The Effects of Dietary Supplementation with Gum Arabic on Blood Pressure and Renal Function in Subjects with Type 2 Diabetes Mellitus

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Thesis presented for the degree of

Doctor of Medicine

Cardiff University 2012

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Acknowledgements

I would like to thank my supervisors Professor Aled Phillps and Dr Steve Riley for their support, knowledge and advice throughout the time spent carrying out the work in this thesis and for their encouragement in getting me to write up and complete this thesis, without which it may have never happened.

A gracious thanks to all of the staff in the Institute who have taught me lab techniques, and to Dr James Chess, whose knowledge of statistics helped greatly. I must thank all of the participants in the study, who took the supplements daily, without whom this research would have never been possible. Also I am grateful for the Diabetes team in Llandough Hospital, for allowing me access to their clinics.

Finally, massive thanks to my wife, Siân, and to my three children, Ben, Josh and Iwan, for supporting me and willing me to finish this manuscript.

Publications

The following publication has resulted directly from the work presented in this thesis:

Acacia(sen) SUPERGUM (TM) (Gum arabic): An evaluation of potential health benefits in human subjects
Glover DA, Ushida K, Phillips AO, Riley SG, *Food Hydrocolloid*, Volume 23, 8 (December 2009) pp.2410-2415

Summary of Thesis

Type 2 diabetes mellitus is associated with a significant increased morbidity and mortality resulting from microvascular and macrovascular complications, in particular diabetic nephropathy and cardiovascular disease. Treatment of these conditions has involved improving diabetic control, reducing blood pressure and addressing other cardiovascular risk factors. Dietary fibre has wide reaching health benefits, including improvement of diabetic control and blood pressure, potentially by alterations in colonic bacterial populations that result in changes in serum short chain fatty acids.

An open labelled study with a washout period was undertaken to examine the potential effects of Gum arabic on blood pressure and renal function.

A daily supplement of gum arabic (25g), a soluble dietary fibre, was administered for a period of 12 weeks. An initial pilot study was conducted in healthy subjects (n=10) and subjects with overt nephropathy (n=14). A follow on study investigated subjects with incipient nephropathy (n=23) in more detail. Measurements of renal function, including isotope GFR and ERPF, blood pressure and vascular stiffness (follow on only), and short chain fatty acids were measured. A significant drop in GFR was seen in the healthy individuals with no associated change in filtration fraction, which could convey some renal protective effect. No changes were seen in the diabetic subjects. Significant drops in blood pressure were seen each of the individual groups. Results of pulse wave analysis and central blood pressure measurements suggest this is not as a result of changes in vascular stiffness. Significant changes in short chain fatty acid production were seen, in particular an increase in acetate ($p=0.033$) in the incipient nephropaths and butyrate ($p=0.03$) in the healthy subjects.

This study suggests that Gum arabic has beneficial effects on blood pressure but no immediate beneficial effects on renal function in either diabetic cohort.

List of Abbreviations

ACE Angiotensin Converting Enzyme

ACR Albumin Creatinine Ratio

AG Augmentation Pressure

AGE Advanced Glycation End Products

AIx Augmentation Index

AIx75 Augmentation index at 75 beats per minute

Ao Aortic

BMI Body mass Index

BP Blood Pressure

CABG Coronary Artery Bypass Graft

CKD Chronic Kidney Disease

CMC Carboxymethylcellulose

CV Coefficient of Variation

eGFR Estimated Glomerular Filtration Rate

ERF Established Renal Failure

ERPF Estimated Renal Plasma Flow

FF Filtration Fraction

GFR Glomerular Filtration Rate

HR Hazard Ratio

IDDM Insulin Dependent Diabetes Mellitus

IPD Intermittent Peritoneal Dialysis

LDL Low Density Lipoprotein

LV Left Ventricle

MAP Mean Arterial Pressure

MDP Mean Diastolic Pressure

MDRD Modification of Diet in Renal Disease

MSP Mean Systolic Pressure

NIDDM Non Insulin dependent diabetes mellitus

NS Non Significant

PAH Para-amino Hippurate

PAS Periodic Acid-Schiff

PCR Protein Creatinine Ratio

PP Pulse Pressure

PWV Pulse Wave Velocity

PYH Pack Year History

RRT Renal Replacement Therapy

SCFA Short Chain Fatty Acid

SD Standard Deviation

SE Standard Error

SEVR Subendocardial Viability Ratio

SMR Standardised Mortality Ratio

TGF-β Transforming Growth Factor Beta

Tr Time for return of reflected wave

UAER Urine Albumin Excretion Rate

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1 INTRODUCTION

1.1 Aims

There are currently numerous medications that have been proven to improve outcomes in patients with diabetes mellitus by reducing cardiovascular and renal complications, but the actual targets of treatment are limited.

Dietary fibre has been documented to have several health benefits and I aim to look at the effects of supplementing the diet with Gum Arabic, a soluble fibre and examine outcomes on renal function and blood pressure. This was carried out firstly in two populations, namely healthy individuals and patients with advanced diabetic nephropathy, and secondly in a population of patients with type 2 diabetes and incipient nephropathy.

1.2 Diabetes Mellitus

Diabetes Mellitus is a disorder affecting blood glucose regulation resulting in chronic hyperglycaemia that in turn can have many serious complications including a significant increase in cardiovascular disease.

1.2.1 Definition

The World Health Organisation and National Diabetes Data Group first recommended uniform diagnostic criteria for diabetes in the late 1970s. This was updated and modified by the World Health Organisation in 1999 [1] and then further in 2006. The criteria now states that a diagnosis of diabetes mellitus is made on a fasting blood glucose of ≥7.0 mmol/L or a blood glucose of ≥ 11.1 two hours after an oral challenge of 75g of glucose [2].

The classification of diabetes is then further split in to Type 1 Diabetes and Type 2 Diabetes depending on the underlying pathogenesis.

1.3 Diabetes Mellitus – Type 1 and Type 2

Type 1 diabetes mellitus, previously referred to as insulin-dependent diabetes (IDDM), is characterised by an absence of insulin production by the beta islet cells of the pancreas, and is usually caused by autoimmune destruction of these cells. The time of onset of Type 1 Diabetes is often, but not always more clearly defined, usually indicated by the presence of polydypsia, polyuria and ketosis.

Type 2 diabetes mellitus, formerly referred to as non insulin dependent diabetes (NIDDM) is a disorder characterised by peripheral insulin resistance and hyperinsulinaemia. The precise time of the onset of type 2 Diabetes is more difficult to define and tends to have a rather insidious onset. During this time, which can last several years, there is hyperglycaemia, leading to organ damage even before the diagnosis of diabetes is made. It is also associated with impairment of fat and protein metabolism as a result of defects of insulin secretion, its action or a combination of the two [3]. Patients are not entirely dependent on administered exogenous insulin, unlike those with Type I Diabetes. However, long term hyperinsulinaemia may ultimately result in a relative impairment of insulin due to pancreatic beta cell "burnout", necessitating the use of exogenous insulin.

Importantly, both type 1 diabetes and type 2 diabetes are often associated with hypertension, hyperlipidaemia and an increased risk of cardiovascular disease.

The vast majority of patients with diabetes have type 2 diabetes and therefore the focus of this thesis will be on patients with type 2 diabetes, it's renal and cardiovascular complications and ways of modifying the course of the disease. However, I will refer at times to data that corresponds to type 1 diabetes to illustrate certain points.

1.4 Epidemiology of Type 2 Diabetes mellitus

1.4.1 Prevalence

Type 2 diabetes mellitus accounts for approximately 90 percent of all diabetes worldwide and is most prevalent in the developed world. Currently there is massive

growth in the number of patients with type 2 diabetes in both the developed and developing world. Engeglau [4] reported in 2004 the increasing prevalence of patients with diabetes in the United States, as shown in Figure 1-1. In 1995 there was an estimated worldwide type 2 diabetes prevalence of 4%. This was expected to rise to 5.4% over the following 30 years. This equates to an increase in absolute numbers from 135 million people in 1995, to approximately 300 million in 2025 [5] as shown in Figure 1-2. The major part of this increase is occurring in developing countries with a 170% increase, from 84 to 228 million. By the year 2025, 75% of people with diabetes will therefore reside in developing countries, as compared with 62% in 1995 [5].

The National Health Interview Survey has shown a 4 to 8 fold increase in the number of patients receiving a diagnosis of diabetes over the last 50 years [6]. By 2002 an estimated 6.3% of the US population had diabetes, this has grown to 8.3% in 2011[7] and this number will continue to grow.

The prevalence of type 2 diabetes in British men has been increasing significantly over the last 3 decades. Thomas et al have suggested that the crude prevalence of diabetes has risen from 1.2% in 1978-1980 to 12.1% in 2005 [8]. This was however in a selected age group of 40-59 year old males. Diabetes UK state the UK prevalence of type 2 diabetes to be 4.26% as of 2010 [9].

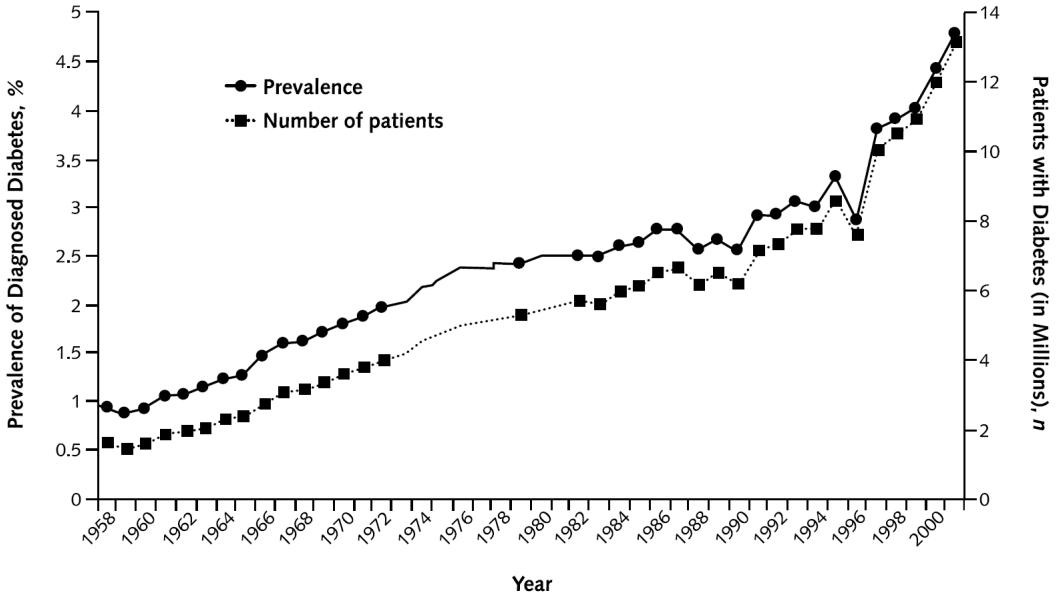


Figure 1-1. US Prevalence of Diabetes 1958-2000

Adapted from Engeglau et al [4]

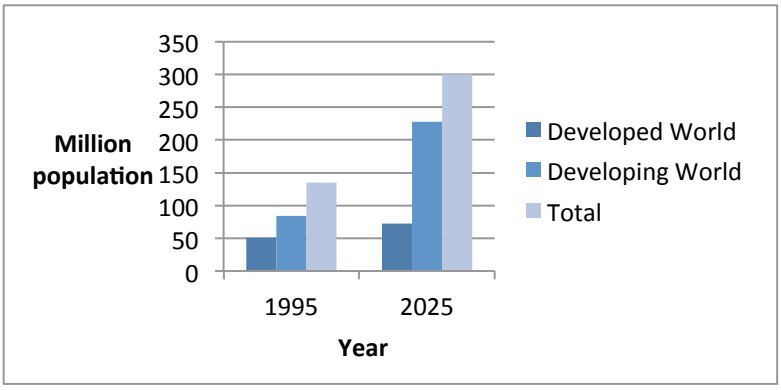


Figure 1-2. Predicted increase in Prevalence of Diabetes

Adapted from King et al [5]

1.4.2 Geographical variation

There is wide global variation in the prevalence of the condition. In the main, Type 2 diabetes mellitus is currently less prevalent in non-Western countries where the diet contains fewer calories and caloric expenditure is higher. Some of these non-Western populations are beginning to adopt Western lifestyles and hence weight gain and type 2 diabetes mellitus are becoming virtually epidemic. Obesity and a lack of exercise are strongly linked to the development of type 2 diabetes [10]. Much of Southern Africa and China have the lowest rates which stand at less than 3%. Europe as a whole has a similar prevalence to Australasia and South America, in the region of 3 to 5%. North America, India and the Philippines have a higher prevalence of 6 to 8%. The highest prevalence areas with rates greater than 8% include parts of North Africa, much of the Middle East and Papua New Guinea [11].

In the UK, as already stated the prevalence of type 2 diabetes is slightly lower. As of 2009, there are an estimated 2.6 million people with the condition, equating to approximately 4.0% of the population. In addition it is estimated there are a further 500,000 people with the condition undiagnosed, due to the known time lag in people presenting with the condition [12].

1.5 Pathogenesis of type 2 diabetes

Hyperglycaemia as defined in Chapter 1.2.1 is as a result of a deficiency of endogenous insulin, either absolute as in Type 1 diabetes or relative as in the case of Type 2 diabetes. The fundamental underlying problem in type 2 diabetes is that of insulin resistance which can be as a result of abnormal insulin molecules [13], an increase in the amount of circulating insulin antagonists or resistance to insulin in target tissues, the latter being by far the most common cause of type 2 diabetes.

In obesity related insulin resistance, the normal response of the β cells of the pancreas is insulin hypersecretion in a process called β cell compensation. Rodent studies suggest there is an increase in β cell mass and enhanced β cell function with increased insulin synthesis. Type 2 diabetes occurs only when the subjects are unable to sustain this compensatory response. Early β cell failure is likely to be caused by numerous mechanisms including mitochondrial dysfunction, oxidative stress and dysfunctional triglyceride cycling amongst others. A self-fulfilling process then takes place in which the hyperglycaemia itself results in processes such as islet cell inflammation, amyloid deposition and O-linked glycosylation as a result of glucotoxicity. This leads to a loss of β cell mass by apoptosis [14]

Environmental factors undoubtedly play a role in its prevalence and known environmental diabetogenic factors include obesity, pregnancy and intercurrent illness. These largely cause insulin resistance. Obesity is one of the most important factors associated with the development of type 2 diabetes and is also increasing in prevalence in the Western World and developing world. It is now reaching epidemic levels [15]. There are however also genetic factors and genetic susceptibility to type 2 diabetes. This is best demonstrated by studies in monozygotic twins which have shown concordance rates of 96% for an abnormality of glucose metabolism [16]. The Pima Indian people have also been extensively studied. They have the highest prevalence of type 2 diabetes of any group in the world. This is thought to be as a result of genetic differences, a so-called “thrifty genotype” which has now been exposed to a Western diet. These genetic factors however are not fully understood but are likely to be related to multiple genes. Increasing age is also an accepted important factor for the development of type 2 diabetes.

Free fatty acids have more recently been implicated in the development of type 2 diabetes mellitus and obesity by mediating insulin resistance. One hypothesis put forward is that increased concentrations of plasma FFA induce insulin resistance in humans through inhibition of glucose transport activity, which appears to be a consequence of decreased insulin receptor substrate-1-associated phosphatidyl inositol 3 kinase activity[17].

1.6 Complications of diabetes

The major complications of diabetes can be broadly split in to those which are either macrovascular or microvascular in origin and are related to glycaemic control. As a result of these complications, particularly cardiovascular disease and diabetic nephropathy, there is an associated significant increase in mortality.

Type 2 diabetes is commonly associated with a triad of medical disorders, these being obesity, hyperlipidaemia and hypertension, which have collectively become known as the “metabolic syndrome” or “syndrome X”. This term has been used for many decades, dating back to at least the 1950s but became increasingly used in the 1970s and was described by Haller in 1977 as a condition that obesity, type 2 diabetes mellitus, hyperlipoproteinemia, hyperuricemia and steato hepatis [18].

1.6.1 Macrovascular complications

The macrovascular complications of diabetes include cardiovascular disease, cerebrovascular disease and an increased incidence of peripheral vascular disease. Macrovascular disease is a major cause of mortality and morbidity in patients with either type 1 or type 2 diabetes. Stamler et al, looked at cardiovascular mortality in the MRFIT study, between 1973 and 1975. The overall risk of cardiovascular mortality was 3 times higher in the diabetic group compared to matched non diabetic subjects. The impact of other risk factors such as hypertension, hypercholesterolaemia and smoking have a larger effect on cardiovascular death rate in the diabetic population than in the non diabetic control group [19].

Data analysed from the NHANES 1 Study shows that ischaemic Heart disease is the major cause of death associated with diabetes [20] in the United States, with a rate two to six times that of the general population, and an age adjusted prevalence of coronary heart disease twice that of the population without diabetes [21]. There has been a decline in the age adjusted mortality from heart disease in the US with time but this decline has not been shown in patients with diabetes. This correlates with a 2-fold increased risk for coronary heart disease in diabetic men, and a 3- to 4-fold increased risk in diabetic women [20]

There is epidemiological evidence to show that there is a correlation between increased rates of cardiovascular disease and chronic hyperglycaemia [22-24].

Selvin et al performed a meta analysis of largely type 2 diabetic patients and demonstrated an increase in relative risk for a cardiovascular event of 1.18 for every percentage point increase in HbA1C [24]. The evidence however on the treatment of blood glucose control on macrovascular outcomes is mixed. In patients with established diabetes, large studies such as the ACCORD study and ADVANCE study [25, 26] have failed to demonstrate that improved glycaemic control is associated with improved macrovascular outcomes. The ACCORD trial enrolled 10,251 patients with longstanding type 2 diabetes, randomising patients to an intensive treatment group, aiming for HBA1C of <6.0%, or to a standard arm aiming for HbA1C of 7.0 to 7.9%. The trial was brought to a halt in 2008 after 3.5 years, due to a higher number of cardiovascular deaths in the intensive treatment group [27]. No significant difference in non fatal cardiovascular events was shown between the groups. Similarly, the ADVANCE trial enrolled 11,140 patients with type 2 diabetes, with an intensive treatment (HbA1C <6.5%) group and a standard treatment group. There was no increased risk of death but also there was no reduction in macrovascular events or death from cardiovascular causes. Both of these studies have looked at intervening in patients with longstanding diabetes. In patients with newly diagnosed diabetes, there may be benefit from stricter control of HbA1C.

The UKPDS 33 study [28] randomised 3867 newly diagnosed patients with type 2 diabetes to either an intensive management group, treated with either a sulphonylurea or insulin aiming for a blood sugar of < 6 mmol/L, or to a conventional group treated with diet aiming for the best achievable blood sugar. The study continued for 10 years and

demonstrated a risk reduction of 10% for any diabetes related endpoint and a 10% reduction for any diabetes related death (NS) for the intensive treatment group. Most of the reduction in diabetes related events however came from the significant reduction in microvascular events. There was a 16% risk reduction in myocardial infarction in the intensive management group but this did not reach statistical significance (p=0.052). The authors postulate that a 10 year follow up may be too short to demonstrate significant differences in macrovascular events.

1.6.2 Microvascular Complications

The microvascular complications of diabetes are diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy. The UKPDS 35 showed that HbA_{1c} is strongly associated with microvascular events and that a 1% reduction in HbA_{1c} was associated with a 37% reduction in microvascular complications [29].

All of the microvascular complications are as a result of microangiopathy, as a result of thickening of the capillary basement membrane, resulting in increased vascular permeability. Metabolism of glucose to glucose alcohol (sorbitol) via the polyol pathway is thought to be key in the process. High glucose levels as a result of diabetes increase the flux of sugar molecules through the polyol pathway, which causes sorbitol accumulation in cells. Accumulation of sorbitol is thought to be an important underlying mechanism in the development of diabetic microvascular complications, including diabetic retinopathy. In animal models, sorbitol accumulation has been linked to microaneurysm formation, thickening of basement membranes [30], glycation of structural proteins and the production of advanced glycation end (AGE) products which become deposited in tissues underlies the structural and functional abnormalities of these diabetic complications [31].

1.6.2.1 Diabetic Retinopathy

Diabetic retinopathy is the leading cause of blindness in the US in persons aged 20 to 64 years [4]. 20% of patients have established retinopathy at the time of diagnosis of

their type 2 diabetes. The earliest, but not macroscopically visible changes of diabetic retinopathy are changes in the capillary bed. With time, and poor diabetic control these progress through the following stages:

- Microaneurysms
- Retinal Haemorrhages
- Hard exudates
- Soft Exudates
- Intraretinal microvascular abnormalities
- Neovascularisation pre-retinal haemorrhage
- Vitreous haemorrhage
- Fibrosis

Good glycaemic control, particularly early on in the disease is vital to reduce the development and progression of retinopathy. The UKPDS 33 study as described above, showed a 29% risk reduction (p=0.0031) in the incidence of photocoagulation for retinopathy with intensive control of blood sugar [28].

1.6.2.2 Diabetic neuropathy

The underlying pathogenesis of diabetic neuropathy is common to all of the microvascular complications. It predominantly affects the somatic nervous system, resulting in complications such as polyneuropathies and mononeuropathies, but also involves the autonomic nervous system resulting in complications such as gastroparesis and postural hypotension. The complication tends to occur relatively early in diabetes and affects up to about 30% of patients. The Diabetes Control and Complications Trial Research Group showed in 1993, albeit in patients with type 1 diabetes, that intensive glycaemic control was associated with a reduction in the incidence of neuropathy by 60% compared to those in the standard treatment group [32].

1.6.3 Diabetic Nephropathy

It was previously a common belief that patients with Type 1 diabetes were at a higher risk of developing nephropathy than those with Type 2 diabetes. It is now widely accepted that the risk is similar [33].

Diabetic nephropathy is a slow and often progressive process, ranging from early glomerular hyperfiltration to overt diabetic nephropathy and end stage renal failure. It is recognised though that about 30% of patients with incipient nephropathy will regress back to normal with an absence of microalbuminuria. The earliest sign of diabetic nephropathy is glomerular hyperfiltration. This is related to glycosuria but does not seem to show any relationship to the development of more advanced diabetic nephropathy. Blood pressure and albuminuria are usually normal. Even at the time of diagnosis of type 2 diabetes, approximately 30 to 40% of patients have an elevated glomerular filtration rate (GFR) [34]. Estimated renal plasma flow (ERPF) however remains unchanged meaning that there is an increased filtration fraction (FF=GFR/ERPF). This would imply that there is glomerular hypertension, which is thought to be an important factor in the progression of diabetic nephropathy. Population based studies also suggest that there is a 5-10% prevalence of nephropathy even at the time of diagnosis of Type 2 Diabetes, due to the lag in making the diagnosis of diabetes.

There is often a latent period during which glomerular filtration rate is at the upper limit of normal, but albumin excretion remains in the normal range. The development of microalbuminuria is often termed “incipient nephropathy”. These patients have a Urine albumin excretion rate (UAER) between 30 and 300mg/day. This occurs approximately 5 to 15 years after the diagnosis of diabetes. Glomerular filtration rate remains within the normal range.

Twenty four hour urine collections have long been, and remain the gold standard for assessment of urine protein or albumin excretion. However they are cumbersome for patients to perform and are often inaccurately collected or incomplete. More recently the level of albumin has been defined by measurement of an Albumin : Creatinine ratio (ACR). The urinary albumin : creatinine ratio is measured using the first morning urine sample where practicable and is a much simpler test for patients to provide a sample for.

Microalbuminuria is diagnosed when there is an albumin: creatinine ratio of:

≥ 2.5 mg/mmol (men) or ≥ 3.5 mg/mmol (women).

Proteinuria is diagnosed by an albumin: creatinine ratio of ≥ 30 mg/mmol.

As the disease progresses there is an increase in albuminuria, along with a progressive fall in glomerular filtration rate and a rise in blood pressure. The decline in GFR is generally quite predictable in a particular patient but varies quite considerably between patients. In untreated diabetic nephropathy the mean rate of decline is 5-10 ml/min/year but the overall range is quite considerable with a rate between 1 and 20 ml/min/year. The patients that decline more rapidly are more likely to have poorer glycaemic control, higher blood pressure, higher albumin excretion rates, hypercholesterolemia and are more likely to be smokers [35]. Overt nephropathy develops between 10 and 15 years after the diagnosis of diabetes and is defined as a UAER of >300mg/day, also termed "proteinuria". This is equivalent approximately to a 24 hour urine protein excretion of 0.5g/24 hours.

In the group of patients who develop nephropathy, end stage renal failure occurs between 15 and 30 years after the diagnosis of diabetes has been made, normally some 5 years after the onset of proteinuria [36]. There is however a state of flux between the stages of diabetic related renal disease. For example, some of the patients who progress to have microalbuminuria can revert back to the normal state.

Approximately 20 years after a diagnosis of type 2 diabetes has been made 25% of those patients will have nephropathy. Of those patients, 20% will require renal replacement over the next 10 years. Type 2 diabetics make up a significantly higher proportion of patients with nephropathy compared to Type 1 diabetics, as type 2 diabetes is 10 to 15 times more common [35].

1.6.3.1 Pathology

The changes within the kidney are the same whether the underlying diagnosis is either type 1 or type 2 diabetes.

The normal kidney consists of approximately 1-1.5x10^6 nephrons, the basic functioning unit of the kidney. A nephron is made up of a glomerulus, which possesses an afferent arteriole, efferent arteriole and a capillary tuft, and a number of tubules lined with epithelial cells. The glomeruli occupy the outer part of the kidney referred to as the cortex and the tubules traverse from the cortex to the inner part of the kidney referred to as the medulla. Tubules in turn become collecting tubules and drain urine in to the renal pelvis [37].

The earliest histopathological change seen is renal hypertrophy, with kidney mass increasing by approximately 15%. Renal hypertrophy is thought to be due to tubular hypertrophy and hyperplasia and an expansion of the tubulointerstitium.

Thickening of the glomerular basement membrane is a common feature of diabetic nephropathy and is almost always a feature of the disease in patients with a history of diabetes of more than 15 years [36]. The thickening of the basement membrane progresses through the course of disease in to the later stages of diabetic nephropathy [38]

Expansion of the mesangium within the Bowman's capsule leads to the pathological hallmark of diabetic nephropathy. This nodular glomerular lesion was originally described by Kimmelstiel and Wilson in 1936. These nodules on light microscopy are well-demarcated, eosinophilic, Periodic acid-Schiff (PAS) positive masses located in the central regions of peripheral glomerular lobules. They are virtually pathognomic, but do not necessarily have to be present to make a diagnosis of diabetic nephropathy. The prevalence of these lesions on renal biopsies occurs in about 20% [38] but in some series suggest they may be present in up to 47% [39].

Diffuse glomerular lesions are more frequent than nodular lesions and occur in approximately 25-50% of patients with type 2 diabetes for more than 10 years. The incidence is higher in patients with type 1 diabetes and nephropathy, at over 90%. These diffuse lesions involve expansion of the mesangium due to accumulation of

matrix. As the disease progresses, glomeruli become progressively sclerosed. This occurs as a result of mesangial expansion and from ischaemic injury. This ischaemic injury is either as a result of hyalinosis of the afferent arteriole, resulting in a reduction in glomerular blood flow or as a result of the mesangial expansion causing capillary occlusion [40]. Hyaline material progressively replaces the entire arteriolar wall of both efferent and afferent arterioles and is a common feature of diabetes. Involvement of the efferent arteriole is a highly specific finding to diabetic nephropathy, in contrast to the changes seen in hypertension in which only the afferent arteriole is affected. Hypertensive changes in the kidney often co-exist in patients with type 2 diabetes.

Early data suggested that it was the glomerular abnormalities that resulted in progressive renal disease, but study data now suggests that the rate of decline in renal function is more closely related to the degree of tubulointerstitial damage rather than that of glomerulosclerosis [41, 42]. Although the aetiologies of progressive renal diseases such as chronic glomerulonephritis and diabetic nephropathy are different there is a final common pathway that results ultimately in glomerulosclerosis, tubular atrophy and interstitial fibrosis as a result of accumulation of extracellular matrix. An inflammatory cell infiltrate is seen within the tubulo-interstitium which is similar to that seen in many other chronic glomerulonephritides. This infiltrate consists of macrophages and monocytes [37].

1.7 Pathogenesis of Diabetic Nephropathy

The underlying cause for the pathological findings described above is multifactorial and are described below.

1.7.1 Glycaemia

In both type 1 and type 2 diabetes it has been shown in observational studies that poor glycaemic control is a significant risk factor for the development of nephropathy. Hyperglycaemia, or more specifically an elevated glycosylated haemoglobin (HbA1c)

has been shown to correlate with a reduction in GFR in patients with type 1 diabetes [43] and the development of proteinuria in patients with type 2 diabetes [44].

Hyperglycaemia is associated with mesangial cell proliferation, mesangial cell matrix production and mesangial cell apoptosis. Mesangial cells play an important role in modulation of glomerular filtration. Mesangial cell expansion seems to be in part as a result of an increase in mesangial cell glucose concentration [45].

Thickening of the glomerular basement membrane is not directly related to the duration of diabetes, urinary albumin excretion or hypertension, but to hyperglycaemia [46].

1.7.2 Hypertension

Diabetes and hypertension occur together at a frequency higher than one would expect by chance. Patients with diabetes are at increased risk of developing hypertension and it is an important factor in both the development of, and progression of diabetic nephropathy. As a result control of hypertension is an important target for treatment.

Both type 1 and type 2 diabetics have an increased incidence of hypertension, it being most marked in type 2 diabetics however, presenting earlier as part of the metabolic syndrome. It has been shown in the Pima Indians, a group at increased risk of diabetes, that blood pressure before the onset of diabetes predicted the UAER after the onset of type 2 diabetes [47]. The Hypertension in Diabetes Study studied 3648 newly diagnosed type 2 diabetic patients found that 39% of patients were hypertensive, or on antihypertensive medications, 2 and 9 months after the diagnosis of type 2 diabetes. They also noted that this same group of patients were more at risk of a cardiovascular event prior to the diagnosis of diabetes [48].

Not only is the risk of developing hypertension increased in a diabetic population but the hypertension is also independently associated with a progressive decline in GFR. In a cohort of 41 type 1 diabetic patients with persistent microalbuminuria, the rate of decline in GFR rose with increasing diastolic blood pressure with an r value of 0.52 ($p<0.0001$) [49]. Hovind et al demonstrated in a population of type 1 diabetics, that an increase in mean arterial blood pressure, systolic or diastolic blood pressure was significantly associated with a worsening of GFR [50]. In a study of 227 type 2 diabetic

patients, a rise in systolic blood pressure of more than 10mmHg was associated with an increased rate of decline in GFR [51]. The occurrence of hypertension in a diabetic individual also increases the risk and accelerates the course of cardiac disease, peripheral vascular disease, cerebrovascular disease and retinopathy [52].

The following three major factors are thought to contribute to the development of hypertension.

- Hyperinsulinaemia

Randeree at al retrospectively studied 80 patients with type 2 diabetes, whom had failed to reach target glucose levels with dietary measures and oral hypoglycaemic agents. After the commencement of insulin, they noted a statistically significant rise in both systolic (131.8 ± 1.7 to 148 ± 1.9 mm Hg, $p<0.05$) and diastolic (80.9 ± 0.9 to 89.2 ± 1.0 mm Hg, $p<0.02$) blood pressure. A control group showed no such rises [53]. The underlying pathophysiology of this is not well understood and has not been demonstrated in all studies. The rise in blood pressure may be related to the associated weight gain caused by hyperinsulinaemia or due to an inherent prohypertensive effect of insulin itself.

- Intravascular volume expansion

It is accepted that sodium retention has a role in the pathophysiology of hypertension in humans. Sodium reabsorption occurs in the kidney, by reabsorption at the distal or proximal tubule, as a result of increased serum glucose and insulin concentrations. Insulin plays a role in the transportation of cations across the cell membrane resulting in increased levels of intracellular sodium. Nosadini et al showed that impaired insulin sensitivity is associated with sodium retention and that insulin resistance is linked with slight elevations in blood pressure and albumin excretion rates [54]. The effect of salt retention on hypertension can be reversed by dietary salt restriction.

- Arterial stiffness

Cruickshank et al studied a population of 397 type 2 diabetic patients and a control group for 10 years. Brachial blood pressure was studied and measures of vascular stiffness were obtained using doppler derived aortic pulse wave velocity (PWV). Mean systolic but not diastolic blood pressure and aortic PWV rose progressively with declining glucose tolerance. Pulse wave velocity was higher, indicating less compliant arteries, in the diabetic population for any given systolic blood pressure. This was associated with a doubling in mortality risk (hazard ratio 2.34, 95% CI 1.5 to 3.74) [55].

1.7.3 Proteinuria

Work initially carried out in animal models suggested that proteinuria was not only a marker of renal disease in diabetes but may actually be responsible for causing progression of the disease.

This has now been confirmed in humans with diabetes mellitus. Early work published by Rossing et al showed that in patients with type 1 diabetes there was a significant correlation between rate of decline in GFR with both diastolic blood pressure and albuminuria [49]. More recent studies have confirmed these findings. Hovind et al have shown in a population of type 1 diabetics that albuminuria has a significant positive correlation with decline in GFR. Univariate analysis in this study of 301 patients, showed that albuminuria had a correlation with decline in GFR of $r=0.41(p<0.001)$ [50]. This relationship also seems to be true of patients with type 2 diabetes. In an average follow up time of 6.5 years, both higher baseline albuminuria and elevated mean albuminuria were associated with a significantly higher rate of decline in renal function [51]. Parving et al have shown that patients with the lowest rates of urine albumin excretion had a rate of decline in GFR less than half that of patients with higher levels of albumin excretion [56]. This data is also supported by the UKPDS 64 study that studied 5097 patients with type 2 diabetes. This showed that patients progressed from a no nephropathy group, to microalbuminuria, to macroalbuminuria to a raised plasma creatinine or RRT at rates shown below [57]. The effect of treatments targeted at urine protein excretion are discussed in Chapter 1.10.3.

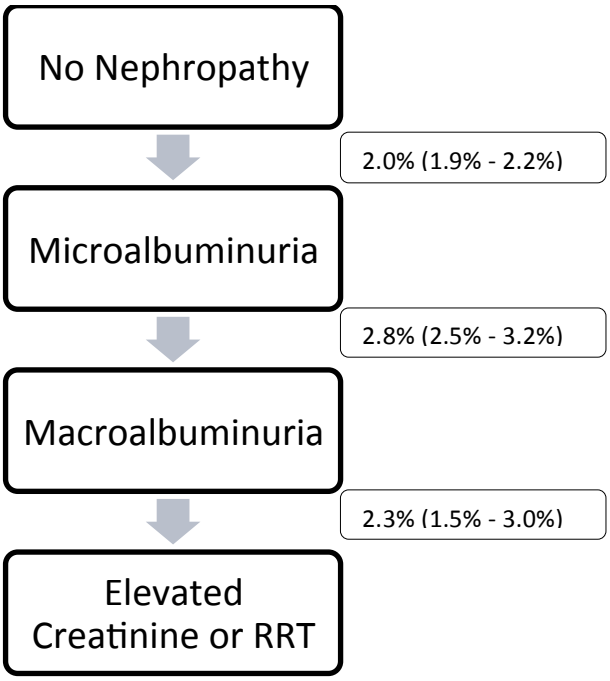


Figure 1-3. Annual Transition Rates (95% CI)[57]

Excessive and sustained protein trafficking has been shown experimentally in both animals and humans to result in progressive renal parenchymal damage [58]. In animal models a reduction in the total nephron mass sets up a series of adaptive events to maintain renal function. The resultant haemodynamic changes are potentially damaging in the long term. As a result of increased glomerular pressure, proteins are filtered and reach the proximal tubule. These proteins then need to be reabsorbed from the proximal tubule and this can lead to interstitial injury by activating intracellular events, including upregulation of vasoactive and inflammatory genes. As a result an interstitial inflammatory reaction is set up which precedes any renal scarring but correlates with a decline in renal function [59].

Albuminuria has therefore become a target for the treatment of diabetic nephropathy and will be discussed later in this chapter.

It has long been recognised that both microalbuminuria and proteinuria are significant independent cardiovascular risk factors, associated with an increase in mortality and morbidity [60, 61]. However, the concept of there somehow being a difference between microalbuminuria and overt proteinuria in terms of progression of renal disease and its associated complications is now being challenged. Ruggenenti et al have shown that the

risk of progression of kidney disease is a continuum even within the nomoalbuminuric range and suggest that we should abandon the concept of microalbuminuria and should view levels of albuminuria in a similar fashion to levels of cholesterol [62]

1.7.4 Genetic susceptibility

The level of risk of developing diabetic nephropathy cannot be explained solely by duration of diabetes or hypertension or by the adequacy of glycaemic control. The chance of developing diabetic nephropathy is significantly increased in individuals who have a sibling or parent with diabetic nephropathy [45, 63]. Further evidence comes from Pettitt et al who have demonstrated that in Pima Indian families with two successive generations of members with type 2 diabetes the risk of the offspring developing proteinuria was 22.9% if one parent had proteinuria and 45.9% if both had proteinuria [64]. Genetics must therefore have an important role.

Advances in molecular genetics and genotyping have now enabled researchers to identify susceptibility genes for microvascular complications of diabetes and for diabetic nephropathy [45]. Loci on chromosomes 3, 7, 9 and 20 have been identified in Pima Indians as being important in microvascular complications and chromosomes 7q21.3, 10p15.3, 14q23.1 and 18q22.3 (cited in [45]).

Numerous studies have looked for candidate genes involved in the development and progression of diabetic nephropathy. Candidate genes that have attracted most interest are those that involve the renin-angiotensin system. In particular the DD (homozygous deletion) polymorphism of the ACE gene has been associated with an increase risk of developing diabetic nephropathy, severe proteinuria, progressive renal failure and mortality in dialysis subjects, in patients with type 2 diabetes [45]. Similar effects have been shown in patients with type 1 diabetes [65].

Other genes encoding the aldose reductase gene, angiotensin II receptor, cytokines, proteins involved in glucose or lipid metabolism and extracellular matrix proteins have also been studied demonstrating the multifactorial nature of the genetic determinants of diabetic nephropathy [45, 66].

1.8 Morbidity and Mortality associated with diabetic nephropathy

There are significant differences in survival of diabetics who develop nephropathy, compared to those who do not. Andersen et al demonstrated that in a follow up of 1471 patients with type 1 diabetes, the 40 year survival of patients (249 patients) who had developed nephropathy was 10%, compared to those who had no evidence of nephropathy whose survival was 70% [67] (See Figure 1-4). This however was prior to the use of angiotensin converting enzyme (ACE) inhibitors, the impact of which will be discussed later.

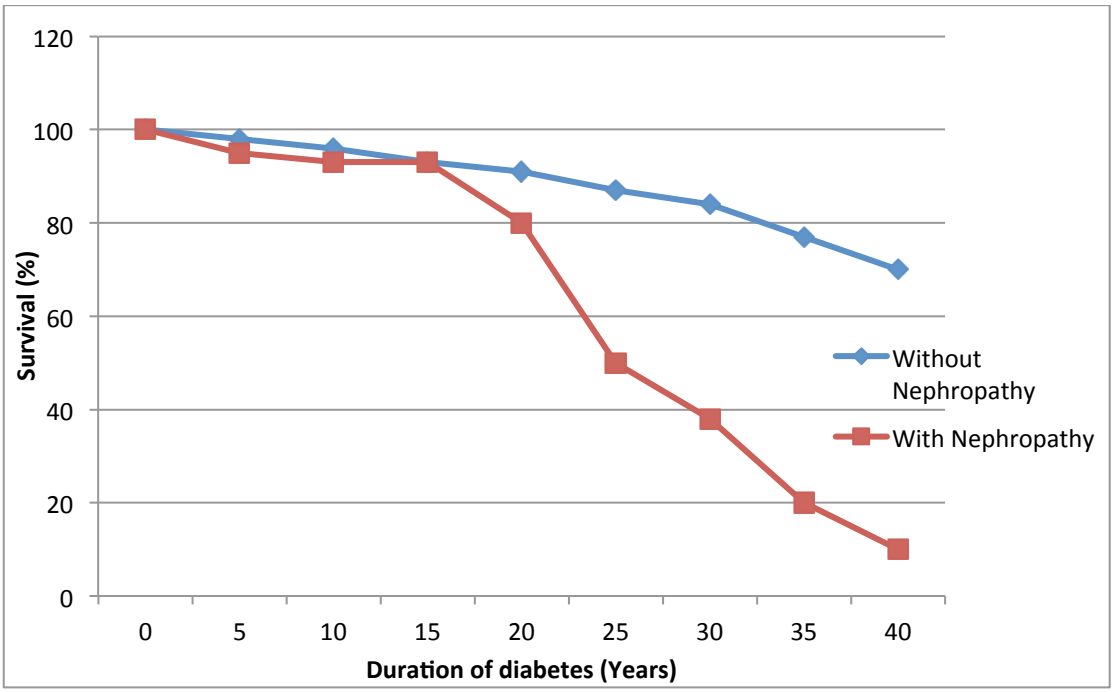


Figure 1-4. Survival Comparison with or without Nephropathy

The presence of either overt proteinuria or microalbuminuria is associated with an excess of cardiovascular mortality and morbidity. The United Kingdom Prospective Disease Study (UKPDS 64) looked at this in detail in a cohort of over 5000 patients with type 2 diabetes. As patients progressed through stages of nephropathy there was a statistically significant ($p<0.0001$) increase in the risk of cardiovascular death [57].

The annual mortality rates as shown below.

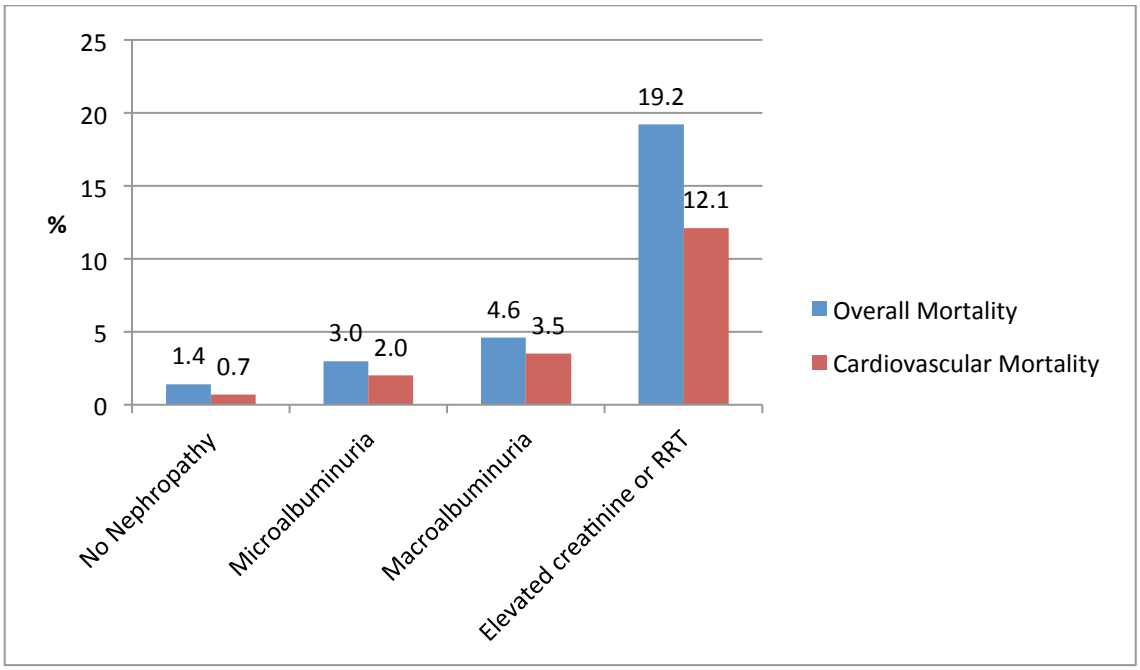


Figure 1-5.Annual Mortality Rates with progression of Diabetic Nephropathy

Gerstein studied data from the HOPE study looking at 1963 patients demonstrated to have microalbuminuria, 1140 of whom had diabetes. In the diabetic group the risk ratio for major cardiovascular events was 1.97 (1.68-2.31, $p<0.001$) and for all cause mortality was 2.15 (1.78-2.60, $p<0.001$). [68]

The hypertension that occurs in association with diabetic nephropathy is further associated with an increased mortality and morbidity, and tends to be more difficult to control than that which occurs in non-diabetic subjects [69].

The World Health Organisation Multinational Study of Vascular Disease in Diabetes looked at the increased risk of cardiovascular mortality in both type 1 and type 2 diabetes, and its relation to hypertension and proteinuria. Over 4700 diabetic patients were included in the study between the ages of 35 and 55. Standardised mortality ratios (SMR) were shown to be higher in the patients with type 1 diabetes compared to those with type 2 diabetes. SMR increased with duration of diabetes. The most significant data to emerge from the study was the increased mortality risk in those patients with both hypertension and proteinuria. In this sub group the increased risk was 11x for men with type 1 diabetes and 18x for women with type 1 diabetes compared to controls. The

risks for patients with type 2 diabetes were not as marked but still greatly increased with a 5 times increased risk for men with type 2 diabetes and 8 times for women with type 2 diabetes [61].

1.9 Economic Impact of Type 2 Diabetes and Diabetic Nephropathy

Type 2 diabetes is associated with significant morbidity and mortality which in turn carry a large financial cost.

The National Health Interview Survey indicates that people with diabetes have a prevalence of physical disability twice that of people without diabetes (66% vs. 29%; P <0.001)[70].

The overall financial burden that this brings to countries is huge although exact cost is very difficult to pin down precisely, but broadly speaking derives from three categories.

- The direct costs of diabetes care itself, such as the cost of insulin and medications. In 2005, the estimated cost in the US of diabetes is $132 billion, $92 billion of which is from direct medical costs.[71] In the UK it has been estimated that diabetes accounts for 9% of the annual NHS expenditure [72]. In 2000, this equated to about £5.2 billion.
- The costs of the complications of diabetes are harder to quantify, but arise from the cost of care of conditions such as ischaemic heart disease and peripheral vascular disease, conditions that are far more common in the diabetic compared with the general population.
- Finally, and perhaps the hardest to put a cost on are the costs associated with its impact on the ability to work and quality of life issues.

The cost to the health service attributable to diabetic nephropathy, again is difficult to quantify. Nichols et al have shown that the healthcare costs associated with diabetes increase with increasing levels of proteinuria [73]. Worldwide an estimated 61 million people undergo renal replacement therapy. Patients with diabetic nephropathy represent the fastest growing group of patients in the US receiving dialysis or requiring

transplantation. More than 100,000 diabetic patients require renal replacement therapy or transplantation each year, accounting for more than 40% of the new cases of end stage renal disease[4]. The UK Renal Registry Data shows that the commonest specific cause of patients starting renal replacement therapy is diabetes. Of all adults commencing renal replacement therapy in 2005, 20% had diabetes as their cause of renal failure. This figure has continued to rise and 2010 Renal Registry data shows that 25.3% of patients have diabetes as their primary cause of end stage renal failure [74, 75]

In the UK there are 49,080 patients undergoing renal replacement therapy. This is distributed amongst the modalities shown in Table 1-1 [74]. The cost of established renal failure (ERF) to the UK NHS amounts to approximately 1-2% of the entire NHS budget, despite ERF only representing 0.05% of the population [76]. For reference the annual costs of haemodialysis and of continuous ambulatory peritoneal dialysis (CAPD) are £35,032 and £15,570 respectively [76].

Haemodialysis	Peritoneal Dialysis	Dialysis	Transplant	Renal Replacement
21,850	3,946	25,796	23,284	49,080

Table 1-1. UK prevalent patients per treatment modality – 2009

1.10 Current Therapeutic Options

The current consensus is that patients with persistent proteinuria will inevitably progress to end stage renal failure if they survive the associated increased cardiovascular risk. Treatment options for diabetic nephropathy are limited and are targeted at both slowing progression of renal disease principally by control of protein excretion and control of blood pressure. It is also important to address any associated cardiovascular risk factors. Current strategies can be viewed in terms of primary, secondary and tertiary prevention or at the targets of treatment.

- Primary prevention will be those treatments targeted at diabetic patients who have not yet developed nephropathy
- Secondary prevention is those treatments aimed at patients who have early signs of being at risk for developing overt nephropathy, such as those with microalbuminuria.
- Tertiary treatment is those treatments in patients who already have nephropathy and aim to reduce or slow progression to end stage renal failure.

1.10.1 Control of blood glucose

There is evidence to show that intensive control of blood glucose reduces the risk of developing incipient nephropathy. The Diabetes Control and Complications Trial (DCCT) was set up to look at both primary and secondary prevention of renal complications in patients with type 1 diabetes. There was a reduction in albumin excretion of 15% ($p<0.001$) after one year of intensive treatment of blood glucose and a mean adjusted reduction in risk of the cumulative incidence of microalbuminuria of 34% ($p=0.04$). In the secondary prevention group, consisting of patients with early complications of diabetes, there was a reduction of mean adjusted risk of cumulative incidence of microalbuminuria of 43% ($p<0.0001$). The risk of developing a more advanced level of microalbuminuria (>70µg/ml) was also significantly reduced [77].

One of the largest studies specifically designed to look at the impact of glycaemic control on the complications in type 2 diabetes is the UKPDS 33 (United Kingdom Prospective Diabetes Study). Patients were randomised to either intensive treatment (sulphonylurea or insulin) or the conventional therapy group (diet control alone) Although not specifically designed to look at progression of albuminuria, development of microalbuminuria was reduced in the intensive treatment group with a relative risk reduction of approximately 0.8 for each of the 3 year study periods. This reduction in progression of albuminuria seems to have been accompanied by a reduced risk for the development of renal failure (Serum creatinine >250 µmol/l or dialysis) [78].

Shichiri et al, showed in a population of 110 Japanese type 2 diabetic patients that intensive control of blood sugar, compared to standard treatment, showed lower rates of progression of nephropathy (microalbuminuria to macroalbuminuria) in the intensively treated group [79].

There have however been some more recent studies that have shown a possible higher mortality in patients with intensively controlled blood glucose. One such study, the ACCORD study published in 2008, showed an increase in mortality in a population of patients with type 2 diabetes randomised to an intensive glucose control treatment group with no reduction on major cardiovascular events [27].

Partly as a result of the concern raised in the ACCORD trial, Hemmingsen et al carried out a meta analysis. Despite the number of patients included, from 14 different trials, the authors were not able to either refute or confirm the fears of an increased cardiovascular mortality in patients with type 2 diabetes, randomised to intensive blood glucose lowering. The analysis failed to show any reduction in the risk of progressive nephropathy [80].

The choice of hypoglycaemic agent may play a role in the reduction in progression of nephropathy. In a 2005 meta analysis of trials using metformin it was found that patients on metformin monotherapy had improved outcomes for glycaemic control, weight, dyslipidaemia and diastolic blood pressure. In obese patients allocated to intensive blood glucose control using metformin, outcomes were better when compared to chlorpropamide, glibenclamide or insulin, for any diabetes related outcome and all cause mortality, and greater benefits than what was seen overweight patients [81]. Rosiglitazone has now been shown in several studies to reduce albuminuria, although some of this effect may have been as a result of rosiglitazone's apparent effect on reducing blood pressure. Two studies, one comparing rosiglitazone to glyburide and the other comparing these two drugs in addition to metformin, showed an increased reduction in urine albumin excretion in the rosiglitazone treated group compared to glyburide [82, 83].

The ADVANCE Trial aimed to see if intensive glycaemic control could improve major macrovascular and microvascular events including new or worsening nephropathy.

Patients were randomised to either a “standard” treatment group or an intensive treatment group to lower blood glucose to 6.5% or lower. After 5 years of follow up, there was a significant reduction in combined major micro and macrovascular events. There was also a reduction in microvascular events alone, largely attributable to the reduction in incidence of nephropathy. There was a reduction in new or worsening nephropathy. The reduction in development of microalbuminuria accounted for much of this improvement. There was also a reduction in new onset microalbuminuria (HR 0.91, p=0.02). There was no reduction in the doubling of serum creatinine [26].

By the time that overt diabetic nephropathy has developed, particularly in type 2 diabetics, there is little evidence to show that tight glycaemic control affects renal outcome, although it may impact on other microvascular complications [56]. Wu et al showed that poor diabetic control prior to dialysis was associated with worse outcomes including mortality and cardiovascular morbidity [84].

1.10.2 Treatment of Hypertension

Overwhelming evidence shows that treatment of hypertension slows the progression of diabetic nephropathy and that it reduces albuminuria.

Hypertension is closely linked to the development of diabetic nephropathy and has an impact on the rate of progression to overt nephropathy as previously discussed. Hypertension also tends to be more difficult to control in this population and is associated with a higher mortality than in a population without diabetes.

Early work carried out by Parving et al in patients with Type 1 Diabetes clearly showed that by reducing blood pressure, using metoprolol, hydralazine and frusemide (or a thiazide), reduced the urine albumin excretion and also the rate of decline in GFR [85, 86].

The UKPDS 38 (United Kingdom Prospective Diabetes Study) [87] demonstrated that strict blood pressure control, using captopril or atenolol, to an average of 144/88 mm Hg, when compared with less tight control (154/87 mmHg) lead to a 30% reduction in microvascular complications including nephropathy. The UKPDS 39 studied 1148 patients with type 2 diabetes and hypertension. They were again randomised to

captopril or atenolol. They showed that captopril and atenolol lowered blood pressure equally effectively. They were also both equally effective at reducing microvascular endpoints that included retinopathy and development of albuminuria [88]. This early study in to the effects of antihypertensives therefore suggested that it was the treatment of hypertension that prevented the development of albuminuria and not a specific characteristic of the medication used, a fact that would be debated and disproven in years to come and demonstrated in the next chapter.

The current targets for the treatment of hypertension in this population are set lower than the normal population at <130/80 mmHg, reflecting the need for aggressive treatment [89]. The latest Chronic Kidney Disease guidelines from the National Institute for Health and Clinical Excellence (NICE) published in 2008 recognise the fact that many guidelines, including the British Hypertension Society's, suggest a target blood pressure of <130/80 mmHg. However, NICE go on to explore possible negative cardiovascular outcomes if blood pressure is lowered too far (<120 mmHg systolic) and therefore suggest a target blood pressure of <130/80 mmHg in patients with diabetes and CKD or those with a urine PCR>100 mg/mmol and a blood pressure of 140/90 mmHg in those without significant proteinuria [90].

1.10.3 Control of proteinuria

Control of proteinuria and control of hypertension are closely linked. There is evidence to show that control of proteinuria and hypertension by blockade of the renin angiotensin system has benefits over and above that seen in control of hypertension with other classes of drugs.

The first such work carried out by Lewis et al showed a renoprotective effect of captopril independent of its effect on blood pressure, as evidenced by a significant reduction in risk of doubling of serum creatinine in patients with Type 1 diabetes. The reasons for this renoprotective effect were not understood at the time but in the authors' discussion they explore that amelioration of the proteinuria may have been linked to the improved renal survival [91].

One of the first primary prevention studies was carried out by Ravid et al in type 2 diabetic patients with normoalbuminuria and normotension. It was shown that during a 6 year follow up of 156 patients, transition to microalbuminuria occurred in 19% of placebo recipients compared to 6.5% of enalapril recipients [92].

In a meta-analysis by the ACE Inhibitors in Diabetic Nephropathy Trialist Group, of 12 placebo controlled trials, ACE inhibitors have been shown to reduce the risk of progression from microalbuminuria to proteinuria in patients with type 1 diabetes. In those patients receiving an ACE inhibitor there was a decreased risk of progression from microalbuminuria to proteinuria with an odds ratio of 0.38 (CI 0.25 to 0.57). At 2 years the albumin excretion rate was 50.5% lower in the treatment group ($p<0.001$), but this was compared to placebo. When this was adjusted for change in blood pressure there was still a significant change in albumin excretion, that was 45.1% lower than the placebo group[43, 93].

The RENAAL Study [94] investigated renal and cardiovascular outcomes in type 2 diabetics with nephropathy using the angiotensin II receptor antagonist (ARB) Losartan. Proteinuria was reduced by 35%. This was associated with a risk reduction of 16% in reaching primary endpoint (doubling of serum creatinine, reaching end stage renal disease or death) and a 25% risk reduction in doubling of serum creatinine alone.

The Irbesartan in Diabetic Nephropathy Trial (IDNT) looked at type 2 diabetic patients with hypertension and established nephropathy. Proteinuria was reduced by 33% in the Irbesartan group compared to a reduction of 10% and 6% in the placebo and amlodipine groups respectively. Compared to either placebo or amlodipine there was a risk reduction of 33% and 37% respectively in doubling of serum creatinine, and a 23% reduced risk of developing end stage renal disease compared to either group [95].

The MICRO-HOPE study showed that the use of ramipril reduced the risk of progression from microalbuminuria to overt nephropathy (proteinuria) by 24% in diabetic patients compared to placebo. It also lowered the primary outcome risk of myocardial infarction, stroke or cardiovascular death by 25% [96].

Most recently researchers from the PREVEND Study Group have shown in an observational study that the efficacy of blood pressure lowering agents in reducing cardiovascular events is dependent on the baseline level of urine albumin excretion, the

higher the level of albuminuria the greater the risk reduction in cardiovascular events. The results also suggest that drugs that affect the renin angiotensin system such as ACE inhibitors and ARBs may have an even more beneficial effect than other classes of antihypertensive [97].

1.10.4 Modification of serum cholesterol

Despite the potential link between lipids and diabetic nephropathy there is as yet no good trial data to show that reduction of serum lipids impacts on the development or progression of diabetic nephropathy. The renoprotective effects of HMG CoA reductase inhibitors in patients with either type 1 or type 2 diabetes, with microalbuminuria or macroalbuminuria is highly variable [43].There is some data from the Heart Protection Study that suggests simvastatin use was associated with a smaller fall in estimated glomerular filtration rate compared to placebo in diabetic patients. Treatment with simvastatin was associated with a smaller fall in eGFR (5.9ml/min in simvastatin group vs 6.7ml/min placebo in group, p=0.0003). Although a small difference it was greater in those with diabetes than in those without [98]. The TNT study, using atorvastatin, showed that after a period of 5 years, there was a statistically significant increase in eGFR. However this study was not carried out purely in patients with diabetes [99]. It is well recognised that HMG-CoA reductase inhibitors have anti-inflammatory effects and the possible improved renal outcomes in these trials may not be as a direct result of lipid lowering but associated with these anti inflammatory properties.

1.10.5 Non-pharmacological interventions

Empowering patients to make a difference to their health can be an important mechanism to improve outcomes [100]. Several approaches are available and discussed in the following chapters.

1.10.5.1 Smoking cessation

Smoking is universally accepted as a major risk factor for the development of cardiovascular disease and that cessation of smoking reverses a significant amount of that risk with time. The data in patients with diabetes has been mixed, with some trials failing to show an association between smoking and poor cardiovascular outcomes in diabetic smokers [101, 102]. There is now good evidence from a large meta analysis to show that there is an increase in cardiovascular mortality in diabetic smokers compared to non smoking diabetics with a risk ratio of 1.36 [103].

There is also an as yet unexplained association between smoking and the risk of developing type 2 diabetes. A meta analysis by Willi et al demonstrated an increased risk in all groups of smokers, with a higher risk in heavy smokers (>20 cigarettes per day, RR 1.61, 95% CI 1.43-1.80) compared to lighter smokers (<20 cigarettes per day, RR 1.29, 95% CI 1.13-1.48) [104].

Christiansen [105] first proposed it as a risk factor for diabetic nephropathy in 1978. Several epidemiological and pathophysiological studies now support the association of smoking with both the onset and progression of diabetic nephropathy in patients with Type 2 diabetes [106]. In 1990, Ekberg et al studied a population of type 1 diabetics and showed that GFR was negatively related to the number of cigarettes smoked per week [107].

Smoking is a promoter of progression of proteinuria in patients with Type 1 or Type 2 diabetes [51, 108]. Sawicki et al showed in a population of type 1 diabetics, progression of nephropathy was less common in non smokers compared to smokers [109].

Not only does smoking increase the risk of progression of renal disease in both diabetic and non-diabetic renal disease, but also in those populations with high cardiovascular risk, and normal renal function, such as the elderly, those with diffuse atherosclerosis and severe hypertension [110].

There is evidence to support cessation of smoking leads to some improvement in renal disease or slowing of progression. Progression of renal disease in patients with type 1 diabetes has been shown to slow in patients who have stopped smoking, and that albuminuria decreases significantly in patients with type 1 diabetes in those who have

stopped smoking (cited in [111]). In a study of 91 patients with type 2 diabetes it was shown that quitting smoking reduced progression to macroalbuminuria and reduced decline in eGFR [112].

1.10.5.2 Modification of protein intake

Dietary manipulation may also impact upon urine albumin excretion and progression of nephropathy.

Gross et al studied 28 patients with type 2 diabetes. They were given either a chicken based diet or a low protein diet, in a crossover design. Results showed that the patients on the chicken diet and low protein diet had lower isotope GFRs compared to the normal diet and patients taking the chicken diet had reduced urine albumin excretion rates compared to the low protein and normal diets (34.3 µg/min vs 52.3µg/min vs 63.8µg/min – $P<0.05$) [113].

There have been concerns though that in restricting protein intake in these patients, there is a risk of these patients becoming malnourished. A Cochrane review, carried out in 2009 included patients with both type 1 and type 2 diabetes. It showed that a non-significant reduction in decline of GFR in type 1 diabetics and one trial in type 2 diabetics demonstrated a small non significant improvement in the rate of decline in GFR. No data was given on level of proteinuria. One study demonstrated the development of malnutrition [114].

Given the lack of evidence that dietary protein restriction has any benefits in diabetes and that there are concerns over it causing malnutrition it is not currently a recommended treatment strategy.

1.10.5.3 Weight loss and Exercise

Many patients with type 2 diabetes are overweight and it is well recognised as a major risk factor for the development of type 2 diabetes.

Both obesity and low fitness levels are associated with worse cardiovascular outcomes. Church et al have shown significantly higher cardiovascular mortality in diabetic patients (type 1and 2 included in the study) with a low fitness level and increasing levels of obesity compared to those with a normal weight and high fitness level (HR 2.8 in the class 1 obese group) [115].

Obesity in diabetes is associated with early onset of glomerulopathy and increased albuminuria [116]. Weight loss by diet has been shown to stabilise renal function and reduce urinary protein excretion in non diabetics and in patients with diabetes [117]. There is currently no good evidence to show that this translates in to improved outcomes such as slowing progression to end stage renal disease [115].

1.10.5.4 Dietary Salt restriction

A recent Cochrane review on dietary salt restriction has improved our understanding of its role [118]. Sodium restriction has been recognised as an important factor in improving blood pressure control in the general population.

By reducing salt intake to an average of 8.5 g/day, blood pressure was reduced in patients with type 1 or type 2 diabetes by 7/3 mmHg. Blood pressure control was improved by an average of 6.9/2.87 mmHg in patients with type 2 diabetes. No convincing change in renal function (Creatinine clearance or GFR) or ERPF was shown. One cited study demonstrates that dietary sodium restriction in type 2 diabetic subjects on Losartan reduced urinary albumin excretion, although this may in part have been as a result of improved blood pressure control in that group [119].

Public health guidelines recommend restricting dietary salt intake to less than 5-6g per day and in patients with diabetes to a level at least as low as this although firm evidence to support a further reduction does not exist [118].

1.11 Dietary fibre intake

My interest in this thesis is focussed on another aspect of dietary manipulation, that of dietary fibre. Dietary fibre as a term was adopted between 1972 and 1976 by Burkitt et al, and Trowell and Painter, but was originally described by Hipsley in 1953 [120]. The term was used to describe the remnants of plant components that were not broken down by hydrolysis in the gastrointestinal tract. Dietary fibre received a formal definition in December 1999

> *"Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and / or blood cholesterol attenuation, and / or blood glucose attenuation"[121]*

The term 'dietary fibre' is commonly defined as plant material that resists digestion by the secreted enzymes of the human alimentary tract but which may be fermented by microflora in the colon [120]. As such, it includes most hydrocolloids, which are hydrophilic polymers, of vegetable, animal, microbial or synthetic origin. These are naturally present or added to control the functional properties of foodstuffs. Hydrocolloids include agar, gelatin, guar gum, locust bean gum and of interest to this thesis, gum arabic.

1.12 The health benefits of dietary fibre

There is a lot of epidemiological data to support that dietary fibre intake has many health benefits. Much of the research in to the use of dietary fibre has specifically looked at its beneficial effects on bowel health. There is an increasing number of studies published looking at additional effects such as lipid lowering, reduction of

cardiovascular risk and on improving blood sugar control in diabetics. These will be discussed in the following chapters.

1.12.1 Fibre and Colorectal Cancer

Colorectal cancer is a major cause of cancer death in the developed world. Hereditary factors, lifestyle and diet all contribute to the disease process. Fibre from grain, cereals and fruit has been shown to reduce the risk of colorectal adenoma [122]. Dietary fibre intake was assessed in a total of 33,971 participants of the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer screening trial. The risk of colorectal adenoma was inversely related to dietary fibre intake. Participants in the highest quintile group of dietary fibre intake, had a 27% lower risk than those in the lowest quintile of fibre intake.

The European Prospective Investigation into Cancer and Nutrition (EPIC) [123] looked at the association between dietary fibre intake and colorectal cancer incidence. The authors demonstrated a hazard ratio for colorectal cancer for those with the highest quintile intake of dietary fibre, of 0.75 (95% CI 0.59-0.95). They also showed a trend in reduction in hazard ratio across the quintile groups of dietary fibre intake showing an 8% reduction in risk for each quintile increase in dietary fibre.

However, not all studies have confirmed the association between increased fibre intake and a reduction in colorectal cancer risk. A pooled analysis of 725,628 individuals from 13 studies did show the associated reduction in colorectal cancer risk with increasing fibre intake but it failed to show a statistically significant association when other risk factors were taken into account except at very low dietary fibre intake [124].

1.12.2 Fibre and risk of developing Type 2 Diabetes Mellitus

In three prospective studies that looked at over 160,000 men and women, studying the relationship of whole grain or cereal-fibre intake, the risk for incident type 2 diabetes was markedly lower in those with a higher fibre intake. In two of the studies, that

focussed on health care professionals, the risk for incident type 2 diabetes was 21-27% lower in the highest quintile of whole-grain intake and 30-36% lower in the highest quintile of cereal fibre intake even when adjusted for lifestyle factors – i.e. physical activity, BMI, waist : hip ratio, smoking, alcohol intake and education [125].

The Nurses Health Study demonstrated a reduction in the incidence of type 2 diabetes of 28% (95% CI 0.53, 0.71, $p<0.0001$) in the group with the high intake of wholegrains over a 10 year follow up period. There was however an associated increased risk of developing type 2 diabetes with increasing quintile intake of refined grain, with a hazard ratio of 1.31 (95% CI 1.36, 1.82, $p<0.0001$) [126]. Similarly, the Health Professionals Study showed that after risk factor adjustment, the relative risk of type 2 diabetes was 0.58 (95% CI: 0.47, 0.70; P for trend < 0.0001) comparing the highest with the lowest quintile of whole-grain intake [127].

The underlying mechanism by which an increased intake of wholegrain may translate in to a decreased risk of developing Type 2 diabetes is not fully understood but there are several accepted hypotheses.

Whole grain products contain more viscous fibre than refined grain products and are digested more slowly. Increased dietary fibre intake has been shown to decrease postprandial glucose and insulin levels in diabetic and non diabetic subjects. Ordinarily, higher levels of postprandial glucose results in increased pancreatic β-cell production of insulin. This hyperinsulinaemia will maintain glucose homeostasis but with time the β-cells become unable to keep up with demand and clinical diabetes results.

Dietary fibre may play a role in reducing oxidative stress that has been associated with reduced insulin dependent glucose disposal and diabetic complications [126]. During the refining process of wholegrains, not only is the outer bran layer removed, but various individual antioxidants, nutrients, and phytochemicals, as well as the interactions between them are also removed which may go some way to explaining the differences between refined grain and whole grain.

1.12.3 Fibre and Effects on Blood Glucose in diabetic subjects

One of the major ways that dietary fibre may impact upon blood glucose levels is by influencing the rate and degree of starch degradation within foods. Soluble fibre in particular has been shown to decrease the rate of starch breakdown and also the rate at which glucose is absorbed [128].

Other than its effect on lowering the risk of developing diabetes there are several papers showing that an increase in dietary fibre intake (and foods with a low glycaemic index) lowers post prandial glucose levels and HbA1c in diabetic and non diabetic populations [129-131].

Data from a meta-analysis of fifteen studies investigated patients with type 2 diabetes randomised to either an increased dietary fibre intake or placebo. Results show a reduction in fasting blood glucose of 0.85 mm/L and a mean decrease in HbA1c of 0.26% [132].

Guar gum is extracted from the Guar bean, and is used extensively in the food industry. It has also been used as a soluble dietary fibre to reduce post-prandial glucose absorption. The method by which either the addition of a fibre such as Guar gum, to the diet, or by actually adding it to food products such as pasta and breads reduces glucose absorption is not fully understood. Brennan suggests that in part it is due to their viscosity altering ability in the small intestine but also by altering the structure of foods and making them less amenable to breakdown by amylase, and therefore effectively reducing their glycaemic index [128].

1.12.4 Fibre and Lipids

Soluble fibres are effective in reducing serum cholesterol by both reducing absorption of fat and cholesterol from the small intestine and absorption of bile acids from the terminal ileum [133]. Another potential mechanism by which serum cholesterol levels may be lowered is as a result of colonic fermentation of dietary fibre. Illman et al showed that in a rat model, feeding with oat bran (a soluble fibre) raised levels of serum

short chain fatty acids, in particular propionate. Propionate in turn can inhibit hepatic cholesterol synthesis [134].

Gerhardt et al have shown that soluble fibre can reduce low density lipoprotein (LDL) cholesterol to a similar extent. In a small study of 44 subjects soluble dietary fibre reduced both total and LDL cholesterol in 78% of the individuals [135].

Similar results have been demonstrated by Haskell et al using water soluble fibres. Individuals with a total cholesterol of greater than 5.17 mmol/L were given supplements of either acacia gum as a sole or primary source, a combination of psyllium, pectin, guar gum and locust bean gums or guar gum alone for a period of 4 weeks. Acacia gum (Gum arabic) alone did not alter serum lipids, however the combination supplement lowered total cholesterol by 8.3% and LDL cholesterol by 12.4%. Similar changes were seen with guar gum [136]. In a longer term study statistically significant sustained reductions in both total cholesterol and LDL cholesterol of 6.4% and 10.5% respectively were seen in a group taking a mixture of psyllium, pectin, guar gum and locust bean gum, compared to the control group that was given acacia gum [137].

1.12.5 Fibre and Cardiovascular Disease

In a review by Liu et al, of data, again from The Nurses Health Study, 729,472 person-years of follow up were studied. Between the highest and lowest quintiles of whole grain intake there was a relative risk reduction of coronary heart disease of 33% when adjusted for age and smoking [138].

A meta-analysis of 11 studies of wholegrain fibre intake shows there is a 17% reduction in cardiovascular risk for each 10g of fibre added to the diet. Much of this effect seems to be associated with insoluble cereal fibre, but less so with soluble fruit or vegetable fibre [139, 140].

Liu et al have provided further evidence to support that a high fibre diet is cardioprotective. With over 230,000 patient follow up years, those patients in the highest, compared to the lowest quintile of fibre intake (26.3g vs 12.5g) had an adjusted relative risk of 0.79 for all cardiovascular disease, and a relative risk of 0.68 for myocardial infarction [140].

Despite the known positive metabolic effects of soluble fibre there is only limited evidence that they have the same beneficial effects on cardiovascular disease that whole grains do. This is most likely as a result of the lack of other metabolically important substances such as minerals, vitamins and antioxidants which are present in bran and the germ [139].

Chronic kidney disease (CKD) is widely recognised as an inflammatory state. CKD is an independent risk factor for cardiovascular disease [141]. An elevation of CRP in patients with CKD is a strong predictor of cardiovascular morbidity and mortality. Research has shown that a high dietary fibre intake is associated with a decrease in inflammation in the general population. [142].

By studying data from the NHANES III trial Krishnamurthy et al have shown an association and possible causal link between levels of dietary fibre intake, inflammation and mortality. This link between fibre and inflammation was demonstrated in both the general population and a population with CKD but the link with mortality was only shown in those with CKD. For each 10g/day increase in fibre intake the hazard ratio for overall mortality was 0.83 for total fibre, 0.77 for insoluble fibre and 0.67 for soluble fibre [142].

1.12.6 Fibre and Blood Pressure Control

Early trial data looking at the effects of dietary fibre showed a reduction in blood pressure in diabetic patients who were placed on a high fibre, high carbohydrate diet. However, there has been much criticism of these papers as the diets, although being high in fibre, were also higher in complex carbohydrate and lower in fat than the diets they were compared with [143].

Whelton carried out a meta-analysis of 25 randomised controlled trials to assess the effect of dietary fibre on blood pressure control. The greatest improvements in blood pressure were in those patients with existing hypertension with a reduction of 5.95mmHg (95% CI -9.50 to -2.40) in systolic blood pressure and a reduction in diastolic blood pressure of 4.20 mmHg (95% CI -6.55 to -1.85) [144].

The DASH (Dietary Approaches to Stop Hypertension) Collaborative Research Group published important data in 1997 [145]. The "combination diet" led to a reduction in systolic blood pressure of 5.5mmHg ($p<0.001$) and 3.0mmHg ($p<0.001$) in diastolic blood pressure, more than in the control group. The "fruit and vegetables diet" led to reductions of 2.8mmHg ($p<0.001$) and 1.1mmHg ($p=0.07$) respectively more than the control group. These reductions in blood pressure were seen in the first 2 weeks of commencing the diet and were maintained for the remaining 6 weeks.

The PREMIER Clinical Trial investigated the effects of combining the diet used in the DASH Trial with further lifestyle modifications and demonstrated further improvements in systolic and diastolic blood pressure [146].

In a meta-analysis of 24 randomised controlled trials by Streppel et al, fibre supplementation of 11.5g/day reduced systolic blood pressure by 1.13mmHg and diastolic blood pressure by 1.26mmHg. They hypothesise that soluble fibre, which has been shown to have beneficial effects on mineral absorption from the gastrointestinal tract, may have indirect beneficial effects on blood pressure [147].

1.12.7 Fibre and Renal Function

Studies that date back more than 20 years suggest that modification of dietary fibre may have beneficial effects on renal function. Locust bean gum is a non digestible polymer of mannose and galactose derived from the seeds of the *ceratonia siliqua* tree. It has been shown to be an efficient sorbent that binds many of the potential toxic metabolites in patients with chronic renal failure [148].

In a case report by Yatzidis, albeit of only 2 patients, serum creatinine was reduced and blood pressure improved with locust bean gum [149].

An increase in dietary fibre intake has been shown to increase the excretion of faecal nitrogen. Stephen and Cummings were amongst the first to show this. In a small study of 6 patients, taking a controlled diet that included 22g of dietary fibre, they were able to show an increase of faecal nitrogen excretion from 1.5g per day to 2g per day. Wheat fibre was shown to alter colonic function by retaining water and increasing the bulk of colonic contents, and thus decreasing transit time. However, they were also able to

show that as a result of fibre being a source of carbohydrate, there was an increase in bacterial composition of the faeces. The bacteria in turn are able to digest nitrogenous (that is, urea forming) compounds to the quantity of about 0.4g per day [150]. Ispaghula husk is a hemicellulosic fibre that may also have a beneficial effect on renal function beyond the associated increased faecal bacterial loss. Rampton showed that a diet supplemented with ispaghula reduced plasma urea by 19% and increased faecal nitrogen excretion by 39% [151].

Experimental data has shown that indoxyl sulphate and p-cresyl sulphate contribute to the progression of renal disease in CKD patients, cardiovascular disease and bone disease, with both of these compounds rising in parallel with decline of renal function. Dietary fibre has been shown to reduce colonic generation of these compounds (by shifting colonic microbial activity from a proteolytic one to a saccharolytic one) and increase short chain fatty acid production , thereby offering up another possible pathway by which dietary fibre may improve renal and cardiovascular outcomes [152].

1.13 Gum Arabic

The specific focus of interest in this thesis will be the dietary supplementation with a modified form of Gum Arabic.

1.13.1 Background

Gum Arabic, otherwise known as Acacia Gum is a naturally occurring gum that is prepared from an exudate from Acacia trees. These trees are harvested predominantly in Sub Saharan Africa. The production of the gum, in a process known as gummosis, is a natural response of the tree to injury of bark. The gum exudes as nodules which are then removed by farmers as a raw product. Generally there are two varieties of the acacia tree that it is harvested from, these being *Acacia senegal* and *Acacia seyal.*

Gum Arabic is a complex and variable mixture of arabinogalactan oligosaccharides, polysaccharides and glycoproteins. The molecular parameters and resulting functionality can vary greatly for different commercial samples [153]. Acacia Senegal tends to have a greater relative proportion of D-galactose compared to L-arabinose. It consists of a lower molecular weight polysaccharide and a higher weight hydroxyproline rich arabinogalactan. As such it belongs to the hydrocolloid group of compounds.

Gum Arabic in a more refined form has many diverse commercial uses including use as a watercolour thickener for artists, in the pharmaceuticals industry and in cosmetics amongst many others. However, its main uses are as an emulsifier in the food industry, and in carbonated drinks to reduce the surface tension of fluids and increase fizzing. Being almost completely soluble in water it makes it an ideal product for use as a stabiliser, emulsifier and thickening agent in foodstuffs.

The Federation of American Societies for Experimental Biology prepared a report for the United States food and Drug Administration in March 1973, looking at the safety profile of Gum Arabic [154]. This committee looked at the safety of Gum Arabic when used in food. A review of the then available literature recommended that Gum Arabic should be "Generally Regarded as Safe" – GRAS. It is accepted as a food additive in the European Union (E414) (Directive 99/77/EC) and by Codex Alimentarius (INS414). Gum arabic is now also officially recognised as a dietary fibre in the EU directive 2008/100/EC [155].

Gum Arabic also has a prebiotic action. A prebiotic is defined as:

"*non-digestible food ingredients which beneficially affect the host by selectively stimulating the growth and / or activity of one or a limited number of bacteria in the colon and thus improve the host health*".

Gum Arabic is a non digestible polysaccharide that has been shown to retard glucose absorption. It has a low glycaemic index and reduces plasma glucose concentrations in healthy individuals [156].

1.13.2 Potential Renal benefits of Gum Arabic

As already discussed, dietary fibre, including soluble fibre, has wide ranging beneficial health effects. To date gum arabic has not been investigated extensively as a product beneficial to health. There is anecdotal evidence to support the use of Gum Arabic to prolong the time needed to commencing renal replacement therapy, particularly in a paediatric population [157, 158]. The mechanisms of this are not fully understood but are at least in part likely to relate to the associated increase in faecal urea nitrogen excretion, with a concomitant decrease in urinary nitrogen excretion [159].

1.13.3 Potential Actions of Gum Arabic in Nephropathy

To date there has been no published work on the use of Gum Arabic in earlier stages of chronic kidney disease, but there are theoretical reasons behind benefits of its use. The areas in which I would like to concentrate are those looking at non renal clearance of urea that is seen with dietary fibres, its effects on glomerular filtration rate and effective renal plasma flow and its potential for affecting transforming growth factor beta (TGF-β) expression that could provide longer term benefits. The action of Gum Arabic on TGF-β may also be of benefit in the shorter term as well as long term by its effects on blood pressure homeostasis.

The use of Gum Arabic in an end stage renal failure population has not been studied in a randomised controlled trial. However, accessibility to renal replacement therapy in the developing world is limited and other treatment modalities that can postpone the time to initiation of renal replacement therapy are of great value. Al-Mosawi describes 4 paediatric patients [157, 158] who had reached end stage renal disease, and in whom access to renal replacement therapy was difficult. All patients' diets were supplemented with powdered acacia gum (Gum Arabic). This was at a dose of 1g/kg in 3 of the patients and 0.5g/kg in the other patient. Intermittent peritoneal dialysis (IPD) was used as an adjunctive therapy if the patients became overtly symptomatic of uraemia. Three of the 4 patients showed stability of their renal function and a probable improvement in urea and creatinine. In one patient, its effect on blood pressure was also considered and,

although only anecdotal evidence appears to show an improvement in blood pressure that was reversed on cessation of treatment.

Nasir showed that gum arabic treatment in mice was associated with an increase in 24 hour urinary creatinine clearance. The mechanism is unclear but suggests a remote effect of gum arabic on the kidney and not that of non renal nitrogen clearance [159].

In a study of patients with CKD, the concentrations of creatinine, urea, phosphate and uric acid have been reported to be significantly lower in subjects give gum arabic at a dose of 50g/day for 3 months Possible mechanisms whereby Gum arabic may have these beneficial effects are discussed in the chapters following.

1.13.3.1 Non renal clearance of nitrogen

Bliss et al conducted a prospective, randomised, placebo controlled, single blinded study of a crossover design in 20 patients with chronic renal failure. The non placebo arm received 25g of Gum Arabic twice daily. They clearly showed that the mean nitrogen content of stool was significantly greater when taking Gum Arabic as compared to placebo or to baseline. In addition, serum urea nitrogen was also lower when compared to placebo or baseline [160]. This effect is dependent on an increase in colonic bacterial growth. These bacteria produce ureases that hydrolyse urea to ammonia and carbon dioxide. The ammonia that is produced is then used by the bacteria and incorporated in to bacterial proteins which are then excreted in the bacterial mass fraction of the faeces [156]. Renal function per se is of course not directly affected, the result being non renal clearance of urea via the bowel.

1.13.3.2 Antioxidant properties

Several studies have also shown a potentially protective effect of Gum Arabic against gentamicin nephrotoxicity in rats [161, 162], possibly in part through inhibition of the production of oxygen free radicals that cause lipid peroxidation.

The renoprotective effect of Gum Arabic seems to be independent of any effects that it may have on faecal bacterial ammonium metabolism [161, 163], although in one of these studies the improvements in serum urea and creatinine did not reach significance.

There is however evidence that contradicts the theory that Gum arabic has significant antioxidant properties. Ali et al demonstrated in a rat model that administration of varying concentrations of Gum arabic in the drinking water did not change significantly levels of free radical scavengers [163].

1.13.3.3 Production of short chain fatty acids

A common feature of all mammals is extensive microbial fermentation of polysaccharides in the hindgut. Large amounts of short chain fatty acids (SCFA) are produced, with concentrations of SCFA generally about 100 mmol/l [164]. Bacterial fermentation of dietary fibre-rich substrates in the colon results in the production of a variety of short chain fatty acids. The proportions of the different SCFAs produced is dependent on the type of dietary fibre ingested. Bourquin et al showed that in human subjects, ingestion of different dietary fibre rich substrates produced different quantities of SCFAs in the faeces. The fibre rich substrates that they tested, in 3 human volunteers, were two varieties of oat hull fibre, Gum Arabic, carboxymethylcellulose (CMC), soy fibre, psyllium, and six blends containing oat fibre, Gum Arabic, and CMC in various proportions. Production of SCFA was directly proportional to the content of Gum Arabic in the substrates and overall, proportions of the SCFAs were acetate, propionate, and butyrate, produced in the molar proportion of 64:24:12 [165].

Gum Arabic when given to caecostomised pigs, has also shown increased levels of short chain fatty acid production, in particular acetate and butyrate. This further supports its potential as a prebiotic agent [166].

Short chain fatty acid production has been shown to have certain health benefits, particularly improved bowel health. Butyrate in particular plays a central role in maintaining the mucosal barrier in the gut. A lack of SCFA may be the cause of ulcerative colitis and other inflammatory conditions. Butyrate has also been shown to increase wound healing and reduce inflammation in the small intestine. Importantly

butyrate is the dominant energy source for epithelial cells and affects cellular proliferation and differentiation by yet unknown mechanisms [167]. It may also reduce the risk of colon cancer, and substrates that can decrease the acetate: propionate ratio may reduce serum lipids and possibly cardiovascular disease risk [168].

Gum arabic is able to selectively raise the proportions of different bifidobacteria in human intestinal microbiota, depending on the exact preparation of Gum arabic used. As a result levels of the short chain fatty acids butyrate and propionate are seen to rise [169].

Kishimoto et al have shown that Supergum™ (a specific preparation of Gum arabic) is fermented to short chain fatty acids by intestinal bacteria and that the dominant bacteria which is likely to be responsible is Prevotella ruminicola [170].

The possible health benefits of fibre as a result of short chain fatty acid production are clear but evidence currently only shows some benefit in the GI tract and possible cardiovascular benefits. It is possible that dietary fibre, including Gum arabic has health benefits beyond the GI tract which could impact on diabetic nephropathy.

1.13.4 Effects of Gum Arabic on TGF-β activity

TGF-β is a key growth factor involved in the development and pathophysiology of blood vessels and increased circulating levels of TGF-β have been found in hypertensive patients. Different isomers of TGF-β are generated but only a small amount of the TGF-β produced by the cells is made available for signalling. Therefore the balance between maturation, sequestration and presentation of TGF-β serves as a regulatory step.

Butyrate has been shown to be a direct inhibitor of the generation and of the signalling of TGF-β1 in a renal epithelial cell line. An in vitro study by Matsumoto et al has shown that the addition of butyrate at increasing doses to renal epithelial cells, suppressed the basal TGF β1 production by these cells. Glucose dependent stimulation of TGF-β1 is also inhibited by butyrate [171]. This of particular interest to diabetic

nephropathy as the glucose dependent alterations in cell function are a well established pathogenic mechanism.

By dietary supplementation with Gum arabic, it may be possible to increase short chain fatty acid production, in particular butyrate, by bacterial fermentation. This increase in serum butyrate may in turn inhibit the production of TGF-β1.

Until relatively recently it was thought that the underlying pathogenesis of essential hypertension was largely as a result of abnormal function of smooth muscle and endothelial cells. Faury et al however have shown that there are other components involved in the cause of essential hypertension. In the murine model they were able to demonstrate that $ELN^{+/-}$ (hemizygous for the elastin gene) mice, had a mean arterial pressure that was 30-40% higher than the wild type heterozygous mice. They concluded that vessel wall proteins, particularly elastin should be considered as causal genes for essential hypertension [172].

Zacchigna et al have studied the role of Emilin 1 (elastin microfibril interface-located protein 1) in the pathogenesis of hypertension in mice. Emilin 1 is a protein of the extracellular matrix of blood vessels, and may be responsible for anchoring smooth muscle cells to elastic fibres, and may also be involved not only in the formation of the elastic fibre, but also in the processes that regulate vessel assembly. Deficiency of Emilin 1 causes systemic arterial hypertension. Emilin 1 only binds to immature proTGF-β and prevents its maturation. The group studied Emilin 1 knockout mice. This inactivation of Emilin 1 leads to increased TGF-β signalling in the vessel wall. These animals had elevated systemic blood pressure, increased peripheral vascular resistance and reduced vessel size, independent of cardiac output. Furthermore, blood pressure returned to normal levels by inactivation of a single TGF-β allele [173].

Modification, by down regulation, of TGF-β expression may therefore play a role in controlling BP and could be a target for treatment with dietary supplements of Gum arabic.

1.13.5 Choice of Gum Arabic preparation

One of the major problems with Gum Arabic is that most commercially available forms have an inherently variable mass due to the fact that it is a natural product. Traditionally, the two widely available forms of Gum Arabic, each from the tree Acacia senegal, have been A.senegal var. Senegal and A.senegal var. karensis. Both of these are approved as food additives by the FAO Joint expert Committee on Food Additives and the Codex Alimenarius Commission. Al-Asaaf et al showed that in 67 commercially available samples there was an extensive variation between individual samples, despite the fact that they were all marketed as "Gum Arabic". Multi angle laser light scattering was used to determine the average molecular mass of the samples which varied from 4.6 to 10.2×10^5 [153].

This wide variation in properties of different types of Gum Arabic could be of major importance when carrying out a clinical study, as the biochemical properties of the different products may well vary. Therefore a product with a consistent molecular make up is required. Supergum™ is a specially produced form of Gum Arabic that is well characterised and can be produced to specific molecular dimensions. The average dietary fibre Supergum is 91% (cited in [156]). The Supergum comes as a dried powder that is stable at room temperature and can be easily dissolved in water to make it an easily ingested product, ideal for use in a human clinical trial.

1.14 The Role of Arterial Stiffness

1.14.1 Historical viewpoint

The measurement of brachial blood pressure with a sphygmomanometer provides a simple recording of systolic and diastolic blood pressure and has been accepted practice in medicine for over 100 years. As a result, measurement of the brachial blood pressure has been used as the gold standard in hundreds of clinical trials and seems to have an impact on outcomes in human trials. Previously little notice has been taken of the actual shape of the pressure waveform and its potential relationship to disease. However, long

before the arrival of the sphygmomanometer, physicians studied the shape of the waveform and understood, to a degree its potential impact.

As far back as Hippocrates, different pulse characters were described in different diseases [174]. In more recent history, Mahomed was one of the pioneers of this science and was able to demonstrate the differences in tracings of asymptomatic hypertension and those associated with nephritis. Mahomed placed great importance on these tracings:

"*Our old ally the pulse ranks the first amongst our guides; no Surgeon can despise its counsel, no Physician shut his ears to its appeal. Since then the information which the pulse affords is of so great importance and so often consulted, surely it must be to our advantage to appreciate fully all it tell us, and to draw from it every detail that it is capable of imparting"*

F.A. Mahomed, 1872 [175]

Mahomed studied the shape of the pressure wave form rather than just isolating and ultimately simplifying a complicated physiological process in to a systolic and diastolic blood pressure. By using a device known as sphygmograph he was able to plot the waveform of a radial artery pulse. As a result of this he was able to describe and recognise the differences between a normal waveform and those generated by individuals with essential hypertension and those with renovascular hypertension.

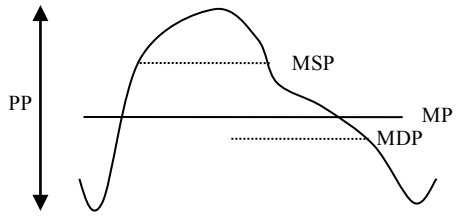
Richard Bright, regarded as the "father" of Nephrology also recognised its importance, identifying increased arterial hypertension from "hardness" of the arterial pulse, and attributed left ventricular hypertrophy and vascular damage to high arterial pressure [175].

With the introduction of the manual sphygmomanometer at the turn of the last century interest was lost in the study of the arterial waveform until recently. It is now becoming increasingly clear that a more complex measure of blood pressure, including an estimation of central blood pressure and shape of the pressure waveform may give a better indication of outcome.

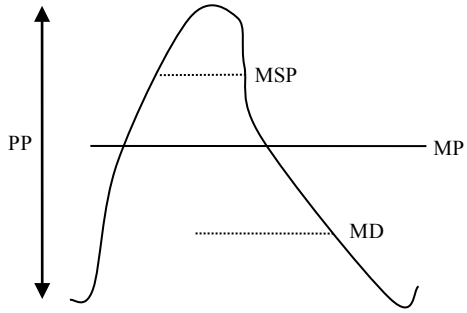
The next major step forward in research was in the 1950s by John Wormersley, a Scottish mathematician who studied the equations of fluid motion [174]. His major collaborator at the time was Donald McDonald who went on to produce Blood Flow in Arteries [176], an important reference manual in this field. One of the most important principles to come out of the research was that of wave reflection. This refers to the outgoing pressure waveform from the heart which travels distally towards the peripheries, then at various bifurcations and narrowings of the arterial tree is reflected back towards the heart. This was first demonstrated experimentally in computer models by Taylor [177] who noted that the major factor influencing transmission along larger vessels was wall elasticity and only a minor factor was fluid viscosity, but in smaller peripheral vessels the major influence became fluid viscosity [177]. Futhermore, O'Rourke and Taylor went on to discover that approximately 90% of the outward pressure wave was reflected back towards the heart from the peripheral vascular bed. [178].

More work carried out by McDonald, Wormersley and Taylor lead to the separation of the mean from the pulsatile components of pressure and flow waves within vessels. Instead of purely looking at changes in systolic and diastolic pressure their work explained how one could view an increase in mean pressure, indicating a change in peripheral resistance, and a change in pulse pressure which would indicate a change in the stiffness of arteries [176]. O'Rourke et al measured central aortic waveforms and recognised significant differences in the arteries of normal individuals from those with hypertension:

Normal aortic pressure wave



Aortic pressure wave in hypertension



MSP	Mean systolic pressure
MDP	Mean diastolic pressure
MP	Pressure over whole cycle

Figure 1-6. Effects of Hypertension on the Ascending Aortic Pressure Wave

[174]

O'Rourke's work then went on to look in animal studies at the shape of the aortic pressure wave in normals and in simulated hypertension using noradrenaline [174]. The resulting waveforms are demonstrated in Figure 1-7.

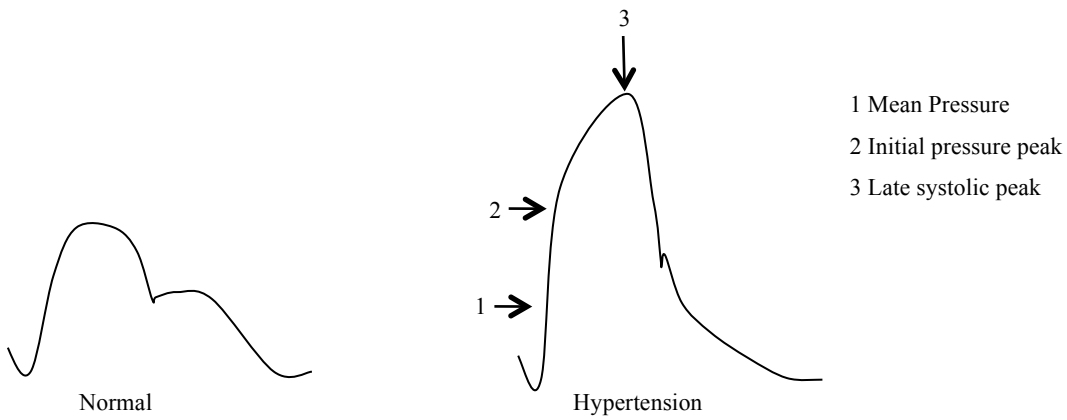


Figure 1-7. Aortic Pressure Waveforms in Normals and in Hypertension

The presence of hypertension makes no difference to the blood flow, but as shown above, it does to the shape of the pressure wave. In Figure 1-7 an increase in peripheral resistance leads to an increase in mean pressure (arrow 1), and increased arterial stiffness leads to an increase in the amplitude of the initial pressure peak (arrow 2). Finally, an earlier return of reflected waves, due to decreased arterial compliance, results in the late systolic pressure peak occurring later (arrow 3).

The peripheral pulse wave that is generated is as a result of the pressure wave that is generated with each contraction of the left ventricle. The size and shape of this wave is dependent on 3 factors:

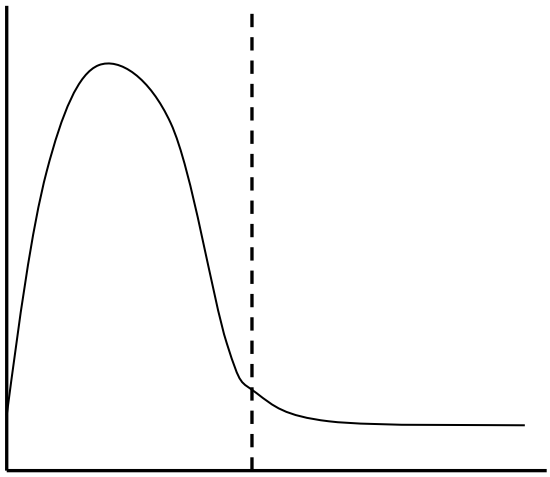
- The magnitude of the left ventricular contraction
- The viscosity of the blood
- Properties of the arterial tree

The pressure wave travels distally during systole until it reaches areas of turbulent or stagnant flow, arterial branches or changes in vessel character. The wave is then reflected back proximally, toward the heart. This is an extremely important physiological point as the timing of this reflected wave impacts on the cardiovascular system greatly. Ordinarily the reflected wave, which itself is a summation of numerous smaller reflected waves, returns to the heart during diastole and increases coronary artery filling, which occurs during the diastolic phase of the cardiac cycle. In a disease state however, peripheral, elastic arteries stiffen and blood flow velocity through those arteries is increased.

1.14.2 The Impact of Arterial Stiffness

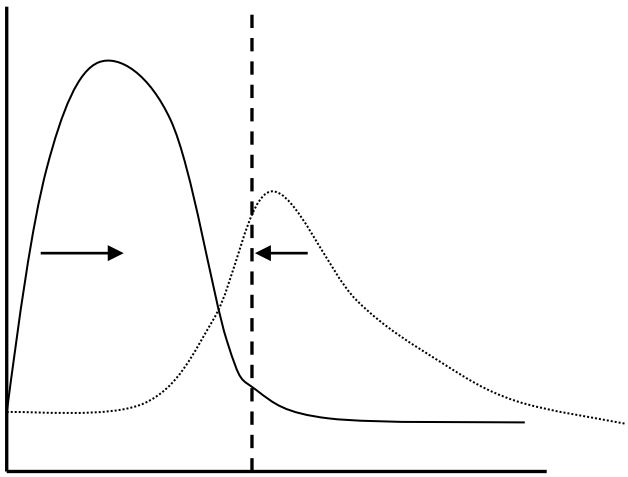
Vessel walls of larger conduit arteries undergo remodelling resulting in arteriosclerosis as a result from repeated stretch and relaxation. This in turn leads to reduced vascular compliance and as a result of this an increase of pulse wave velocity down the conduit artery. The impact of the reflected wave on the aortic waveform is best represented graphically and has several important clinical implications. These are described be

Figure 1-8. Simple Waveform



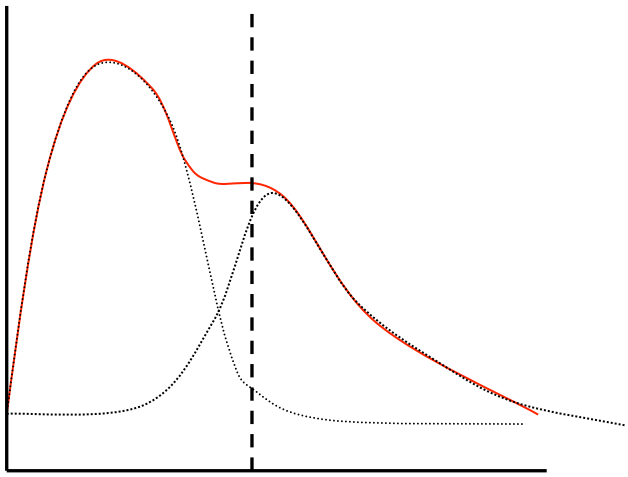
This simple graph represents what the pressure waveform would look like if there were no reflection of the wave from the peripheries.

Figure 1-9. Simple Waveform and Reflected Wave



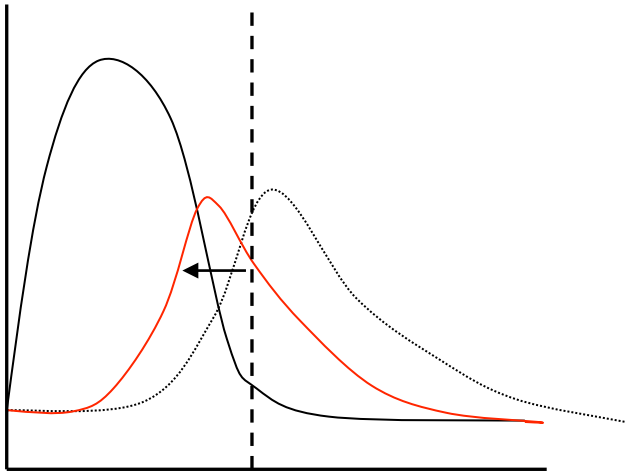
As well as the waveform generated from LV contraction, represented here is the reflected waveform from the peripheries, returning in diastole.

Figure 1-10. Composite Waveform



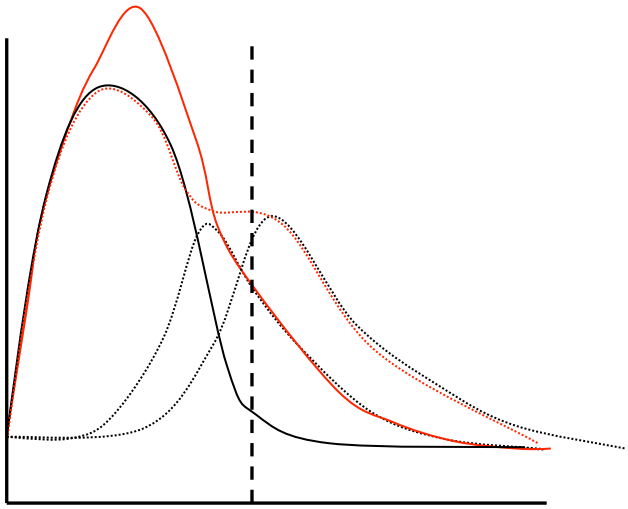
The pressure wave (shown in red) in the aortic root is therefore composed of the outgoing and reflected waves and increases coronary artery perfusion during diastole

Figure 1-11. Reflected Waveform in Stiff Vessels



In a subject with stiff peripheral vessels, transmission of the outgoing and returning waves will occur faster (shown in red). The reflected wave therefore returns quicker, during systole.

Figure 1-12. Aortic Waveform in Stiff Vessels

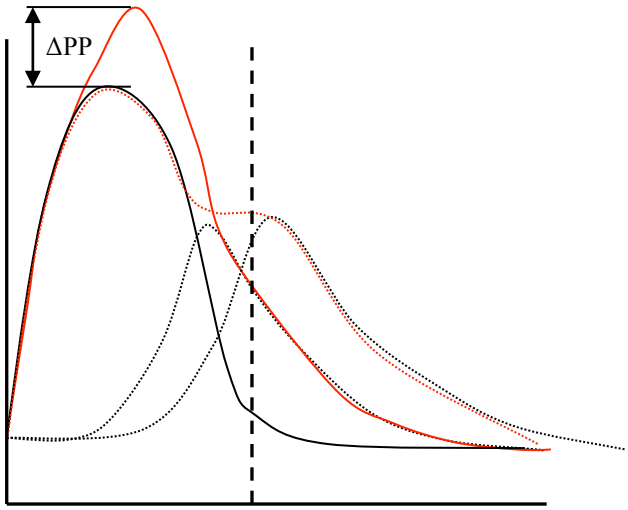


The sum of the 2 pressure waveforms at the aortic root now looks very different than previously and has 3 important clinical implications.

Normal composite waveform

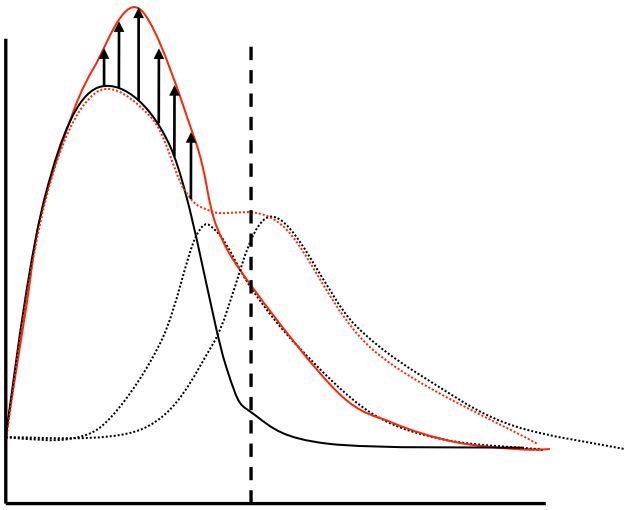
Abnormal composite waveform

Figure 1-13. Increased Central Pulse Pressure



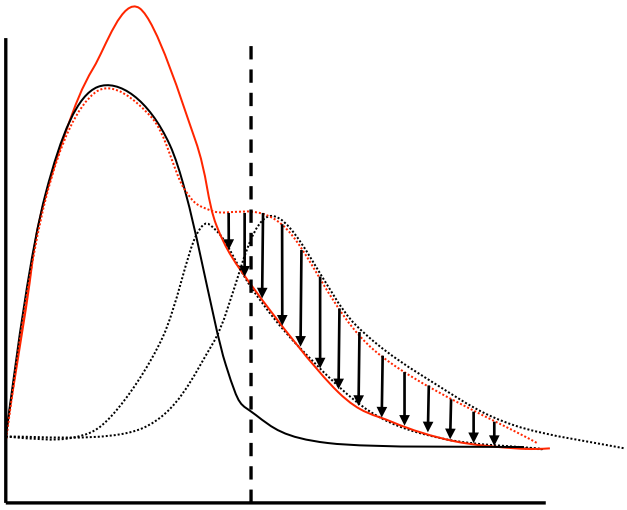
1. There is an increase in central pulse pressure that can occur independently of peripheral systolic pressure

Figure 1-14. Increased Left Ventricular Load



2. There is an increase in Left Ventricular load, increasing the risk for Left Ventricular Hypertrophy.

Figure 1-15. Decreased Coronary Artery Perfusion



3. There is decreased coronary artery perfusion filling pressure in diastole, potentially leading to myocardial ischaemia.

Adapted from [179]

Pulse pressure is a marker of vascular stiffness. Schram et al studied 2484 patients, of which 208 had type 2 diabetes. They demonstrated that pulse pressure was associated with increased cardiovascular mortality among the diabetic individuals with an adjusted relative risk of cardiovascular mortality (95% confidence interval) per 10 mmHg increase in pulse pressure of 1.27 (95% CI 1.00-1.61) . There was no associated increase in mortality in the non diabetic population [180].

The impact of vascular stiffness has been investigated using pulse wave velocity (PWV) [181-184] and augmentation index (AIx) [184, 185] as surrogate markers and have been shown to be associated with an increased cardiovascular risk. Many of these studies have been carried out in patients with end stage renal disease, a group of patients already with a high cardiovascular mortality.

Smith et al studied the relationship between Aortic pulse wave velocity (Ao-PWV) and albumin creatinine ratio (ACR) in 134 patients with type 2 diabetes without overt renal impairment (serum creatinine <150 µmol/L). Patients with raised ACR (>/=3 mg/mmol) were associated with a higher Ao-PWV, poorer diabetic control, higher pulse pressure and systolic BP (all $P < 0.05$) than those with normal ACR. Ao-PWV was also negatively associated with GFR ($P<0.01$). They suggest that even modest decline in renal function may affect the viscoelastic properties of arteries [186].

However not all studies have shown similar results. Diabetic patients have been shown to have increased pulse wave velocity, a measure of increased vascular stiffness, when compared to non-diabetic patients, but peripheral and central augmentation index were not different between the groups. Pulse pressure is a good marker of vascular stiffness and explains much of the widening of pulse pressure with ageing, it is also a good predictor of outcome, along with pulse wave velocity [187].

The greatest amount of evidence currently, for the role of vascular stiffness impacting on cardiovascular outcomes is from the measurement of carotid-femoral pulse wave velocity. This has been shown in several papers to be independently predictive for all-cause and cardiovascular mortality, cardiovascular disease, fatal and non fatal coronary events and fatal strokes [55, 182, 188-191]

Despite many studies showing equal control of brachial blood pressure using different antihypertensive agents, there have been differences in outcome. The outcome from

studies such as HOPE[192], ASCOT-BPLA[193] and CONSENSUS[194] have not been explained purely on the basis of brachial blood pressure and have often been attributed to the "non BP lowering effect" of some of these classes of medication. The CAFÉ study[195], a sub-investigation of 2199 of the ASCOT Study [196] showed a poorer outcome in a beta blocker treated group compared to an amlodipine treated group. Despite similar brachial blood pressure control in each group, there were substantial reductions in central aortic systolic pressure and central aortic pulse pressure in the amlodipine treated group. In post-hoc analysis, central pulse pressure was shown to be associated with total cardiovascular events/procedures and the development of renal impairment.

This reinforces previous studies, that have shown that different classes of antihypertensive agent have different effects on central blood pressure [197].

Due to the relative reduced effect of beta blockers on central blood pressure compared to other antihypertensives, as a class of antihypertensive they have recently fallen out of favour and are now not regarded as first line therapy in the management of hypertension, except in certain circumstances [198].

1.15 Aim of the study

I have shown Gum Arabic is a type of soluble dietary fibre for which there is at least theoretical evidence and some anecdotal evidence for it having a beneficial effect on renal function and blood pressure. Dietary fibre has been shown to have beneficial effects on cardiovascular outcome, blood pressure and diabetes. I have shown that patients with diabetes have a significantly increased cardiovascular risk, and have a high rate of decline in renal function.

Gum Arabic, as a source of modified dietary fibre may lead to increased production of serum short chain fatty acids, in particular butyrate. This SCFA could potentially lead to a reduction in production of the profibrotic cytokine TGF-β1, and hence improve

renal function. By as yet undefined pathways, Gum Arabic may also improve blood pressure, which may also in turn impact on renal function.

The current therapeutic options to improve cardiovascular risk and slow progression of renal failure are quite limited. From the data presented I have shown that Gum Arabic may well be a beneficial dietary addition to this group of patients.

It was therefore hypothesised that by giving Gum Arabic to 3 groups of patients, renal function, blood pressure, and various other biochemical parameters may be improved.

A pilot study was initially run to look broadly at the Healthy Individuals and the Overt Nephropaths. Following results from this smaller study, a larger more in depth study was set up to look at a high risk population of Incipient Nephropaths.

The 3 groups investigated are :

1. Healthy Individuals – *Pilot Study*
2. Patients with Type 2 Diabetes and abnormal renal function ("Overt Nephropaths") – *Pilot Study*
3. Patients with Type 2 Diabetes and normal renal function (as calculated by Cockroft-Gault creatinine clearance) ("Incipient Nephropaths") – *Follow on Study*

2 METHODS

2.1 Introduction

The aims of the study were to examine the effects of modified Gum Arabic (SUPER GUM™) on renal and arterial function in addition to serological markers of cardiovascular risk and short chain fatty acid metabolism in individuals with type 2 diabetes.

A pilot study was carried out in normal healthy individuals and in patients with Type 2 Diabetes and overt diabetic nephropathy. Healthy individuals were volunteers from the Department of Nephrology at the University Hospital of Wales. Patients with overt diabetic nephropathy were recruited from the Nephrology outpatients department at the University Hospital of Wales.

Following the results of the pilot study, a follow up study was undertaken to investigate a group of patients who are well documented to have an increased cardiovascular risk, which may well be modifiable. These patients had type 2 diabetes and incipient nephropathy and were recruited from a Diabetes outpatients department. Patients who fitted the predefined entry criteria were invited to take part in the study during an outpatient visit. They were then provided with a patient information sheet (See Appendix). A follow up telephone call was made several days later. If the patient was willing to take part in the study they were then invited to attend the investigation unit for a further screening test.

Approval for the study was gained from the local Ethics Committee, the local Research and Developments Committee and from the Medicines and Healthcare products Regulatory Agency (MHRA).

Funding for this study was from a research grant provided by San-Ei Gen Inc, Japan, who manufacture SuperGum.

The objectives of the follow on study were as follows:

- **Primary objective:** To assess effects of a diet supplemented with modified Gum Arabic on renal physiology and arterial function.
- **Secondary objective:** To observe effects of modified Gum Arabic on serum lipids, short chain fatty acid production and glucose metabolism.

2.2 Study Design

The Pilot Study consisted of an initial study visit following which subjects were commenced on the gum arabic supplementation for a period of 12 weeks. Following this 12 week period the subjects returned for another study visit. An optional third post washout visit was also included.

The Follow On study was a single centre, open labelled prospective study with a washout period. The total duration of the study was 24 weeks and is described in detail in section 2.5. All measurements were recorded by DG

A randomised placebo controlled double blind study design would have been ideal but was not carried out, largely due to the technicalities and cost of designing a robust trial of this design.

2.3 Participants

Patients with normal renal function were selected from a group of healthy volunteers. Urine dipstick was negative and had a creatinine clearance of >60 ml/min. The patients with overt diabetic nephropathy were selected from a specialist Nephrology clinic and had a creatinine clearance of <60ml/min and had overt proteinuria. Recruitment of patients with type 2 diabetes and incipient nephropathy for the follow on study was from a specialist diabetes clinic. Patients were identified with stable renal function and no overt proteinuria, microalbuminuria however was acceptable.

Renal function was calculated using the Cockroft and Gault formula for creatinine clearance [199] and used as a screening tool prior to formal testing of renal function with an isotope GFR. The Cockroft-Gault equation was used to assess this population as it is probably more accurate in this population than the now more widely used MDRD (Modifications of Diet in renal Disease) equation in patients with a GFR of greater than 60 ml/min [200].

The original Cockroft-Gault equation was derived from the measurement of the mean of two 24 hour urine creatinine clearances in 236 patients, aged 18-92, predominantly male. Mean creatinine clearances varied in each 10 year age group from 114.9 in the youngest to 37.4 in the oldest.

The original formula is as follows [199]:

$$C_{cr} = \frac{(140 - Age) \times (wt)}{72 \times S_{cr}(mg/100ml)}$$

Ccr = Creatinine clearance

Scr = Serum creatinine (mg/dl)

wt = Weight (kg)

This formula has been modified since to take account of differences in body composition between males and females and measurement of serum creatinine in µmol/l.

$$C_{cr} = \frac{(140 - Age) \times wt \times cons}{S_{cr}}$$

Cons = constant, where cons = 1.23 for men and 1.04 for women.

Patients were accepted to the follow up study if the estimated creatinine clearance was > 60 ml/min, and this had been stable for a period of 6 months or more.

Patients were identified as having or not having microalbuminuria, as previously described in Chapter 1.6.3.

2.4 Study Settings

All investigations were carried out in the Investigation Unit in the Institute of nephrology, University Hospital of Wales, Cardiff.

2.5 Study Time Points and Interventions – Follow on Study

Following study entry all patients had a 4-week run in period during which blood pressure was monitored. If necessary additional antihypertensive medication was added to maximise blood pressure control to a target blood pressure of 140-150 mmHg systolic and 75-90 mmHg diastolic. This was felt to be an achievable target in the period given, although not in line with current ideal targets. All groups of antihypertensive medication were allowed during the run in period with a preference for maximising angiotensin blocking drugs.

Following the run in period subjects were invited back to the investigation unit for the commencement of the study – Test Point 1 (see Figure 2-1).Each of the Test Points were identical in the way in which they were carried out and the tests performed, which will be detailed later in this chapter.

Patients were then commenced on the study treatment, Supergum for a period of 12 weeks and then recalled to the investigation unit for Test Point 2. There was then a washout period of 8 weeks, followed by a further visit to repeat the previous investigations.

All patients had baseline characteristics measured prior to the run in.

The overall design of the study is best represented graphically in Figure 2-1.

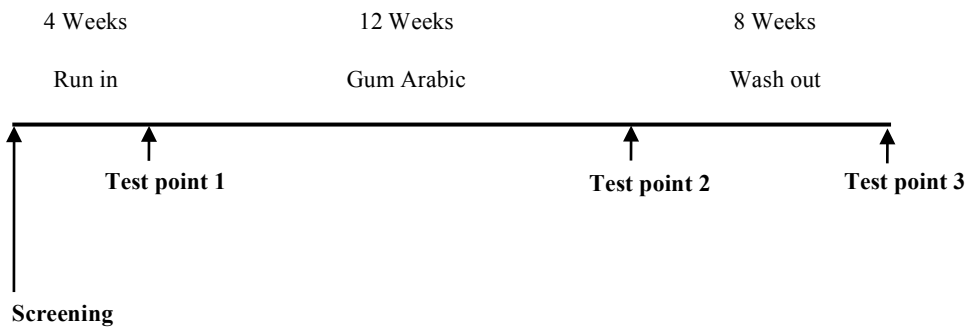


Figure 2-1. Timeline of Study

2.6 Investigations carried out

The investigations performed at each of the test visits were very similar. At the initial screening visit the following investigations were performed:

- Height
- Weight
- Sitting office blood pressure
- Serum urea, creatinine, electrolytes, glucose
- HbA_1C
- Serum Lipids
- 24 hour urine protein

These acted as baseline measurements and as a screening tool for patients to enter the study.

The following 3 visits, Test Points 1,2 and 3, had identical tests performed on each occasion, as follows. All data was recorded on a pro forma (See appendix)

- Height
- Weight
- Sitting office blood pressure
- Serum urea, creatinine, electrolytes, glucose
- HbA_1C
- Serum Lipids
- 24 hour urine protein
- Urine for short chain fatty acid analysis *(Follow on study only)*
- Stool collection for bacterial DNA analysis *(Follow on study only)*
- Pulse Wave Analysis *(Follow on study only)*
- Isotope GFR and ERPF *(Follow on study only)*

2.7 Treatment Formulation

Cherbut et al have shown that gum arabic has a prebiotic action at a dose of 10g per day and that digestive tolerance was not different to that of sucrose up to a dose of 30g per day [201]. After the first test point, subjects were commenced on a daily supplementation of 25g of Gum Arabic, a dose at which it will have a prebiotic action and will be tolerable. This was supplied in pre-weighed sealed foil sachets that could be stored at room temperature. Subjects were asked to add a sachet of Gum Arabic to 200ml of water in a small bottle of water and shake to mix. If needed, flavouring such as orange juice or orange squash was added to improve palatability. The solution could be drunk in one sitting but could also be kept in the fridge to be drunk throughout the day. Adherence was measured by patients returning all sachets, both empty and full at the end of the 12 week period.

2.8 Inclusion and Exclusion criteria

Inclusion criteria

1. Healthy individuals with normal renal function or subjects with type 2 diabetes with stable renal function in the 6 months prior to study start.
2. Females of childbearing age must have had a negative pregnancy test prior to the inclusion period. Effective contraception must have been used during the trial.
3. Patients who were willing and able to participate in the full course of the study and from whom written consent was obtained.

Exclusion criteria

1. Individuals with poorly controlled blood pressure (systolic > 160mmHg and/or diastolic>100mmHg).
2. Individuals with overt proteinuria. *(For follow on study)*
3. Individuals intolerant of Gum Arabic.

Patients were invited to attend the research facility for an 8:00am start. This enabled fasting samples to be tested and so that repeated measurements of vascular stiffness and blood pressure were performed at approximately the same time of day at subsequent visits in the follow on study. Patients were requested to fast from midnight the night before and abstain from smoking prior to attendance as this could interfere with the measurement of vascular stiffness. They were permitted water to drink on the morning of the investigations.

2.9 Baseline demographics

The following parameters were recorded on an initial screening visit pro forma (see Appendix)

2.9.1 Cardiovascular Risk

A detailed history of the subjects' diabetes history and cardiovascular risk data was recorded in the follow on study. The particular factors that were recorded were the following:

- Date of diagnosis of diabetes
- Presence of microalbuminuria and duration if known
- History of ischaemic heart disease
- History of myocardial infarction
- History of coronary artery bypass graft (CABG)
- Smoking history including number of pack years
- Presence of hypertension
- History of hyperlipidaemia
- Family history of cardiovascular disease
- History of cerebrovascular disease
- Presence of peripheral vascular disease
- Presence of diabetic neuropathy
- Presence of diabetic retinopathy

2.9.2 Other Parameters measured

Height and Weight

Each subject's height and weight were recorded at each visit. Height was measured in metres using a Seca measuring rod (Seca gmbh and Co, Hamburg, Germany) and patients' weight was recorded using a calibrated Seca Model 761 mechanical weighing scales, recorded in kilograms.

The Body Mass Index (BMI) was calculated using the following formula:

$$BMI = \frac{Weight\ (kg)}{Height\ (m^2)}$$

Sitting office blood pressure

Patients were sat in a chair to rest for 10 minutes prior to measurement of the brachial blood pressure. This was measured in the right arm with the patient seated using an A and D Medical UA-767 Plus, British Hypertension Society approved automated sphygmomanometer. Two recordings were taken and used if the readings were within 10mmHg of one another and an average of the two then calculated. If they were not the recordings were repeated to ensure accurate results.

Biochemical assays

An intravenous cannula was placed in a suitable vein in the arm of the patient, preferably in the antecubital fossa. Blood was drawn from the cannula, using a 3 way tap, for all necessary biochemical investigations.

Samples of venous blood taken from the cannula were placed in to appropriate Vacutainer (Beckton Dickinson, USA) bottles. The samples were analysed at the University Hospital of Wales biochemistry laboratory. All assays, apart from HbA_1C were measured on an Abbott Aeroset Clinical Chemistry System (Abbott Industries, Illinois, USA). Coefficients of variation (CV)of the assays as stated by the manufacturer as given below.

Sodium and Potassium

These are measured by an indirect measuring electrode and expressed in mmol/l.
Sodium CV 0.7% at 148.7 mmol/L, 1.1% at 133.0 mmol/L
Potassium CV 2.0% at 4.3 mmol/L, 0.9% at 6.6 mmol/L

Urea

This was measured by a urease reaction and expressed in mmol/L.

Urea CV 1.8% at 5.5 mmol/L, 2.0% at 17.1 mmol/L

Creatinine

This was measured using a two part liquid format based on the Alkaline-Picrate Jaffe reaction. Units used are µmol/L.

Creatinine CV 3% at 115 µmol/L, 2.6% at 592 µmol/L

Lipids

Cholesterol was measured using a cholesterol oxidase method and triglycerides by an enzymatic lipase method. Units are mmol/l.

Total cholesterol CV 1.6% at 3.36 mmol/L, 0.8% at 6.95 mmol/L

Triglycerides CV 1.1% at 1.20 mmol/L, 0.8% at 2.13 mmol/L

Urine protein

24 hour urine protein was measured using an aliquot from the sample using a turbidimetric method and expressed as g/24 hour.

HbA1c

This was measured using a Tosoh (Tosoh Corporation, Minato-Ku, Japan) automated glycohaemoglobin analyser using cation exchange high performance liquid chromatography. HbA1c has the units %.

Stored samples

Twenty millilitres of venous blood was taken and prepared for future analysis. The 20ml sample was evenly divided in to two 5ml Vacutainer tubes containing a clot activator. The remaining 10 ml was placed in to two Lithium-heparin containing tubes. All 4 of these samples were then placed in a MSE Mistral 3000i centrifuge. The samples were centrifuged at 2500g, at $4^{O}C$, for a period of 10 minutes. Following this period the samples were removed from the centrifuge and the supernatant removed with a pipette and placed into four 2ml aliquots. The samples from the clot activator tubes provided serum, and those from the Lithium-heparin tubes provided plasma. The

samples were labelled and then stored in a Thermco Scientific Revco Ultima II temperature controlled freezer, at -80°C.

24 hour urine protein

To obtain an accurate measure of urinary protein excretion, patients were requested to bring a urine collection with them from the previous 24 hours. This was placed in a plain 2.5l container. Urine protein excretion is best measured with a 24 hour collection. The instructions provided to the patient are in the Appendix.

2.9.3 Measurement of Renal Function

To accurately assess renal function, isotopic measurements of both Glomerular Filtration Rate and Effective Renal Plasma Flow were undertaken. Both assays were undertaken with a single injection including 2 necessary isotopes. Blood samples were drawn from the intravenous cannula. The cannula was then flushed with 5ml of 0.9% sodium chloride solution.

GFR

Measurement of GFR was done using an injection of ^{51}Cr-EDTA (Chromium 51 labelled ethylenediaminetetraacetic acid). The injection contained a known quantity of the isotope that had been counted in the medical physics laboratory, this was nominally 1MBq. Counting was undertaken in a Sodium Iodide scintillation well counter. The isotope was injected directly in to a suitable vein on the dorsum of the hand, preferably the one on the opposite side to the cannula used for blood sampling. A stopwatch was commenced at the time of injection, to enable accurate timing of the subsequent blood tests. The used syringe and needle were then placed in a plain tube which was placed in a lead lined transport box.

GFR was calculated using a 3 time point method. Blood samples were taken at 2, 3 and 4 hours following injection of the isotope. These samples were taken from the cannula by first withdrawing and discarding 10ml of blood. A further 10ml was then taken and evenly distributed in to 3 Vacutainer bottles and stored in the lead lined transport box.

The cannula was then flushed with 5ml of 0.9% sodium chloride to maintain its patency.

All samples were sent to the medical physics department for calculation of glomerular filtration rate. The empty syringe and needle was counted again on the Panax manual well counter. The blood samples were then centrifuged and the plasma counted using the Wallac Compugamma automatic well counter, along with diluted aliquots from the standard. All counts are then background subtracted. The difference between the counts from the full syringe and the empty syringe is equal to the amount of isotope entering the patient's circulation.

The GFR was then corrected for body surface area and expressed as GFR per 1.73m^2 using the Mosteller technique [202] defined by the following formula:

$$\sqrt{\frac{[Height(cm) \times Weight\ (kg)]}{3600}}$$

The coefficient of variation of isotope GFR measurement is given as between and 8% and 10% [203, 204].

ERPF

Renal blood flow accounts for approximately one fifth of cardiac output, despite the kidneys only accounting for about 0.4% of total body mass. On average therefore, renal blood flow is approximately 4ml/g/min [205].

Measurement of renal plasma flow has been made possible by the availability of substances that have tubular secretion and glomerular filtration. Classically measurement of ERPF has been measured using Para-amino hippurate (PAH), an

inorganic acid that undergoes glomerular filtration and is actively secreted by the proximal tubule. It is completely removed from the plasma on a single pass through the kidney. Therefore the amount of PAH passing through the kidney in a given time period is equal to the amount of PAH that is present in the urine. This can be expressed by the following:

$$RPF = \frac{(U_{PAH} \times V)}{P_{PAH}} = PAH\ Clearance$$

RPF = Renal Plasma Flow, U= Urine, V= volume, P= Plasma

[35]

However this method can potentially underestimate renal plasma flow as the actual extraction ratio of PAH is approximately 0.92, therefore underestimating RPF by about 10% [206]. The method therefore used for this study was isotopic measurement of renal plasma flow using the radioiodine labelled PAH analogue ortho-iodo-hippuran. This technique was first described by Tauxe et al, using ^{123}I-orthoiodohippurate [207]. Calculation of ERPF following a single injection, as described above, of 1MBq of I-orthoiodohippurate, is taken from the measurement of 1 sample of blood taken at 44 minutes.

The original calculation used the formula:

$$ERPF = a + bx + cx^2$$

$x = {}^{1}/_{C_t}$ (a theoretical volume of distribution)

$Coefficients\ a\ and\ b, and\ constant\ (c) were\ calculated$

This method tends to underestimate high ERPF values, as the study population did not include individuals with a high ERPF. An expanded series of 116 subjects was studied resulting in a modified formula that is now used [208].

Leach et al have described the measurement of renal plasma flow using a three sample method. However they importantly demonstrated a close correlation of renal plasma flow using 3 different isotopes of I-orthoiodohippurate, these being ^{125}I, ^{123}I and ^{131}I [209]. For the purposes of this study, due to availability of radioisotopes, 1MBq of ^{123}I-orthoiodohippurate was used.

There is not widely published data on the coefficient of variation of ERPF. The medical physics laboratory at the University Hospital of Wales state a CV of ± 5% (Personal communication with Dr Beth Jones, Principal Physicist, Medical Physics, University Hospital of Wales). In 2 papers by Tauxe et al the standard error of the tests were 30.7 ml/min and 31.2 ml/min [207, 208]. Average ERPF in these studies was approximately 600ml/min, giving a CV in the region of 5%.

Filtration Fraction

The filtration fraction (FF) is a measure of the proportion of fluid that is filtered and passes in to the renal tubules.

Filtration fraction is simply calculated as

$$FF = GFR/_{ERPF}$$

Plasma for short chain fatty acid analysis

Plasma was stored as previously described. These samples were then sent to Japan for analysis of concentrations of short chain fatty acids. Following thawing of the samples, they were sub-sampled in to microfuge tubes and deproteinised by the addition of 0.1 vol of perchloric acid (60% w/v). These were then centrifuged at 15000g for 5 minutes

at 4º C and subjected to High performance liquid chromatography (HPLC) analysis as detailed by Kishimoto et al [170].

Urine for 3-methyl histidine analysis

3-methyl histidine, a product of protein metabolism was measured in a fresh early morning urine sample at each study visit.

Approximately 20ml of urine was placed in a 30ml flask on a magnetic stirrer. An electronic pH metre probe was placed in the urine. Concentrated (38%) hydrochloric acid was then added to the urine until a pH of 2.0 was reached. This was to ensure that any bacteria present were killed. The sample was then placed in to a clean container, labelled and stored at -80°C.

Stool collection for bacterial DNA analysis and SCFA Analysis

A sample of faeces was an optional part of the study. Faeces were stored for bacterial DNA analysis and SCFA production. The faeces was prepared and stored in 2 separate ways.

Storage in Acid

Approximately 1ml of faeces was placed in to a 2.5m Eppendorf tube. Fours drops of concentrated hydrochloric acid were added to the faeces in the container. These samples were stored at -80°C to stop bacterial activity. The samples would be used for faecal SCFA analysis in Japan by first thawing them and then sub-sampling in to microfuge tubes and centrifuging at 15000g for 5 minutes at 4º C. The samples were then subjected to HPLC analysis.

Storage in Alcohol

The remaining sample of stool was placed in a 50ml sealed container containing 40ml of 95.6% ethanol. The container was labelled and stored in a refrigerator at $4^{O}C$. These samples would be used for faecal bacterial DNA analysis at a future date.

2.9.4 Measurement of the central waveform

An invasive method of recording central pressure waveforms is not suitable for routine investigation of human subjects. O'Rourke therefore used a method known as applanation tonometry [174], which provides an accurate calculation of central blood pressure and vascular stiffness. A tonometer, which is effectively a pressure transducer, was developed to enable measurement of the pressure waveform at either the radial pulse or the carotid pulse, by compressing, but not occluding, the artery against a firm tissue, such as the radius at the wrist (see Figure 2-2). When these surfaces are flattened, the circumferential pressures are equalised and an accurate waveform recorded [210].

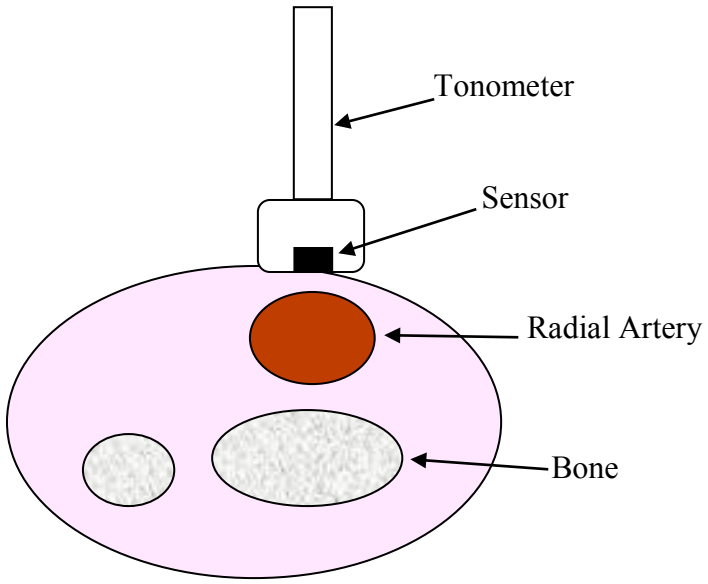


Figure 2-2. Applanation Tonometry at the Radial Artery

There is amplification of the pressure between central and peripheral arteries, and therefore a pressure measured at the wrist would give an inaccurately high estimation of central pressure. Karamangolu et al therefore studied 14 patients and compared pressure recordings from cardiac catheterisation and those obtained from tonometry at the radial artery. A mathematical transfer function was then devised to calculate an aortic pressure from a brachial pressure [211]. The equation for the transfer function takes in to account the pressure waves at the two sites the frequency of the wave and the shape of the waveforms.

Since the calculation of the original equation, more patients have been studied, to confirm its validity. The Association for the Advancement of Medical Instrumentation have set criteria that need to be met for systems measuring arterial pressures by different methods, set out in AAMI SP10. The values can be different by no more than 5mmHg with a SD of 8mmHg or less in 25 or more patients. Pauca et al studied 62 consecutive anaesthetised patients undergoing cardiac surgery. They demonstrated that the equation was valid for aortic systolic, pulse, mean and diastolic pressures [212]

This formula has been implemented in the SphygmoCor system (AtCor Medical, Sydney, Australia), the equipment used for the purpose of measuring pressure waveforms in this study.

2.9.5 The SphygmoCor System

For the purposes of this study the SphygmoCor Vx system was used. This system is able to non-invasively measure both the shape of the aortic pulse waveform and the velocity of the pulse waveform. It uses a high fidelity micromanometer (SPC-301, Millar Instruments, Texas USA) to record a pulse waveform at an arterial site, usually the radial, femoral or carotid, depending on the parameter studied. Data is then automatically transferred in to the software for the system (SphygmoCor 2000 ver 7.1).

There are several data series looking at the reproducibility of the data that can be acquired, which is very important given the time period over which this studied was carried out. Wilkinson et al have studied the system's ability to produce reliable data for both Pulse Wave Velocity (PWV) and Augmentation Index (AIx). This study showed

there was no statistically significant intra- or inter-observer difference [210]. Savage et al carried out a study in a population of patients with renal impairment. In the population of 188 subjects they included 23 healthy controls, 71 pre-dialysis patients, 67 dialysis patients and 27 renal transplant patients. Central aortic mean blood pressure, AIx, Time for reflected Wave (TR) and Subendocardial viability ratio (SEVR) were all shown to be reproducible with only small, statistically non-significant differences in inter and intra-observer measurements [213].

Although this has been shown to be a reliable tool in other trials as outlined above, I carried out my own small study to make sure that my own intra-observer variation was not statistically significant.

2.9.5.1 Using the SphygmoCor System

The SphygmoCor system is attached to a laptop computer that runs the Sphygmocor 2000 software. A Standard Operating Protocol (SOP) was developed to make sure that the measurements were carried out the same way, on each patient and at each visit to reduce variability. Inter-operator variability was eliminated as I was the only operator.

All measurements were carried out in a quiet room. Patients were fasted from midnight the night before and had been asked to abstain from smoking. All studies were performed early in the morning to again reduce any variability that could be introduced if studies were repeated at different times of day.

2.9.5.1.1 Carrying out Pulse Wave Analysis

Patients were rested for approximately 10 minutes prior to the start of any measurements. They were in a semi recumbent position, with the forearm placed comfortably on an arm rest. The forearm was placed in a supine position with the wrist slightly extended, the aid of a rolled bandaged was sometimes used to make this easier.

Baseline data was entered, including height, weight, date of birth and brachial blood pressure. The radial artery was palpated between 2 fingers and the tonometer placed between them over the artery, whilst applying slight downward pressure. Slight

adjustments were made until a clear recording of the pulse waveform was displayed on the monitor. Ten seconds of recording was necessary for the programme to analyse the data. Using the sequential waveforms recorded in that time, an average radial waveform is generated.

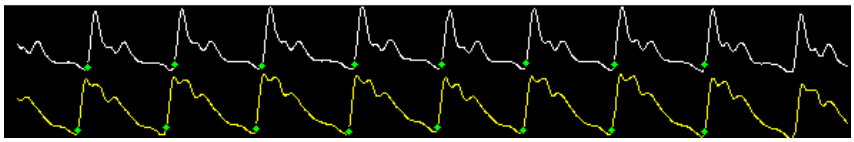


Figure 2-3. Screen showing sequential radial waveforms (upper) and calculated aortic waveforms

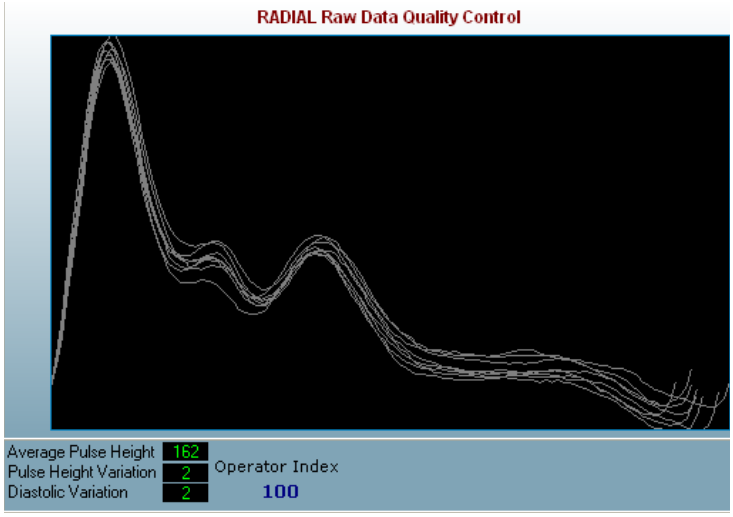


Figure 2-4. Screen showing overlaid individual radial waveforms and Quality Data

The software has a quality control system, whereby it assesses the individual waveforms and looks for variability within them, and scores them on a scale of 0-100, termed the Operator Index.

Operator Index	
95-100%	Excellent
90-94%	Good
85-89%	Acceptable
75-84%	Borderline
<74%	Unacceptable

Table 2-1. Operator Index Measurements

Data was rejected if the Operator Index was <74%, indicating unacceptably variable wave form measurements. If the Operator index was between 75% and 84% the raw data waveforms (see Table 2-1) were examined visually to look for similarities of the waveforms to decide if they were of acceptable quality.

For the purposes of measurement the Augmentation index corrected for a heart rate of 75 (AIx75) beats per minute was examined. The study was repeated to obtain 2 recordings of AIx75 within ± 4% of one another and of acceptable quality as described.

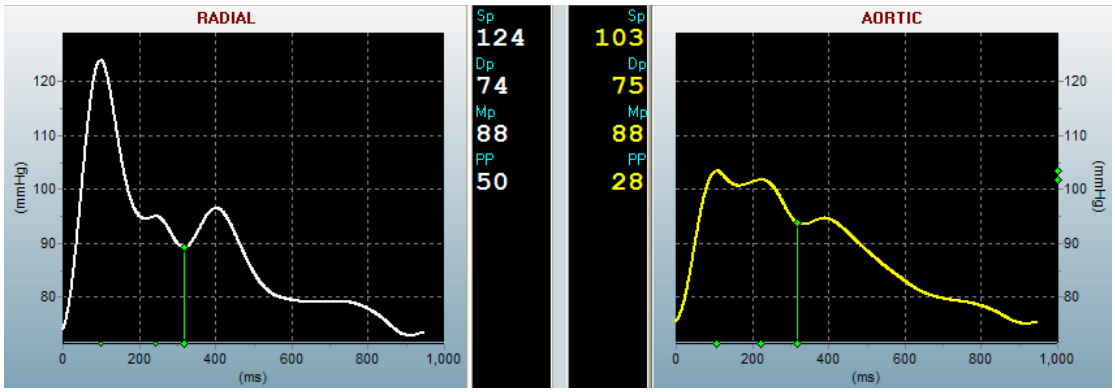


Figure 2-5. Screen showing averaged radial waveform (left) and calculated average aortic waveform

2.9.5.2 Measurements Obtained of the Pulse Waveform

For the purposes of the study, data on the following parameters was collected and analysed.

Central aortic pressures

Having calibrated the radial pulse measurements by measuring brachial blood pressure using a sphygmomanometer, the software calculates, using the averaged aortic pressure waveform central aortic blood pressures. The recorded values are for central systolic blood pressure, central diastolic pressure and aortic pulse pressure – the difference between systolic and diastolic pressures.

Augmentation index AIx

Each arterial pulse waveform is generated by the sum of the forward pressure wave created by left ventricular ejection and a backward wave that is the summation of numerous reflected from peripheral sites. The time point at which these forward and backward waves merge and the amplitude of the reflected wave affect the level of central BP. Augmentation pressure (AG) is actually measured with the tonometer by calculating the contribution of the reflected wave to systolic pressure. Augmentation index is then calculated to give an indirect measure of arterial stiffness. Augmentation index is calculated by the following formula [214]:

$$AIx = \frac{AG}{PP} \times 100$$

PP = Pulse pressure

Augmentation index is therefore expressed as a percentage.

Augmentation index corrected for heart rate Aix75

Due to the possibility of numerous patients being on rate controlling medications such as Beta blockers, augmentation index corrected for a heart rate of 75 beats per minute was measured. Heart rate itself can impact upon augmentation index, due to an increased wave reflection at lower heart rates, leading to higher augmentation indices for lower heart rates [215]

Subendocardial viability ratio SEVR

SEVR is a measure of the arterial systems ability to supply the myocardium with sufficient blood. Buckberg et al first described a measurement – the SEVR to look at the perfusion of the subendocardium, thought to be more sensitive to ischemia than the subepicardium. The SEVR is the ratio of area of the diastolic phase to that of the systolic phase measured in the central aorta. This has been shown to correlate well with the blood supply to the subendocardium. A lower SEVR is indicative of poorer delivery of blood. Pulse rate and age have been shown also to be shown to correlate with SEVR [216].

Time for return of reflected wave Tr

This is simply crude data recorded, showing the actual time for the reflected waveform to return from the peripheries to the central aorta.

2.9.5.3 Carrying out Pulse Wave Velocity measurements

Pulse wave velocity was measured using the carotid – femoral method. A standard operating protocol was written. After performing the pulse wave analysis, as described above, subjects were reclined in the chair. Blood pressure was repeated as previously described. Measurements from the femoral artery recording site to the umbilicus, from the umbilicus to the suprasternal notch and also from the carotid artery recording site to the suprasternal notch were made in millimetres (mm) and entered in to the Sphygmocor system. Three ECG recording electrodes were applied to the patient. Recordings of the pulse waveform were made at the distal site initially. A good quality 10 second recording was made. The Sphygmocor system indicated the quality of the

trace and whether this was suitable. The Sphygmocor system then calculates a Pulse Wave Velocity. All pulse wave analysis investigations were carried out by investigator DG.

Wilkinson et al studied the reproducibility of this method. Reproducibility assessed through the Bland-Altman method with calculation of the repeatability coefficient was, following intraobserver comparison, for Brachial PWV: 1.64m/sec (for a mean value of 8.65±1.58 m/sec); and for aortic PWV:2.34m/sec (for mean value of 8.15±3.01 m/sec) [210].

2.9.6 Statistical analysis

Statistical analysis and data collection was carried out using SPSS version 15.0 (SPSS Inc. Illinois, USA). Data was analysed as a whole group and within individual groups of patients. Data was analysed for normality of distribution by means of inspection of a histogram. Further details of statistical methods will be discussed in the Results chapter.

3 RESULTS

3.1 Introduction

This chapter will discuss the results of the investigations firstly carried out during the pilot study. Secondly it will present the results from the follow on study and look at the combined results of all subjects. As discussed in the introduction, diabetes has a strong association with cardiovascular disease and with this comes an associated morbidity and mortality. Dietary fibre intake has a wide range of health benefits, with evidence to show it can improve these cardiovascular outcomes. There are also theoretical ways by which it may improve renal outcomes.

These results explore what happens in the three groups of subjects when they are given the dietary fibre supplement gum arabic in terms of changes in renal function, changes in blood pressure and cardiovascular risk, and other metabolic parameters. The results will be discussed in sections, determined by the study group populations, that is: Healthy Individuals and Overt nephropaths in the pilot study and finally the Incipient Nephropaths in the follow on study.

3.2 Recruitment

The initial pilot study recruited a total of 10 healthy volunteers with normal renal function from members of staff in the Institute of Nephrology. All individuals screened met the entry criteria for the study. Patients with known overt diabetic nephropathy were recruited from the University Hospital of Wales Hospital Nephrology clinics. Fourteen patients expressed an interest in taking part in the study and met the entry criteria.

Following the pilot study a power calculation was undertaken to ascertain the sample size for the follow on study. In order to obtain an 80% chance of detecting a 10% difference in GFR at the 5% level it was calculated that 30 patients would need to be recruited in to the study. The standard difference in the GFR measurements calculated from the pilot study were used and applied to a nomogram to achieve the power calculation.

The subjects with incipient nephropathy were recruited from a diabetes clinic within the same trust. A total of 242 patients met the entry criteria of which 36 agreed to the initial screening visit. A total of 27 subjects agreed to commence the study following the initial screening. Four subjects withdrew from the study resulting in 23 incipient nephropaths completing the study. Recruitment for the study is shown in Figure 3-1.

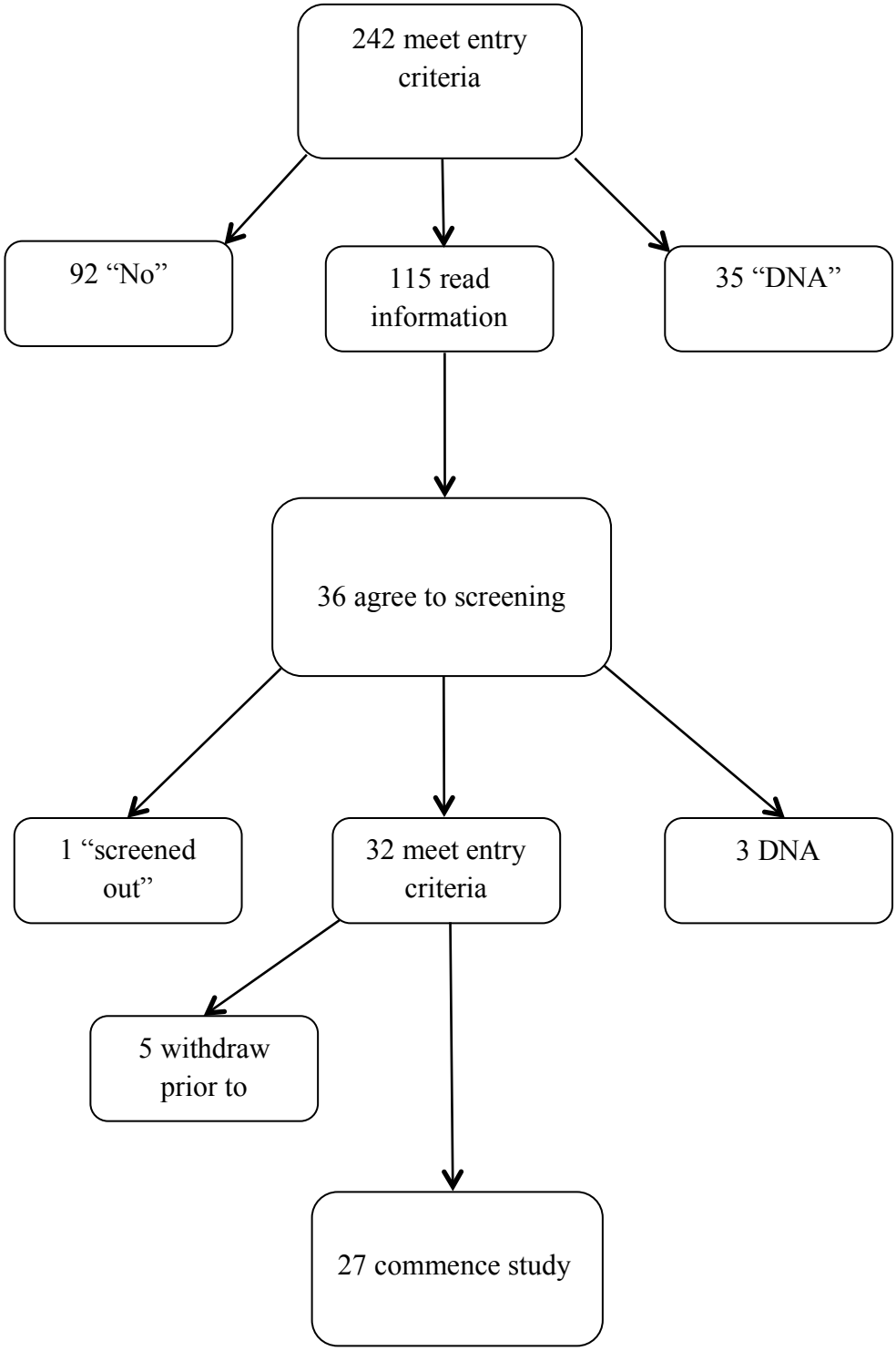


Figure 3-1. Recruitment to Follow on Study

3.3 Demography

The data presented in Table 3-1 shows baseline demographic data for all of the 3 individual study groups and for all the groups as a whole.

	Value			
Parameter	**Total Group**	**Healthy**	**Incipient Nephropath**	**Overt Nephropaths**
n	47	10	23	14
Sex (M:F)	40:7	8:2	18:5	14:0
Age (years)	58.07 ± 12.68	39.74 ± 6.24	59.95 ± 8.74	68.09 ± 6.33
Weight (kg)	90.21 ± 17.21	82.3 ± 14.40	88.63 ± 17.13	98.46 ± 16.82
BMI (kg/m^2)	29.42 ± 4.43	25.20 ± 2.42	29.83 ± 3.11	31.78 ± 5.39
Systolic blood pressure (mmHg)	138.5 ± 18.90	129.10 ± 8.23	141.52 ± 18.72	140.07 ± 23.18
Diastolic blood pressure (mmHg)	79.43 ± 12.13	75.5. ± 11.21	86.09 ± 7.26	71.29 ± 13.53

Table 3-1. Baseline Demographics – All Groups

All data is mean ± standard deviation. Mean age and weight were highest in patients with overt nephropathy and lowest in the healthy volunteers.

3.4 Completion of Study and Side Effect Profile

All subjects completed the 12 week period on the Gum arabic supplement and there were no withdrawals once the study had commenced. Side effects were minimal and tended to be gastrointestinal in origin. Four patients noted some bloating with the Gum arabic supplement and one subject noted an increased bowel frequency. Compliance with the supplement was measured by the number of full and empty Gum arabic sachets returned. Four full sachets of Gum arabic were returned suggesting good compliance.

3a

Results of Pilot Study

3.5 Effects of Gum Arabic on Biochemistry and Renal Function

3.5.1 Methods

The data in Table 3-2 is baseline biochemical data taken at the first study visit. This includes measures of baseline electrolytes and renal function taken from simple blood measurements. All biochemical parameters were repeated at each study visit. Any significant changes in these measurements are discussed. The primary method of measurement of renal function in this study was by way of isotope GFR and ERPF, as detailed in the Chapter 2.9.3.

The data for the biochemical results and isotopic measures of renal function was normally distributed so a paired samples t-test was carried out, comparing changes in the following parameters between Visit 1 and Visit 2 (pre and post administration of gum arabic), and Visit 2 and Visit 3 (post administration of gum arabic and post washout phase). Significance was taken to be a p value < 0.05. Data was analysed on subjects who had completed data rather than on an "intention to treat basis" as in some of the studies, data was limited for study visit 3 due to the limited numbers of patients returning for the third study point.

It should therefore be noted that in some of the following tables, the values for measured GFR and ERPF at "visit 2" may be different when comparing them with values from visits 1 and 3. This is because data was not available for some patients at study visit 3.Therefore, when comparing measurements between visits 2 and 3, only patients who had both recorded values were included for analysis.

Parameter	Healthy subjects			Overt nephropaths		
	N	**Mean**	SD	N	**Mean**	SD
Sodium (mmol/L)	10	**140.7**	1.16	14	**139.93**	2.97
Potassium (mmol/L)	10	**4.10**	0.29	14	**4.72**	0.59
Urea (mmol/L)	10	**6.01**	1.13	14	**18.68**	7.93
Creatinine (µmol/L)	10	**87.10**	10.56	14	**214.14**	64.45

Table 3-2. Baseline Data

3.5.2 Effects of Gum Arabic on electrolytes and simple measures of Renal Function

Data on electrolytes and simple measures of renal function was normally distributed and therefore analysis was undertaken using a Student's t-test.

3.5.2.1 Healthy Individuals

	Visit 1	Visit 2	n	p	Visit 2	Visit 3	n	p
Sodium (mmol/l)	140.70	139.30	10	0.055	139.75	139.50	4	0.82
Potassium (mmol/l)	4.1	4.26	10	0.20	4.175	4.00	4	0.21
Urea (mmol/l)	6.01	5.63	10	0.21	5.87	6.14	7	0.58
Creatinine (µmol/L)	87.10	82.80	10	**0.016**	84.14	84.71	7	0.66

Table 3-3. Changes in biochemistry - Healthy Individuals

The only significant change in the simple biochemistry of the healthy individuals was a drop in serum creatinine from 87.1 µmol/l to 82.8 µmol/l (p=0.016). Only 7 of the 10

subjects had a third visit sample for urea and creatinine recorded, of those who did there was a no significant rise in creatinine after the washout period.

3.5.2.2 Overt Nephropaths

	Visit 1	Visit 2	n	p
Sodium (mmol/l)	139.93	139.57	14	0.74
Potassium (mmol/l)	4.72	4.47	14	0.07
Urea (mmol/l)	18.68	16.99	14	0.21
Creatinine (µmol/L)	214.14	215.43	14	0.83

Table 3-4. Changes in Biochemistry - Overt Nephropaths

In the overt nephropath group, there was no change in the biochemical parameters after taking gum arabic. No subjects in the overt nephropath group in the pilot study returned for the third visit.

3.5.3 Effects of Gum Arabic on isotopic measures of renal function

All patients had a baseline measurement of isotope glomerular filtration rate performed as previously described at the first study visit. Data was normally distributed and analysis was undertaken using a Student's t-test.

			Units = ml/min			
Patient group		**n**	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Healthy	GFR	10	100.0	141.0	**113.0**	13.33
	ERPF	10	381.0	541.00	**474.6**	47.96
Overt nephropaths	GFR	14	21.0	46.0	**32.1**	7.74
	ERPF	11	96.0	266.0	**158.9**	49.68

Table 3-5. Baseline Measures of Isotopic Renal Function

The mean GFR of the healthy individuals was 113 ml/min compared to 32.1 ml/min in the overt nephropathy group. Only 11 of the overt nephropathy patients had an isotope ERPF as a result of difficulties obtaining the isotope as a result of manufacturing and supply problems.

3.5.3.1 Healthy Individuals

	Visit 1	**Visit2**	**n**	**P**	**Visit 2**	**Visit 3**	**n**	**p**
GFR (ml/min)	113.00	99.40	10	**0.016**	97.00	106.86	7	**0.030**
ERPF (ml/min)	489.75	463.00	8	**0.041**				
FF (%)	24	22	8	0.21				

Table 3-6. Changes in Renal Function - Healthy Individuals

The healthy individuals demonstrated a statistically significant drop in GFR (113.0 ml/min to 99.4 ml/min, p=0.016) following dietary supplementation with gum arabic. GFR then increased again from 97.0 ml/min to 106.9 ml/min following the washout period (p=0.03). A statistically significant fall in ERPF was also seen (489.75 ml/min to 463.0, p=0.041) with no significant change in filtration fraction.

3.5.3.2 Overt Nephropaths

	Visit 1	**Visit2**	**n**	**P**
GFR (ml/min)	32.07	33.50	14	0.55
ERPF (ml/min)	158.00	163.00	4	0.35
FF (%)	19	19	4	0.85

Table 3-7. Changes in Renal Function - Overt Nephropaths

Glomerular filtration rate and ERPF both increased a small, non-statistically significant amount. GFR increased from 32.07 to 33.50 and ERPF from 158.00 to 163.00, but numbers were small for measurement of ERPF as the isotope was unavailable on the day of the study visit. There was no change in filtration fraction.

3.6 The Effects of Gum Arabic on Blood Pressure and Cardiovascular Risk

3.6.1 Methods

Baseline blood pressure measurements were recorded in all 3 groups of patients at baseline and at subsequent study visits. This chapter studies the effects that the administration of gum arabic had on the 3 groups of subjects.

As previously, data was analysed for normality of distribution by means of inspection of a histogram. Peripheral blood pressure was normally distributed for the whole group and a paired samples t-test was used for statistical analysis. For individual group analysis, the data was not normally distributed and therefore non-parametric analysis of the data used a Wilcoxon Signed Ranks Test.

3.6.1.1 Healthy Individuals

Data for the individual groups of subjects blood pressures was not normally distributed. Therefore a Wilcoxon Signed Ranks test was undertaken.

Variable	Mean (mmHg)	SD	Significance
Systolic BP Visit1	129.10	8.25	**0.038**
Systolic BP Visit 2	123.60	11.51	
Diastolic BP Visit 1	75.50	11.21	0.86
Diastolic BP Visit 2	74.60	10.54	
Pulse Pressure Visit 1	53.60	9.34	0.39
Pulse Pressure Visit 2	49.00	11.38	
MAP Visit 1	93.37	9.33	0.11
MAP Visit 2	90.93	9.46	

Table 3-8.Changes in Blood Pressure - Healthy Individuals

Following administration of Gum Arabic there was a clinically and statistically significant drop in systolic blood pressure from 129.10 mmHg to 123.60 mmHg (p=0.038) in healthy individuals. Reductions in diastolic pressure, pulse pressure and mean arterial pressure were also seen but these did not reach statistical significance.

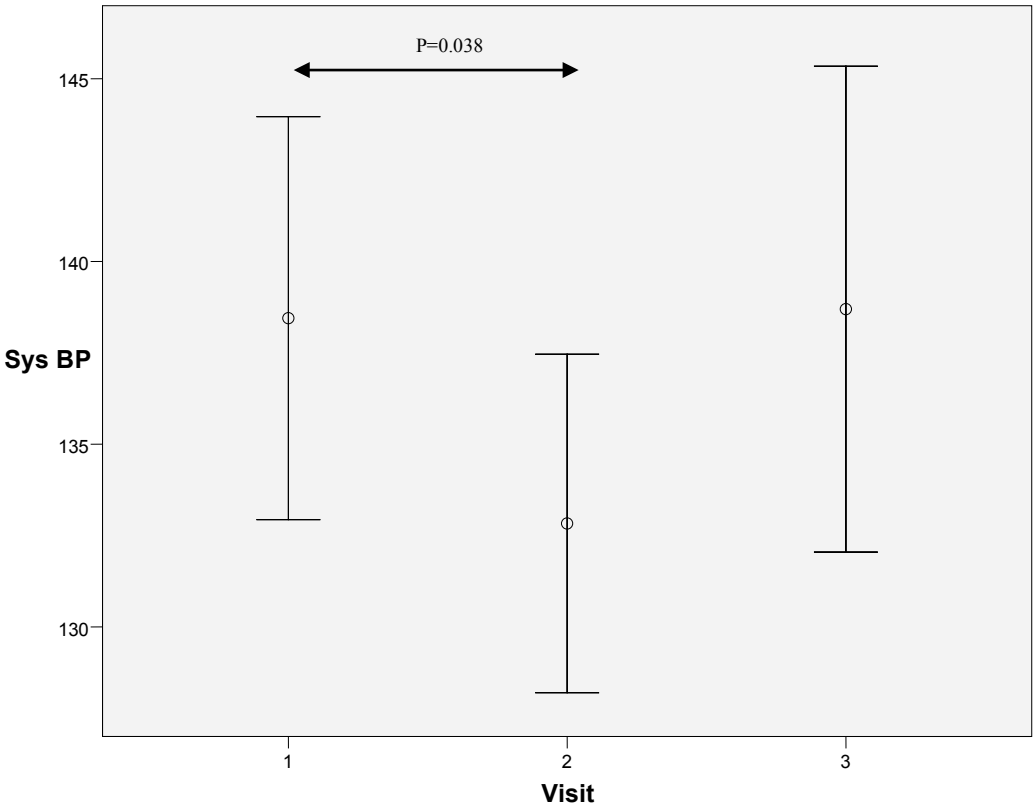


Figure 3-2. Changes in Systolic Blood Pressure - Healthy Individuals

3.6.1.2 Overt Nephropaths

Variable	Mean (mmHg)	SD	Significance
Systolic BP Visit1	140.07	23.18	0.13
Systolic BP Visit 2	131.07	12.13	
Diastolic BP Visit 1	71.29	13.52	0.19
Diastolic BP Visit 2	66.07	12.26	
Pulse Pressure Visit 1	68.79	22.28	0.75
Pulse Pressure Visit 2	65.00	12.10	
MAP Visit 1	94.21	13.81	**0.026**
MAP Visit 2	87.74	10.80	

Table 3-9. Changes in Blood Pressure - Overt Nephropaths

Despite there being a clinically important drop in systolic blood pressure in the overt nephropath group from 140.07 mmHg to 131.07 mmHg, this did not reach statistical significance. There was also a fall in diastolic blood pressure but again this did not reach a level of statistical significance. The combined effect of reductions in both systolic and diastolic pressure resulted in a drop in mean arterial blood pressure from 94.21 mmHg to 87.74 mmHg, reaching statistical significance (p=0.026).

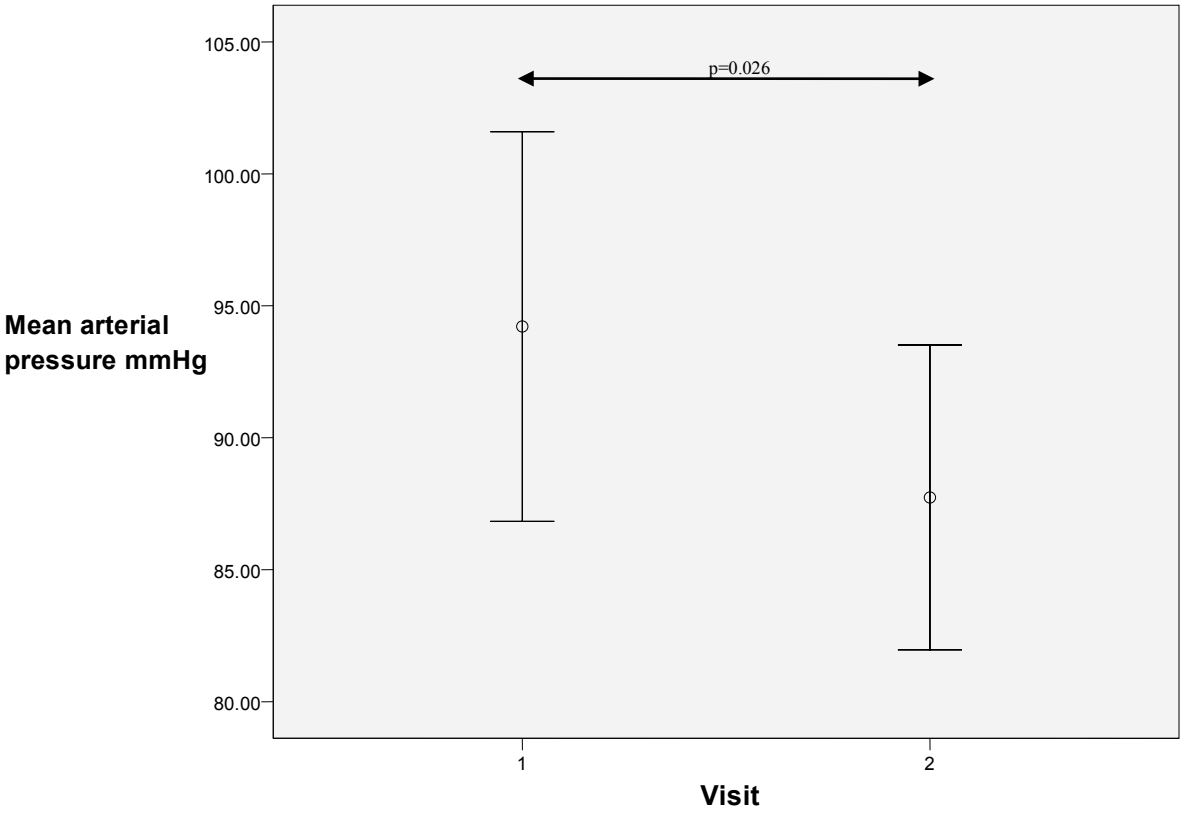


Figure 3-3. Changes in MAP - Overt Nephropaths

3.7 Metabolic Parameters

Baseline metabolic parameters are recorded in Table 3-10. Cholesterol is noted to be higher in the healthy individuals as compared to the overt nephropaths. This is likely to be due to the fact that these groups, with a recognised increased cardiovascular risk will be treated with a lipid lowering agent. Urinary protein excretion was measured by a 24 urine sample.

Parameter	**Healthy**			**Overt Nephropaths**		
	N	**Mean**	**SD**	**N**	**Mean**	**SD**
Glucose visit 1 (mmol/L)	10	**5.22**	0.54	14	**9.26**	4.19
HbA1C Visit1 (%)	10	**5.09**	0.26	12	**8.05**	1.22
Cholesterol (mmol/l)	8	**4.81**	0.99	14	**3.94**	0.68
Triglycerides (mmol/L)	8	**0.90**	0.37	14	**2.30**	1.45
24 hour urine protein (g/24hrs)	9	**0.21**	0.15	14	**0.91**	0.68

Table 3-10. Baseline metabolic parameters

3.7.1 The effects of Gum Arabic on Metabolic Parameters

Statistical analysis was carried out using a Students t-test.

3.7.1.1 Healthy Individuals

	N	Value	SD	P
HbA1C Visit 1 (%)	10	5.09	0.26	0.67
HbA1C Visit 2 (%)	10	5.12	0.27	
HbA1C Visit 2 (%)	7	5.14	0.32	0.02
HbA1C Visit 3 (%)	7	4.97	0.26	
Total Cholesterol Visit 1 (mmol/l)	8	4.81	0.99	0.39
Total Cholesterol Visit 2 (mmol/l)	8	4.68	0.94	
Total Cholesterol Visit 2 (mmol/l)	7	4.59	1.00	0.18
Total Cholesterol Visit 3 (mmol/l)	7	4.83	1.26	
Triglycerides Visit 1 (mmol/l)	8	0.90	0.37	0.23
Triglycerides Visit 2 (mmol/l)	8	1.00	0.47	
Triglycerides Visit 2 (mmol/l)	7	0.93	0.29	0.26
Triglycerides Visit 3 (mmol/l)	7	0.79	0.23	
24 hr urine protein Visit 1 (g/24hr)	8	0.22	0.16	0.11
24 hr urine protein Visit 2 (g/24hr)	8	0.12	0.08	
Weight Visit 1 (kg)	10	82.30	14.40	0.83
Weight Visit 2 (kg)	10	82.39	15.24	
Weight Visit 2 (kg)	7	81.64	16.60	0.08
Weight Visit 3 (kg)	7	80.89	16.03	

Table 3-11. Effects of Gum arabic on metabolic parameters - Healthy Individuals

Following the study period on Gum arabic, no significant changes in metabolic data are seen in the group of healthy individuals. There was however a statistically significant drop in HbA1C following the washout period (5.14 to 4.97, p=0.02). The reasons for

this are not clear. Data for 24 hour urine protein excretion at visit 3 was not complete. Weight remained static during the study period. There was a drop in weight in the washout period that did not reach statistical significance.

3.7.1.2 Overt nephropaths

	N	Value	SD	P
HbA1C Visit 1 (%)	12	8.05	1.22	0.24
HbA1C Visit 2 (%)	12	7.68	1.32	
Total Cholesterol Visit 1 (mmol/l)	14	3.94	0.68	0.41
Total Cholesterol Visit 2 (mmol/l)	14	4.09	0.70	
HDL Cholesterol Visit 1 (mmol/l)	7	0.79	0.09	**0.05**
HDL Cholesterol Visit 2 (mmol/l)	7	0.86	0.08	
LDL Cholesterol Visit 1 (mmol/l)	7	2.10	0.57	0.32
LDL Cholesterol Visit 2 (mmol/l)	7	2.34	0.64	
Triglycerides Visit 1 (mmol/l)	14	2.30	1.45	0.66
Triglycerides Visit 2 (mmol/l)	14	2.34	1.60	
24 hr urine protein Visit 1 (g/24hr)	14	0.91	0.68	0.46
24 hr urine protein Visit 2 (g/24hr)	14	0.99	0.64	
Weight Visit 1 (kg)	14	98.46	16.82	0.16
Weight Visit 2 (kg)	14	99.14	16.56	

Table 3-12. Effects of Gum arabic on metabolic parameters - Overt Nephropaths

There were no changes in the metabolic parameters in the overt nephropath group following the intervention period. A small but statistically significant rise in HDL cholesterol, from 0.79 to 0.86 mmol/l (p=0.05) was seen. A small and non-statistically significant rise in weight is seen (98.46kg to 99.14 kg, p=0.16).

3.7.2 The effects of Gum Arabic on Short Chain Fatty Acid Production

3.7.2.1 SCFA Production in Normal Individuals

Data on SCFA (butyrate and propionate) production was collected in the healthy individual group.

Levels of serum butyrate increased significantly from baseline levels to post gum arabic ingestion levels, with no associated change in serum propionate levels. Levels of butyrate rose form a baseline of 0.49 ± 0.08 to 0.71 ± 0.11 µmol/l (P=0.03).

3b Results

Follow on Study

3.8 Extended Baseline Demographics

The follow on study consisted entirely of subjects with incipient nephropathy. In addition to the baseline demographic data presented, data on the complications of diabetes and other cardiovascular risk data were also collected and are presented in Table 3-13.

Risk factor	Value	Percentage
Duration of diabetes	9.99 ± 8.39 years	NA
History of microalbuminuria recorded	9	39.1
Duration of microalbuminura	1.33 ± 0.34 years	NA
Ischaemic heart disease	4	17.4
Previous myocardial infarction	3	13.0
Previous CABG	0	0
Smoker	11	47.8
Pack year history (years)	33.91 ± 22.87	NA
Hypertension	16	69.6
History of total cholesterol >5.00 mmol/L	19	82.6
Family history of cardiovascular disease	10	43.5
Previous CVA	1	4.3
Peripheral vascular disease	3	13
Diabetic neuropathy	3	13.0
Diabetic retinopathy	6	23.0

Table 3-13. Baseline cardiovascular risk data - Incipient Nephropaths

Duration of diabetes was taken as the time as the period from diagnosis until the time of screening. This may underestimate the true duration of diabetes due to the time lag form onset to diagnosis. Average duration of diabetes was 9.99 ± 8.39 years.

17.4% of this population had ischaemic heart disease, defined as any patient who had been given a clinical diagnosis of angina, which may or may not have been proven with cardiac investigations. 47.8% were smokers, defined as anyone who had smoked

regularly in the past or continued to do so. Pack year history (PYH) was defined as: 1 pack year being equivalent to smoking 20 cigarettes daily for 1 year, the average PYH being 33.91 ± 22.87 years.

All patients in the diabetes clinic undergo annual diabetic retinopathy screening. The most recent report was referred to, to make a diagnosis of diabetic retinopathy, showing that 23.0% had a diagnosis of diabetic retinopathy.

39.1% of the population had a documented history of microalbuminuria with an average duration of 1.33 ± 0.34 years. ACR measurement was not part of the study, 24 hour urine protein excretion was and all of the patients had levels of protein excretion that would make a diagnosis of "microalbuminuria", and therefore incipient nephropathy.

In the follow on study data was also collected regarding medications that were prescribed to the incipient nephropath subjects and is presented in Figure 3-4.

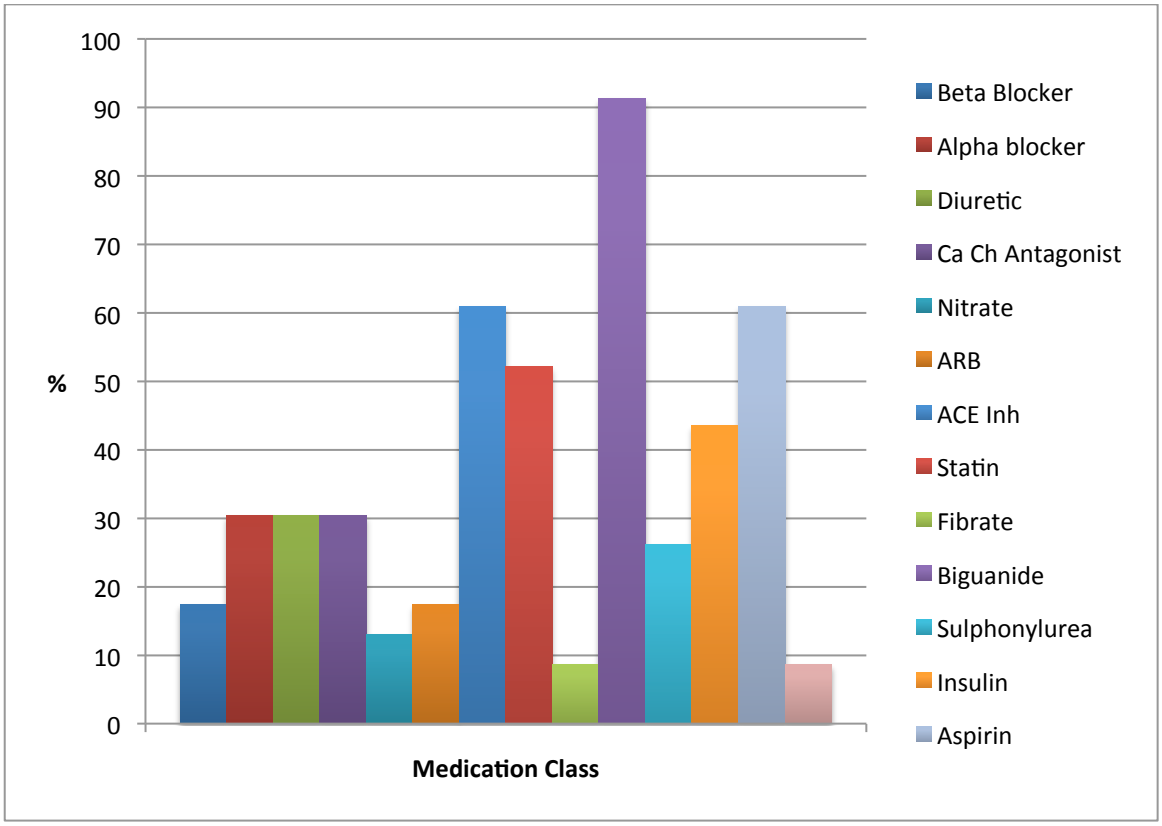


Figure 3-4. Concurrent Medication - Subjects with Incipient Nephropathy

3.9 Baseline Data

The data in Table 3-14 is data taken from the first study visit and includes simple measures of renal function and electrolytes.

Parameter	Incipient nephropaths		
	N	Mean	SD
Sodium (mmol/L)	23	**140.13**	2.14
Potassium (mmol/L)	23	**3.97**	0.35
Urea (mmol/L)	23	**5.94**	1.18
Creatinine (µmol/L)	23	**78.30**	13.18

Table 3-14. Baseline Biochemical Data - Incipient Nephropath

3.10 Effects of Gum Arabic on Biochemistry and Renal Function

3.10.1 Incipient Nephropaths

	Visit 1	Visit 2	n	p	Visit 2	Visit 3	n	p
Sodium (mmol/l)	140.13	140.13	23	1.0	140.13	140.65	23	0.30
Potassium (mmol/l)	3.97	3.99	23	0.79	3.99	4.01	23	0.57
Urea (mmol/l)	5.94	6.13	23	0.53	6.13	5.85	23	0.30
Creatinine (µmol/L)	78.30	81.96	23	**0.044**	81.96	83.17	23	0.56
Bicarbonate (mmol/l)	26.09	24.50	22	**0.011**	24.50	23.00	22	**0.03**

Table 3-15. Changes in Biochemistry - Incipient Nephropaths

Data was normally distributed and analysed using a Student's t-test. All patients in the incipient nephropath group had the parameters measured at each visit. There was a statistically significant rise in creatinine from visit 1 to visit 2, from 78.30 to 81.96 (p=0.044), the period during which Gum Arabic was taken. Similarly there was a progressive and statistically significant decline in serum bicarbonate throughout the duration of the study.

3.10.2 Whole group

	Visit 1	Visit 2	n	P	Visit 2	Visit 3	n	p
Sodium (mmol/l)	140.19	139.79	47	0.33	140.07	140.48	27	0.37
Potassium (mmol/l)	4.22	4.189	47	0.59	4.02	4.01	27	0.93
Urea (mmol/l)	9.75	9.26	47	0.25	6.07	5.92	30	0.52
Creatinine (µmol/L)	120.64	121.89	47	0.52	82.47	83.53	30	0.50

Table 3-16. Changes in biochemistry - Whole group

There were no significant changes in simple measures of renal function (urea and creatinine) or electrolytes following the intake of gum arabic or any changes following the washout period. Values stated for visit 2 are different in the comparisons with visits 1 and 3 as not all patients had complete data for visit 3.

3.10.3 Effects of Gum Arabic on isotopic measures of renal function

All subjects had a baseline measurement of isotope glomerular filtration rate performed as previously described at the first study visit. Data was normally distributed and analysis was undertaken using a Student's t-test.

			Units = ml/min			
Patient group		**n**	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Incipient nephropaths	GFR	23	69.0	139.0	**100.5**	20.05
	ERPF	23	278.0	489.0	**382.0**	62.11
Whole Group	GFR	47	21.0	141.0	**82.8**	37.17
	ERPF	44	96.0	541.0	**347.3**	128.53

Table 3-17. Baseline Measures of Isotopic Renal Function

The mean GFR of the incipient nephropath group was 100.5 ml/min with an ERPF of 382.0 ml/min.

3.10.4 Incipient Nephropaths

	Visit 1	**Visit2**	**n**	**P**	**Visit 2**	**Visit 3**	**n**	**p**
GFR (ml/min)	100.68	98.23	22	0.34	98.23	99.05	22	0.79
ERPF (ml/min)	382.00	388.00	23	0.68	380.82	366.23	22	0.29
FF (%)	27	26	22	0.58	26	27	22	0.20

Table 3-18. Changes in renal Function - Incipient Nephropaths

Amongst the incipient nephropaths GFR dropped from 100.68 ml/min to 98.23 ml/min, between visit 1 and visit 2 but this was non-significant (p=0.34). There was no significant change in ERPF or filtration fraction. One subject did not have data on GFR at visit 2 due to problems with analysis of the sample in the laboratory.

3.10.5 Whole Group

	Visit 1	Visit2	n	P	Visit 2	Visit 3	n	p
GFR (ml/min)	82.48	78.78	46	0.054	97.93	100.93	29	0.24
ERPF (ml/min)	381.03	379.42	35	0.87	380.81	366.23	22	0.29
FF (%)	25	24	34	0.24	26	27	22	0.20

Table 3-19. Changes in Renal Function - Whole Group

Isotope GFR dropped from 82.48 ml/min to 78.78 ml/min in the whole group following the intake of gum arabic. This approached statistical significance (p=0.054). Following the washout period there was a small rise in GFR from 97.93 ml/min to 100.93 ml/min although this did not reach significance. Data was not complete for visit 3 or for ERPF data. This was due to difficulties in sourcing the radioisotope (particularly in the pilot study) and not due to withdrawals from the study. There were no significant changes in ERPF or filtration fraction.

3.10.6 Effect of Gum Arabic on Peripheral Blood Pressure

The following analyses look specifically at the changes in peripheral blood pressure following administration of Gum Arabic.

3.10.7 Incipient Nephropaths

Variable	Mean (mmHg)	SD	Significance
Systolic BP Visit 1	141.52	18.72	0.26
Systolic BP Visit 2	137.91	17.85	0.93
Systolic BP Visit 3	138.70	15.94	
Diastolic BP Visit 1	86.09	7.23	**0.044**
Diastolic BP Visit 2	82.57	9.43	0.94
Diastolic BP Visit 3	82.74	10.89	
Pulse Pressure Visit 1	55.44	16.35	0.78
Pulse Pressure Visit 2	55.35	14.68	0.92
Pulse Pressure Visit 3	55.96	11.70	
MAP Visit 1	104.57	9.62	0.08
MAP Visit 2	101.01	10.84	0.96
MAP Visit 3	101.39	11.54	

Table 3-20. Changes in Blood Pressure - Incipient Nephropaths

A small drop is observed in systolic pressure (141.52 mmHg to 137.91 mmHg) following the intervention period but this does not reach statistical significance. A statistically significant reduction in diastolic blood pressure is observed from 86.09 mmHg to 82.57mmHg (p=0.044). No significant rise in diastolic blood pressure was seen following the washout period. Mean arterial pressure also fell from 104.57 mmHg to 101.01 mmHg but was not statistically significant.

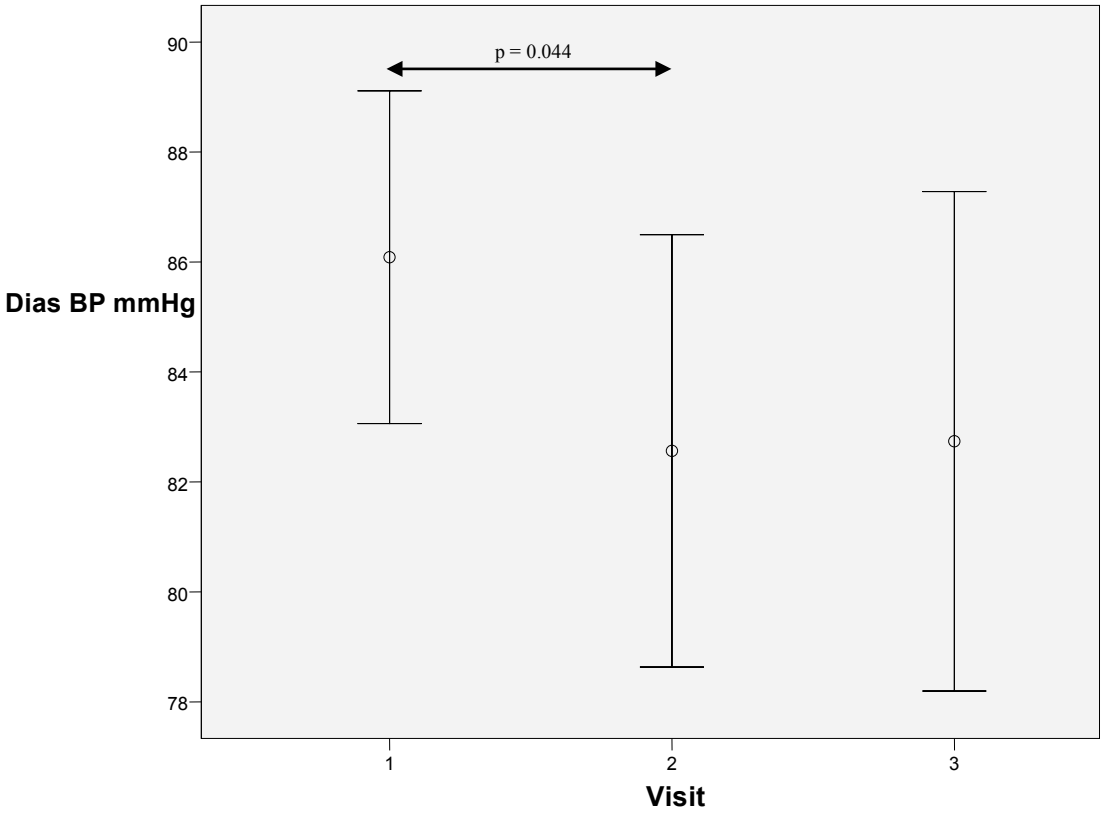


Figure 3-5. Changes in Diastolic Blood Pressure - Incipient Nephropaths

3.10.8 Whole Group

The whole group was analysed to look for changes in peripheral blood pressure on and off treatment with Gum Arabic. Statistical analysis was with a Student t-test.

Variable	Mean (mmHg)	Std. Deviation	Significance
Systolic BP Visit 1	138.45	18.90	**0.024**
Systolic BP Visit 2	132.83	15.88	
Diastolic BP Visit 1	79.43	12.13	0.071
Diastolic BP Visit 2	75.96	12.60	
Pulse Pressure Visit 1	59.02	18.09	0.34
Pulse Pressure Visit 2	56.87	14.30	
Mean Arterial Pressure Visit 1	100.00	12.02	**0.023**
Mean Arterial Pressure Visit 2	94.91	12.03	

Table 3-21. Changes in Blood Pressure – Whole Group

Statistically significant changes were seen in systolic and mean arterial blood pressure with a reduction in systolic blood pressure of nearly 6 mmHg (shown in Figure 3-6) from 138.45 mmHg to 132.83 mmHg after dietary supplementation with gum arabic (p=0.024).

There was also a statistically significant drop in MAP of just over 5mmHg (p=0.023) shown in Figure 3-7.

A clinically relevant fall in diastolic blood pressure in the whole group is seen after the study period on Gum Arabic which trended towards significance with a reduction from 79.43 mmHg to 75.96 mmHg (p=0.071).

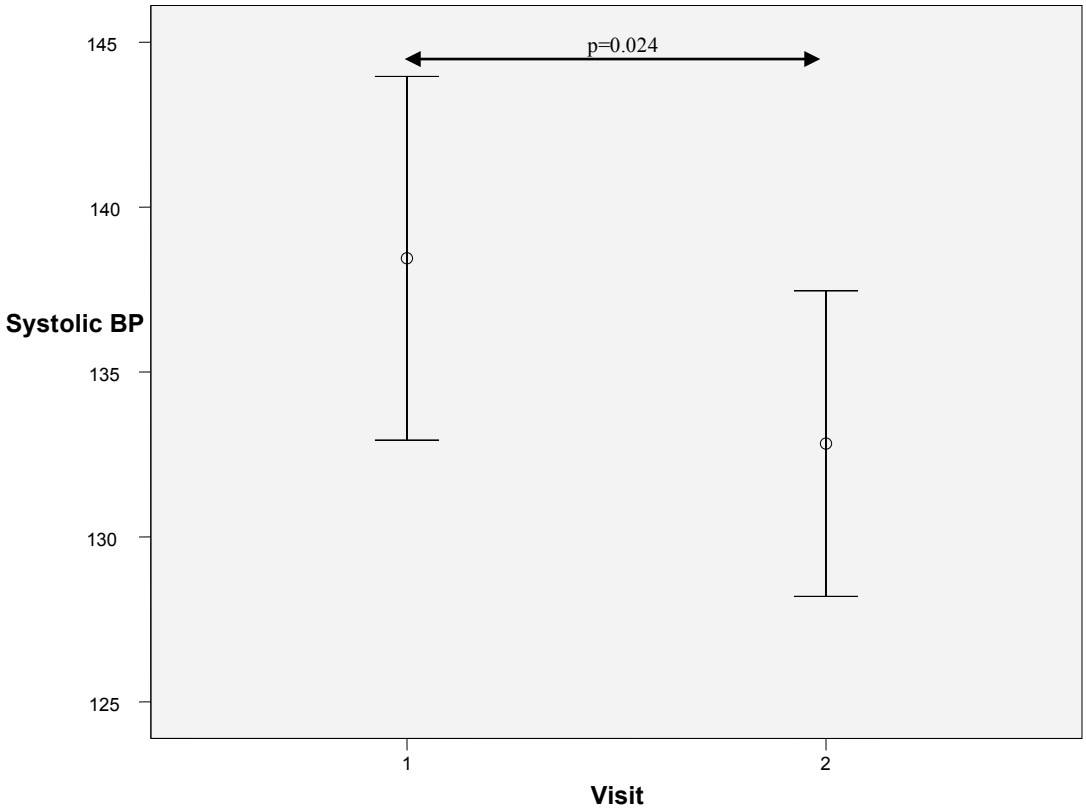


Figure 3-6. Changes in Systolic Blood Pressure - Whole Group

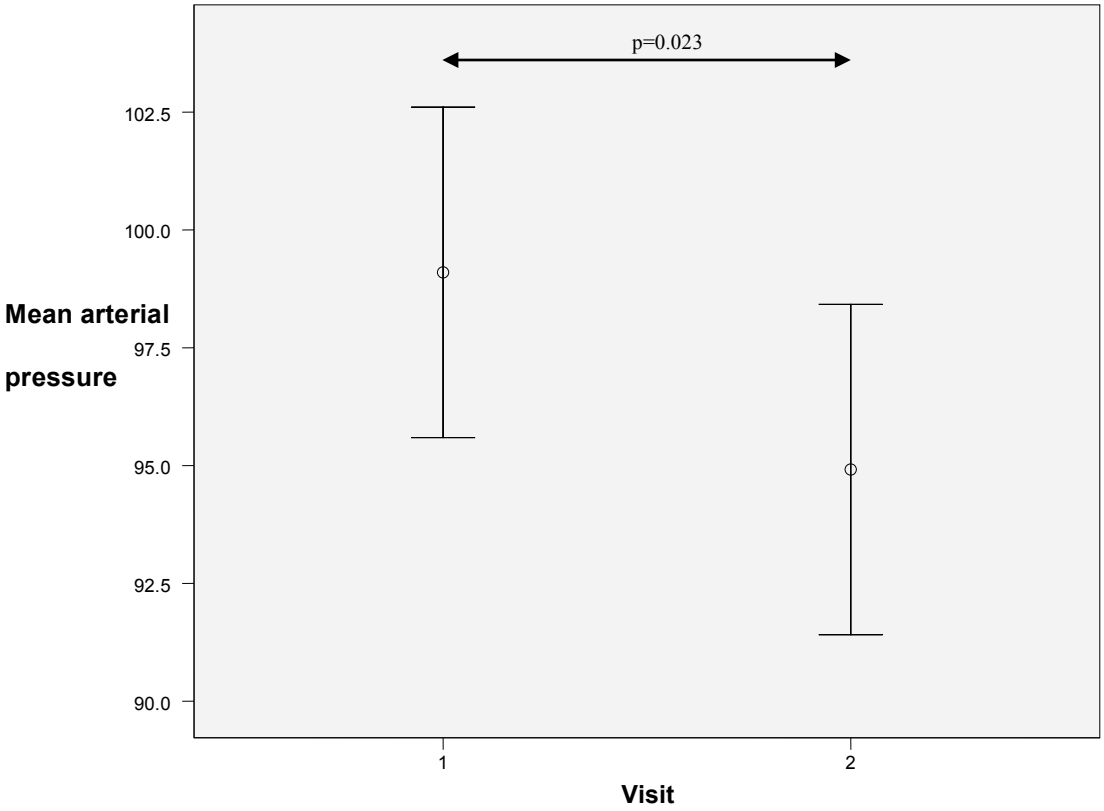


Figure 3-7. Change in Mean Arterial Pressure - Whole Group

3.10.9 The Effect of Gum Arabic on Central Blood Pressure and Cardiovascular Risk

This chapter will discuss the effects that Gum arabic has on central blood pressure. Measurements of vascular stiffness are accepted to be markers of cardiovascular risk. Baseline data on other more traditional markers of cardiovascular risk and complications of diabetes were also recorded in this group and have been presented in Table 3-13. Central blood pressure and measures of vascular stiffness were calculated in the follow on study using the Sphygmocor system in the group of incipient nephropaths.

Incipient nephropaths are a group of patients with an increased cardiovascular risk which is potentially modifiable and treatable. To further examine this group in detail the effects of dietary supplementation with Gum Arabic, the effects on central blood pressure and vascular stiffness were looked at in this sub group of incipient diabetic nephropaths taking part in the main study. Data for this group was normally distributed and statistical analysis was carried out using a comparison of means using a paired t-test.

3.10.10 Central blood pressure

	Mean BP mmHg	Std. Error Mean	p value
Aortic Systolic Pressure Visit 1	129.65	4.18	0.62
Aortic Systolic Pressure Visit 2	128.13	3.80	
Aortic Systolic Pressure Visit 2	128.13	3.80	0.33
Aortic Systolic Pressure Visit 3	126.89	3.52	
Aortic Diastolic Pressure Visit 1	86.52	1.60	0.68
Aortic Diastolic Pressure Visit 2	84.96	2.00	
Aortic Diastolic Pressure Visit 2	84.96	2.00	0.38
Aortic Diastolic Pressure Visit 3	83.80	2.36	
Aortic Pulse Pressure Visit 1	42.85	3.58	0.89
Aortic Pulse Pressure Visit 2	43.17	2.83	
Aortic Pulse Pressure Visit 2	43.17	2.83	0.97
Aortic Pulse Pressure Visit 3	43.09	2.31	

Table 3-22**.** Changes in Central Blood Pressure

Following treatment with Gum Arabic, there were no statistically significant changes are observed in aortic (central) blood pressure. There is a small drop in aortic systolic and diastolic blood pressure following the study period on gum arabic but with no associated increase in these pressures following the washout period.

3.10.11 Effect of Gum Arabic on measures of Vascular Stiffness

	Mean	N	SE Mean	p value
AIx @75bpm Visit1 (%)	22.07	23	2.49	0.42
AIx @75bpm Visit 2 (%)	23.11	23	2.11	
AIx @75bpm Visit 2 (%)	23.11	23	2.11	0.89
Aix @75bpm Visit 3 (%)	23.30	23	1.89	
AIx Visit1 (%)	24.24	23	2.45	0.86
AIx Visit 2 (%)	24.50	23	2.32	
AIx Visit 2 (%)	24.50	23	2.32	0.41
AIx Visit 3 (%)	25.87	23	1.76	
PWV Visit 1 (m/sec)	10.47	22	0.58	0.61
PWV Visit 2 (m/sec)	10.32	22	0.57	
PWV Visit 2 (m/sec)	10.32	22	0.57	0.68
PWV Visit 3 (m/sec)	10.53	22	0.71	
Tr Visit1 (ms)	136.48	23	2.67	0.57
Tr Visit 2 (ms)	135.24	23	2.4	
Tr Visit 2 (ms)	135.24	23	2.46	0.51
Tr Visit 3 (ms)	136.52	23	2.10	
SEVR Visit 1 (%)	159.52	23	6.59	0.60
SEVR Visit 2 (%)	157.89	23	6.47	
SEVR Visit 2 (%)	157.89	23	6.47	0.42
SEVR Visit 3 (%)	160.78	23	7.01	

Table 3-23. Changes in Parameters of Vascular Stiffness

The measures of vascular stiffness detailed in Table 3-23 show that there are no changes vascular stiffness following the administration of Gum arabic to subjects with incipient nephropathy. This would suggest that the blood pressure lowering effect that is seen is not as a result of changes to vascular compliance.

3.11 The Effects of Gum Arabic on Metabolic Parameters

Baseline metabolic parameters are recorded in Table 3-24. Urinary protein excretion was measured by a 24 urine sample of urinary protein excretion.

Parameter	Incipient Nephropaths		
	N	**Mean**	**SD**
Glucose visit 1 (mmol/L)	22	**9.13**	2.16
HbA1C Visit1 (%)	23	**7.54**	1.15
Cholesterol (mmol/l)	23	**3.97**	0.86
Triglycerides (mmol/L)	23	**1.52**	0.63
24 hour urine protein (g/24hrs)	23	**0.18**	0.13

Table 3-24. Baseline Metabolic Parameters - Incipient Nephropaths

3.11.1 The effects of Gum Arabic on Metabolic Parameters

3.11.1.1 Incipient Nephropaths

	N	Value	SD	P
HbA1C Visit 1 (%)	23	7.54	1.15	0.25
HbA1C Visit 2 (%)	23	7.73	1.30	
HbA1C Visit 2 (%)	22	7.71	1.33	0.18
HbA1C Visit 3 (%)	22	7.51	1.28	
Total Cholesterol Visit 1 (mmol/l)	23	3.97	0.86	**0.045**
Total Cholesterol Visit 2 (mmol/l)	23	4.13	0.89	
Total Cholesterol Visit 2 (mmol/l)	23	4.13	0.89	0.66
Total Cholesterol Visit 3 (mmol/l)	23	4.08	0.95	
HDL Cholesterol Visit 1 (mmol/l)	17	0.99	0.30	0.33
HDL Cholesterol Visit 2 (mmol/l)	17	0.97	0.27	
HDL Cholesterol Visit 2 (mmol/l)	19	0.95	0.19	0.85
HDL Cholesterol Visit 3 (mmol/l)	19	0.95	0.21	
LDL Cholesterol Visit 1 (mmol/l)	17	2.21	0.81	0.43
LDL Cholesterol Visit 2 (mmol/l)	17	2.28	0.80	
LDL Cholesterol Visit 2 (mmol/l)	19	2.24	0.84	0.56
LDL Cholesterol Visit 3 (mmol/l)	19	2.17	0.89	
Triglycerides Visit 1 (mmol/l)	23	1.52	0.63	0.03
Triglycerides Visit 2 (mmol/l)	23	1.70	0.58	
Triglycerides Visit 2 (mmol/l)	23	1.70	0.58	0.69
Triglycerides Visit 3 (mmol/l)	23	1.74	0.75	
24 hr urine protein Visit 1 (g/24hr)	23	0.18	0.13	0.26
24 hr urine protein Visit 2 (g/24hr)	23	0.21	0.12	
24 hr urine protein Visit 2 (g/24hr)	21	0.19	0.11	0.56
24 hr urine protein Visit 3 (g/24hr)	21	0.18	0.11	
Weight Visit 1 (kg)	23	88.63	17.13	0.85
Weight Visit 2 (kg)	23	88.59	17.28	
Weight Visit 2 (kg)	23	88.59	17.28	0.50
Weight Visit 3 (kg)	23	88.74	17.46	

Table 3-25. Effects of Gum arabic on metabolic parameters - Incipient Nephropaths

Statistical analysis was carried out using a Student's t-test as data was normally distributed. No change in HBa1C is seen in the group of incipient nephropaths following the study period on gum arabic. Due to problems in the biochemistry lab, data for some subjects was only available for total cholesterol and triglycerides. There are however statistically significant increases in total cholesterol (3.97 mmol/l to 4.13 mmol/l, p=0.05) and in serum triglycerides (1.52 mmol/l to 1.70 mmol/l, p=0.03). This could once again relate to a possible appetite stimulation effect. However, this is not supported by any change in weight of the subjects.

3.11.1.2 Whole Group

	N	Value	SD	p
HbA1C Visit 1 (mmol/l)	45	7.13	1.52	0.99
HbA1C Visit 2 (mmol/l)	45	7.13	1.57	
HbA1C Visit 2 (mmol/l)	29	7.09	1.61	0.09
HbA1C Visit 3 (mmol/l)	29	6.9	1.57	
Total Cholesterol Visit 1 (mmol/l)	45	4.11	0.88	0.15
Total Cholesterol Visit 2 (mmol/l)	45	4.21	0.85	
Total Cholesterol Visit 2 (mmol/l)	30	4.24	0.92	0.83
Total Cholesterol Visit 3 (mmol/l)	30	4.26	1.06	
Triglycerides Visit 1 (mmol/l)	45	1.65	1.04	0.02
Triglycerides Visit 2 (mmol/l)	45	1.78	1.08	
Triglycerides Visit 2 (mmol/l)	30	1.52	0.62	0.93
Triglycerides Visit 3 (mmol/l)	30	1.52	0.78	
24 hr urine protein Visit 1 (g/24hr)	45	0.41	0.51	0.54
24 hr urine protein Visit 2 (g/24hr)	45	0.44	0.52	
24 hr urine protein Visit 2 (g/24hr)	21	0.19	0.11	0.56
24 hr urine protein Visit 3 (g/24hr)	21	0.18	0.11	

Table 3-26. Effects of Gum arabic on metabolic parameters - Whole Group

Statistical analysis was done using a Students t-test. Gum arabic did not change levels of HbA1C in the whole group. There was no change in levels of total cholesterol either. Following the study period on Gum arabic, an increase in serum triglycerides (1.65 mmol/l to 1.78, p=0.02) is seen. This could be as a result of improved appetite. No change in urine protein excretion was seen.

3.12 The effects of Gum Arabic on Short Chain Fatty Acid Production

3.12.1 SCFA Production in Incipient nephropaths

Data on an expanded series of plasma short chain fatty acids was collected for 22 of the incipient diabetic nephropath group at all 3 study points. The following short chain fatty acids were quantified:

Lactate	Acetate
Propionate	Isobutyrate
Butyrate	Isovalerate
Succinate	Valerate
Formate	

Statistical analysis was undertaken to ascertain whether there had been a change in the levels of SCFAs. A paired t-test was used for the total short chain fatty acids as the levels were normally distributed. A Wilcoxon Signed Ranks Test was used for the individual SCFAs as these levels were not normally distributed.

	Mean (µmol/l)	Std. Error Mean	p value
Plasma Acetate Visit 1	22.85	6.27	**0.033**
Plasma Acetate Visit 2	39.19	10.16	
Plasma Acetate Visit 2	39.19	10.16	0.55
Plasma Acetate Visit 3	32.21	6.52	
Plasma Succinate Visit 1	24.51	4.03	0.91
Plasma Succinate Visit 2	27.59	5.70	
Plasma Succinate Visit 2	27.59	5.70	0.90
Plasma Succinate Visit 3	26.64	4.08	
Plasma Lactate Visit 1	3652.32	360.71	0.31
Plasma Lactate Visit 2	3816.12	406.97	
Plasma Lactate Visit 2	3816.12	406.97	0.32
Plasma Lactate Visit 3	3896.77	276.98	
Plasma Isobutyrate Visit 1	33.22	4.23	0.73
Plasma Isobutyrate Visit 2	36.61	5.96	
Plasma Isobutyrate Visit 2	36.61	5.96	0.78
Plasma Isobutyrate Visit 3	39.67	6.72	
Plasma Isovalerate Visit 1	33.07	4.87	0.53
Plasma Isovalerate Visit 2	33.00	6.93	
Plasma Isovalerate Visit 2	33.00	6.93	0.71
Plasma Isovalerate Visit 3	25.90	4.55	
Plasma Butyrate Visit 1	36.72	5.90	**0.031**
Plasma Butyrate Visit 2	23.79	4.79	
Plasma Butyrate Visit 2	23.79	4.79	0.10
Plasma Butyrate Visit 3	36.58	6.72	
Plasma Formate Visit 1	46.30	13.60	0.64
Plasma Formate Visit 2	83.66	28.45	
Plasma Formate Visit 2	83.66	28.45	0.14
Plasma Formate Visit 3	46.90	12.25	
Plasma Valerate Visit 1	27.55	4.28	0.43
Plasma Valerate Visit 2	34.65	6.16	
Plasma Valerate Visit 2	34.65	6.16	0.76
Plasma Valerate Visit 3	40.03	6.59	
Plasma Propionate Visit 1	50.86	11.19	0.59
Plasma Propionate Visit 2	51.92	15.92	
Plasma Propionate Visit 2	51.92	15.92	0.14
Plasma Propionate Visit 3	58.72	10.78	
Total SCFA Visit 1	275.04	24.86	0.12
Total SCFA Visit 2	330.37	39.42	
Total SCFA Visit 2	330.37	39.42	0.56
Total SCFA Visit 3	306.63	25.94	

Table 3-27. Changes in Production of SCFAs – Incipient Nephropaths

Only changes in the levels of plasma acetate, butyrate, formate and total SCFA were observed following administration of Gum Arabic with a subsequent fall towards baseline after the washout period. Data for the other SCFAs was complete but no clinically relevant or statistically significant changes were seen in their levels.

Plasma acetate rose from 22.85 to 39.19 (p=0.033) with a fall towards baseline after the washout period (p=NS). Conversely plasma butyrate fell from 36.72 to 23.79 (p=0.031) with a subsequent rise back to baseline (NS, p=NS). Plasma formate levels increased greatly after the study period on Gum Arabic, from 46.30 to 83.66, with a fall towards baseline but this did not reach statistical significance. There was an increase in total plasma SCFAs after administration of Gum Arabic from 275.04 to 330.37 with a drop towards baseline (306.63) following the washout period. Neither of these changes reached statistical significance.

The original study design allowed for collection of faeces to measure both faecal short chain fatty acids and faecal bacteria composition. The collection was undertaken in a number of patients and stored for future analysis. This analysis has not yet taken place, in part due to cost limitations of the study.

3.13 Further data analysis

Although many of the patients showed little or no change in the measurements presented, this could be for numerous reasons. One of the major compounding factors, particularly in the group of incipient nephropaths may be the fact that 78.3% of this group were taking an ACE inhibitor or ARB. This group of medications is well documented to have effects on GFR and vascular function and it may not have been possible to improve these parameters further by the addition of Gum arabic. Another unexplored confounding factor may have been that of pre-existing dietary fibre intake. No measurement of concurrent dietary fibre intake was recorded and it is feasible that some of the subjects were already on a high fibre diet. The addition of further fibre in the form of gum arabic may not have had any further impact on the parameters recorded. By looking for a group of subjects that may have responded to the Gum Arabic, by way of a reduction in blood pressure, it might be possible to see if this group has also had improvements in other parameters measured such as changes in renal haemodynamics or in measures of arterial stiffness.

3.13.1 Blood Pressure Responders

To further study the effects of gum arabic on blood pressure the data was analysed to see if there was a sub-group of patients who responded to gum arabic. This group was defined as any individual who had a fall in their mean arterial blood pressure. There was no significant difference in the number of responders in each group.

	MAP Responder		
	No	**Yes**	**% responders**
Healthy Individuals	3	7	70
Incipient Nephropaths	8	15	65.2
Overt Nephropaths	3	11	78.6
Total	14	33	70.2

Table 3-28 Distribution of patients with significant fall in blood pressure - Classified by MAP response

As expected, these "responders" had a statistically significant drop in both peripheral systolic and diastolic blood pressure (p=0.000). When the 3 patient groups were analysed separately only the incipient nephropaths had a significant drop in both systolic and diastolic blood pressure (p=0.002 and p=0.001 respectively). The healthy individuals had a significant drop in diastolic blood pressure (p=0.027) and the overt nephropaths only in systolic blood pressure (p=0.013). These significant changes may be as a result of selection bias.

3.13.1.1 Changes in Central Blood Pressure and Vascular Stiffness

Central blood pressure was of course only measured in the group of incipient nephropaths using the Sphygmocor system, and I have already demonstrated that they had a significant drop in their peripheral diastolic blood pressure and a trend to a drop in systolic blood pressure but there was no associated drop seen in central blood pressure which may be a better marker of cardiovascular risk in these patients.

Patient Group	Ao Sys BP (pre)	Ao Sys BP (post)	P value
Non-responders	124.38 ± 4.85	135.31 ± 5.19	**0.032**
Responders	132.47 ± 5.85	124.30 ± 4.97	**0.015**

Units are mmHg

Table 3-29. Changes in Central Systolic Blood Pressure

When the data is analysed further, comparing responders, that is those with a drop in MAP, and non responders some marked differences are seen. The responder group show a significant fall in central aortic systolic blood pressure with a fall from 132.47 mmHg to 124.30 mmHg (p=0.015) compared with the non responder group who actually showed a significant rise in their aortic systolic pressure from 124.83 mmHg to 135.31 mmHg (p=0.032).

Patient Group	Ao Dias BP (pre)	Ao Dias BP (post)	p value
Non-responders	84.63 ± 2.71	89.63 ± 3.39	**0.031**
Responders	87.53 ± 2.00	82.47 ± 2.29	**0.007**

Units are mmHg

Table 3-30. Changes in Central Diastolic Blood Pressure

Similar changes are demonstrated when aortic diastolic blood pressure is analysed. The responder group showed a significant fall in pressure from 87.53 mmHg to 82.47 mmHg (p=0.007) and the non responders showed a significant rise in pressure from 84.63 mmHg to 89.63 mmHg (p=0.031). Analysis of parameters of arterial stiffness failed to show any significant changes as demonstrated below in Table 3-31.

	Pre	SD	Post	SD	P value
AIx (%)	24.87	12.95	25.27	12.47	NS
AIx @75 (%)	22.47	12.30	23.30	11.77	NS
PWV (m/sec)	10.07	2.86	10.14	2.84	NS

Table 3-31 Analysis of Diabetic responders - Classified by MAP response

3.13.1.2 Changes in Renal Function in responder group

Looking at all of the subjects, data was analysed to see if there was any change in renal function within the groups of responders or non responders. As discussed earlier, there was a drop in GFR following administration of Gum Arabic in the whole group that did not quite reach statistical significance (p= 0.054). The non-responder group showed no

real change in the GFR (88.85 ± 9.91ml/min to 87.54 ± 10.14 ml/min). The MAP responder group show a greater drop in GFR (79.97 ± 6.69 ml/min to 75.33 ± 5.97 ml/min) with a trend towards statistical significance ($p= 0.053$) which is likely to be directly related to the change in blood pressure.

For the three separate patient groups the data was further analysed, looking at the responder and non-responder groups. There were no significant changes in renal function when the groups were split down in to these fairly small numbers, other than a significant drop in the GFR ($p<0.05$) in the sub-group of healthy "non-responders". However this group consisted of only 3 patients and may be inappropriate to infer anything from this.

4 DISCUSSION

4.1 Baseline Demographics

The vast majority of the patients recruited to the two studies were male. The reasons for this are not clear. There were no female patients in the overt nephropathy group.

Comparison of age, weight and BMI was calculated using the One-way ANOVA method. Both age and weight were statistically significantly different ($p<0.001$ and $p=0.038$ respectively) between the 3 groups. This study will be unable to answer why this difference in weight existed but studies do show that obesity is an independent risk factor for progression and worse outcomes of kidney disease [116]. This difference in weight is also reflected in a statistically significant ($p=0.001$) difference in BMI between the groups. It is likely that the difference in age between the 2 diabetic populations is that it has taken time to progress to overt nephropathy and hence the greater age. The healthy population was younger as they were selected from a population who were of working age.

4.2 The Effects of Gum Arabic on Biochemistry and Renal Function

Baseline biochemical data for the group of healthy individuals and for the incipient nephropaths was very similar, with no statistically significant differences between them. Overall there was little in the way of change in the simple biochemical parameters following the ingestion of Gum Arabic when looking at the group as a whole.

The results show that there is a drop in isotope GFR in the group as a whole, following the intake of gum arabic but this did not reach statistical significance ($p=0.054$). There was no associated change in filtration fraction which is important. It is likely that most of this change is as a result of the statistically significant change in GFR seen in the group of healthy individuals, with a drop in GFR of 13.60 ml/min ($p=0.016$). As a result of a drop in EPRF in the same period there was no change in filtration fraction. Following the washout period the GFR increased again suggesting a reversible effect that was likely to have been caused by the gum arabic. Despite this drop in GFR, the results show that there was actually a drop in serum creatinine in the group of healthy individuals (87.10 to 82.80 ml/min, $p=0.016$). This is difficult to reconcile given that a

decrease in GFR should lead to an increase in serum creatinine. One possible explanation is that there was an increase in the tubular secretion of creatinine during the gum arabic supplementation. This compensation by a tubular process could have led to these results. Similar changes are not shown in the group of incipient nephropaths. In this group there was climb in serum creatinine after taking gum arabic supplements (78.3 µmol/l to 81.96 µmol/l, p=0.044). There was no associated change in isotope GFR. A possible explanation is a potential effect on appetite, though this is not supported by a change in weight remained constant in the throughout the study. No food diary was recorded in the study to look at either fibre intake or protein intake. If there had been an increase in protein intake this may be sufficient to account for the change in serum creatinine.

There was no significant change in either ERPF or GFR in either group of diabetic subjects although there did appear to be a trend towards a reduction in GFR in the incipient nephropath group following administration of Gum Arabic. The reasons for this are unclear but may relate to the poly pharmacy that these patients are taking, in particular drugs affecting the renin angiotensin system (see Figure 3-4). There may have also been an element of "self selection". Many of the patients willing to take part in the study are likely to already be the type of patient who takes an interest in their health. This is reflected by relatively good diabetic control, good control of lipids and urine protein excretion. Blood pressure control in all 3 groups was also good (see Table 3-1). It was not known what the subjects' diets were as a food diary was not kept. It is quite possible that there was a subset of patients who were on a high fibre diet already and the addition of 25g of Gum Arabic would not have been able to alter bacterial short chain fatty acid production further. There may not therefore have been any scope for improving renal function further by the addition of gum arabic to the diet.

The small changes that have been observed may be clinically important and may have been better demonstrated if the study period was longer and if more patients had been recruited to the study. In this particular study, a dose of 25g of Gum arabic was chosen. Ali et al [217] have however chosen a higher dose of 50g of gum arabic, for a similar time period, in a study of patients with chronic renal failure. A higher dose may have lead to an increased effect but may have worsened its side effect profile and compliance.

4.3 The Effects of Gum Arabic on Blood Pressure and Cardiovascular Risk

Analysis of peripheral blood pressure at baseline demonstrates that the diastolic and mean arterial pressure in the group of incipient nephropaths were statistically higher than the healthy individuals and the overt nephropaths. Diastolic blood pressure was significantly higher at 86.09mmHg in the incipient nephropath group compared to the healthy individuals who had a mean diastolic BP of 75.50 mmHg (p=0.028) and the overt nephropaths who had a diastolic blood pressure of 71.29 mmHg (p=0.000). It would not be unexpected that the blood pressure would be higher in the incipient group compared to the healthy group, due to the association of hypertension and diabetes. Given the association of high blood pressure and progressive diabetic nephropathy, it would be likely that the group of overt nephropaths would have higher blood pressures than those with incipient nephropathy [47]. One possible explanation is purely that the overt nephropath group have been more aggressively treated for their hypertension as their increased cardiovascular risk has been recognised in the specialist nephrology clinics treating them.

By analysing the group as a whole Gum arabic is shown to have a clinically beneficial effect on peripheral blood pressure. There was nearly a 6 mmHg reduction in systolic blood pressure (p=0.024) and just over a 4mmHg drop in mean arterial pressure (p=0.023). Despite a potentially clinically important reduction in diastolic blood pressure of over 3 mmHg, it did not reach statistical significance (p=0.071).

Looking at the individual groups of patients, there was a significant drop in the systolic blood pressure of healthy individuals, with some rise back towards baseline following the washout period. This partial rise back to baseline could indicate a prolonged effect of the Gum Arabic and that not a long enough washout time was used.

The use of antihypertensive agents is seen extensively in the incipient nephropath group. As previously discussed there is good evidence for the use of drugs that block the renin angiotensin system and hence 78% of the patients were on either an ACE inhibitor or an angiotensin II receptor antagonist (Figure 3-4). Fourteen patients were on ACE inhibitors, 4 patients were on angiotensin II receptor antagonists but none of them were on dual therapy. A total of 18 or 78.3% of the patients were on a drug that inhibited the renin angiotensin system. Many other classes of antihypertensive agent

were also commonly prescribed as monotherapy or in combination other medication. The number of antihypertensive medications taken by individual patients is shown in Figure 4-1, with the majority of patients being on 2 drugs.

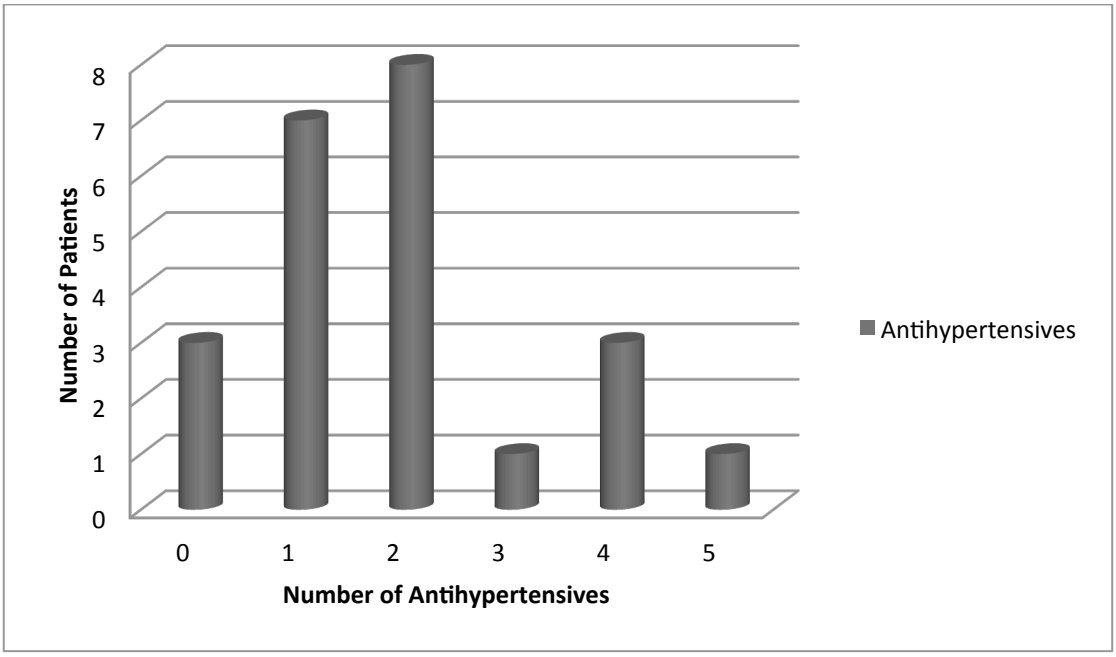


Figure 4-1. Number of Antihypertensives - Incipient Nephropaths

In the incipient nephropaths, there was a significant drop in the diastolic blood pressure, again with a possible trend to return to baseline following the washout period. There was a non-statistically significant drop in systolic and mean arterial pressure. A possible criticism of this study is that not large enough numbers of subjects were recruited. With greater numbers of patients there is a possibility this could be shown to be significant. These individuals had less than adequately controlled blood pressure despite being on multiple medications and were not reaching a target blood pressure of 130/80mmHg at the start of the study. The improvements in blood pressure following gum arabic suggest gum arabic could have an important role in lowering peripheral blood pressure further in this population of subjects.

Despite some improvements in the peripheral blood pressure measurements of the incipient nephropaths, this did not translate in to changes in measures of central blood

pressure or measures of vascular stiffness. This would suggest that the blood pressure lowering effect of Gum arabic seen in this study is unlikely to be as a result in changes in endothelial function. This is similar to what was demonstrated in the CAFE study [195]. An antihypertensive regime of amlodipine ± perindopril versus atenolol ± thiazide showed similar reductions in peripheral blood pressure but the amlodipine based regime showed significant improvements in central blood pressure and improvements in cardiovascular outcomes.

Looking at the overt nephropath group there was a clinically important drop in systolic blood pressure of 9 mmHg, but this was not statistically significant. Mean arterial pressure did improve, with a reduction of over 6 mmHg and this has clear clinical importance.

The reasons for the differences between the groups are not entirely clear and cannot be explained on the basis of this study. Numbers in the different groups were markedly different and this may have an impact on the results and statistical analysis. Compliance is difficult to record accurately in a study design such as this. An attempt was made to record compliance in the incipient nephropath group by recording the number of sachets of Gum arabic used. A total of 4 unused sachets of Gum arabic were returned. This would suggest excellent compliance but unfortunately does not prove that study subjects actually took the supplements.

By looking at the data available more closely a group of patients have been potentially identified who appear have responded to gum arabic. One potential limitation of the study is that a food diary was not obtained. Some of the subjects may have already been taking a high fibre diet, and therefore the addition of further fibre to the diet may not have shown any beneficial effect. By selecting a group of patients who have had an improvement in their blood pressure it is possible that we have selected subjects who were in some way able to respond to the effects of gum arabic. It was decided to select this group on the basis of a drop in their mean arterial pressure. Mean arterial pressure was taken to represent a change in “average” blood pressure, reflecting changes in either systolic or diastolic blood pressure. Lowering of mean arterial pressure has been shown to reduce cardiovascular risk [218]. This group did have significant changes in peripheral systolic and diastolic blood pressures as a whole and as individual groups with some changes in either systolic or diastolic blood pressure or both. This is likely to

be purely as a result of selection bias, in as much as they have been selected by the fact they had a change in blood pressure. In the group of incipient nephropaths, the group who had further assessment of vascular stiffness, this change also translated in to a change in central systolic and diastolic blood pressure but not in to a change in measurements of vascular stiffness. This would suggest that the changes in blood pressure seen are not as a result of alterations vascular stiffness.

There are also other limitations of this study that need to be recognised. The design was unblinded and there was no placebo arm. It is possible that the blood pressure recordings were improved at the second study visit purely as a result of the subjects being more relaxed at this visit. All recordings using the Sphygmocor were taken by one investigator, but due to the unblinded nature of the study, a bias could also have been introduced.

This study suggests that Gum Arabic may have beneficial effects on peripheral blood pressure that are not only statistically significant changes that could translate in to clinically relevant changes that might result in improved cardiovascular outcomes.

4.4 The Effects of Gum Arabic on Metabolic Parameters

Although there is good evidence to show that an increased intake of dietary fibre can result in improved glycaemic control [129-132], use of Gum arabic in these populations has not shown any change in glycosylated haemoglobin. The average HbA1C of the incipient nephropaths was 7.54±1.15% at baseline, which would be regarded above target of 7%. Many of these patients were already on multiple medications. The most commonly prescribed oral hypoglycaemic agent was metformin. Twenty one of the 23 patients in the group were on this as a single hypoglycaemic agent or in combination with insulin, gliclazide or a thiazolidinedione. Improving diabetic control further by the addition of dietary fibre in the form of Gum arabic has not been demonstrated in this study. The combinations of medication are shown Figure 4-2

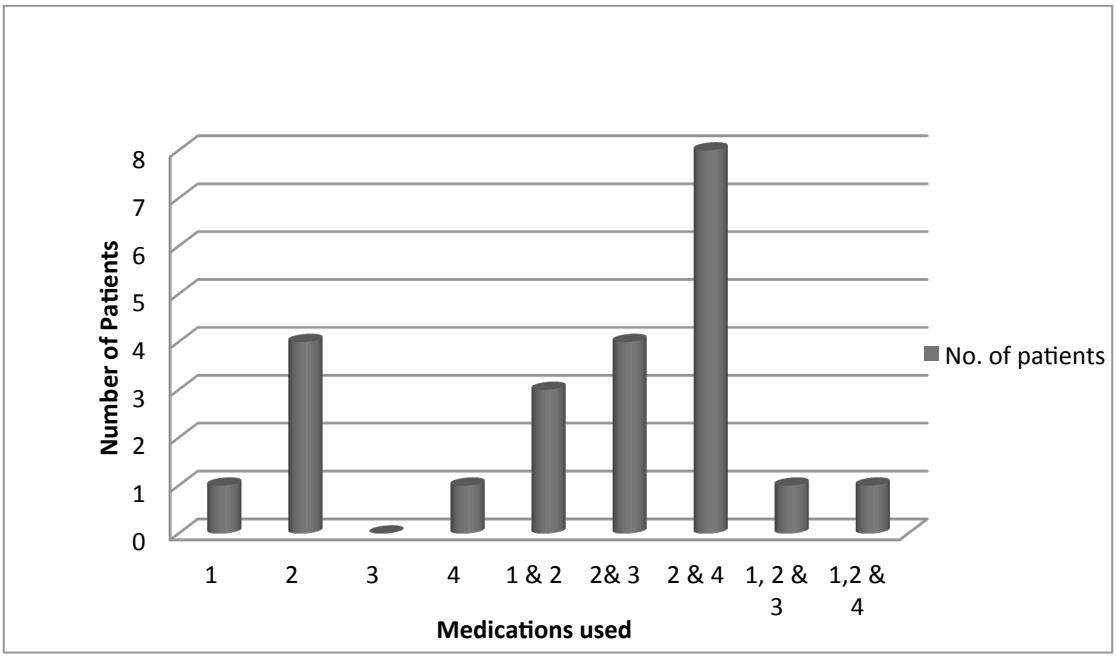


Figure 4-2. Number of hypoglycaemic medications used - Incipient Nephropaths

1 = Sulphonylurea
2 = Biguanide
3 = Thiazolidinedione
4 = Insulin

Although there was a statistically significant fall in HbA1C in the washout period in the healthy subjects, who have normal physiological control of their blood sugars, this is not likely to be of clinical importance and the results remained within normal limits.

Measurements of serum lipids at baseline show that serum total cholesterol was higher in the healthy individuals compared to the incipient nephropaths and overt nephropaths (p=0.06 and p=0.035 respectively). The healthy individuals are not a population at risk of cardiovascular disease and have probably not been previously screened or treated for dyslipidaemia. The lower cholesterol value in the group of incipient nephropaths is likely to be due to the extensive use of lipid lowering agents in this group. Eighteen of the patients, due to their history of hyperlipidaemia were on a cholesterol lowering agent, 12 of them being on an HMG CoA reductase inhibitor. Only 2 of the patients were on a fibrate and 4 of the patients were on ezetimibe. This drug acts at the small intestine brush border and inhibits absorption of cholesterol from the diet.

There was a clear rise in the serum total cholesterol of the incipient nephropath subjects and a rise in triglycerides in the same group and in the group as a whole. A rise in serum HDL is seen in the group of overt nephropaths. The changes in serum lipids are contrary to what much of the literature would suggest, as ingestion of fibre tends to have a lipid lowering affect as previously discussed [135]. However, it must be noted that Haskell et al showed that Gum arabic in particular resulted in no change on serum lipids [136]. This study of Gum arabic has shown a rise in cholesterol in this group. This could add evidence to the hypothesis that Gum arabic may have an appetite stimulatory effect which could explain the rise in serum creatinine also seen. Another possible cause for the rise in cholesterol in the incipient nephropath group is associated with the rise in serum acetate, discussed later in this chapter.

Urinary protein excretion was higher in the healthy group (0.21g/24hr) compared to the Incipient Nephropathy group (0.18g/24hr). However, this is not clinically significant in the healthy group and is regarded as within the normal range. It is however clinically significant in the incipient nephropath group and sufficient to make a diagnosis of microalbuminuria. 24 hour urinary protein excretion did not change significantly throughout the study. The data have shown that Gum arabic did not have a statistically significant effect on renal haemodynamics and is therefore unlikely to change urinary protein excretion. There is a possible suggestion of an effect in the healthy individuals, with a drop in urinary protein excretion (p=NS) and a drop in GFR and ERPF. It is possible that the study period needed to be longer to see a more significant effect.

There is a clear rise in serum acetate following the administration of Gum Arabic in the incipient nephropath group (p=0.033), with a fall back to baseline after the washout period, however this drop did not reach statistical significance. During the same period the serum butyrate levels fell in this group (p=0.031) with a non significant rise back towards baseline in the washout period. There was also a rise and subsequent fall in the serum formate levels and total amount of short chain fatty acids during this time but these did not reach statistical significance. This is clearly different than what happened in the group of healthy individuals in which I have shown a rise in the serum butyrate levels following the administration of Gum Arabic.

The rate and amount of individual serum short chain fatty acids is dependent on the amounts and species of microflora in the colon, the source of the substrate and also the transit time. SCFAs produced in the colon are readily absorbed across the bowel mucosa in to the peripheral circulation. It has previously been demonstrated that acetate is the principal SCFA in the colon. Acetate is absorbed in to the peripheral circulation and has been shown to increase cholesterol synthesis. Propionate on the other hand has been shown to be an inhibitor of cholesterol synthesis [168].The changes seen in this study suggest a change in production of short chain fatty acids from bowel flora, reflected by the changes in serum levels. I have already shown earlier in this chapter that in this same cohort of patients there was a small but significant rise in total cholesterol but also, more importantly this rise was due largely to an increase in HDL cholesterol. It could be postulated that this rise in HDL cholesterol may be due in part to the rise in serum acetate. There was no associated fall in the levels of propionate to further account for this rise in cholesterol.

The findings from this study are somewhat similar to what has been previously demonstrated. Bourquin et al [165] showed that Gum Arabic lead to a rise in production of acetate, propionate and butyrate, with acetate being the greatest. However, this was in a study of patients taking a variety of different fibre supplements, Gum Arabic being just one of them.

5 CONCLUSIONS

This thesis has discussed the fact that Type 2 Diabetes Mellitus is on a global increase in prevalence. Type 2 diabetes is associated with significant morbidity and mortality, largely as a result of the increase in macrovascular and microvascular complications compared to the normal population. This will ultimately result in a worldwide economic health burden unless steps are either taken to reduce the risk of developing type 2 Diabetes Mellitus or to reduce the risks of developing its complications. Diabetic nephropathy and the associated increase in cardiovascular risk are two of the causes of the increased health and economic burden.

The pathogenesis of diabetic nephropathy has been studied extensively. There is a lot of evidence to suggest a role of various growth factors in the development and progression of diabetic nephropathy. In particular TGF-β seems to be a key factor in the final common pathway of tissue fibrosis in many tissues, including liver, heart, lung, skin, and importantly to the work in this thesis the kidney. However there has been no real clinical application to modify TGF-β production in an attempt to reduce the progression of diabetic nephropathy.

Control of glycaemia, the associated hypertension that almost invariably occurs with diabetic nephropathy and control of proteinuria, particularly with the use of medications that alter the renin angiotensin system have all been shown to reduce the risk of progression of diabetic nephropathy. However, the current therapeutic options for preventing or even slowing the progression of diabetic nephropathy are largely limited to these target areas, that is, strict control of blood pressure, reduction of proteinuria and tight glycaemic control. Cardiovascular risk factors must also be addressed and treated aggressively

Dietary fibre has wide reaching health benefits, including a reduction in colorectal cancer, an improvement in cardiovascular risk and improvements in blood pressure. Dietary fibre intake has also correlated in a reduced risk in the development of type 2 diabetes and improved glycaemic control.

The focus of this thesis has been on Gum Arabic, a naturally occurring gum exudate from Acacia trees. In particular I have used SuperGum™, a modified form of Gum Arabic designed such that it has a consistent molecular mass. There are anecdotal reports of the use of Gum Arabic in patients with end stage renal failure showing that it

slowed the progression of disease and reduced the need for dialysis, possibly by non renal nitrogen clearance.

Colonic bacteria produce ureases that hydrolyse urea from dietary proteins to ammonia and CO_2. The resultant ammonia is then incorporated in to bacterial proteins which are then excreted as the bacterial mass fraction of the faeces. The net result is an increase in nitrogen in the faeces, and therefore a reduction in the need for the renal clearance of these nitrogenous compounds. There is also an increase in bacterial short chain fatty acid production which is absorbed in to the systemic circulation in varying proportions. Short chain fatty acids have a variety of beneficial effects on health and it may be these that could lead to an improvement in renal function, improvements in blood pressure and in other metabolic factors associated with cardiovascular risk. Addition of gum arabic to the diet has certainly had effects on bowel flora which has resulted in a change in short chain fatty acid production. There does seem to be a difference in the way the individual patient groups have responded though. The healthy individuals have shown a significant increase in the levels of butyrate, whereas the incipient nephropaths have shown a significant rise in levels of acetate with a concurrent significant drop in the levels of butyrate. This rise in acetate has previously been demonstrated. There was also a rise in formate levels but this was not statistically significant. There was an overall increase in levels of short chain fatty acids following gum arabic which is likely to be secondary to bacterial fermentation of the fibre. Serum butyrate has been shown to reduce TGF-β1 production, an important profibrotic cytokine implicated in diabetic nephropathy.

In the group of healthy individuals, following administration of Gum Arabic there was a statistically significant drop in GFR and ERPF, with no change in filtration fraction in the same period. This could represent a beneficial drop in intraglomerular pressure which could translate in to long term benefits on renal health in subjects with type 2 diabetes. This may well have been due to the rise in serum butyrate. However, similar changes were not seen in the 2 groups of subjects with type 2 diabetes. A rise in serum acetate and not butyrate was seen in the incipient nephropaths. It must be noted that the vast proportion of these patients were already on appropriate treatment for diabetic nephropathy, in particular drugs that block the renin angiotensin system. Further improvements to renal haemodynamics may be small and difficult to demonstrate in a relatively small population.

Evidence suggests one may expect a small drop in serum cholesterol in a diet supplemented by soluble fibre, but there is evidence to show that gum arabic may not possess this property. This study seems to confirm this, with in fact a statistically significant rise in cholesterol and triglycerides in the group of incipient nephropaths. Serum acetate was shown to rise in the group of incipient nephropaths and this is documented to lead to an increase in hepatic cholesterol synthesis. This is a potentially negative effect of the Gum Arabic. All subjects were requested not to change their diet during the study period but this was not recorded by means of a food diary. One other possible explanation would be that gum arabic has an as yet unrecognised stimulatory effect on appetite. If this were the case it could also explain the rise in serum creatinine in the same group but this is not supported by an increase in BMI, which remained unchanged. Gum arabic did not have any significant effect on HbA1C which has been seen in others study of dietary fibre, including soluble fibre. This could reflect the short time period over which the study took place or the relatively good diabetic control of the patient cohort prior to commencing the study.

Control of blood pressure has a well established close correlation with progression of renal disease and furthermore there is a large body of evidence to suggest that aggressive management of hypertension has profound beneficial effects in delaying its progression, not to mention its effects on the reduction of overall cardiovascular risk. This study has demonstrated several improvements in peripheral blood pressure. Healthy individuals showed a drop in systolic blood pressure and incipient nephropaths in diastolic blood pressure, with a trend towards a drop in systolic pressure. A drop in MAP was shown in the overt nephropath group. These are all short term changes demonstrated over a period of 12 weeks and are likely to translate in to marked improvement in long term renal and cardiac outcomes if the gum arabic was continued. The direct mechanism by which this has occurred is not clear, but data from this study would suggest that it is not as a direct result of changes in levels of vascular stiffness. Vascular stiffness, in addition to peripheral blood pressure has been shown to be an independent predictor of all cause and cardiovascular mortality, in the general, diabetic, hypertensive and renal populations [55, 186, 191, 219, 220]. But, despite significant reductions in blood pressure in the incipient nephropaths, there have been no changes in the measurements of vascular stiffness or in measures of central blood pressure. This would therefore suggest the mechanism by which peripheral blood pressure is lowered

following dietary supplementation with SuperGum™ is independent of specific alterations in vascular stiffness.

5.1 Limitations of the Study

Ideally this study would have been conducted as a randomised controlled double blind study, rather than an unblinded study with a washout period and no placebo arm. This however would have been prohibitively expensive to have undertaken. Patient recruitment to the main study of incipient nephropaths was difficult and resulted in smaller patient numbers than what was calculated in the power calculation. Ideally 30 patients should have been recruited to the follow on study, rather than the 23 that were recruited, meaning the study was underpowered. The study required numerous lengthy visits for investigations and the requirement to take a supplement every day, both of which I believe were barriers to joining the study to some patients. Patient compliance with taking the SuperGum™ was very good and the side effects, as discussed, were minimal with occasional bloating being the most common symptom. This did not however lead to any withdrawals from the study. Attempts were made to record compliance by counting the number of foil sachets of gum arabic returned, but measuring true compliance is difficult and it is possible that not all patients took all of the supplements which again would significantly affect the results. Due to the relatively small sample size, it has been difficult to draw conclusions from some of the investigations. For instance many of the groups have shown changes in blood pressure and renal function (GFR and EPRF) but these have not reached statistical significance potentially due to the small numbers. It would have also been beneficial to ask the participants in the study to keep a food diary. As discussed, it is possible some of the subjects were already taking a high fibre diet and the addition of further fibre may have not been of any benefit.

There appear to have been changes in blood pressure from the first study visit to the second visit, following dietary supplementation with gum arabic. Blood pressure was only recorded with a sitting office blood pressure. A potential bias has been introduced as the subjects' blood pressures may well have "regressed to the mean" purely by virtue of the fact that they could have been more relaxed on a second visit. A potential way

around this would be to ideally use 24 hour blood pressure monitoring to measure blood pressure, or by means of a randomised placebo controlled study.

As stated earlier this study was supported by a grant by San-Ei Gen, the company that manufacture SuperGum™ and the study could be criticised for this as a potential bias could be introduced.

It is not clear form any studies how long the effects of TGF-β or other profibrotic cytokines take to cause a noticeable effect on renal function and indeed how long these changes may take to reverse. A period of 12 weeks was taken to supplement the diets with SuperGum™ but it is entirely feasible that this study has not allowed sufficient time to make meaningful and detectable differences in renal function, particularly the benefits that are likely to arise as a result of the potential blood pressure lowering effect that has been seen.

A potential criticism of the study would be that a significance level of $p<0.05$ was chosen for both the primary and secondary outcomes. One could argue that a more stringent level of significance would be taken for secondary outcomes, for instance $p<0.01$. This may well be reasonable for some outcomes. However, for changes in short chain fatty acids, this was an expected change and key to the hypothesis of the action of gum arabic and a significance level of $p<0.05$ is reasonable. For some of the other metabolic parameters, if a level of $p<0.01$ were chosen, it would have actually meant that there were no significant changes in levels of glucose control or of lipid metabolism.

5.2 Future directions

There evidence presented in this thesis suggests that dietary supplementation with SuperGum™ may have some clinically important effects on renal function and on blood pressure in certain populations, which could translate in to long term health benefits. In particular it is interesting to note that the healthy individuals were the only group to have had a drop in GFR and ERPF, and indeed a change in systolic blood pressure, and are also the only group to have had a rise in serum butyrate. A clinically and statistically significant change in diastolic pressure was seen in the incipient

nephropaths, a group of individuals with a high cardiovascular risk. I believe there is enough evidence to support further investigation in to the use of Gum Arabic compounds. The group who are most likely to see a potential benefit are the incipient nephropaths. However, a larger sample size would be required and ideally studied for a longer period of time. Changes in blood pressure and small changes in renal function are only likely to show beneficial effects on outcome in the long term.

A further study, ideally of a double blind randomised placebo controlled design should be undertaken to confirm these potential health benefits. Again, a population of subjects with type 2 diabetes and incipient nephropathy should be chosen as I believe these are an at risk group who may have the potential to benefit. Combination of the Gum Arabic powder in to foodstuffs such as bread and biscuits would make this an easier substance to ingest on a regular long term basis.

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APPENDIX

The Effects of Acacia (sen) SUPER GUM™ (Gum Arabic) on renal physiology and arterial function in Type II diabetic patients

PATIENT INFORMATION SHEET

You are being invited to take part in a research study. Before you decide to take part it is important for you to understand why the study is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Consumers for Ethics in Research (CERES) publish a leaflet entitled 'Health Research and You'. The research nurse coordinating this study can give you a copy of this leaflet.

1. What is the purpose of the study?

Good control of blood pressure and blood sugar has been shown to help in slowing the progression of diabetic kidney disease. There is also some evidence from laboratory studies that the addition of extra fibre in the form of "Gum Arabic" may be able to stabilise kidney function. Gum Arabic is used as a stabilising additive in many processed foods and is regarded as very safe.

2. Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive. If you decide not to take part, you do not have to give a reason and the care you receive now or in the future will not be affected.

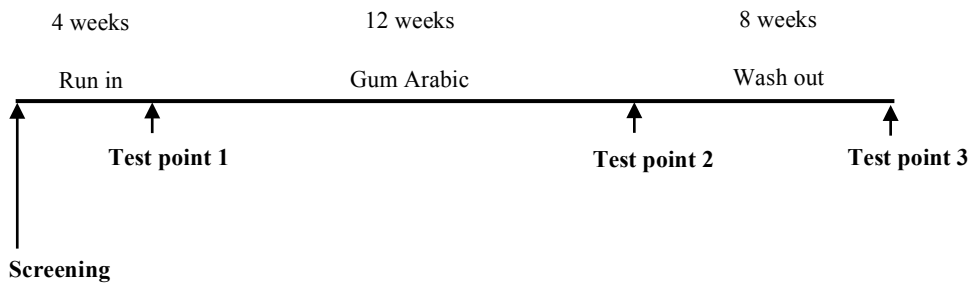
3. What will happen to me if I take part?

If you agree to take part in this study, the doctor will examine you and take a blood sample to make sure you can take part. Each patient will be part of the study for a total of 24 weeks, 12 weeks of which you will be taking the fibre supplement. At the start of the study there will be a screening visit where a doctor will go through the study with you, measure your blood pressure and do a basic physical investigation. This screening visit will last approximately 30 minutes.

This will be followed by a 4 week run in period if the initial examination shows that you are

eligible to enroll in the study. During this time you will have to make a visit to the hospital when your blood pressure will be monitored and your kidney function assessed to make certain that you will be able to take part in the study. This visit will last about 20 minutes.

After the initial screening visit you will need to attend the Clinical Investigation unit a further 3 times, at 4 weeks, 12 weeks after that and then after a further 6-8 weeks.



These visits (Test Points) will each take about five hours and we will ask you to bring a 24-hour collection of urine with you each time.

- **Test point 1**

This visit will consist of having a thin plastic tube inserted into a vein in your arm, which will be used to inject low dose radioactive material into your blood stream. The amount of radioactivity in this injection is around the same as you would get from a flight to Rome or a 2 week holiday in Cornwall. This test measures the blood flow to your kidneys by measuring the radioactivity in blood samples (10ml or about 2 teaspoons will be taken on 4 occasions from the thin plastic tube). The total amount of blood that will be drawn during each visit will be approximately 40 ml (8-10 teaspoons). We will also take an extra 10 ml of blood for other biochemical tests that are necessary for the study. In addition we would also like to assess the effects of the study medication on the blood vessels. This is done with a special ultrasound machine and does not involve the use of any needles. A probe is placed on an artery at the wrist, neck and top of the thigh. Measurements are then made with the help of a computer. The only thing you will feel is slight pressure when the probe is placed on the skin and the procedure is not painful. It will take about 20 minutes to perform and can be done during the assessment of your kidney function. You will also be fitted with a 24 hour blood pressure monitor which will check your blood pressure at home every hour automatically. We will provide you with a stamped addressed envelope to post this back to us the following day.

You will then be commenced on the Gum Arabic. The study period during which you take the Gum Arabic is scheduled to last 12 weeks and during this time you will be given a drink to take every morning, which has the Gum Arabic in it. The Gum Arabic is supplied as a dried powder and we will provide a bottle to allow you to mix the powder with water and flavour of your choice e.g. orange squash. During the study you will be phoned every two weeks by the research team coordinating the study who will check that you are alright.

- **Test point 2**

Following taking the Gum Arabic for 12 week period, the same tests as Test Point 1 will be done.

- **Test Point 3**

In order to be sure that any effects seen are due to the Gum Arabic a final test of kidney function will be arranged 6-8 weeks after you have finished taking the study material.

The tests performed will be as Test point 1

To further assess the mechanism behind the potential benefit of the Gum Arabic we would like some volunteers to provide a stool sample to be analyzed for chemicals and natural microorganisms that are usually present. These may change with the Gum Arabic treatment. We would provide a small container for you to collect the sample and then we would send this off to Japan for analysis. This part of the study is completely voluntary and if you felt that you were unable to help with this particular aspect of the study that would be ok.

You can decide to stop the study at any time. This will not affect your future care in any way. You may also be taken out of the study by the doctor if he thinks it is necessary.

4. What are the possible disadvantages and risks of taking part?

Having a needle in your arm to take blood can be uncomfortable. Apart from this we would not expect you to suffer in any way. Gum Arabic is a food additive used in many products which are eaten every day, however it is possible that you may feel a bit bloated or have 'wind' while taking part in the study. In our experience this is usually very mild and infrequent. To the best of our knowledge there are no known interactions with your regular medication.

5. What are the possible benefits of taking part?

Taking part in this study will not help you directly, however we hope it may help us to understand diabetic kidney disease more clearly.

6. Will my taking part in this study be kept confidential?

If you agree to take part in the study, any of your medical records may be inspected by the investigators carrying out the research. They may also be looked at by people from the Regulatory Authorities to check that the study is being carried out correctly. Your name, however, will be kept secret outside the hospital. With your permission, your doctor will inform your General Practitioner that you are taking part in the study.

7. What will happen to the results of the research study?

A report containing the results of this study will be written and submitted for publication in a medical journal. Your identity will not be revealed in this report.

8. Who is organising and funding the research?

The research is being funded by San-Ei Gen FFI, Osaka, Japan who have provided a grant to the Institute of Nephrology to cover a research doctor salary and the cost of all the research study tests

9. Who has reviewed the study?

This study has been reviewed and approved by the South East Wales Research Ethics Committee.

10. Costs/Reimbursement

All travel expenses (including parking) will be paid back.

11. Contact for further information

Now or during the course of the study, if you have any needs or questions concerning this study or your rights as a patient or in case of emergency, you should contact the doctor or nurse at the number below. You will be given a copy of the information sheet and a signed consent form to keep.

Contact:	**Dr Dave Glover:**	**Tel 02920 748435**
	Dr Steve Riley:	**Tel 02920 748467**

Thank you for taking the time to read this information sheet.

Screening Assessment

Date: ……//……//…….

Name:
Address:
Affix label here

Type II DM diagnosis date:……..

Microalbuminuria? Yes / No If yes, diagnosis date……

ACR……(>2.5 male, >3.5 female : <30)

DM medications …….

…….

…….

…….

Insulin? Yes / No

Medications (Cardiovascular)

Diuretic……	Ca channel blocker…….
Beta-blocker……	ARB……
ACE inh……..	Nitrate…….
Statin……...	Other……

Other Risk Factors

Angina	Y / N	
CABG	Y / N	
FHx of CV disease	Y / N	
Previous CVA / TIA	Y / N	
Hypertension	Y / N	
Previous MI	Y / N	
Dyslipidaemia	Y / N	
Smoker (current or past = Y)	Y / N	PYH…….
Retinopathy	Y / N	
Neuropathy	Y / N	
Peripheral Vascular Disease	Y / N	

Other Past Medical History

Relevant Family History

Examination

Height…….

Weight……

Pulse…….AF?…

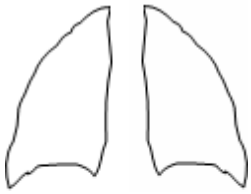
BP……...

HS……...

JVP…….

Oedema……..

RS



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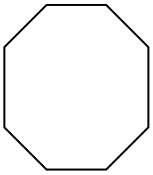

Notes / Additional Comments

Bloods to do etc.

U+E, bicarb

HbA1C

Lipid Profile

24 hour urine protein

Sign consent form (copy x3)

Fill out expenses form (copy x3)

Give instructions on 24 hr urine

24 hr urine bottles x 2

Instructions for 24 hour urine collection

The following are instructions for the proper collection of a 24-hour urine specimen.

The aim is to collect every all of your urine during the specified 24-hour period. It does not matter what the volume of the urine is, so long as it represents all of the urine you pass. If you need to have a bowel movement, you must collect the urine separately. If unable to do so you should abandon the collection and start again on another day.

- Begin the collection at the usual time that you awaken.
- At that time, pass your urine, flush it down the toilet and **note the exact time**. You will now have an empty bladder and an empty bottle. The collection of urine will start from this time.
- Collect all of the urine you pass during the day and night and finish the collection by passing urine at exactly the same time the next morning; this specimen of urine should also be collected in the bottle.
- The time you void the last urine specimen should not vary by more than 5 or 10 minutes from the time of starting the collection the previous day. If you have to urinate one hour before the appointed time, drink a full glass of water or more so that you can void again at the appropriate time. If you have to urinate 20 minutes before try and hold the urine until the proper time. This is necessary in order to accurately interpret the results of the test.
- You can store the bottle at normal room temperature for a day or two, but ideally hand the sample in to the Institute of Nephrology at the Hospital. The bottle should be kept cool or refrigerated for longer periods of time.

Pulse Wave Analysis / Isotope GFR – Patient Record

Date: ……//……//…….

Name:

Address:

Affix label here

Visit: Initial – after run-in ☐

Post Gum Arabic ☐

Post Washout ☐

Compliance……..(not taken)

Side effects……

Medications (Cardiovascular)

Diuretic…… Ca channel blocker…….
Beta-blocker…… ARB……
ACE inh…….. Nitrate…….
Statin……... Other……

DM medications …….
…….
…….
…….

Insulin? Yes / No

Notes

Pulse Wave Analysis – AIx

BP (average): ……./…....mmHg

Height: ……cm

Weight: ……kg

AIx
(Circle 2 to be used – within 4%)

1:…… 2:…… 3:…… 4:…… 5:…… 6:……

Pulse Wave Velocity

BP (average): ……./…....mmHg

Distances:

Femoral to Umbilicus to Suprasternal notch:.....mm

Radial to Suprasternal notch:……mm

Carotid to Suprasternal notch:……..mm

PWV (*Radial to Carotid*)
(Circle 2 to be used – within 0.5m/sec)

1:…… 2:…… 3:…… 4:…… 5:…… 6:……

PWV (*Femoral to Carotid*)
(Circle 2 to be used – within 0.5m/sec)

1:…… 2:…… 3:…… 4:…… 5:…… 6:……

Isotope GFR / ERPF

Time commenced…….