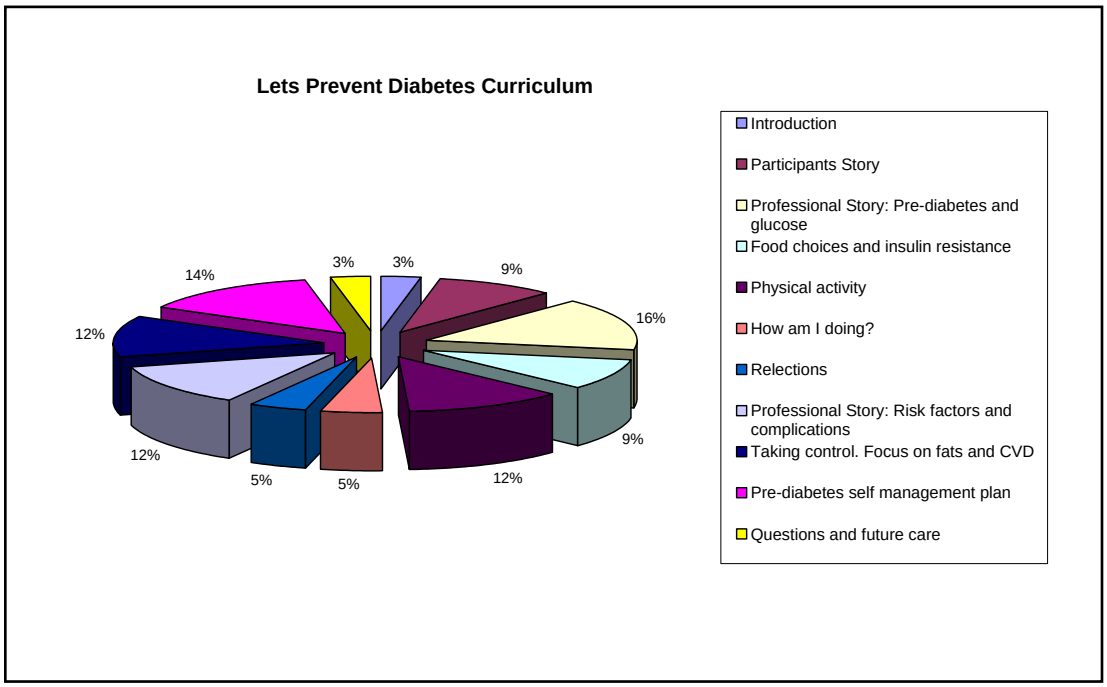


Table 4-1: Clinical assessments and measures

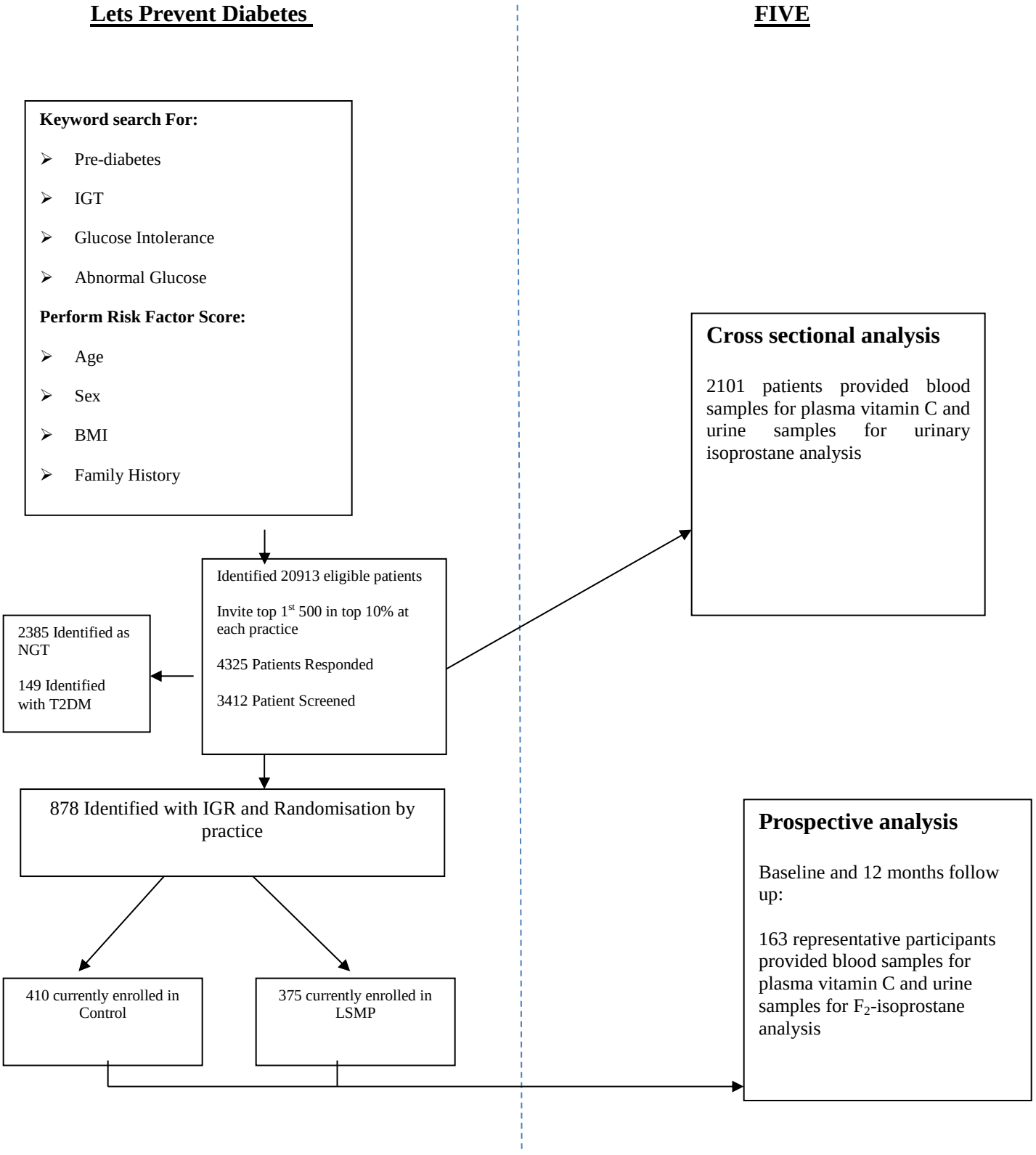
Measurements	Time points				
	0[Ψ]	6	12[Ψ]	24	36
Clinical Assessment					
Medical History	x		x	X	x
Medication History	x		x	X	x
Physical Exam	x		x	X	x
Cardiovascular Risk Score	x	x	x	X	x
Metabolic Syndrome Assessment	x	x	x	X	x
Anthropometric Measures					
Blood Pressure (mmHg)	x	x	x	X	x
Height (m)	x				
Weight (kg)	x	x	x	X	x
Waist Circumference (cm)	x	x	x	X	x
Blood Tests					
OGTT (which includes a fasting and 2 hour post glucose challenge test) (mmol/l)	x		x	X	x
HbA1c (%)	x	x	x	X	x
Triglycerides (mmol/l)	x	x	x	X	x
HDL & LDL (mmol/l)					
Urea & Electrolytes	x		x	X	x
Liver Function Tests	x		x	X	x
***Plasma Vitamin C (µmol/l)**	**x**		**x**	**x**	**x**
Questionnaires & Lifestyle Measures					
DINE	x	x	x	x	x
IPAQ	x	x	x	x	x
BIPQ	x	x	x	x	x
HADS	x	x	x	x	x
15D	x	x	x	x	x
7 day step count	x	x	x	x	x
***Urine Sample**	**x**		**x**	**x**	**x**

***Samples taken specifically for FIVE, highlighted in yellow**

[Ψ]Time points of FIVE

HADS – Hospital Anxiety and Depression
DINE – Dietary instrument for Nutrition Education
IPAQ – International physical activity questionnaire
BIPQ – Brief illness Perception questionnaire
15D – The Health State Descriptive System to assess quality of Life

Figure 4-4: Study procedure



4.6.5 Follow up clinics

Participants were followed up at 6 months to have anthropometric measures taken along with blood samples for HbA1c and lipid levels. Individuals were also asked to complete the same lifestyle questionnaires completed at the screening session. CV risk score and presence of metabolic syndrome was also evaluated. At the annual clinic participants provided all blood, urine and questionnaires completed in the screening session. The same will occur at second and third year clinics.

4.7 Chapter summary

Chapter 4 is the first of three chapters devoted to the FIVE study. This chapter has provided the rationale for the study, stated study aims and the study design. I have also described the methodology for both clinical and laboratory assessment measures. The chapter aims to show how FIVE is a sub study of a large screening and intervention study, Let's Prevent Diabetes, to provide currently unavailable information on fruit and vegetable intake and glucose regulation.

Chapter 5

Fruit and Vegetable Intake and Glucose Control Study (FIVE) – Results

5.0 Chapter overview

In this chapter I will present all the findings from the FIVE study. I will begin by describing the cross sectional population which make up the data set and then explore the results to answer the first three aims of the study, as determined in the previous chapter (section 4.3). The final part of this chapter will provide the data from the prospective section of the FIVE study; the population will be described and results presented to determine whether structured education increases fruit and vegetable intake more successfully than routine care.

5.1 Baseline characteristics of individuals enrolled to FIVE: cross sectional study

Between July 2009 and November 2010, 2878 participants were screened as part of Let's Prevent Diabetes; of these 2101 participants provided both blood samples for analysis of plasma vitamin C and urine samples for urinary F_2-isoprostane analysis as described in chapter 4; these individuals make up the cross sectional cohort for the first section of FIVE. No significant differences were seen between those who provided plasma vitamin C and urine samples compared to those who did not provide samples (data provided in appendix 5).

Baseline characteristics of the participants enrolled into FIVE are presented in Table 5-1. The mean age of the participants was 63 years (SD 7.9) with a range of 29 to 87 years. Mean BMI and WC were 32kg/m^2 (SD 5.7) and 109cm (SD 13.0) respectively. Mean HbA1c, fasting glucose and 2 hour glucose were 5.9% (SD 0.5), 5.3mmol/l (SD 0.8) and 6.7mmol/l (SD 2.6) respectively.

Overall 60% (n=1272) of the participants were male and 40% (n=823) female. Eighty six percent (n=1797) of the population were WE, 12% (n=248) SA and 2% (n=56) from other ethnic groups. Twenty one percent of the population (n=422) reported a previous history of CVD, including MI, angina, stroke, atrial fibrillation or angioplasty. Thirty seven percent (n=646) of those screened had a first degree family history of T2DM. Only 9% (n=164) of the population were current smokers (Table 5-1).

Table 5-1: Baseline characteristics of the FIVE cohort

	Mean	SD
Age (years)	63	7.9
BMI (kg/m^2)	32	5.7
Waist circumference (cm)	109	13.0
TG (mmol/l)	1.6	0.9
HDL (mmol/l)	1.4	0.4
LDL (mmol/l)	3.1	1.2
Systolic blood pressure (mmHg)	146	19.2
Diastolic blood pressure (mmHg)	86	10.5
HbA1c (%)	5.9	0.5
Fasting glucose (mmol/l)	5.3	0.8
2 hour glucose (mmol/l)	6.7	2.6
	Percentage	Number
Male	60	1272
History of CVD	21	422
Family History of T2DM	37	646
Current Smoker	9	164

The majority of the population screened were diagnosed with NGT (70.5%). 29.5% were diagnosed with IGR, as defined in section 4.5.2. Of these, 4.3% were diagnosed

with T2DM, 3.5% and 17.5% were diagnosed with IFG and IGT respectively and 4.2% had both IGT and IFG. (Table 5-2)

Table 5-2: Classification of glucose tolerance of participants in FIVE

Category of glucose tolerance	Percentage	Number
Normal glucose tolerance (NGT)	70.5	1475
Impaired fasting glucose (IFG)	3.5	74
Impaired glucose tolerance (IGT)	17.5	367
IFG & IGT	4.2	88
Type 2 diabetes (T2DM)	4.3	90
Impaired glucose regulation (IGR)*	*29.5*	*619*

*IGR = Participants with T2DM, IFG, IGT and both IFG & IGT combined

5.2 Aim 1: To determine whether fruit and vegetable intake, as assessed by plasma vitamin C is associated with baseline glycaemic state of a population identified as being high risk for T2DM.

5.2.1 Vitamin C concentrations of the FIVE cross sectional population

Mean plasma vitamin C of the population was 39.3µmol/l (SD 21.8). Female participants had significantly higher plasma vitamin C compared to males (42.6µmol/l (SD 21.9) *vs.* 37.2µmol/l (SD 21.5) $p \leq 0.0001$). A plasma vitamin C concentration of ≥50µmol/l is considered a reflection of consuming 5 portions fruit and vegetables a day.[277] Less than a third (29%, n=599) of the total population consumed the recommended 5 portions of fruit and vegetables per day. A greater percentage of females as compared to males consumed 5 portions of fruit and vegetables per day,

37% (n=298) compared to 24% (n=297) respectively. No interaction existed between plasma vitamin C and sex (p=0.8) therefore the observed differences between the sexes indicate differences in intake.

A state of vitamin C deficiency is classified as a plasma vitamin C level below 11.4µmol/l and marginal deficiency as between 11.5µmol/l and 20µmol/l.[278] Over 20% of the population had some form of vitamin C deficiency, 11.8% (n=247) had deficient levels and 8.7% had marginal deficiency of plasma vitamin C (Table 5-3).

Mean vitamin C levels were significantly different between individuals who reported high, medium and low fat, fibre and unsaturated fatty acid intake levels as assessed by the DINE questionnaire, (p=0.008, 0.008 and 0.8 respectively) (Table 5-4).

Table 5-3: Plasma vitamin C status of participants in FIVE

	Total population	Males	Females
	%	%	%
Individuals with vitamin C deficiency[1]	11.8	13.3	9.5
Individuals with marginal vitamin C deficiency[2]	8.7	9.2	8.0
Individuals who consume at least 5 portions fruit and vegetables a day or more[3]	29	24	37

1=plasma vitamin C <11.4µmol/l
2=plasma vitamin C 11.5µmol/l - 20µmol/l
3= plasma vitamin C ≥50µmol/l

Table 5-4: Mean plasma vitamin C concentrations of individuals with self reported low, medium and high fat, fibre and unsaturated fatty acid intake levels[§]

		Number	Mean Vitamin C(µmol/l)	SD	P value*
Fat Intake	Low	415	40.9	21.2	
	Medium	185	37.6	21.2	
	High	72	33.2	19.8	0.008
Fibre Intake	Low	346	36.4	21.7	
	Medium	317	40.5	19.9	
	High	274	39.1	21.0	0.008
USFA Intake	Low	51	33.2	19.9	
	Medium	750	39.8	21.7	
	High	804	40.1	20.8	0.08

*P value corresponds to the unadjusted difference in mean plasma vitamin C between the low, medium and high intake groups for fat, fibre and USFA

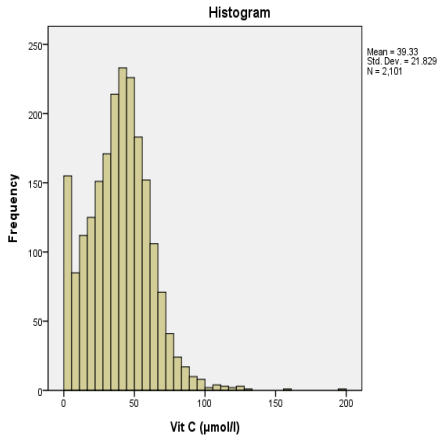
[§]Fat, fibre and USFA intake assessed by DINE

5.2.2 Exploration of plasma vitamin C (1): Vitamin C as a continuous variable

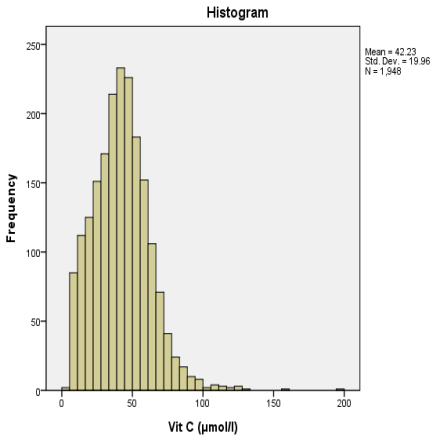
Assessment of normality showed 7% of the population had plasma vitamin C levels less than 5µmol/l, resulting in skewed distribution of normality (Figure 5-1). Analysis of all data was carried out including and excluding these participants, however no difference in results was seen. Furthermore these participants have true low values of vitamin C, therefore all data presented is from analysis including these individuals.

Figure 5-1: Histograms to show normality of plasma vitamin C concentrations

Histogram A
(includes all participants in FIVE)



Histogram B
(excludes those with plasma vitamin C <5µmol/l)



Plasma vitamin C was independently and significantly associated with HbA1c, fasting and 2 hour glucose, p ≤0.0001 for all three variables. To determine a more realistic change in vitamin C through fruit or vegetable intake, analysis was re-run on all three glucose parameters using the SD of mean plasma vitamin C (21.8µmol/l). In the unadjusted model, an increase of 21.8µmol/l plasma vitamin C was associated with a decrease of 0.05% HbA1c, a decrease of 0.08mmol/l fasting glucose and with a decrease of 0.2mmol/l in 2 hour glucose (table 5-5, 5-6 and 5-7).

Adjustment for demographic variables and factors found to influence each glycaemic parameter did not significantly alter the results (model 1 and 2). Model 2 demonstrated that an increase of 21.8µmol/l plasma vitamin C was associated with a 0.04% lower HbA1c, 0.05mmol/l lower fasting glucose and 0.2mol/l lower 2 hour glucose.

Table 5-5: Association between 1SD plasma vitamin C (21.8µmol/l) and HbA1c

	Regression co-efficient	95% confidence intervals		P value
		Lower bound	Upper bound	
Unadjusted model	-0.05	-0.07	-0.03	<0.0001
Model 1	-0.05	-0.07	-0.03	<0.0001
Model 2	-0.04	-0.07	-0.2	0.001
Model 3	-0.02	-0.05	0.002	0.07
Model 4	-0.09	-0.17	0.007	0.03

Model 1: Age, sex, BMI, ethnicity
Model 2: Model 1, plus HDL, TG, CVD history and family history of T2DM
Model 3: Model 2 plus smoking status, vitamin use, and $\log^{10}$[urinary isops]
Model 4: Model 3 plus deprivation score, fat and fibre group

Table 5-6: Association between 1SD plasma vitamin C (21.8µmol/l) and fasting Glucose

	Regression co-efficient	95% confidence intervals		P value
		Lower bound	Upper bound	
Unadjusted model	-0.08	-0.11	-0.05	<0.0001
Model 1	-0.06	-0.09	-0.03	<0.0001
Model 2	-0.05	-0.09	-0.02	0.005
Model 3	-0.04	-0.08	-0.006	0.02
Model 4	-0.06	-0.16	0.03	0.19

Model 1: Age, sex, BMI, ethnicity
Model 2: Model 1 plus BP, HDL, TG and family history of T2DM
Model 3: Model 2 plus smoking status, vitamin use, and $\log^{10}$[urinary isops]
Model 4: Model 3 plus deprivation score, fat and fibre group

Table 5-7: Association between 1SD plasma vitamin C (21.8µmol/l) and 2 hour glucose

	Regression co-efficient	95% confidence intervals		P value
		Lower bound	Upper bound	
Unadjusted model	-0.21	-0.33	-0.10	<0.0001
Model 1	-0.22	-0.33	-0.10	<0.0001
Model 2	-0.22	-0.34	-0.10	<0.0001
Model 3	-0.17	-0.30	-0.03	0.01
Model 4	-0.40	-0.80	0.003	0.05

Model 1: Age, sex, BMI, ethnicity
Model 2: Model 1, plus HDL, TG, CVD history and family history of T2DM
Model 3: Model 2 plus smoking status, vitamin use, and $\log^{10}$[urinary isops]
Model 4: Model 3 plus deprivation score, fat and fibre group

5.2.3 Summary of findings

Analysis of baseline plasma vitamin C concentrations as a continuous variable demonstrated that lower plasma vitamin C was associated with a higher HbA1c, fasting and 2 hour glucose in this population. The relationship remained after adjustment for demographic variables and factors seen to influence each glycaemic parameter (model 2). Models 3 and 4 resulted in attenuation of the results.

5.3 Exploration of plasma vitamin C (2): Vitamin C as a categorical variable

5.3.1 Characteristics of individuals in the plasma vitamin C tertiles

To determine differences between those with low and high fruit and vegetable intake, tertiles of intake were created. Those in the highest group of plasma vitamin C (T3) were older, 65 years (SD 7.7) compared to 63 years (SD 7.90) for those in the other two tertiles (T1 and T2). Those in T3 had the lowest BMI and WC, 32kg/m^2 (SD 5.7) and 106cm (SD12.0) compared to 33 kg/m^2 (SD 6.3) and 111cm (SD 13.7) and 32kg/m^2 (SD 5.6) and 109cm (SD 12.6) for T1 and T2 respectively. Male participants made up 67% of T1, 65% of T2 and 51% of T3 ($p \leq 0.0001$). However separate analysis of males and females did not significantly affect the results.

In addition HbA1c, fasting and 2 hour glucose were lower for those in T3 as compared to both T1 and T2, 5.9% (SD 0.5), 5.2mmol/l (SD 0.7) and 6.5mmol/l (SD 2.5) compared to 6.0% (SD 0.6), 5.4mmol/l (SD 0.9) and 6.9mmol/l (SD 2.8) for T1 and 5.9% (SD 0.5), 5.3mmol/l (SD 0.7) and 6.9mmol/l (SD 2.4) for T2 (Table 5-8).

Table 5-8: Characteristics of individuals in the plasma vitamin C tertiles

	T1 – low plasma vitamin C (≤30µmol/l)		T2 – medium plasma vitamin C (31-48µmol/l)		T3 – High plasma vitamin C (≥49µmol/l)		P value*
	Mean	SD	Mean	SD	Mean	SD	
Number	701		702		698		
Mean plasma Vitamin C (µmol/l)	15.7	9.3	39.6	5.1	62.8	14.5	<0.0001
Age (years)	63	7.9	63	7.9	65	7.7	<0.0001
BMI (kg/m^2)	33	6.3	32	5.6	32	5.7	<0.0001
Waist circumference (cm)	111	13.7	109	12.6	106	12.0	<0.0001
HbA1c (%)	6.0	0.6	5.9	0.5	5.9	0.5	<0.0001
Fasting glucose (mmol/l)	5.4	0.9	5.3	0.7	5.2	0.7	<0.0001
2 hour glucose (mmol/l)	6.9	2.8	6.9	2.4	6.5	2.5	0.02

*P values are associated with the unadjusted mean differences in characteristics between the tertiles

5.3.2 Analysis of plasma vitamin C tertiles and glycaemic measures

In the unadjusted models participants with the greatest fruit and vegetable intake (those in the highest tertile of plasma vitamin C (T3)) compared to those with the lowest intake (those in the lowest tertile of plasma vitamin C (T1) had a reduced HbA1c, fasting and 2 hour glucose (0.1%, 0.17mmol/l and 0.4mmol/l). The association was slightly attenuated but remained significant after inclusion of demographic variables and factors associated with the different glycaemic measures (models 1 and 2). Model 3 showed attenuation of the data.

Table 5-9: Differences in HbA1c between those in T3 (high plasma vitamin C) and T1 (low plasma vitamin C)

	Regression co-efficient	95% confidence intervals		P value
		Lower bound	Upper bound	
Unadjusted model	0.10	0.05	0.15	<0.0001
Model 1	0.10	0.04	0.15	<0.0001
Model 2	0.07	0.02	0.13	0.01
Model 3	0.05	-0.02	0.11	0.14

Model 1: Age, sex, BMI, ethnicity
Model 2: Model 1, plus HDL, TG, CVD history and family history of T2DM
Model 3: Model 2 plus smoking status, vitamin use, and $\log^{10}$[urinary isops]

Table 5-10: Differences in fasting glucose between those in T3 (high plasma vitamin C) and T1 (low plasma vitamin C)

	Regression co-efficient	95% confidence intervals		P value
		Lower bound	Upper bound	
Unadjusted model	0.17	0.08	0.25	<0.0001
Model 1	0.12	0.04	0.20	0.005
Model 2	0.10	0.01	0.19	0.02
Model 3	0.08	-0.009	0.17	0.07
Model 4	0.10	-0.10	0.31	0.33

Model 1: Age, sex, BMI, ethnicity
Model 2: Model 1 plus BP, HDL, TG and family history of T2DM
Model 3: Model 2 plus smoking status, vitamin use, $\log^{10}$[urinary isops]
Model 4: Model 3 plus deprivation score, fat and fibre score

Table 5-11: Differences in 2 hour glucose between those in T3 (high plasma vitamin C) and T1 (low plasma vitamin C)

	Regression co-efficient	95% confidence intervals		P value
		Lower bound	Upper bound	
Unadjusted model	0.40	0.12	0.67	0.004
Model 1	0.40	0.12	0.68	0.005
Model 2	0.33	0.03	0.63	0.03
Model 3	0.17	-0.16	0.49	0.31

Model 1: Age, sex, BMI, ethnicity
Model 2: Model 1 plus HDL, TG, CVD history and family history of T2DM
Model 3: Model 2 plus smoking status, vitamin use and $\log^{10}$[urinary isops]

5.3.3 Summary of plasma vitamin C tertile analysis

Individuals enrolled to FIVE who were in the highest tertile of plasma vitamin C had lower levels of HbA1c, fasting and 2 hour glucose as compared to those in the lowest tertile of plasma vitamin C. The association between tertiles showed the greatest clinical association with 2 hour glucose, in the adjusted model (model 2) being in T3 as compared to T1 was associated with a 0.33mol/l lower 2 hour glucose.

5.4 Exploration of data (3): Is plasma Vitamin C associated with impaired glucose regulation?

Mean plasma vitamin C was significantly different across the glucose classification groups (p= 0.001); those with T2DM had the lowest plasma vitamin C of the different categories, (Table 5-12).

Logistic regression demonstrated that increasing 1 SD of plasma vitamin C (21.8µmol/l) resulted in a 16% reduced risk of IGR (OR = 0.84, 95% CI = 0.77 to 0.93, p=0.001). Adjustment for confounding variables attenuated the association marginally but this was still significant (OR = 0.85, 95% CI = 0.75 to 0.95, p = 0.005) (Table 5-13). Incorporation of factors found to influence plasma vitamin C (model 4) resulted in the significant association being lost.

Logistic regression was repeated to calculate the associated risk of being diagnosed with IGR if participants consumed the recommended 5 a day (plasma vitamin C ≥50µmol/l). In the unadjusted model, consumption of at least 5 a day was associated with a 23% lower risk of being diagnosed with IGR at screening as compared to not

consuming 5 portions a day (OR = 0.77, 95% CI = 0.63 to 0.96, p=0.02) and in the adjusted model the relationship remained (model 3) however inclusion of factors associated with vitamin C attenuated the results (Table 5-14).

Table 5-12: Mean plasma vitamin C of glucose classification groups

Glucose classification	Percentage	Number	Plasma Vitamin C (µmol/l)	
			Mean	SD
NGT	70.4	1474	40.3	22.2
IFG	3.5	74	38.9	22.2
IGT	17.5	367	37.9	20.3
IFG & IGT	4.2	88	35.2	20.6
T2DM	4.3	90	31.7	21.1

Table 5-13: Association of 1SD plasma vitamin C (21.8µmol/l) and risk of being diagnosed with IGR at screening

	Exp(B) - OR	95% confidence intervals		P value
		Lower bound	Upper bound	
Unadjusted model	0.84	0.77	0.93	0.001
Model 1	0.86	0.78	0.95	0.003
Model 2	0.84	0.75	0.94	0.003
Model 3	0.85	0.75	0.95	0.005
Model 4	0.89	0.78	1.02	0.08
Model 5	0.75	0.46	1.21	0.24

Model 1: Age, sex, BMI, ethnicity
Model 2: Model 1, plus BP, CVD history and family history of T2DM
Model 3: Model 2, plus HDL, LDL, TG
Model 4: Model 3 plus smoking status, vitamin use and $\log^{10}$[urinary isops]
Model 5: Model 4 plus deprivation score, fat and fibre group

Table 5-14: Association of not consuming 5 portion of fruit and vegetables a day (plasma vitamin C <50µmol/l) and risk of being diagnosed with IGR at screening

	Exp(B) - OR	95% confidence intervals		P value
		Lower bound	Upper bound	
Unadjusted model	0.77	0.63	0.96	0.02
Model 1	0.80	0.64	0.99	0.05
Model 2	0.74	0.57	0.95	0.02
Model 3	0.76	0.59	0.98	0.03
Model 4	1.18	0.88	1.59	0.26

Model 1: Age, sex, BMI, ethnicity
Model 2: Model 1, plus BP, CVD history and family history of T2DM
Model 3: Model 2, plus HDL, LDL, TG
Model 4: Model 3 plus smoking status, vitamin use and $\log^{10}$[urinary isops]

5.4.1 Summary of plasma vitamin C and the association of IGR

The OR of being diagnosed with IGR at screening was significantly greater in those with lower baseline plasma vitamin C levels. Adjusted analysis showed that a 21.8µmol/l lower plasma vitamin C concentration was associated with a 15% greater risk of being diagnosed with IGR at screening. Furthermore consumption of 5 portions of fruit and vegetables a day was associated with an adjusted OR of 0.76 (95% CI = 0.59 to 0.98) as compared to not consuming 5 portions a day.

5.5 Exploration of co-linearity

Co-linearity between factors included in the regression models (model 3 and 4) may have resulted in attenuation of results. However correlations between factors were not strong[279] (Table 5-15). Furthermore the variation inflation factor was less than 10; statistically this is not considered a concern but it is known that nutritional factors are causally related to one another[190] and co-linearity should not be ruled out.

Table 5-15: Correlations between plasma vitamin C and confounding factors

Potential confounding factor	r value	P value
Smoking	-0.08	0.001
Fat intake	-0.2	0.002
Fibre intake	0.1	0.005
Deprivation score	-0.1	<0.0001
Vitamin use	-0.2	<0.0001

5.6 Serum potassium (K^+) as a potential mechanism for the effect of fruit and vegetable consumption on glycaemic control

Serum potassium was not significantly different between individuals with NGT, IFG, IGT, both IFG and IGT or T2DM (p=0.07) (Table 5-16). Serum potassium showed no significant association with HbA1c or fasting glucose. However there was a significant association between serum potassium and 2 hour glucose. In the adjusted model a one unit increase in K^+ was associated with a 0.73mmol/l decrease in 2 hour glucose (model 2). Incorporation of factors associated with serum K^+ resulted in attenuation of the association (model 3) (Table 5-17). There was only a weak correlation between plasma vitamin C and serum potassium, r = -0.06 (p=0.005).

Table 5-16: Serum potassium across the glucose classification groups

Glucose Category	Number	Potassium	
		Mean	SD
NGT	1450	4.3	0.4
IFG	362	4.3	0.5
IGT	73	4.3	0.4
IFG & IGT	88	4.3	0.3
T2DM	89	4.2	0.5

(P = 0.07)

Table 5-17: Association between serum K^+ and 2 hour glucose

	Regression co-efficient	95% confidence intervals		P value
		Lower bound	Upper bound	
Unadjusted model	-0.71	-1.0	-0.4	<0.0001
Model 1	-0.72	-1.0	-0.5	<0.0001
Model 2	-0.73	-1.0	-0.5	<0.0001
Model 3	-0.4	-1.1	0.3	0.25

Model 1: Age, sex, BMI, ethnicity
Model 2: Model 1, plus HDL, TG, CVD history and family history of T2DM
Model 3: Model 2 plus smoking status, plasma vitamin C levels, eGFR and use of hypertensive medication

5.6.1 Summary of serum K^+ findings

Serum K^+ was significantly associated with 2 hour glucose. Fruit and vegetables are the main source of dietary potassium; however in this population plasma vitamin C was not correlated to serum K^+.

5.7 Aim 2: To determine whether urinary F_2-isoprostanes are related to baseline plasma vitamin C and to glycaemic control

5.7.1 Baseline urinary F_2-isoprostanes concentrations

Mean concentration of urinary isoprostanes was 1.6mM/L mM/L creatinine (SD 3.5). Urinary isoprostanes were not normally distributed therefore data was log transformed, resulting in a mean log concentration of 0.1mM/L mM/L creatinine (SD 0.2), geometric mean of 1.3mM/L mM/L creatinine. There were no significant differences in urinary isoprostanes between individuals in the different glucose classification groups (Table 5-18).

Table 5-18: Geometric means of [urinary Isops] across glycaemic groups

Diagnosis	Geometric mean [urinary Isops] mM/L mM/L creatinine	SD
Normal glucose tolerance (NGT)	1.3	1.6
Impaired fasting glucose (IFG)	1.1	1.6
Impaired glucose tolerance (IGT)	1.2	1.6
IFG & IGT	1.1	1.6
Type 2 diabetes (T2DM)	1.3	2.0
Chi square showed significant difference between glucose classification groups, P = 0.06		

5.7.2 Urinary F_2-isoprostanes and glucose control

Log^{10} [Urinary Isops] was not associated with HbA1c, (p = 0.64). However there was a trend towards an association with 2 hour glucose, (p=0.08) and a significant association with fasting glucose (p=0.01). Adjustment for age and BMI did not significantly affect the associations with fasting glucose, however sex and ethnicity attenuated the relationship (p=0.14). The association between log^{10} [urinary Isops] and 2 hour glucose was strengthened after adjustment for demographic variables, (p=0.03). However incorporation of factors found to influence 2 hour glucose (model 2) resulted in attenuation and loss of a significant relationship between urinary isoprostanes and 2 hour glucose, TG levels and systolic blood pressure had the greatest influence on the regression model.

Table 5-19: Association of urinary F_2-isoprostane concentration and fasting glucose

	Regression co-efficient	95% confidence intervals		P value
		Lower bound	Upper bound	
Unadjusted model	-0.2	-0.4	-0.05	0.01
Model 1	-0.1	-0.3	0.04	0.14

Model 1: Age, BMI, sex and ethnicity

Table 5-20: Association of urinary F_2-isoprostane concentration and 2 hour glucose

	Regression co-efficient	95% confidence intervals		P value
		Lower bound	Upper bound	
Unadjusted model	-0.5	-0.9	0.05	0.08
Model 1	-0.6	-1.1	-0.04	0.03
Model 2	-0.3	-0.9	0.3	0.30

Model 1: Age, BMI, sex and ethnicity
Model 2: Model 2, plus HDL, TG, BP, CVD history and family history of T2DM

5.7.3 Urinary F_2-isoprostanes and plasma vitamin C

Plasma vitamin C concentrations demonstrated a trend towards association with urinary isoprostane concentrations, however this was not significant (p = 0.07). An increase of 1 SD plasma vitamin C (21.8µmol/l) was associated with a 0.009mM/L mM/L creatinine greater $\log^{10}$[urinary isops]. The association was lost when taking into account, age, BMI, sex and ethnicity (p = 0.93) (Table 5-21). Further analysis showed that those who consumed the recommended 5 portions of fruit and vegetables a day, as compared to those who did not, had an increased urinary isoprostane concentration; however the weak association was lost with incorporation of demographic variables (Table 5-22).

Table 5-21: Association of 1SD plasma vitamin C (21.8µmol/l) and urinary F_2-isoprostane concentration

	Regression co-efficient	95% confidence intervals		P value
		Lower bound	Upper bound	
Unadjusted model	0.04	-0.004	0.02	0.07
Model 1	0.000	-0.1	0.01	0.93

Model 1: Age, BMI sex and ethnicity

Table 5-22: Association of consuming 5 portions fruit and vegetables a day and urinary F_2-isoprostane concentration

	Regression co-efficient	95% confidence intervals		P value
		Lower bound	Upper bound	
Unadjusted model	0.02	0.000	-0.05	0.05
Model 1	0.000	-0.02	0.2	0.99

Model 1: Age, BMI sex and ethnicity

5.7.4 Summary of F_2-isoprostane analysis

Urinary F_2-isoprostane concentration was not significantly associated with HbA1c or 2 hour glucose. The significant association between urinary F_2-isoprostanes and fasting glucose was lost once demographic variables were incorporated into the model. Furthermore the weak association between fruit and vegetable consumption was attenuated with adjustment for demographic variables.

5.8 Aim 3: To determine whether any differences exist in fruit and vegetable intake between South Asian individuals screened for T2DM as compared to White Europeans

5.8.1 Study characteristics

Overall 98% (n=2050) of the total screened cohort were either WE or SA origin, 86% (n=1797) were WE and 12% (n=248) SA. Baseline characteristics of the two ethnic groups are shown in Table 5-23. SAs were significantly younger than WEs, 60 years (SD 8.3) as compared to 64 years (SD 7.6). SAs had lower BMI and WC, 31kg/m^2 (SD 5.5) and 105cm (SD 12.0) as compared to 33kg/m^2 (SD5.8) and 109cm (SD 13.0) in WEs. HbA1c, fasting and 2 hour glucose were significantly greater in SAs (6.2% (SD 0.6), 5.4mmol/l (SD 0.8) 7.5mmol/l (SD3.0)) compared to WEs (5.9% (SD 0.5), 5.3mmol/l (SD 0.8) and 6.6mmol/l (SD 2.5)). There was no difference in the levels of oxidative stress between ethnic groups. Geometric mean urinary F_2-isoprostane level of WE was 1.3mM/L mM/L creatinine (SD 1.6) compared to 1.2mM/L mM/L creatinine (SD 1.6) in SA (p=0.35).

5.8.2 Plasma vitamin C concentrations

Mean plasma vitamin C of the cohort was 39.3µmol/l (SD21.8). SAs had significantly lower mean plasma vitamin C compared to WE, 34.5µmol/l (SD 19.8) compared to 39.8µmol/l (SD 21.8), ($p \leq 0.0001$) (Table 5-23). In addition 79% of SA had vitamin C levels below 50µmol/l compared to 70% of WEs, (p=0.003), indicating fewer SA individuals are meeting the 5 a day target. In addition 15% of SA individuals had deficient levels of plasma vitamin C (<11µmol/l) compared to 11% of WEs (p=0.04) (Table 5-24).

Females in both ethnic groups had greater plasma vitamin C concentrations compared to their male counterparts. WE females had the greatest unadjusted mean plasma vitamin C, 43.2µmol/l (SD 22.1). WE males had a mean plasma vitamin C concentration of 37.7µmol/l (SD 21.3) SA females had a mean plasma vitamin C concentration of 37.1µmol/l (SD 20.4). SA males had the lowest mean plasma vitamin C levels of 32.8µmol/l (SD 19.2).

Table 5-23: Baseline characteristics of White European and South Asian participants

	White European (n=1797)		South Asian (n=248)		P value*
	Mean	SD	Mean	SD	
Age (years)	64	7.6	60	8.3	<0.0001
BMI (kg/m^2)	33	5.8	31	5.5	0.001
Waist Circumference (cm)	109	13.0	105	12.0	<0.0001
Total Cholesterol (mmol/l)	5.1	1.0	4.9	1.0	<0.0001
LDL (mmol/l)	3.1	1.2	3.0	0.8	0.04
HDL (mmol/l)	1.4	0.4	1.3	0.4	<0.0001
TG (mmol/l)	1.6	0.9	1.5	0.8	0.51
HbA1c (%)	5.9	0.5	6.2	0.6	<0.0001
Fasting glucose (mmol/l)	5.3	0.8	5.4	0.8	0.02
2hr Glucose (mmol/l)	6.6	2.5	7.5	3.0	<0.0001
Vitamin C (µmol/l)	39.8	21.8	34.5	19.8	<0.0001
Geometric means[Urinary IsoPs] (mM/L mM/L creatinine)	1.3	1.6	1.2	1.6	0.35

*P value corresponds to the unadjusted difference between WE and SA individuals

Table 5-24: Vitamin C status of ethnic groups

	White Europeans	South Asian
	%	%
Individuals with vitamin C deficiency[1]	11	15
Individuals with marginal vitamin C deficiency[2]	8	11
Chi Square showed significant difference between the ethnic groups P=0.04		
Individuals who consume 5 a day or more[4]	30	21
Chi Square showed significant difference between the ethnic groups P=0.003		

1=plasma vitamin C <11.4µmol/l
2=plasma vitamin C 11.5µmol/l - 20µmol/l
3= plasma vitamin C ≥21µmol/l
4= plasma vitamin C ≥50µmol/l

5.8.3 Association between plasma vitamin C and ethnicity

Linear regression showed that SAs had significantly lower mean plasma vitamin C compared to WEs (34.5µmol/l (SD 19.8) *vs.* 39.4µmol/l (SD 22.1), ($p \leq 0.0001$) (Table 5-25). The relationship between ethnicity and vitamin C remained significant after adjusting for age, gender and BMI ($p \leq 0.0001$). Adjustment for BP, HDL, 2 hour glucose, history of CVD and family history of T2DM attenuated the results but the relationship remained significant ($p=0.03$). Incorporation of factors found to influence vitamin C status (smoking, vitamin use, deprivation score, urinary isoprostane concentration) strengthened the relationship between vitamin C and ethnicity ($p=0.003$).

Table 5-25: Association between ethnicity (WE *vs.* SA) and plasma vitamin C

	Regression co-efficient	95% confidence intervals		P value
		Lower bound	Upper bound	
Unadjusted model	-5.32	-8.18	-2.46	<0.0001
Model 1	-5.66	-8.56	-2.76	<0.0001
Model 2	-6.48	-10.62	-2.34	0.002
Model 3	-7.05	-11.84	-2.26	0.004
Model 4	-6.34	-13.61	0.93	0.09
Model 5	-6.28	-18.48	5.92	0.31

Model 1: Age, sex, BMI,
Model 2: Model 2 plus smoking status, vitamin use, deprivation score and [urinary isops]
Model 3: Model 1 plus BP, HDL, CVD history, family history T2DM, 2 hour glucose
Model 4: Model 3 plus Fibre score
Model 5: Model 4 plus Fat score

5.8.4 Relationship between fat score and vitamin C

Table 5-26 presents the number of participants who completed the fat, fibre and USFA sections of the DINE questionnaire; corresponding mean plasma vitamin C is also presented. Inclusion of Fat Score, as determined by the DINE questionnaire resulted in the significant relationship between ethnicity and plasma vitamin C being lost (p=0.31), (Table 5-25). However the accuracy of this score should be regarded with caution. In the overall cohort, those with the greatest fat intake had the lowest plasma vitamin C levels, the low fat intake group had a mean of 40.8µmol/l (SD 21.3) plasma vitamin C compared to 37.5µmol/l (SD 21.3) and 33.1µmol/l (SD 19.9) for the medium and high fat intake groups respectively. Independent analysis of the two ethnic groups showed that the relationship between plasma vitamin C concentration and fat group in SAs was not significant (p=0.11), (Table 5-25). However the number of individuals who accurately completed DINE was low, only 17% of SA completed the section on fat intake accurately. Thus only 35 SA individuals actually completed the fat section of DINE, resulting in only 4 SA participants reporting high fat intake (Table 5-26). Indeed overall completion of DINE was poor in the whole cohort, only

32% (35% of WE and 17% of SA). Furthermore a greater percentage of SA individuals reported low fat intake (77%) compared to WEs (58%).

5.8.5 Relationship between plasma vitamin C and reported fruit and vegetable intake

The correlation between plasma vitamin C concentrations and reported fruit and vegetable intake as determined by DINE was also explored. In the total cohort plasma vitamin C was weakly correlated to reported fruit and vegetable intake, r = 0.24 and 0.17 respectively. Although statistically significant these correlations are small suggesting that DINE is not an accurate tool for measuring fruit and vegetable intake. The correlations were even weaker in the SA population, again suggesting that the DINE questionnaire is not suitable for use in this population. (Table 5-27). These correlations between plasma vitamin C and fruit and vegetable intake as assessed by DINE are reliable as 76% and 73% of SA individuals and 94% and 93% of WE individuals accurately completed the questions on fruit and vegetable intake respectively.

Table 5-26: Plasma vitamin C levels of individuals in the three reported fat, fibre and USFA intake groups

	Total cohort				White European				South Asian			
	Number	Mean vitamin C (µmol/l)	SD	P value*	Number	Mean vitamin C (µmol/l)	SD	P value*	Number	Mean vitamin C (µmol/l)	SD	P value *
Total completed DINE (%)	672 (32)	39.1	21.1		624 (35)	39.5	21.3		35 (14)	32.5	19.8	
Low fat intake	415	41.0	21.2		378	41.3	21.5		29	34.7	18.2	
Medium fat intake	185	36.9	20.9		180	37.9	21.2		2	4.3	3.3	
High fat intake	72	32.0	19.7		66	33.2	21.3		4	31.0	27.3	
Missing	1429	~	~	0.008	1179	~	~	0.009	213	~	~	0.1
Total completed DINE (%)	937 (46)	39.1	21.0		841(47)	39.6	21.2		72 (29)	31.6	18.1	
Low fibre intake	346	36.3	21.6		312	36.4	22.0		26	30.7	17.9	
Medium fibre intake	317	40.4	19.9		283	41.0	20.0		25	33.4	19.3	
High fibre intake	274	41.1	21.2		246	42.0	21.3		24	30.6	17.6	
Missing	1164	~	~	0.008	962	~	~	0.003	176	~	~	0.9
Total completed DINE (%)	1605 (76)	98.8	21.2		1426 (80)	40.2	21.6		147 (60)	35.9	18.3	
Low USFA intake	51	33.2	19.9		46	33.6	20.2		4	23.7	15.1	
Medium USFA intake	750	39.8	21.7		676	39.9	22.1		61	38.6	18.2	
High USFA intake	804	40.1	20.7		704	40.6	21.1		82	34.5	18.3	
Missing	496	~	~	0.08	377	~	~	0.1	101	~	~	0.2

*P value corresponds to the statistical difference of plasma vitamin C between fat, fibre or USFA groups as determined by DINE

Table 5-27: Correlations between plasma vitamin C and reported fruit and vegetable intake

	Total cohort			White Europeans			South Asians		
	Number	r value	P value	Number	r value	P value	Number	r value	P value
Plasma vitamin C and reported fruit intake	2550	0.24	<0.0001	1699	0.25	<0.0001	188	0.12	0.12
Plasma vitamin C and reported vegetable intake	2507	0.17	<0.0001	1674	0.16	<0.0001	179	0.05	0.5

5.8.6 Summary of differences found between SA and WE individuals

SA individuals had significantly lower mean plasma vitamin C compared to WE individuals. Levels of urinary F_2-isoprostanes were not significantly different between the two groups, thus differences in vitamin C likely reflect differences in intake. Furthermore fewer SA individuals consumed the recommended 5 portions fruit and vegetables a day compared to WE individuals. Completion rates of the DINE questionnaire were poor, and this tool may not be valid in the SA population.

5.9 Aim 4: To determine whether structured education for those at risk of T2DM can increase fruit and vegetable intake as measured by plasma vitamin C

5.9.1 Baseline characteristics of participants enrolled to prospective section of FIVE

A total of 153 participants were included in the prospective section of FIVE; of these, 73 participants were enrolled into the education intervention arm and 80 randomised to control. There were 49 male participants in the intervention arm and 48 in the routine care arm. HbA1c at baseline was statistically significantly greater in the routine care arm of the study, 6.2% (SD 0.4) compared to 6.0% (SD 0.4) for those in the intervention arm. However, clinically this difference is not significantly different. There were no other significant differences between the two groups as baseline (Table 5-28).

Table 5-28: Baseline characteristics of the study groups enrolled to FIVE

	Overall Mean SD		Routine Care Arm Mean SD		Education Arm Mean SD		P value* (RC v INT)
Age	64	7.9	63	8.1	65	7.5	0.1
BMI (kg/m^2)	32	5.7	32	6.2	31	5.0	0.4
Waist circumference (cm)	109	13.5	110	13.8	107	13.1	0.2
HbA1c (%)	6.1	0.4	6.2	0.4	6.0	0.4	0.03
Fasting Glucose (mmol/l)	5.6	0.6	5.6	0.7	5.6	0.6	1.0
2 Hour Glucose (mmol/l)	8.8	1.6	8.9	1.6	8.8	1.7	0.7
Vitamin C (µmol/l)	35.7	18.3	35.7	19.0	35.1	17.7	0.8
Geometric[Urinary Isop] mM/L mM/L creatinine	1.1	1.6	1.2	1.6	1.0	1.3	1.0

*P Value corresponds to mean differences between individuals in RC and INT arms at baseline

5.9.2 Characteristics of participants in the study groups enrolled to FIVE at 1 year

Characteristics of each group at baseline and 1 year post intervention are presented in table 5-29. Mean change from baseline in BMI, WC and glucose parameters are presented in table 5-30. After 1 year there was no significant difference between groups in the mean change of BMI, WC or glucose parameters (table 5-30). These variables are secondary outcomes of FIVE; when the data is included in the analysis of Let's Prevent Diabetes study there may be a significant difference between groups.

Table 5-29: Characteristics of participants in the study groups enrolled to FIVE at 1 year

	Routine Care Arm				Education Arm			
	Baseline		12 months		Baseline		12 months	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BMI (kg/m^2)	32	6.2	32	6.2	31	5.0	32	5.5
Waist circumference (cm)	110	13.8	108	14.4	107	13.1	107	12.7
HbA1c (%)	6.2	0.4	6.2	0.6	6.0	0.4	5.9	0.5
Fasting Glucose (mmol/l)	5.6	0.7	5.6	0.9	5.6	0.6	5.6	0.7
2 Hour Glucose (mmol/l)	8.9	1.6	7.4	2.1	8.8	1.7	7.4	2.3
Vitamin C (µmol/l)	35.7	19.0	29.9	20.3	35.1	17.7	36.1	20.7
Geometric[Urinary Isop] mM/L mM/L creatinine	1.2	1.6	1.0	2.0	1.0	1.3	-1.0	1.6

Table 5-30: Change in mean BMI, WC and glycaemic measures of study groups 12 months post intervention

Mean Change	Routine Care Mean change(SD)	Education Arm Mean change (SD)	Regression Co-efficient	95% CI Lower	Upper	P value*
BMI (kg/m^2)	0.5 (1.3)	0.2 (2.5)	-0.3	-0.69	0.11	0.16
Waist circumference (cm)	-1.0 (4.7)	-0.8 (4.8)	-0.07	-1.75	1.62	0.94
HbA1c (%)	-0.01 (0.3)	-0.07 (0.2)	-0.07	-0.19	0.06	0.29
Fasting Glucose (mmol/l)	-0.007 (0.6)	0.05 (0.5)	0.06	-0.12	0.25	0.52
2 hour Glucose (mmol/l)	-1.5 (2.5)	-1.3 (2.3)	0.13	-0.18	0.53	0.54

*P value corresponds to difference in mean change between intervention groups from baseline to 12 months follow up

5.9.3 Fat and fibre intake of participants in FIVE

The percentage of participants with low, medium and high fat, fibre and unsaturated fatty acid intakes are presented in table 5-31 below. No statistical analysis was carried out due to the small numbers of participants who successfully completed the questionnaire. The percentage of individuals in each fat tertile in the intervention arm has remained relatively stable yet those reporting high fat intake has increased in the routine care arm, (an increase from 4% to 19.2%). A greater percentage of participants in the intervention arm reported medium and low fibre intake after 12 months compared to an increase in high fibre intake reported in the routine care arm. In the education arm there was a 7.4% increase in participants who reported high USFA consumption with little change in the routine care arm. This may reflect one of the main messages of the curriculum which discusses how USFA can influence cholesterol levels.

Table 5-31: Table of fat, fibre and unsaturated fatty acid intake of participants

	Intervention Arm				Routine Care Arm			
	Baseline		1 Year		Baseline		1 year	
	Percentage	(n)	Percentage	(n)	Percentage	(n)	Percentage	(n)
Fat Intake								
Low	53.6	15	52.4	11	64.0	16	57.7	15
Medium	35.7	10	38.1	8	32.0	8	23.1	6
High	10.7	31	9.5	2	4.0	1	19.2	5
Fibre Intake								
Low	41.9	13	33.3	10	31.3	10	33.3	13
Medium	25.8	8	50.0	15	37.5	12	25.6	10
High	32.8	10	16.7	5	31.3	10	41.0	16
USFA intake								
Low	3.6	2	2.1	1	5.5	3	1.6	1
Medium	46.4	26	40.4	19	40.0	22	44.3	27
High	50.0	28	57.4	27	54.5	30	54.1	33

5.9.4 Effect of intervention on plasma vitamin C levels

Twelve months post intervention those in the intervention arm of the study had greater plasma vitamin C levels compared to those in the routine care arm, 36.1µmol/l (SD 20.7) compared to 29.9µmol/l (SD 20.3), however analysis showed that this relationship was not significant (Table 5-32). Concentrations of urinary F_2-isoprostanes were also not significantly different between the two groups after 12 months (Table 5-33).

Table 5-32: Effect of intervention on plasma vitamin C

	Routine Care Arm Mean (SD)	Education Mean (SD)	Regression Co-efficient	95% CI Lower	Upper	P value
Baseline Vitamin C (µmol/l)	35.7 (19.0)	35.1 (17.7)				0.8
12 months Vitamin C (µmol/l)	29.9 (20.3)	36.1 (20.7)				~
Mean Change (µmol/l)	-6.0 (17.1)	0.9 (21.3)	5.75	-2.08	13.58	0.15

*P value corresponds to difference in mean change between intervention groups from baseline to 12 months follow up

Table 5-33: Effect of intervention on Log urinary isoprostane concentration (geometric means presented)

	Routine Care Arm Mean (SD)	Education Mean (SD)	Regression Co-efficient	95% CI Lower	Upper	P value
Baseline [IsoPs]	1.2 (1.6)	1.0 (1.6)				0.03
12 months [Isops]	1.0 (2.0)	1.1 (2.0)				~
Mean Change	-2.5 (4.0)	-2.5 (3.2)	0.06	-0.02	0.13	0.14

*P value corresponds to difference in mean change between intervention groups from baseline to 12 months follow up

5.9.5 Summary of findings of the prospective section of FIVE

Mean plasma vitamin C was greater in the intervention arm 12 months after receiving the lifestyle education programme compared to those who received routine care; however the difference was not statistically significant.

5.10 Summary of results

Greater consumption of fruit and vegetables was associated with lower HbA1c, fasting and 2 hour glucose in this cohort. Consuming the recommended 5 portions of fruit and vegetables a day was associated with a reduced risk of diagnosis with IGR. An increase in serum K^+ was also associated with lower 2 hour glucose, however there was only a weak correlation between K^+ and plasma vitamin C.

Individuals of SA ethnicity had significantly lower plasma vitamin C levels compared to WE, furthermore fewer SA individuals consumed the recommended 5 portions of

fruit and vegetables a day. Differences between the two ethnic groups remained after adjustment for confounding variables. Greater consumption of dietary fat may be associated with reduced consumption of fruit and vegetables however the DINE questionnaire used may not be valid in the SA population.

Urinary F_2-isoprostanes were weakly associated with fasting glucose, 2 hour glucose and plasma vitamin C. Once demographic variables were adjusted for, all relationships were lost.

The intervention part of FIVE found no significant differences at 12 months in HbA1c, fasting or 2 hour glucose between individuals who received the education or those who received routine care. Mean plasma vitamin C was greater in the intervention arm after 12 months; however the difference between treatment arms was not significant.

Chapter 6

Fruit and Vegetable Intake and Glucose Control Study (FIVE) – Discussion

6.0 Chapter overview

Chapter 6 will discuss the implications of the results presented in chapter 5. The main findings from the cross sectional part of FIVE will be explored and how these results relate to clinical practice considered. The chapter will then examine how the results of the prospective section of FIVE relate to current evidence, eliciting how the data may impact on public health messages.

6.1 Glucose classification of participants

Almost a third of the screened population (n=2101) were identified with IGR, 4.3% with T2DM, 3.5% with IFG, 17.5% with IGT and 4.2% with both IFG and IGT. Therefore 25% of participants had IGR, but not overt diabetes. Diabetes UK estimates 15% of the UK population has IGT, IFG or both.[280] Furthermore a study in Australia reported prevalence of IGT and IFG to be 10.6% and 5.8% respectively.[281] A European study also reported prevalence of IGT and IFG of just 6.5% and 3.0%.[282] Therefore prevalence of IGR in the FIVE cohort is greater than previous population estimates. This reflects the two staged strategy used to identify high risk individuals for the study. It may also reflect the multi-ethnic population of FIVE. These factors may also explain the relatively high observed prevalence of T2DM (4.3%). Previous screening studies in Leicester found those with undiagnosed T2DM made up 3.3% of the population.[52] In addition a study from Australia which compared different screening strategies found undiagnosed diabetes in 3.6% of the population.[283]

6.2 Fruit and vegetable intake of participants

Analysis of plasma vitamin C demonstrated that only 29% of the population in the cross sectional analysis consumed the recommended 5 portions of fruit and vegetables

a day (as determined by plasma vitamin C concentration of ≥50µmol/l). The percentage of males (24%) consuming 5 portions of fruit and vegetables reflects the national average (25%).[22] However the percentage of females in the FIVE study found to consume the recommended 5 portions of fruit and vegetables a day is greater than the national average, 37% compared to 28% respectively.[22] The results from FIVE are also slightly above the global average intake, the World health Survey recently estimated that only 22% of men and women met the 5 a day target.[197] The data from FIVE is novel in that consumption is measured by a plasma vitamin C concentration of equal to or greater than 50µmol/l/l.[277] The comparative estimates are based on self report and therefore likely to be less accurate.

FIVE provides up to date estimates of fruit and vegetable intake. Intake levels are greater than previous estimates which may reflect the average age of the population (63 years (SD7.9)). Older populations have previously been shown to have higher consumption levels than younger generations.[278] In spite of higher intake rates than in earlier reports, low consumption remains prevalent. Low intake appears to occur despite reported increases in awareness of the 5 a day messge.[284] UK and worldwide initiatives aimed at promotion of greater fruit and vegetable intake[285, 286] appear to be relatively well recognised yet are failing to effectively increase consumption levels. There is a need to investigate why these programmes are failing and how individuals can be encouraged to successfully consume greater amounts of fruit and vegetables.

6.3 Aim 1: To determine whether fruit and vegetable intake, as assessed by plasma vitamin C is associated with baseline glycaemic state of a population identified as being high risk for T2DM.

6.3.1 Cross sectional analysis of plasma vitamin C concentration

Unadjusted mean plasma vitamin C of the population was 39.3µmol/l (SD21.8). The mean concentration is lower than reported in previous studies. EPIC-Norfolk reported a mean plasma vitamin C concentration of 57.8µmol/l (SD20.3) and 46.2µmol/l (SD 19.5) in women and men respectively.[190] In addition the latest NHANES study found the age adjusted mean plasma vitamin C to be 49.0µmol/l.[278] Disparity may reflect the different demographic make-up between study participants. Individuals included in FIVE were identified due to common risk factors for T2DM. However individuals in both NHANES and EPIC-Norfolk were recruited to large observational studies from the general population. Thus participants in these two studies were wider ranging in factors such as age and BMI. Furthermore EPIC-Norfolk and NHANES may suffer from non-response bias, that is healthy responders are more likely in observational studies than unhealthy ones.[287]

6.3.2 Association between plasma vitamin C and glycaemic state

Analysis of plasma vitamin C as a continuous variable showed that an increase in plasma vitamin C was associated with a significantly lower HbA1c, fasting and 2 hour glucose. Exploration of data using 1SD (21.8µmol/l) was carried out to reflect a realistic change in plasma vitamin C through fruit and vegetable intake, 21.8µmol/l being roughly equivalent to the consumption of 1 additional orange.[190] Adjusted analysis showed that an increase in 1SD vitamin C was associated with a 0.04%

reduction in HbA1c, 0.05mmol/l in fasting glucose and 0.22mmol/l in 2 hour glucose. This observed reduction in the glucose parameters may not in itself be clinically significant; clinical studies designed with the intention of reducing diabetes incidence of a population aim to reduce 2 hour glucose by approximately 1mmol/l.[124] However an increased consumption of just one piece of fruit or vegetable should be considered a small lifestyle change which could potentially contribute to greater overall improvements in glucose regulation. A greater increase in intake levels could additionally contribute to larger changes in glucose regulation; further supporting the importance of small lifestyle changes. The Finnish Diabetes Prevention study found a strong inverse correlation between the number of successful goal changes and incidence of diabetes.[64] In addition lower overall mortality has been observed in those who have a greater number of combined positive health behaviours.[277] The data from FIVE contributes to the evidence that small lifestyle changes can contribute to overall reduction in risk of developing IGR. Future health campaigns should emphasis this message.

FIVE provides novel, robust information on fruit and vegetable intake in those with IGR. Indeed little data is available on plasma vitamin C and glucose control in any population. However the EPIC-Norfolk study, a large prospective study found in their adjusted analysis that an increase of 20µmol/l in plasma vitamin C was associated with a 0.08% (95% CI: -0.11 to -0.0) lower HbA1c in men and a 0.05% (95%CI: -0.07 to -0.03) lower HbA1c in women,[190] demonstrating comparable results to those observed in FIVE (0.04% (95% CI: -0.07 to -0.2) reduction in HbA1c). The similarity in data strengthens the evidence of an association between plasma vitamin C and HbA1c, regardless of the population examined. EPIC-Norfolk is a large

observational study which includes healthy, free living individuals compared to those in FIVE who were identified as high risk for T2DM. Furthermore the plasma vitamin C status differed between studies; for example, those in the lowest category of plasma vitamin C in FIVE had a mean plasma vitamin C of 15.7µmol/l (SD 9.3) as compared to 23µmol/l (SD8.3) in EPIC-Norfolk. FIVE also adds novelty by including a range of ethnic groups compared to those in EPIC-Norfolk who were predominately of WE background. In addition results from FIVE provide up to date data, EPIC-Norfolk data is over 15 years old, as it was collected between 1995 and 1997. As stated previously the data supports recommendation for those at risk of T2DM and the general population to increase fruit and vegetable consumption.

6.3.3 Fruit and vegetable intake and prevalence of IGR

Analysis found that increasing plasma vitamin C by 21.8µmol/l was associated with a 15% (OR=0.85, 95%CI: 0.75 to 0.95) reduction in prevalence of IGR. In comparison the EPIC-Norfolk study observed that each 20µmol/l increase in plasma vitamin C was associated with a 30% (OR=0.70, 95%CI: 0.52 to 0.95) reduction in the diagnosis of undiagnosed hyperglycaemia.[190] A potential explanation for the greater observed association seen in EPIC-Norfolk may be due to the categorisation of the IGR groups. EPIC-Norfolk calculated OR between those with NGT and those with previously undiagnosed hyperglycaemia, (individuals with HbA1c ≥7%). The FIVE study calculated risk between those with NGT and those with IGR (individuals with T2DM, IGT, IFG, both IGT and IFG). Therefore EPIC-Norfolk compared two categories with a greater difference between them. Mean HbA1c for men and women in the previously undiagnosed hyperglycaemia category was 8.3% (SD 1.3) and 8.7% (SD

1.9) respectively[190] compared to mean HbA1c in the FIVE IGR group of 6.2% (SD 0.6) in both men and women.

Current nutritional guidelines for the prevention of T2DM recommend that individuals should consume five portions of fruit and vegetables a day.[133, 134] However evidence supporting this recommendation is lacking. FIVE provides novel data on the association between diagnosis of IGR and the consumption of fruit and vegetables. Categorical analysis showed that those who consumed the recommended five a day had a 24% associated reduced risk of developing IGR (OR = 0.76, 95%CI: 0.59 to 0.98) compared to those who did not meet the five a day target. Although this supports current recommendations, analysis of vitamin C as a continuous variable did not determine an upper threshold of benefit; each 21.8μmol/l increase was associated with 15% reduction in risk. Therefore consuming more than five pieces of fruit and vegetables a day could potentially be recommended. Indeed a recent report on risk of heart disease reported a 22% reduced risk (RR = 0.78, 95%CI: 0.65 to 0.95) of fatal ischaemic heart attack in those who consumed eight portions of fruit and vegetables a day compared to those who consumed less than three portions a day.[288]

Therefore the results add to the limited evidence that consuming the recommended intake of fruit and vegetables is associated with reduced risk of developing IGR; however consumption of more than five portions per day may well be beneficial. The question arises whether changing current public health messages to recommend consumption of more than five portions of fruit and vegetables a day would prompt individuals to attempt to consume more, and thus potentially reach five portions, or would individuals feel such a target is unrealistic and thus consume less?

6.4 Aim 2: To determine whether urinary isoprostanes are related to baseline plasma vitamin C and to glycaemic control

6.4.1 Antioxidant effects of fruit and vegetables

One potential benefit of greater fruit and vegetable consumption is their high content of antioxidants. Fruit and vegetables are rich sources of antioxidants such as vitamin C, β-carotene, lycopene, leutin, plus a range of polyphenols and flavinoids.[289] It has been demonstrated that an increase in consumption of fruit and vegetables results in increased antioxidant status of individuals.[257] A four week intervention study demonstrated that participants who consumed 500g of fruit and vegetables per day (equivalent to about six portions) had a significant increase in α-carotene, β-cryptoxanthin, leutin and β-carotene.[204] Increased levels of antioxidants are suggested to protect against oxidative stress, implicated in the development and progression of T2DM and CVD as discussed in section 1.5 Thus exploration of the second aim of FIVE may help to determine whether oxidative stress, as measured by urinary F_2-isoprostanes explains the observed relationship between fruit and vegetable consumption and risk of T2DM.

6.4.2 Urinary F_2-isoprostanes and glucose control

Urinary F_2-isoprostane concentrations were not significantly different between those with NGT, T2DM, IGT, IFG or IGT and IFG. Furthermore F_2-isoprostanes were not independently associated with HbA1c. There was a weak association between fasting glucose and urinary F_2-isoprostanes but this relationship was lost once age, sex, BMI

and ethnicity were incorporated into the model. Urinary F_2-isoprostanes were weakly associated with 2 hour glucose, (p=0.08).

Measurement of F_2-isoprostanes is considered the gold standard assessment of lipid peroxidation.[261, 290, 291] Furthermore measurement of urinary F_2-isoprostanes in a single morning sample is thought to adequately represent daily excretion.[261] Thus data from FIVE should be considered accurate; however it has been proposed that ELISA techniques, as used in FIVE may be less sensitive than mass spectrometry.[291] Therefore small differences between participants may not have been successfully identified.

Previous studies which have investigated oxidative stress and glycaemic control have produced conflicting results. F_2-isoprostanes have been observed to be greater in those with diabetes than those with NGT.[263, 292, 293] However other studies have suggested that F_2-isoprostanes are inversely related to the development of T2DM.[294] Inconsistencies may arise due to measures of F_2-isoprostanes being carried out in plasma and urine[263] or different analysis methods.[291] Therefore further validation studies may be required.

6.4.3 Urinary F_2-isoprostanes and plasma vitamin C

Urinary F_2-isoprostanes were weakly associated with plasma vitamin C and incorporation of demographic variables resulted in attenuation of the relationship. Therefore age, BMI, sex and ethnicity were found to be stronger predictors of oxidative stress than fruit and vegetable intake in this cohort. Previous studies have also shown strong associations between F_2-isoprostanes, age, BMI and WC.[109, 294,

[295,261, 296] There was no significant association between the consumption of 5 portions of fruit and vegetables a day and urinary F_2-isoprostanes (p=0.99).

Previous studies have demonstrated consistent associations between fruit and vegetable consumption and oxidative damage. Participants given a prescribed diet for 14 days of either 3.6 or 12 servings fruit and vegetables per day were seen to have reduced urinary F_2-isoprostane levels. The extent of reduction was greater in those consuming 12 portions of fruit and vegetables per day.[259] A further study showed that consumption of two glasses of 500ml orange juice per day reduced plasma F_2-isoprostane concentrations of participants.[297] FIVE is unique in that plasma vitamin C and urinary F_2-isoprostane concentrations were measured in free living individuals, as compared to being prescribed fruit and vegetable intake. In addition effects on oxidative stress may be dependent on baseline levels. A study which prescribed vitamin C supplements to participants found a 13.8% reduction in F_2-isoprostanes but the effect was only seen in those with high baseline levels of F_2-isoprostanes.[295] It should be considered whether the measurement of F_2-isoprostanes in the cohort was not sensitive enough to detect small but true differences. However differences may simply not exist in this population. The cohort has a limited spectrum of participants, in that all individuals were identified due to similar characteristics which identified them all as high risk for future T2DM. None-the-less this data is important; without reporting negative findings, the potential for publication bias exists.

6.4.4 Oxidative stress and β-cell dysfunction

The results from FIVE do not show associations between oxidative stress and glucose control; however the oxidative stress hypothesis cannot be eliminated. A weak

association was observed in FIVE between urinary F_2-isoprostane concentrations and 2 hour glucose, implicating beta-cell dysfunction. It is well documented that beta-cells are particularly susceptible to ROS due to their low antioxidant enzyme capacity.[70, 73, 298] In muscle cells raised ROS due to substrate overload in the citric acid cycle can be minimised by reduction of GLUT-4 transporters and insulin resistance. Beta-cells are not insulin dependent for glucose uptake therefore excessive ROS production cannot be controlled and the low levels of antioxidant enzymes are quickly depleted.[70] The association between plasma vitamin C and 2 hour glucose observed may suggest that high fruit and vegetable intake supplements beta-cell antioxidant levels, potentially protecting beta-cells from damaging ROS. A limitation to this study is that Beta cell function was not measured; further studies are required to investigate this potential theory.

Evaluation of urinary F_2-isoprostane data may suggest that more sensitive techniques are required. However mass spectrometry is expensive and time consuming which may not be feasible in large scale studies. Therefore the use of F_2-isoprostanes in future studies may not be appropriate. Indeed the data from FIVE does not consolidate any proposed hypothesis on the role of oxidative stress in development of T2DM or as a beneficial mechanism of fruit and vegetable consumption. Future prospective studies should investigate other proposed benefits of fruit and vegetable consumption.

6.5 Aim 3: To determine whether any differences exist in fruit and vegetable intake between South Asian individuals screened for T2DM as compared to White Europeans

6.5.1 Fruit and vegetable intake in WE and SA individuals

SAs had significantly lower mean plasma vitamin C compared to WEs (34.5µmol/l (SD 19.8) *vs.* 39.4µmol/l (SD 22.11), $p \leq 0.0001$). This is consistent with significantly fewer SA consuming the recommended five portions fruit and vegetables a day (21% SA *vs.* 30% WE). Furthermore a greater percentage of SAs than WEs had both deficient plasma vitamin C (15% *vs.* 11%) levels and marginal deficiency levels (11% *vs.* 8%). The results highlight that the majority of both WE (70%) and SA (79%) individuals do not consume the recommended five portions of fruit and vegetables a day. Results reflect the need for future investigation into the failure of current government initiatives aimed at increasing fruit and vegetable intake. Particular additional research into why fewer SA individuals are acting upon the recommendations is required.

The data from FIVE provides much needed current information on the fruit and vegetable intake of SAs in the UK. The results are supported by previous data on plasma vitamin C in the SA population; however this data is over 10 years old and from a different geographical area of the UK.[299] The original study found WEs to have greater plasma vitamin C concentrations than SA individuals. SA men and women had plasma vitamin C concentrations of 38.2µmol/l (SD1.6) and 50.8µmol/l (SD 1.5) respectively in the original study[299] as compared to 33.2µmol/l (SD 1.5) and 39.3µmol/l (SD 1.6) in SA men and women in FIVE.

6.5.2 The relationship between plasma vitamin C concentrations and ethnicity

Significant differences in mean plasma vitamin C between the two ethnic groups remained after adjustment for potential confounding variables (models 1 to 4). The significant association between plasma vitamin C and ethnic group did not remain once fat score, as determined by DINE was incorporated into the regression model. This suggests that fat intake may be a significant predictor of plasma vitamin C levels, not ethnicity. However due to low completion rate of the questionnaire, especially in the SA population results should be regarded with caution.

Analysis of urinary F_2-isoprostanes in the FIVE cohort did not show any association with plasma vitamin C concentrations. However it is generally acknowledged that under high levels of oxidative stress antioxidant levels may become depleted.[92] Plasma vitamin C is an antioxidant as well as a biomarker for fruit and vegetable intake. Therefore concentrations in the SA population may become depleted due to underlying higher oxidative stress compared to WE individuals. Previous data has shown that SAs have greater levels of urinary F_2-isoprostanes than WE (11.02nM/mMcreat vs. 7.78nM/mMcreat).[300] However urinary F_2-isoprostane concentration in the FIVE population were not significantly different between the two ethnic groups. We can be confident this is a true measure as the previous study was also carried out at Leicester using the same laboratory and analysis techniques. Thus we may suggest that differences in plasma vitamin C observed between ethnic groups in this cohort likely reflect differences in intake not depletion via oxidative stress, supporting the proposal that SA are consuming fewer portions of fruit and vegetables than their WE counterparts. It cannot be excluded that different cooking methods between ethnic groups influences vitamin C intake. Vitamin C is water soluble and

easily depleted from foods with prolonged cooking. It has been reported that prolonged cooking of vegetables is common practice in SA households.[188] However, only qualitative research into cooking practices will enable determination of these potential differences between ethnic groups.

6.6 Assessment of DINE

Incorporation of the Fat Score as determined by DINE into the regression model resulted in the attenuation of the association between plasma vitamin C and ethnicity. This suggests that fat intake influences plasma vitamin C more than ethnic group. Indeed examining the whole cohort showed that those with the lowest fat intake had the greatest plasma vitamin C concentrations. Vitamin C is a water soluble vitamin and is therefore not affected by varying fat intake levels. High fat intake and low plasma vitamin C reflect poor dietary status; therefore plasma vitamin C may be acting as a marker for overall dietary pattern.

Independent analysis of the two ethnic groups showed that the relationship between plasma vitamin C and fat group was not significant in the SA population. A smaller sample size inevitably reduced power to detect associations. A strong possible explanation for lack of association is the poor completion rates of the questionnaire. Only 17% of the SA population accurately completed the fat section of DINE. Yet only 32% of WEs completed the questionnaire. DINE was chosen as it is validated for self completion and is considered a simple tool. However such low completion rates by both ethnic groups suggests either the questionnaire is too long, too complicated or has poor instructions for self completion. To understand the reasons why participants did not complete the DINE questionnaire qualitative research with

participants is required. A recent lifestyle intervention used "The Smart Diet Score" questionnaire and reported 95% completion rates.[301] It is important to determine why the DINE questionnaire has not been successfully completed and adoption of simpler tools may be required.

DINE may not be suitable for use by SA individuals. The version used in FIVE is the one adapted for use in a SA community as part of the Health Survey for England; however it has not been validated in this population. DINE was originally validated in Oxford, England in a predominately white population.[265] DINE may not be capable of capturing ethnic specific fat consumption or the result may reflect limited knowledge about fat content of specific foods in the SA population.

A greater proportion of SAs reported low fat intake as compared to WEs yet a number of studies have demonstrated that SA individuals consume high fat diets. A study in Glasgow found only 8.6% of SAs achieved the recommendation of ≤35% of total energy intake from fat as compared to 20% of the general population. Furthermore 50% of the SA population had 15% of total food energy from saturated fat.[183] Another study showed SAs from a range of ethnic groups ate both traditional snacks high in fat but had also adopted westernised snacks which were also rich in fat.[184] However there is conflicting data on SA diets, other studies have shown SAs to have lower fat intake than WEs.[182] The inconsistent data on dietary fat intake may be due to studies being carried out in small non-comparable groups; there is a great deal of heterogeneity in the SA population within the UK. Individuals with Indian, Pakistani and Bangladeshi backgrounds consume a range of different foods.[178] Observed differences may also reflect limited knowledge of food content or reflect poor

measurement techniques. It has also been suggested that biased reporting is higher in ethnic minority groups.[302] Research is required to determine optimum methods of recording dietary intake, what the dietary habits of SA groups are, and also to examine how dietary practices can be successfully manipulated to increase fruit and vegetable consumption.

6.7 Plasma vitamin C as a biomarker *vs.* reported fruit and vegetable intake

In FIVE plasma vitamin C was used as a biomarker for fruit and vegetable intake as self report is open to a number of errors. DINE is only validated for its use in determining fat and fibre intake levels; however it contains one question on fruit intake and another on vegetable intake. Analysis of the fruit and vegetable scores and plasma vitamin C showed a stronger correlation between reported fruit intake and plasma vitamin C than between reported vegetable intake and plasma vitamin C, Pearson's correlation = 0.24 and 0.17 respectively. These correlation values only represent a low level of correlation,[279] therefore DINE should not be used as a true reflection of fruit and vegetable intake. Correlations were weaker still in the SA population, again supporting evidence that this questionnaire is not suitable for use in the SA population. The continued use of plasma vitamin C in nutritional studies is required as a reliable biomarker for fruit and vegetable consumption over and above dietary questionnaires.

6.8 Aim 4: To determine whether structured education for those at risk of T2DM can increase fruit and vegetable intake as measured by plasma vitamin C

6.8.1 Effect of intervention on plasma vitamin C, urinary F_2-isoprostane concentrations and glucose control

Twelve months post intervention those individuals who had received structured education had mean plasma vitamin C levels of 36.1µmol/l (SD 20.7) compared to those in the routine care arm whose plasma vitamin C levels had fallen to 29.9µmol/l (SD 20.30). However statistical analysis showed that the difference between the two groups was not significant (p=0.15). Analysis of urinary F_2-isoprostanes also showed no significant difference between the two arms post intervention.

There were no observed differences in change in HbA1c, fasting or 2 hour glucose between the two arms of the study. However Let's Prevent Diabetes has a larger sample size than FIVE thus numbers in this analysis may be too small to detect a difference.

The differences in fat and fibre intake between the two intervention arms was not analysed due to such small numbers of completion rates, emphasising the need to carry out qualitative work aimed at finding a simple, effective tool for the assessment of diet in large cohorts.

6.8.2 The Let's Prevent Diabetes Intervention: fruit and vegetable intake

The Let's Prevent Diabetes curriculum was designed to educate participants on the risk of being identified with IGR and aid understanding of potential ways an individual can reduce their risk. Only 21% of the curriculum is designated to dietary habits; within this the benefits of consuming the recommended five portions of fruit and vegetables a day are discussed. Thus the proportion of the curriculum devoted to the benefits of fruit and vegetables may be too small to have seen an effect in this population. Furthermore participants are encouraged to select lifestyle changes which they believe they can successfully modify. Participants may not have chosen to alter fruit and vegetable intake.

Let's Prevent Diabetes is not an intervention designed specifically to increase the consumption of fruit and vegetables of a population. Potentially more emphasis on fruit and vegetable intake is needed to see a greater change in plasma vitamin C of participants. A recent study in Germany found that dietary advice which emphasised the consumption of five portions of fruit and vegetables a day found significant improvements in plasma leutin, lycopene, β-carotene, α-carotene, B6 and plasma vitamin C after only three months.[303] After just 12 weeks individuals' baseline plasma vitamin C had increased from 55.1µmol/l (SD 20.9) to 63.6µmol/l (SD 21.8).[303]

A pilot study should be developed which tailors the current Let's Prevent Diabetes curriculum to include greater emphasis on the importance of fruit and vegetables to determine if improvements in consumption levels can be made. The development of an additional dietary module could be developed and incorporated in to a revised

curriculum. Additionally a study solely designed to increase fruit and vegetable intake should be developed.

The German study also demonstrates that plasma vitamin C concentrations can be increased over short periods,[303] illustrating that the length of intervention of FIVE does not explain the small differences observed between treatment groups. However if participants made initial changes immediately following the education session, then reverted to pre-education levels, no change in plasma vitamin C at one year would be detected.

The number of participants (n=153) recruited to the intervention section of FIVE was based on a power calculation which took into consideration the cluster randomised design of the trial. However it has been suggested that if intervention effects vary between clusters more than anticipated, then sample size calculations may underestimate the numbers needed to detect a true effect.[304] In addition clusters which differ in size may make the statistical model weaker, making associations difficult to detect. Analysis of the remaining Let's Prevent Diabetes participants is warranted to determine whether the sample size is currently too small.

6.9 Potential benefits of fruit and vegetables

The relationship between fruit and vegetable intake and oxidative stress remains inconclusive from the results of FIVE. However it is important to remember that in this study plasma vitamin C was measured as a biomarker for fruit and vegetable intake not as a measure of antioxidant status. Thus the associations observed between plasma vitamin C and glycaemic control may not be due to the antioxidant ability of

fruits and vegetables. Fruit and vegetables provide a range of plausible components that may influence glycaemic control.

Potassium rich diets have been associated with reduced CVD risk due to blood pressure lowering effects.[305] However more recently studies have reported that increased risk of T2DM is associated with both low serum K^+ and low dietary K^+ intakes.[306, 307] Low serum K^+ may restrict the potassium sensitive ATP channels and thus inhibit insulin secretion from the beta-cell.[308] Supporting the role of K^+, the data from the cross sectional part of FIVE demonstrated a significant association between serum K^+ and 2 hour glucose. Fruit and vegetables are the main source of dietary potassium, yet only a weak correlation was seen between plasma vitamin C and serum K^+. However serum K^+ does not reflect intake; 24-hour urinary excretion would be required to measure dietary intake levels of K^+. In addition, the range of plasma vitamin C in the FIVE population may not be great enough to show a relationship with serum K^+. Further research into this area is required to help understand these findings.

Fruit and vegetables may also influence glucose control through a number of mechanisms not measured in FIVE. Any future work on fruit and vegetable intake and the risk of T2DM should concentrate on the measurement of novel factors found in fruit and vegetables; for example, fruit and vegetables provide a supply of dietary magnesium. A recent meta-analysis found a significant inverse association between magnesium intake and incidence T2DM.[243] Magnesium is a co-factor for multiple enzymes involved in glucose metabolism.[309] Precise mechanisms between magnesium and glucose homeostasis are yet to be determined however low

intracellular magnesium may alter insulin sensitivity by impairment of signalling pathways.[309]

Dietary nitrate has also been suggested as a mechanism by which fruit and vegetables may reduce the risk of T2DM.[310] Diets rich in fruit and vegetables supply inorganic nitrate which can be converted to nitric oxide, thus supplying a supplementary source which can add to endothelial derived nitric oxide.[311] Nitric oxide may affect glucose transporters and thus improve glucose transport.[312] Any future studies should measure magnesium and nitrate to investigate whether these components of fruit and vegetables do indeed influence glucose control.

Fruit and vegetable consumption may influence the inflammatory response irrespective of antioxidant function. A cross sectional study in the US found a significant inverse dose response between fruit and vegetable intake and plasma CRP. Prevalence of high CRP levels were significantly greater among subjects in the lowest quintile of fruit and vegetable intake relative to those in the highest quintile.[313] A further study enrolled participants who consumed low levels of fruit and vegetables. Diets rich in fruit and vegetables were then randomly allocated to half of the participants whilst others remained on low fruit and vegetable diets. Those who moved to the diet rich in fruit and vegetables had significant reductions in plasma CRP levels after four weeks as compared to those who continued to consume low amounts of fruit and vegetables.[314] Future studies investigating the role of fruit and vegetables and risk of T2DM should consider eliciting the role of inflammation and glycaemic control by inclusion of such measures as CRP.

Fruit and vegetables come from a range of botanical families and contribute much complexity to the diet.[315] It is clear that there are a number of potential mechanisms which may act alone or in combination to influence glucose homeostasis. Data from FIVE suggests that fruit and vegetable intake may not be beneficial specifically due to effects on oxidative stress. A number of clinical trials have been carried out to investigate the independent effects of antioxidant supplements and had disappointing results.[246, 247, 316] FIVE supports the evidence from these studies which suggest that antioxidants themselves are not the sole reason greater fruit and vegetable consumption has been associated with reduced risk of CVD, cancer and T2DM.[41, 201, 317] The data contribute to the evidence that foods provide a plethora of beneficial components. A range of different nutrients and a combination of mechanisms may be at work when consumption of fruit and vegetables is high. Consumption of different foods may influence the actions of different nutrients. Research into food combinations may help to answer these questions.

6.10 Future work

A RCT designed to give a realistic dietary intake of fruit and vegetables will allow for more exact evidence about whether greater consumption can be beneficial for reducing risk of T2DM. Measurement of a range of plausible mechanisms such as those discussed above should be included to determine how fruit and vegetables may provide health benefits. Food combination studies could be developed to investigate the role of fruit and vegetables when eaten in conjunction with other foods. In addition screening studies could incorporate additional plasma and urine samples to measure a range of potential beneficial components such as magnesium and nitrate.

There is a need for qualitative research into why participants do not complete dietary questionnaires accurately. It may be necessary to find simpler tools and conduct focus groups to evaluate which tools are likely to give higher completion rates. Pilot studies should be carried out to determine which questionnaires are more successfully completed and truly reflect dietary intake. Comparison with both diet diaries and nutritional biomarkers should be incorporated to validate this work.

Research is also greatly needed in the SA population; it is imperative to determine dietary habits across SA groups and across different age groups, as acculturation may be more apparent in younger generations.[178] It is also important to carry out focus groups within the community to determine how dietary practices can be manipulated to increase fruit and vegetable intake and provide an overall healthy dietary profile. Following this work pilot studies which are tailored to the SA population should be conducted to see if dietary habits can be effectively altered.

6.11 Strengths and limitations

FIVE provides robust biomarker data on fruit and vegetable consumption from a large cohort. FIVE is a novel population based study which recruited high risk individuals and included a large number of SA participants. Nutritional biomarkers remove reliance on self-report and can be used across ethnic groups. Plasma vitamin C has consistently been shown to accurately reflect both short term and habitual fruit and vegetable intake.[255, 258, 318] Vitamin C can be unstable, however the protocol used ensured that whole blood was kept in a dark, cool box. Evidence has shown that vitamin C remains stable in whole blood up to 24 hours after collection.[264] Analysis

was carried out under stringent conditions using an assay validated by the National Institute of Standards and Technology.

The data from FIVE add to the limited evidence that fruit and vegetable consumption may be beneficial for the improvement of glycaemic control. However this part of the study is only cross sectional by design and associations cannot claim cause and effect. Currently the prospective data from FIVE does not show any difference between fruit and vegetable intake and glucose parameters between treatment groups. However Let's Prevent Diabetes is a three year study, at the end of the trial it may be possible to investigate changes in plasma vitamin C in relation to glucose measures between the treatment arms.

To attempt to investigate the independent effect of plasma vitamin C regression models were carried out. The independent association between plasma vitamin C and each glucose parameter was investigated by adjustment for age, sex, BMI and ethnicity. Further adjustment was made by inclusion of each individual factor found to be independently associated with each glycaemic measure (model 2). Incorporation of these factors attenuated the relationships but significance remained between plasma vitamin C and both fasting and 2 hour glucose. Thus regardless of factors found to influence an individual's HbA1c, fasting or 2 hour glucose in this cohort, plasma vitamin C remained a significant independent variable.

The final regression model used to explore the relationship between plasma vitamin C and each glucose parameter included factors found to be independently associated with plasma vitamin C (model 3). Inclusion of these factors resulted in attenuation of

the association between plasma vitamin C and fasting and 2 hour glucose. However factors causally related to plasma vitamin C are not considered true confounders[190] and co-linearity between factors can give flawed results as the assumptions of the statistical model may not be met. Therefore data from model 3 has not been used to report findings. Furthermore the inclusion of data from the DINE score should be regarded with caution due to very low completion rates. However it cannot be excluded that vitamin C is acting not only as a biomarker for fruit and vegetable intake but as a biomarker for overall dietary pattern. Furthermore it is well documented that healthy behaviours are often interrelated.[319] In addition not all confounders may have been measured or adjusted for in the analysis; for example poor sleep quality, an emerging risk factor for diabetes risk was not measured. Further research should be developed to try and disentangle these factors. Full data sets from the Let's Prevent Diabetes study may aid to determine if fruit and vegetable intake directly and independently influences glucose control.

The work investigating differences in fruit and vegetable consumption between SAs and WEs adds to the much needed data on dietary habits of the SA population. FIVE included a large number of SA individuals. Furthermore the SA population in this cohort is fairly homogenous, with the majority (96%) originating from India. It is plausible that low fruit and vegetable intake is contributing to the increased prevalence of T2DM in the SA population. However the small number of participants from other ethnic groups limits the statistical power of the study to provide meaningful data across different ethnic groups.

The DINE questionnaire was included in FIVE as part of the Let's Prevent Diabetes protocol to estimate fat and fibre intake of participants. However dietary questionnaires rely on accurate self report, full completion of questionnaire, unbiased reporting and knowledge of fat and fibre sources. Poor completion of DINE, a tool chosen as it is classed as a simple tool, highlights the fact that accurate measurement of habitual diet is perhaps the most difficult area of nutritional research.[254] In addition DINE has been validated only in a WE community.[265, 267] The results from this questionnaire in FIVE should be regarded with caution and support continued use of nutritional biomarkers in future studies.

6.12 Conclusion

Fruit and vegetable intake is still low in the UK despite government initiatives to encourage consumption. Investigation into why these campaigns are failing is required. Development of new programmes to promote greater intake of fruit and vegetables across the UK may be needed; however associations observed in cross sectional studies cannot be used to develop public health policies. Further investigation, including trials which specifically increase fruit and vegetables of individuals, are required to determine true associations between consumption levels and glycaemic control.

FIVE observed that the consumption of five portions fruit and vegetables a day was associated with a 24% reduced risk of being diagnosed with IGR as compared to those who did not meet the recommended intake of five5 a day. Thus intake of fruit and vegetables should be encouraged for reducing the risk of developing T2DM.

However no upper threshold was determined and promotion of intake levels greater than five portions fruit and vegetables per day is warranted.

Fewer SA individuals consumed five portions of fruit and vegetables a day compared to WEs. The data suggest that poor dietary choices may contribute to SA higher risk of T2DM. Public health messages aimed at SAs should be developed and the effects of tailored advice to this high risk group should be investigated.

Fruit and vegetable intake is likely to be a marker for overall diet quality and may also potentially reflect healthy lifestyle patterns. Fruit and vegetable intervention studies are required to disentangle the exact role that fruits and vegetables play in the prevention of T2DM.

The DINE questionnaire is not suitable for self completion in large scale studies. Development of a simple, user friendly tools, which are ethnically diverse are required for future studies. The use of nutritional biomarkers should be encouraged when developing dietary studies.

Thesis overview

The thesis has provided evidence to support the current dietary recommendations that individuals at risk of T2DM should consume five portions of fruit and vegetables a day. The thesis includes novel data which shows convincing evidence that greater consumption of GLV is associated with a reduced incidence of T2DM. Furthermore the FIVE study demonstrated a strong association between fruit and vegetable intake, as measured by plasma vitamin C and glycaemic parameters. However cross sectional studies cannot infer cause and effect; therefore randomised studies which supplement fruit and vegetables into an individual's diet are required to determine the exact role that fruit and vegetables have on the prevention of T2DM.

Chapter 7

Executive Summary

7.0 Introduction

This thesis starts by discussing the current diabetes epidemic and examines the role of obesity and oxidative stress as potential causative mechanisms. The thesis evaluates current LSMP which have been developed for the prevention of T2DM. The influence that dietary change plays within LSMP is discussed, demonstrating that the exact roles of different dietary factors have yet to be elucidated.

Current recommendations for the dietary prevention of T2DM are critically examined in relation to available observational and clinical evidence. Recommendations appear appropriate yet the evidence largely originates from observational studies which cannot exclude underlying confounders. There is a need for future RCTs which provide realistic intakes of dietary components to understand true mechanistic actions.

7.1 Fruit and Vegetable Intake and Incidence of Type 2 Diabetes Mellitus: systematic review and meta-analysis

A number of dietary patterns which are characterised by high intakes of fruit and vegetables have been associated with reduced risk of developing T2DM. However the independent role of fruit and vegetables is unclear. The systematic review and meta-analysis conducted in chapter 3 demonstrated convincing benefits for greater consumption of GLV. A trend towards benefit was also observed for fruit and vegetables; however the results were not significant. Heterogeneity between studies and the reliance on FFQ in observational studies included in the review may explain the inability to determine true relationships between diet and disease. Investigation into the protective role of GLV is warranted. A pilot study which supplements GLV into the diet could be conducted to evaluate mechanistic actions. Furthermore there is

a need for nutritional biomarkers in both observational studies and randomised controlled trials. Thus the remainder of this thesis was based on plasma vitamin C as a biomarker for fruit and vegetables in the relation to risk of developing T2DM.

7.2 The Fruit and Vegetable Intake and Glucose Control Study (FIVE)

The FIVE study comprises the remaining part of the thesis. FIVE is a sub study of the Let's Prevent Diabetes Study, a large screening and prevention study. The first study of FIVE was a cross sectional analysis of the baseline screening visit of Let's Prevent Diabetes participants. Individuals from a multi ethnic population were identified from GP practices using a risk score for T2DM. 2101 participants provided fasting blood samples for the analysis of plasma vitamin C and a spot urine sample for the analysis of F_2-isoprostanes. Participants were also requested to complete a dietary questionnaire.

The results demonstrated that only 29% of the population consumed the recommended five portions of fruit and vegetables a day (determined by a plasma vitamin C of ≥50μmol/l). In addition fewer SA individuals consumed the recommended five a day than WEs (21% *vs*. 30%). FIVE illustrates inadequacies of current initiatives aimed at increasing fruit and vegetable intake. Research to try and understand why individuals are not acting on health messages around diet is needed. It is also necessary to explore why the SA community appears not to have been reached in these campaigns.

FIVE showed a clear association between fruit and vegetable intake and HbA1c, fasting and 2 hour glucose. Adjusted analysis found that for each additional piece of

fruit or vegetable consumed (as measured by plasma vitamin C of 21.8μmol/l) there was an associated reduction of 0.04% in HbA1c, 0.05mmol/l in fasting and 0.22mol/l in 2 hour blood glucose; demonstrating how small changes can significantly contribute to an overall change in glucose regulation. Although not clinically significant in itself, the public should be encouraged to make small changes to contribute to overall improvements in glucose control.

Participants who consumed the recommended five portions a day compared to those who did not had a 24% associated lower risk of being diagnosed with IGR (OR = 0.76, 95% CI: 0.59 to 0.98). No upper threshold of benefit was observed, for each additional portion of fruit or vegetable consumed (each increase in plasma vitamin C of 21.8μmol/l) there was a 15% associated reduced risk of diagnosis with IGR (OR = 0.85, 95% CI: 0.75 to 0.95). Therefore recommendation of more than five portions fruit and vegetables per day may be warranted. It is important to investigate how the general public may react to any changes to current advice about intake of fruit and vegetables. It may be necessary to determine whether a recommendation to consume more than five portions of fruit and vegetables is likely to increase consumption levels or simply overwhelm individuals, potentially leading them to consume less, believing it is impossible to reach such levels. Attitudes to conflicting advice that the public already receive about diet and health should be explored if future campaigns are to succeed.

In this cohort fewer SAs met the five a day target compared to WEs, furthermore SA individuals had lower mean plasma vitamin C compared to WEs (34.5μmol/l (SD 19.8) *vs.* 39.4μmol/l (SD 22.1). This relationship remained significant regardless of

factors such as age, sex and BMI. However adjustment for dietary fat resulted in attenuation of the data, suggesting poor overall diet quality may contribute to SAs increased risk of T2DM. There is an urgent need for up to date research into the dietary habits of SA individuals, including cooking techniques and how these can be manipulated to increase fruit and vegetable intake. Tailored dietary education for the SA population should be developed.

The dietary questionnaire used in FIVE was poorly completed (32% in WE and 17% in SA); therefore no firm conclusions can be made using these data. Research into the associated problems of self completion is required. Implementation of a simpler tool is necessary. The results further support continued use of nutritional biomarkers for dietary research rather than reliance on self report.

Assessment of urinary F_2-isoprostanes found no significant association with fruit and vegetable intake or glucose regulation. The results do not support the hypothesis that fruit and vegetable intake is beneficial for reducing the development of diabetes by reducing oxidative stress. The potential mechanistic actions by which fruit and vegetables do confer benefit requires further investigation.

The second study in FIVE was a sub study of the Let's Prevent Diabetes RCT. Participants identified with IGR were randomised to receive either usual care or invited to attend a six hour group education programme, the Let's Prevent Diabetes Study. The educational curriculum included sessions on glucose regulation, risks for diabetes, health complications of IGR, benefits of dietary change, physical activity and weight loss. Participants in FIVE (n=153) provided plasma vitamin C at baseline

and at 12 months. The follow up data showed those in the intervention arm had greater levels of plasma vitamin C than those in the usual care arm (36.1μmol/l (SD 20.7) *vs.* 29.9μmol/l (SD 20.3)). However no statistical difference in mean change between intervention arms was seen. The educational arm contained messages about dietary change but it was not an intervention solely aimed at increasing fruit and vegetable intake. Indeed participants were encouraged to select behaviour changes they felt most appropriate to themselves. Modification of the curriculum or a study which focuses on increasing fruit and vegetable intake is warranted.

The thesis adds to the limited evidence on fruit and vegetable intake and its association with risk of IGR and T2DM. The thesis contains a systematic review and meta-analysis which supports increasing consumption of GLV to reduce the risk of T2DM. FIVE is the first study to include plasma vitamin C as a biomarker for fruit and vegetable intake in a multi-ethnic, at risk population. The use of a nutritional biomarker provides robust information on dietary intake; the study supports continued use of biomarkers as the self completed questionnaire was poorly used by participants. The study highlights how small changes in lifestyle are associated with improvements in glucose regulation.

Appendix

8.0 Appendix 1: Publications & abstracts from thesis

Journal of Nutrition and Metabolism 2011; doi:10.1155/2011/847202
Dietary Recommendations for the Prevention of Type 2 Diabetes: What are They Based on? Patrice Carter, Kamlesh Khunti, Melanie J Davies.

BMJ 2010; 431: c4229
Fruit and vegetable intake and incidence of type 2 diabetes mellitus: a systematic review and meta-analysis. Patrice Carter, Laura J Gray, Jacqui Troughton, Kamlesh Khunti & Melanie J Davies

Poster presentation 21st World Diabetes Congress, Dubai, December 2011: pd27
South Asian individuals at high risk of type 2 diabetes have lower plasma vitamin C levels than White Europeans
P. Carter, L.J. Gray, K. Khunti, M.J. Davies

Diabetic Medicine 2011, P143; 28 (S1) 76
South Asian individuals at high risk of Type 2 diabetes have lower plasma vitamin C levels than White Europeans
P. Carter, L.J. Gray, K. Khunti, M.J. Davies

Diabetic Medicine 2010, P;243 27 (S1) 109
Increasing green leafy vegetable consumption can decrease the risk of type 2 diabetes
P. Carter, J.Troughton, L.Gray, K.Khunti, N.G.Forouhi, M.J.Davies

Due to third party copyright restrictions the following published articles have been removed from Appendix 1 of the electronic version of this thesis:

Journal of Nutrition and Metabolism 2011; http://dx.doi.org/10.1155/2012/847202
Dietary Recommendations for the Prevention of Type 2 Diabetes: What are They Based on? Patrice Carter, Kamlesh Khunti, Melanie J Davies.

BMJ 2010; 431: c4229 http://dx.doi.org/10.1136/bmj.c4229
Fruit and vegetable intake and incidence of type 2 diabetes mellitus: a systematic review and meta-analysis. Patrice Carter, Laura J Gray, Jacqui Troughton, Kamlesh Khunti & Melanie J Davies

The unabridged version can be consulted, on request, at the University of Leicester's David Wilson Library.

8.1 Appendix 2: Protocol Amendment for the Let's Prevent Study – Vitamin C

Diets characterized by a high fruit and vegetable content have been associated with improvements in glucose control (1,2), we therefore intend to measure fruit and vegetable intake. The term "fruit and Vegetables" covers a wide range of food groups which differ between and even within different cultures (3) making dietary assessment difficult. However as well as reflecting short term intake of Vitamin C (4), plasma Vitamin C has consistently been shown to be correlated with habitual, reported intake of fruit and vegetables (5). The use of Vitamin C as a biomarker also has the added benefit of not relying on self reported food intake (6).

It has previously been demonstrated that recommendations to increase fruit and vegetable intake can produce an increase in Vitamin C levels (7,8) and subjects in the LSM group of the study will be encouraged to do so. Therefore the use of plasma vitamin C as a biomarker will not only provide information about differences in intake between subjects at baseline but will also provide a useful tool that can demonstrate whether subjects in either control or to LSM groups alter their fruit and vegetable intake after enrolment in the study.

Oxidative Stress (OS) occurs when an imbalance between pro-oxidants and antioxidants occur in a biological system and it is widely accepted that oxidative stress acts as a participant in the development and progression of T2D (9). It is proposed that the accelerated complications and increased risk of coronary heart disease seen in T2D is due to the presence of oxidative stress. Diabetic subjects show both an increase in levels of free radicals, substances which promote oxidative damage and a decrease in antioxidants (10). Antioxidants are substances which prevent or delay oxidation (11). Increasing fruit and vegetable intake may beneficially increase essential antioxidant levels and thus reduce oxidative stress. It has been demonstrated that patients who consume two to three portions of fruit daily have lower levels of lipid peroxidation (10). Plasma and urinary F_2isoprostanes are established biomarkers of lipid peroxidation in vivo (12). Therefore we also intend to measure urinary F_2-isoprostanes. This will allow for the assessment of oxidative stress in relation to fruit and vegetable intake as well as glucose tolerance.

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(4) Sargeant LA, Wareham NJ, Bingham SA, Day NE, Luben RN, Oakes S, et al. Vitamin C and Hyperglycemia in the European Prospective Investigation Into Cancer-Norfolk (EPIC-Norfolk) Study. A population-based study. Diabetes care 2000;23:726-732.
(5) Block G, Norkus E, Hudes M, Mandel S, Helzlsouer K. Which Plasma Antioxidants Are Most Related to Fruit and Vegetable Consumption? American Journal of Epidemiology 2001;154:1113-1118.

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(8) van het Hof kH, Tijburg LBM, Pietrzik K, Westsrate JA. Influence of feeding different vegetables on plasma levels of carotenoids, folate and vitamin C. Effect of disruption of the vegetable matrix. British Journal of Nutrition 1999;82:203-212.
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(11) Halliwell B, Gutteridge JMC. Antioxidant defences: endogenous and diet derived. In Free Radicals in Biology and Medicine 4th Edition 2007. Oxford University Press.
(12) Higdon JV, Frei B. Obesity and Oxidative Stress, A Direct Link to CVD? Arterioscler.Thromb.Vasc.Biol. 2003;23:365-367.

8.2 Appendix 3: DINE (plus scores)

Section G: Eating Habits

Purpose

The purpose of this questionnaire is to get an idea of your usual eating habits. For the listed foods, we would like to know how many servings you eat in a typical day or week. A serving is an average portion that would be served at a meal. If you usually eat more than one serving of the food at a time, you should count all the servings you eat.

Instructions

For each food listed, tick the box that describes the number of servings that you usually eat. If you never eat a particular food, tick the box under "None".

Please do not leave any lines blank.

About how many **pieces or slices per day** do you eat of the following types of bread, rolls, or chapatis? (Please tick one box on each line)

	Breads & Rolls	None	Less than 1 a day	1 to 2 a day	3 to 4 a day	5 or more a day
1.	White bread or rolls	0	1	4	9	13
2.	Brown or granary bread or rolls	0	2	7	15	22
3.	Wholemeal bread or rolls	0	3	8	18	26

About how many **servings per week** do you eat of the following types of breakfast cereal or porridge? (Please tick one box on each line)

	Breakfast cereals	None	Less than 1 a week	1 to 2 a week	3 to 5 a week	6 or more a week
4.	Sugared type: Frosties, Coco Pops, Ricicles Sugar Puffs Rice or Corn type: Corn Flakes, Rice Krispies, Special K	0	0	0	1	2
5.	Porridge or Ready Brek Wheat type: Shredded Wheat, Weetabix, Fruit 'n Fibre, Puffed Wheat, Nutri-grain, Start Muesli type: Alpen, Jordan's	0	1	2	5	7
6.	Bran type: All-Bran, Bran Flakes, Sultana Bran	0	2	5	12	18

About how many **servings per week** do you eat of the following foods?
(Please tick one box on each line)

	Vegetable foods	None	Less than 1 a week	1 to 2 a week	3 to 5 a week	6 to 7 a week	8 to 11 a week	12 or more a week
7.	Pasta or rice	0	0	1	3	4	6	8
8.	Potatoes	0	0	1	3	5	8	10
9.	Peas	1	1	4	10	15	20	30
10.	Beans (baked, tinned, or dried) or lentils	1	1	4	10	15	20	30
11.	Other vegetables (any type)	0	0	1	2	3	5	6
12.	Fruit (fresh, frozen, canned)	0	0	1	3	5	8	10

FIBRE SECTION ENDS HERE. TOTAL FIBRE SCORE=

About how many **servings per week** do you eat of the following foods?
(Please tick one box on each line)

		None	Less than 1 a week	1 to 2 a week	3 to 5 a week	6 or more a week
13.	Cheese (any except cottage)	1	1	2	6	9
14.	Beefburgers or sausages	1	1	2	6	9
15.	Beef, pork, or lamb (for vegetarians: nuts)	1	1	2	6	9
16.	Bacon, meat pie, processed meat	1	1	2	6	9
17.	Chicken or turkey	0	0	1	3	5
18.	Fish (NOT fried fish)	0	0	0	1	2
19.	ANY fried food: fried fish, chips, cooked breakfast, samosas	1	1	2	6	9
20.	Cakes, pies, puddings, pastries	1	1	2	5	8
21.	Biscuits, chocolate, or crisps	1	1	2	4	6
		None	Less than 1 a week	1 to 2 a week	3 to 5 a week	6 or more a week

About how much of the following types of milk do you yourself use **in a day**, for example in cereal, tea, or coffee? (Please tick one box on each line)

	Milks	None	Less than a quarter pint	About a quarter pint	About half a pint	1 pint or more
22.	Full cream (silver top) or Channel Islands (gold top)	0	1	3	6	12
23.	Semi-skimmed (red striped top)	0	0	1	3	6
24.	Skimmed (blue checked top)	0	0	0	0	0

About how many **rounded teaspoons per day** do you usually use of the following types of spreads, for example on bread, sandwiches, toast, potatoes, or vegetables?

	Spreads	None	1 a day	2 a day	3 a day	4 a day	5 a day	6 a day	7 or more
25.	**Regular margarine or butter** or **Reduced fat spread** such as sunflower or olive spread, Flora, Vitalite, Clover, Olivio, Stork, Utterly Butterly	0	4	8	12	16	20	24	28
26.	**Low fat spread** such as Flora Light, St. Ivel Gold, Olivite, Half-fat butter, Flora Pro-activ, Light spread	0	2	4	6	8	10	12	14

FAT SECTION ENDS HERE. TOTAL FAT SCORE=

What type of fat do you usually use for the following purposes? (Please tick one box on each line)

		Butter, lard, or **dripping**	**Solid cooking fat** (White Flora, Cookeen) **Half-fat butter** **Hard margarine** (Stork)	**Soft margarine** (sunflower, soya) **Reduced fat spread** (olive, Flora Buttery, Olivio)	**Vegetable oil** or **Low fat spread** (Flora Light, Olivite, St. Ivel Gold)	**No fat used**
27.	On bread and vegetables	1	2	3	4	3
28.	For frying	1	2	3	4	3
29.	For baking or cooking	1	2	3	4	3

UNSATURATED FAT SECTION ENDS HERE. TOATAL SCORE=

8.3 Appendix 4: The Let's Prevent Diabetes Curriculum

A: Introduction and Housekeeping (10mins)
B: Participants Story (30mins) – The educator elicits information from each participant to explore their health beliefs in relation to diagnosis of pre-diabetes.
C: Professional Story. Pre-diabetes and glucose (50mins) –Participants are asked open questions to determine how the body maintains healthy glucose levels and to understand what glucose and insulin are. Participants learn the differences between pre-diabetes, T1DM and T2DM. Understanding how pre-diabetes may change over time and discover what factors contribute to causing pre-diabetes and the different options available to decrease risk of going onto develop T2DM.
D: Food Choices and Insulin Resistance (30mins) – Participants learn which dietary factors affect insulin resistance and become aware of the effects SFA and central obesity may have. Individuals develop an understanding of energy balance and learn that small changes in calorie intake can make a significant difference on weight management and insulin resistance.
E: Physical Activity (40mins) – Participants learn how physical activity can improve blood glucose and reduce their risk of CVD. Individuals learn the current recommendations for activity levels, discuss barriers to implementing activity and develop strategies to become more active in their daily lives. Participants will understand how pedometers can be a useful tool for action planning and the importance of setting small, achievable goals.
F& G: How am I doing? (5mins) and Reflections (15mins) – Participants have time to review what lifestyle changes may benefit themselves based on the information covered so far. Facilitators ask participants to express any concerns and questions they may have.
H: Professional Story. Risk Factors and Complications (40mins) – Participants learn that pre-diabetes is associated with an increased risk of CVD risk. They learn potential ways to reduce blood pressure, cholesterol and weight, in addition to discussing the harmful effects of smoking and how physical activity may reduce risk factors. Depression and its impact on pre-diabetes and self-management are also explored. Participants will identify their own risk factors and explore the role they play in improving their own risk factors by potential lifestyle changes.
I: Taking Control. Focus on Fats and CVD (40mins) – Participants will identify different fat types and hidden fats in food. Discussion on how to identify foods high in fat and how to select lower fat alternatives will be carried out. Participants will link fats to risk factors for insulin resistance, lipid profile, blood pressure and weight. Participants will be informed on the benefits of eating five portions of fruit and vegetables a day. There will also be discussion on selecting higher fibre foods.
J: Pre diabetes Self-Management Plan (45mins) – Participants will list their current risk factors in relation to development of long term complications and identify at least one behavioural goal they can aim to change in order to improve their risk profile. Participants are encouraged to recognise their personal barriers to change in relation to achieving their goal and think about how to overcome them. Participants are encouraged to write a plan of action using SMART goal setting processes.
K: Questions and Future Care (10mins) – Participants should now have the answers to their key questions from the Participants Story. Information on how to access on-going care and support that Let's Prevent has to offer will also be discussed.

8.4 Appendix 5: T-Test data between those who provided plasma vitamin C and those who did not

Group Statistics: Baseline data of those who provided plasma vitamin C (vit C data) and those who did not provide plasma vitamin C (no vit C data)

	withorwithoutvitC	N	Mean	Std. Deviation	Std. Error Mean
Age at attendance	no vitC data	754	62.63	8.489	.309
	vitC data	2094	59.33	159.107	3.477
AvSystolic	no vitC data	755	143.92	46.091	1.677
	vitc data	2095	145.30	19.211	.420
AvDiastolic	no vitC data	754	86.32	10.531	.384
	vitc data	2095	86.02	10.520	.230
BMI	no vitC data	753	32.7317	5.92117	.21578
	vitc data	2094	32.2535	5.78817	.12649
Waist	no vitC data	754	109.14	13.437	.489
	vitc data	2095	108.97	22.910	.501
LDL	no vitC data	720	3.0687	.86570	.03226
	vitc data	2071	3.0883	1.15766	.02544
TC	no vitC data	726	5.7036	14.91099	.55340
	vitc data	2095	5.1186	1.04149	.02275
HDL	no vitC data	726	1.4044	.43312	.01607
	vitc data	2092	1.3832	.43580	.00953
TG	no vitC data	726	1.5817	.86465	.03209
	vitc data	2094	1.5759	.91426	.01998
HbA1c	no vitC data	720	5.9658	.62751	.02339
	vitc data	2085	5.9429	.50087	.01097
0 Glu	no vitC data	754	4.0706	36.59405	1.33268
	vitc data	2094	5.3313	.78195	.01709
120 Glu	no vitC data	748	5.6349	36.89447	1.34900
	vitc data	2093	4.7756	44.01055	.96199

Independent Samples Test: Statistical difference in characteristics between those who provided plasma vitamin C and those who did not. Column labelled “Sig (2.tailed)” gives the p value between groups.

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Age at attendance	Equal variances assumed	.679	.410	.569	2846	.569	3.301	5.798	-8.067	14.670
	Equal variances not assumed			.946	2125.855	.344	3.301	3.491	-3.544	10.147
AvSystolic	Equal variances assumed	2.380	.123	-1.126	2848	.260	-1.380	1.226	-3.783	1.024
	Equal variances not assumed			-.798	850.165	.425	-1.380	1.729	-4.774	2.014
AvDiastolic	Equal variances assumed	.032	.858	.665	2847	.506	.297	.447	-.579	1.174
	Equal variances not assumed			.665	1329.404	.506	.297	.447	-.580	1.175

BMI	Equal variances assumed	1.468	.226	1.932	2845	.053	.47816	.24746	-.00705	.96338
	Equal variances not assumed			1.912	1302.355	.056	.47816	.25012	-.01252	.96885
Waist	Equal variances assumed	.228	.633	.190	2847	.849	.168	.885	-1.566	1.903
	Equal variances not assumed			.240	2262.265	.810	.168	.700	-1.204	1.541
LDL	Equal variances assumed	1.591	.207	-.414	2789	.679	-.01952	.04715	-.11198	.07294
	Equal variances not assumed			-.475	1667.089	.635	-.01952	.04109	-.10010	.06107
TC	Equal variances assumed	4.212	.040	1.784	2819	.075	.58497	.32795	-.05808	1.22801
	Equal variances not assumed			1.056	727.453	.291	.58497	.55387	-.50240	1.67233
HDL	Equal variances assumed	.033	.855	1.133	2816	.257	.02123	.01874	-.01552	.05798
	Equal variances not assumed			1.136	1269.603	.256	.02123	.01869	-.01543	.05789

TG	Equal variances assumed	.000	.982	.149	2818	.881	.00580	.03884	-.07036	.08195
	Equal variances not assumed			.153	1326.933	.878	.00580	.03780	-.06836	.07995
HbA1c	Equal variances assumed	6.923	.009	.988	2803	.323	.02291	.02318	-.02254	.06836
	Equal variances not assumed			.887	1052.589	.375	.02291	.02583	-.02778	.07359
0 Glu	Equal variances assumed	7.264	.007	-1.576	2846	.115	-1.26077	.79995	-2.82931	.30777
	Equal variances not assumed			-.946	753.248	.344	-1.26077	1.33279	-3.87719	1.35565
120 Glu	Equal variances assumed	.169	.681	.477	2839	.633	.85926	1.80000	-2.67019	4.38871
	Equal variances not assumed			.519	1556.230	.604	.85926	1.65687	-2.39067	4.10919

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