The Relationship between 24-hour Ambulatory Blood Pressure Patterns and Urinary Albumin Excretion in Type 2 Diabetes and Hypertension

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I certify that all material in this thesis which is not my own work has been identified and that any material that has previously been submitted and approved for the award of a degree by this or any other University has been acknowledged.

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Abstract

Albuminuria is a risk factor for kidney disease progression and cardiovascular events. There are strong links between high blood pressure (BP), diabetes and increased albuminuria. Studies suggest that loss of the normal night time dip in BP (non-dipping) may alter albuminuria and could be a treatment target to slow kidney damage and reduce cardiovascular risk. Damage to a lining of the blood vessels called the glycocalyx is thought to be involved in the development of albuminuria and could further understanding of albuminuria pathophysiology.

This thesis aims to further understanding of these closely linked conditions by exploring the relationship between 24-hour blood pressure patterns, albuminuria and glycocalyx integrity in newly diagnosed hypertensive patients and in type 2 diabetes (T2DM). The primary aim is to determine whether loss of the normal night time blood pressure dip contributes to urine albumin excretion using an albumin assay which enables quantification of urine albumin in all participants.

Twenty-four hour BP, sublingual glycocalyx integrity and urinary albumin data were derived from two studies. Data were acquired prospectively from 34 participants with T2DM within the observational BEAT-DKD study and a retrospective analysis was undertaken on data collected from 54 treatment naïve patients with grade II hypertension within the 18 week interventional DASHER trial (2015-16).

The treatment protocol significantly reduced albumin excretion rate (AER) **(p=0.0311).** There were no observed relationships with night time blood pressure dipping and AER in the hypertensive or T2DM cohort. In the hypertensive cohort, systolic, diastolic and mean arterial BP were associated with AER. In the diabetic cohort there were no correlations with AER and average systolic BP **(Rs: 0.2072, p=0.2397)** and pulse pressure **(Rs: 0.2398, p=0.1719)**, but strong correlations with night time BP variability in those with moderately increased albuminuria (AER >20 µg/min) **(Rs: 0.6553, p=0.0032).** Glycocalyx integrity showed no relationship with albuminuria or 24-hour blood pressure.

In conclusion, loss of normal night time BP dipping showed no relationship with albuminuria. Sublingual glycocalyx integrity showed no relevance to albuminuria or blood pressure. Systolic, diastolic, mean arterial pressure and pulse pressure show the strongest relationship with albuminuria but BP variability may be informative of albuminuria risk in diabetes.

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Authors Declaration

I carried out all re-analysis and discussion of data described within this thesis. I conducted all ambulatory blood pressure measurements within the BEAT-DKD study, microvascular assessments were undertaken by Andrew Forbes-Brown. The DASHER study was designed by Dr Andrew Jordan, microvascular assessments were carried out by Dave Mawson and ambulatory blood pressure measurements by research nurses. I organised the re-analysis of DASHER and BEAT-DKD urine samples which was undertaken by Rachel Nice at the central Royal Devon and Exeter hospital laboratory drawing on work by Dr Daniel Chapman.

Acknowledgements

List of definitions and abbreviations

ACE - Angiotensin receptor blockers

ACR – Albumin creatinine ratio

AER – Albumin excretion rate

AGE – Advanced glycation end product

AN – Autonomic neuropathy

ANS – Autonomic nervous system

ARB – Angiotensin receptor blocker

BMI- Body mass index

BP –Blood pressure

CAN – Cardiac autonomic neuropathy

CCB - Calcium channel blocker

CKD – Chronic kidney disease

DBP- Diastolic blood pressure

DCT – Distal Convoluted tubule

DKD – Diabetic kidney disease

DNA – Deoxyribonucleic acid

DP – Double Product

eGFR – Estimated glomerular filtration rate

ESRD – End stage renal disease

GAG – Glycosaminoglycan

GBM – Glomerular basement membrane

GFB – Glomerular filtration barrier

GFR – Glomerular filtration rate

HbA1c – Glycosylated haemoglobin A1c

HR – Heart rate

KDIGO – Kidney disease improving global outcomes

MAP – Mean arterial pressure

MBPS – Morning blood pressure surge

MDRD - Modification of diet in renal disease formula

MIA – Moderately increased albuminuria

NO – Nitric oxide

OGTT - Oral glucose tolerance test

PBR – Perfused boundary region

PCT – Proximal Convoluted Tubule

PGI_2 – Prostacyclin

PP – Pulse pressure

PPP- Pulse Pressure Product

PSNS – Parasympathetic nervous system

RAAS – Renin angiotensin aldosterone system

RBC – Red blood cell

ROS – Reactive oxygen species

SBP – Systolic blood pressure

SIA – Severely increased albuminuria

SNS – Sympathetic nervous system

T2DM – Type 2 diabetes mellitus

UAE – Urine albumin excretion

VEGF – Vascular endothelial growth factor

WHO – World health organisation

1. Introduction

Type 2 diabetes mellitus (T2DM) is a common risk factor for the development of diabetic kidney disease (DKD) and often goes hand in hand with hypertension. Diabetes and hypertension are the two main causes of chronic kidney disease (CKD) worldwide [1]. CKD may progress to end-stage renal disease (ESRD) requiring kidney transplant or renal replacement therapy such as dialysis. The need for renal replacement therapy substantially shortens survival and reduces quality of life.

Not only is CKD a huge burden on the patient but also on the healthcare system. The NHS spent an estimated £1.454 billion directly and indirectly on CKD in 2009 alone. Over half of this (£780 million) was spent on renal replacement therapy for those who had reached ESRD [2].

DKD currently accounts for around 30% of ESRD cases in the UK [3]. However, the prevalence of T2DM has more than doubled in the last 20 years to 3.7 million cases and is predicted to increase to 5 million cases by 2025 [4]. This is likely as a result of the increasing obesity levels, poor diet and inactive lifestyle of modern society. If these environmental factors are not tackled, the prevalence of DKD and ESRD are likely to rise alongside T2DM.

It is therefore vital that we increase our understanding of the development of DKD in order to find ways of slowing or preventing progression to ESRD, reducing the patient and healthcare burden.

This thesis will focus on the complex relationship between blood pressure (BP), diabetes and kidney damage. It is known that albuminuria is an independent indicator of poor prognosis in CKD [5]. Concurrent hypertension is a negative prognostic factor for CKD and has a close relationship with levels of albuminuria[6]. Diabetic patients may also have a tendency to lose the normal 24-hour blood pressure patterns. This introduction will give background information on each of these concepts and provide context for the proposed research.

1.1 Thesis Overview and Structure

1.1.1 Overview

This study will hypothesise that there is a relationship between albuminuria and pathological 24-hour blood pressure patterns in T2DM patients. The study will examine the relationship between pathological 24-hour blood pressure patterns, particularly non-dipping, and albuminuria into the normal albuminuric range in type 2 diabetes and hypertensive patients. The study will also assess whether the protective glycocalyx layer of the endothelium is affected by these blood pressure patterns or related to albuminuria. This examination will further understanding of the relevance of 24-hour blood pressure patterns in the development of diabetic kidney disease.

1.1.2 Structure

Chapter 1: Introduction – This chapter provides background information on the complex relationship between diabetes, kidney disease, hypertension and albuminuria. The introduction will then analyse current literature around the relationship of 24-hour blood pressure patterns to albuminuria to provide justification for the proposed research.

Chapter 2: Methods – This chapter will describe the methods common to the two studies within this thesis.

Chapter 3: The DASHER Study – This section will analyse 24-hour blood pressure, urine albumin excretion and glycocalyx integrity data taken from the DASHER study of hypertensive patients and discuss the findings.

Chapter 4: The BEAT-DKD Study – This section will analyse 24-hour blood pressure, urinary albumin excretion and glycocalyx data taken from the BEAT-DKD study of type 2 diabetic patients and discuss the findings.

Chapter 5: Overall discussion and conclusion.

1.2 Type 2 Diabetes Mellitus

Diabetes literally translates as 'passing too much urine' and mellitus as 'sweet'. It is now known that this is a symptom of an underlying condition which causes chronic hyperglycaemia as a result of reduced insulin sensitivity and or production.

The World Health Organisation (WHO) recommends that diabetes be diagnosed by symptoms of diabetes (polyuria, polydipsia) alongside:

- A random venous plasma glucose concentration ≥11.1mmol/L
  OR
- A fasting plasma glucose concentration ≥7mmol/L (whole blood ≥6.1mmol/L)
  OR
- A two hour plasma glucose concentration ≥11.1 mmol/L two hours after 75g of anhydrous glucose – Oral glucose tolerance test (OGTT).
  OR
- A Haemoglobin A1c (HbA1c) ≥ 48mmol/mol (6.5%).

If no symptoms are present then two separate diabetic range glucose readings are needed from separate days, or a repeat of the HbA1c. If the second HbA1c is below 48mmol/mol then the person is classed as high risk of diabetes[7, 8].

Although there are rare monogenic forms of diabetes, there are two main forms; Type 1 diabetes (failure to produce insulin) and Type 2 diabetes (reduced insulin sensitivity and consequent reduced production of insulin). This paper will focus on Type 2 diabetes.

1.2.1 Epidemiology

T2DM constitutes over 85% of cases of diabetes and is increasing in prevalence due to its close association with obesity, inactivity and age. The lifetime risk for developing T2DM in the U.S. population is 40% [9].

There are many risk factors for developing T2DM but the most significant include obesity, increasing age, physical inactivity, high glycaemic index diet and family history [10].

1.2.2 Pathophysiology

In T2DM insulin resistance precedes hyperinsulinaemia and then reduced production of insulin. Although individuals may have a genetic predisposition to developing T2DM, levels of obesity show a direct correlation to insulin resistance [11].

It is thought that insulin resistance may be due to excess adipokines, released from excess adipose tissue in those that are overweight. One significant site of insulin resistance is the liver. In normal physiology, the liver responds to decreased blood glucose and insulin levels by breaking down glycogen in to glucose (glycogenolysis) hence increasing blood glucose. When liver cells become resistant to insulin, glucose is secreted in spite of hyperglycaemia, raising blood glucose even further. Resistance of peripheral muscle cells to insulin leads to reduced glucose clearance into muscle cells [11].

In response to high glucose levels in the urine, the sodium-glucose cotransporter-2 within the kidney is upregulated which leads to increased glucose reabsorption into the blood from the kidney [12].

Hyperglycaemia has been shown to have a toxic effect on insulin producing β-cells in the pancreas [11]. This leads to a vicious cycle of increasing dysfunction of insulin production as chronic hyperglycaemia ensues. This accounts for the cases where people with type 2 diabetes eventually require treatment with insulin.

1.2.3 Mechanism of diabetic vascular complications

People with diabetes experience many complications as a result of hyperglycaemia, hyperinsulinaemia and haemodynamic factors. These can be divided into microvascular and macrovascular complications, although their pathophysiologic mechanisms may be closely related.

Macrovascular complications include coronary artery disease, peripheral vascular disease and stroke. These complications arise as a result of development of atheroma within arterial walls. Diabetes is a direct contributor to atheroma formation alongside risk factors such as obesity, hypertension and hyperlipidaemia which are often present in diabetic individuals [13].

Microvascular complications include nephropathy, retinopathy and neuropathy. These occur due to damage to the small blood vessels which are vital to the structure of these organs.

These complications arise from a complex series of mechanisms which are not fully understood.

An important factor in the mechanism behind these complications is the formation of advanced glycation end-products (AGEs) [14]. AGEs are produced when plasma proteins or lipids are glycated as a result of hyperglycaemia and are thought to be important within the pathogenesis of diabetic microvasculopathies [14-16]. When AGEs bind to cell membrane receptors they can increase the production of pro-inflammatory molecules and reactive oxygen species (ROS) [17]. AGEs may modify low-density lipoproteins leading to accelerated atherosclerosis [18]. Importantly, when AGEs are bound to cell membranes they increase endothelial permeability to macromolecules. This is thought to be a key factor in the development of diabetic kidney disease which occurs in 5-20% of T2DM patients[19].

AGEs alongside oxidative stress caused by ROS may decrease endothelial derived nitric oxide (NO) [18]. NO leads to vasodilation, immune response modulation, prevention of platelet aggregation and leukocyte adhesion [20]. Therefore reduced availability of NO may be an important contributor to vascular complications in diabetes [20, 21].

1.3 Diabetic Kidney Disease

Diabetic kidney disease (DKD) is a microvascular complication of diabetes caused primarily by chronic hyperglycaemia and is the most common cause of chronic kidney disease in the world [1].

There are four main histological changes to the glomerulus in DKD [22]:

- Podocyte injury
- Glomerular basement membrane thickening
- Expansion of the mesangium
- Glomerulosclerosis

Many of these changes may originate from the previously described oxidative stress as a result of hyperglycaemia in diabetes. This theory is supported by evidence showing that blockade of AGE receptors improves structural and functional signs of DKD [23].

Increased glomerular permeability resulting in abnormal albuminuria is one of the earliest clinical signs of DKD [22]. This is accompanied by progressive expansion of the basement membrane and mesangium through accumulation of extra cellular matrix components [24, 25]. The expansion of the mesangium leads to stiffening and narrowing of the glomerular arterioles/capillaries and eventually scarring of the kidneys (glomerulosclerosis) [22]. Albuminuria and the glomerular basement membrane (GBM) will now be discussed in more detail, in the context of type 2 diabetes.

Afferent arteriole

Juxtaglomerular cells

Glomerular
capillaries

Distal convoluted
tubule

Proximal
convoluted tubule

Macula densa

Bowman's capsule

Collecting duct

Efferent arteriole

Loop of Henle
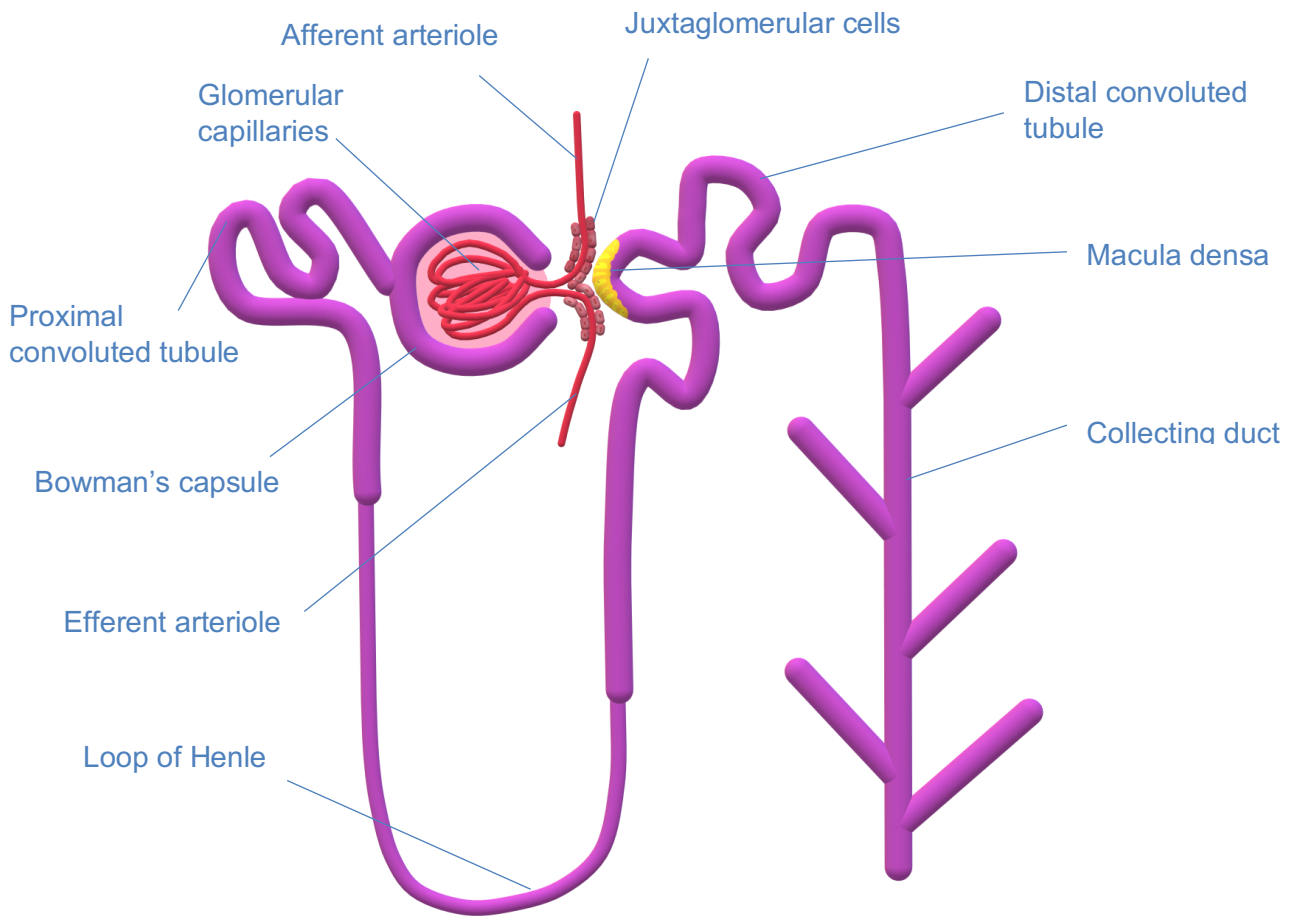
Figure 1 - The nephron.

1.3.1 Albuminuria

Albumin is a globular protein found in the blood with multiple functions including regulation of the oncotic pressure of blood.

Albumin, alongside other molecules in the blood, is selectively filtered through the glomerulus. In a healthy individual, almost no albumin should be filtered into the urine due to the filtration barriers of the glomerulus. Pathological albuminuria was, until recently, defined as microalbuminuria or macroalbuminuria but is now defined as 'moderately increased albuminuria (MIA)' and 'severely increased albuminuria (SIA)'.

Normal albuminuria is <30mg/day, MIA is 30-300mg/day, SIA is >300mg/day. The concentration of albumin in the urine can vary depending on how dilute the urine is. Therefore, urine albumin excretion must be measured using an albumin to creatinine ratio (ACR) of a random urine sample or by a timed urine collection. ACR gives an approximation of albumin excretion in relation to creatinine excretion which is fairly consistently and exclusively filtered by the kidneys. Timed urine collections allow an albumin excretion rate (AER) to be calculated.

Albuminuria is a hallmark of DKD and is present in around 20-25% of T2DM patients [26]. The presence of albuminuria is accepted to be a poor prognostic indicator as it seems to directly exacerbate kidney damage (discussed in more detail in 1.3.4). Albuminuria has also been found to be an independent risk factor for cardiovascular mortality even in the normal albuminuria range [27, 28]. Doubling the urine albumin concentration gives a relative risk of 1.29 (with a 95% confidence interval between 1.18-1.40) for cardiovascular mortality and a relative risk of 1.12 (95% confidence interval 1.04 to 1.21) for non-cardiovascular mortality[27]. This indicates a robust and significant relationship between increased albuminuria and mortality with particular relevance to cardiovascular risk. It is not understood why albuminuria is associated with cardiovascular events. It could be that albuminuria is a sensitive marker of kidney function decline or of endothelial dysfunction, both of which contribute to cardiovascular morbidity.

As CKD patients with albuminuria showed a faster rate of decline in glomerular filtration rate (GFR) than those without [29], KDIGO guidelines recommend that albuminuria is considered independently of GFR when monitoring kidney function [5]. Even in a patient with normal GFR, a moderately or severely increased albuminuria indicates a high risk state.

Studies have shown that GFR may decline two to three times faster in those with increased albuminuria than the general population [30, 31].

There are likely to be several factors which contribute to the development of albuminuria in T2DM. These include structural changes to the glomerular filtration barrier (GFB), increased blood pressure and intra-glomerular pressure [26].

Prognosis of CKD by GFR and Albuminuria Categories: KDIGO 2012			Persistent albuminuria categories Description and range		
			A1	A2	A3
			Normal to mildly increased	Moderately increased	Severely increased
			<30 mg/g <3 mg/mmol	30-300 mg/g 3-30 mg/mmol	>300 mg/g >30 mg/mmol
GFR categories (ml/min/ 1.73 m²) Description and range	G1	Normal or high	≥90		
	G2	Mildly decreased	60-89		
	G3a	Mildly to moderately decreased	45-59		
	G3b	Moderately to severely decreased	30-44		
	G4	Severely decreased	15-29		
	G5	Kidney failure	<15		

Figure 2 *KDIGO 2012 Glomerular filtration rate guidelines (Colour indicates risk stratification: green shows low risk, yellow moderate risk, orange high risk and red is very high risk)* [5].

1.3.2 The Glomerular filtration barrier

1.3.2.1 The Glomerulus and Bowman's capsule

The glomerulus is constituted of an afferent arteriole which gives rise to multiple loops of capillaries which then re-join to form the efferent arteriole. As blood travels through the glomerular capillaries some of its constituents pass across a filtration barrier into a space called the Bowman's capsule where they become the urinary filtrate. The filtration barrier is made up of three distinct layers and restricts movement of solutes based on size, charge and sterical configuration [32]. Each layer of the membrane is negatively charged which repels other negatively charged molecules like albumin but favours the passage of small positively charged ions such as sodium and potassium. Recently, research has suggested that a lining of the endothelium called the glycocalyx plays a more significant role in filtration than charge selectivity [33].
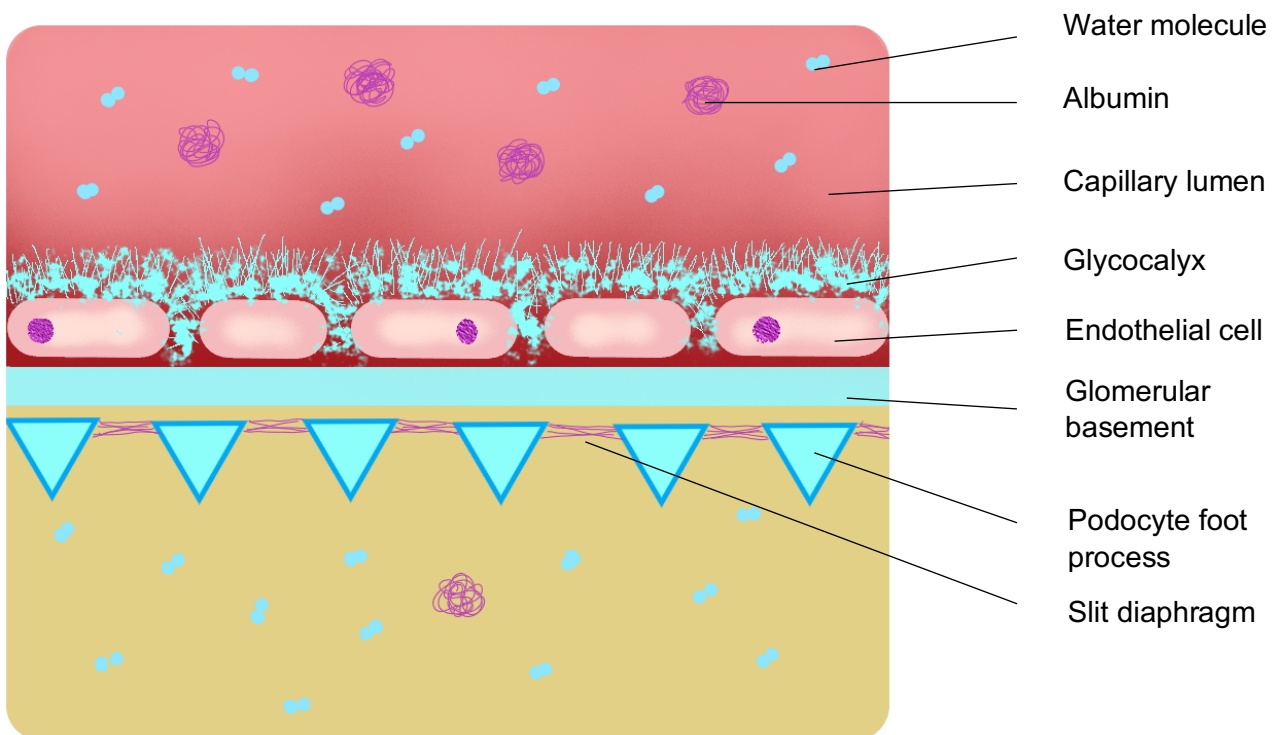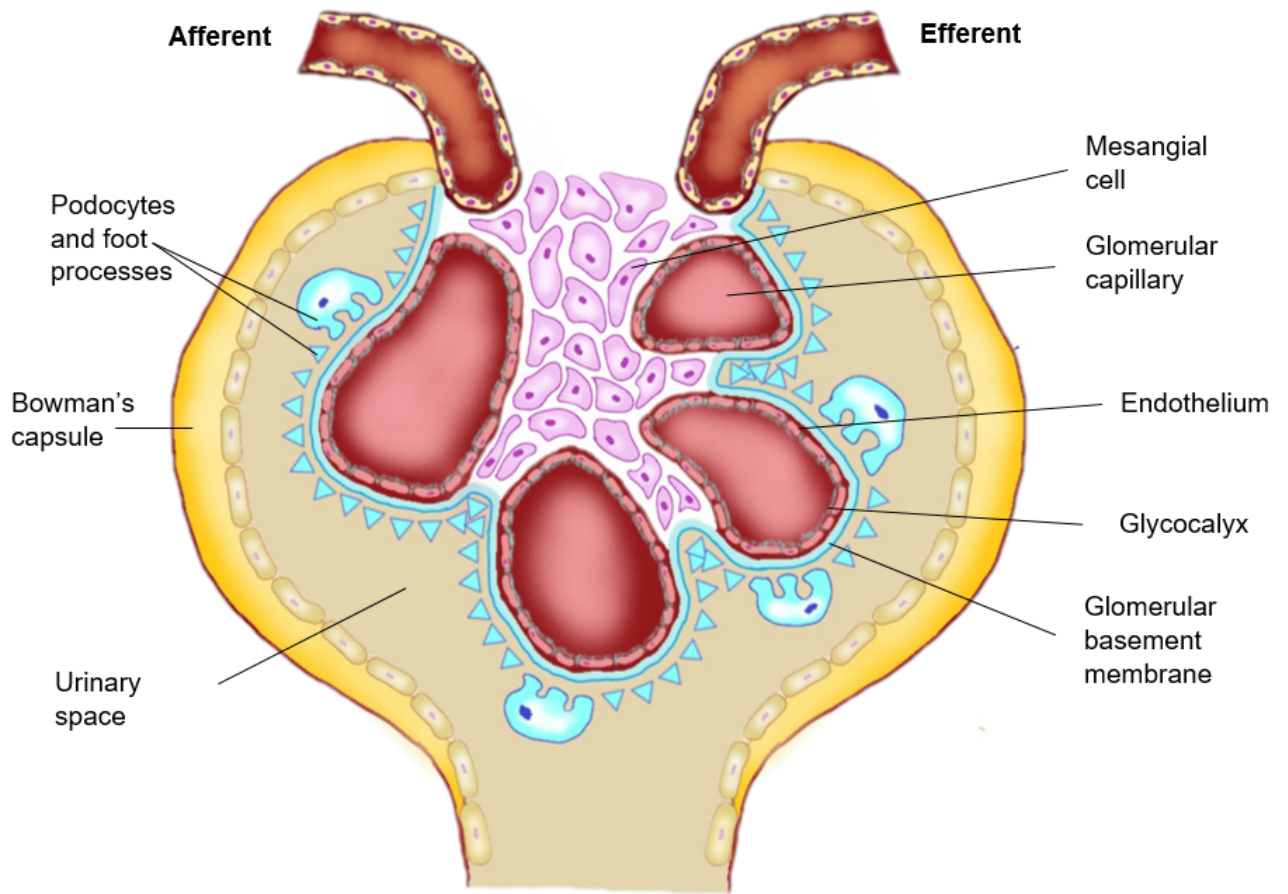
Figure 3 *The Glomerulus (above) and the layers of the glomerular filtration barrier (below).*

1.3.2.2 Fenestrated endothelium of the glomerular capillaries

Glomerular capillaries are unique due to their many large fenestrations which allow the vast majority of solutes in the blood (up to 0.1µm) to pass through the capillary endothelium to the glomerular basement membrane [34]. The entire epithelium is lined with glycocalyx which extends into the fenestrations themselves [35].

1.3.2.3 The Glycocalyx

The glycocalyx is a gel-like structure which lines the lumen of all capillaries, including the glomerulus.

It is made up, primarily, of glycoproteins and proteoglycans [36, 37]. Proteoglycans are macromolecules with a protein core and various glycosaminoglycan (GAG) side chains. The main GAG side chain in the glycocalyx is heparan sulphate (50-90%) which is also present in the GBM [37]. Hyaluronic acid is another GAG present in the glycocalyx which binds to water to give the glycocalyx its gel-like consistency [38].

The main functions of the glycocalyx within the glomerulus include:

- Adhesion of molecules to glycoproteins leading to vital mechanisms such as coagulation and fibrinolysis.
- The binding of enzymes which remove ROS and reduce oxidative stress.
- Protection of the endothelium from shear stress of plasma flow.
- Stimulation of NO production.
- Molecular sieve [37].

In protecting the vascular epithelium from the shear stress of plasma flow, the glycocalyx itself is degraded and so its thickness constantly fluctuates as it is reformed [37].

It is thought that the negative charge of molecules, including heparan sulphate, within the glycocalyx contribute to its ability to stop the passage of albumin through the glomerulus. It is also suggested that albumin has areas of positive charge along its structure which allow it to bind strongly to the glycocalyx [37].

Dysfunction of the glycocalyx is considered as a strong link between cardiovascular risk and renal risk, particularly in those with diabetes [36]. Hyperglycaemia may cause damage to the glycocalyx through degradation by ROS [39]. As a result, in diabetic patients, the endothelial response to shear stress may be diminished and so flow induced vasodilation may in turn become impaired [40].

One of the most important factors in development of albuminuria could be damage to the glycocalyx [26, 36, 41, 42]. The glycocalyx could therefore be the common pathway linking albuminuria with cardiovascular mortality. It is known that the endothelial glycocalyx can be damaged by acute hyperglycaemia and ROS [26]. Endothelial activation resulting from diabetes or hypertension can also lead to the release of enzymes which break down parts of the glycocalyx such as hyaluronic acid and heparan [36]. In relation to this, plasma levels of hyaluronic acid negatively correlate with renal function and could be used as a marker of glycocalyx breakdown [43].

In relation to its function, damage to the glycocalyx provides a convincing mechanism behind the link between DKD, albuminuria, hypertension and cardiovascular morbidity.
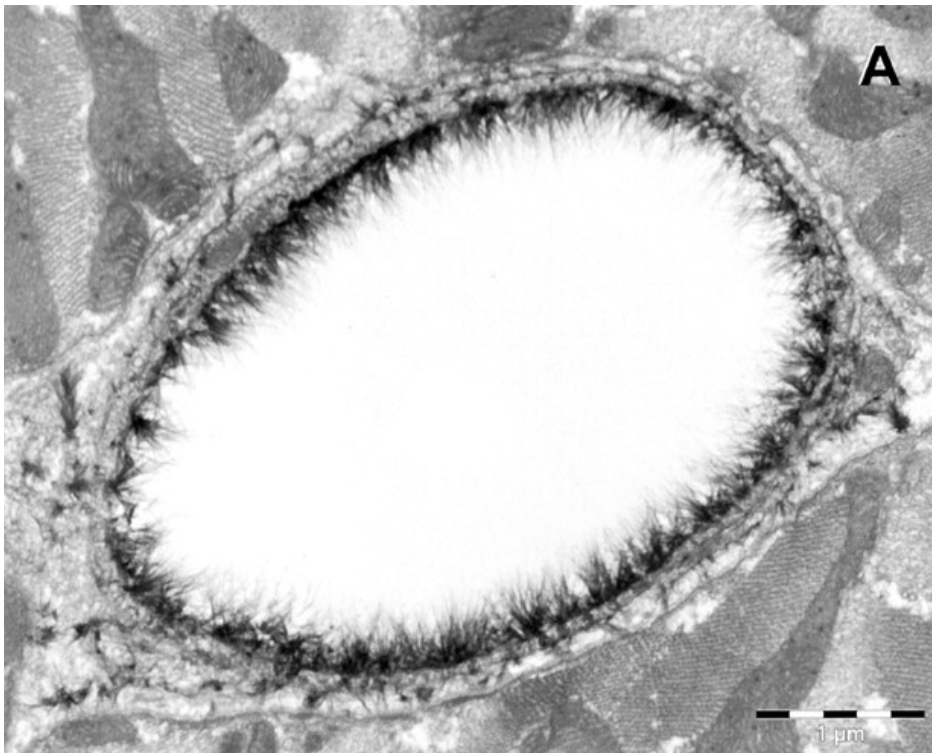
Figure 4 an electron micrograph of the endothelial glycocalyx layer of a rat [44].

1.3.2.4 The Glomerular basement membrane

The glomerular basement membrane (GBM) is a mesh-like structure formed from four macromolecules; laminin, type IV collagen, nidogen and the specific heparan sulphate proteoglycan agrin [32, 33]. It was thought that agrin and the other negatively charged constituents of the GBM played a major role in repelling negatively charged molecules, such as albumin, from being filtered through the membrane [32]. However, several studies showed that removal of agrin and other molecules from the GBM resulted in a large decrease in negative charge of the GBM but no significant change in albumin filtration [45-47]. This has led to the theory that charge is less important in filtration than previously believed and that the glycocalyx could play a more significant role. In diabetic kidney disease the GBM becomes thickened through deposition of extracellular matrix. It is thought this deposition is linked with podocyte injury [25].

1.3.2.5 The Podocytes and slit diaphragm

Overlying the basement membrane are many cells called podocytes. Podocytes take their name from their foot like processes. The foot processes of each of these cells interdigitate. A very thin membrane forms across the minute gaps between these interdigitations called the slit diaphragm. Little is known about the structure of the slit diaphragm but it is hypothesised to be formed from rods of nephrin into a zip-like structure [32]. The slit diaphragm only allows passage of molecules smaller than 0.007µm into the Bowman's capsule which means that albumin (diameter 0.007µm) is rarely filtered in healthy individuals [34]. Widening of the podocyte slit diaphragms could be an early sign of DKD and loss of nephrin within the slit diaphragm may be an important factor in albuminuria development [48]. It is thought that in the very early stages of persistent hyperglycaemia, podocytes are stimulated to produce GBM matrix molecules. The resultant thickening of the GBM is thought to be one of the earliest stages of DKD [25].

1.3.3 Glomerular hyperfiltration

In the early stages of DKD, many patients develop a hyperfiltration through high pressure in the glomerulus. The hydrostatic pressure within the glomerulus can be affected by fluctuations in the systemic blood pressure. On average, both kidneys filter 120ml of fluid per minute through the glomeruli, in young healthy individuals [34]. This filtration rate relies on the hydrostatic pressure within the glomerular capillaries. Up to 45% of people with T2DM will demonstrate hyperfiltration with a GFR averaging 117-133mL/min) [49]. The rise in GFR may contribute to glomerular hypertrophy and glomerular basement membrane thickening and albuminuria. There are several mechanisms involved in regulating glomerular pressure such as myogenic autoregulation, tubuloglomerular feedback and the action of angiotensin which is discussed in more detail in 1.4.3.4. These mechanisms may be affected by high blood pressure and diabetes.

When glomerular arterioles are over stretched by higher blood pressures their smooth muscle contracts to reduce blood flow through the glomerulus [34]. This is called myogenic autoregulation and helps to maintain a stable level of tissue perfusion at varying levels of blood pressure. This mechanism is present throughout the body but is particularly important within cerebral blood vessels and the glomerulus. Myogenic autoregulation may be reduced in diabetic patients and therefore may contribute to abnormal glomerular filtration [50].

Tubuloglomerular feedback is the mechanism by which the macula densa cells detect changes in the concentration of sodium within the kidney tubule and consequently inhibit dilation of the glomerular arterioles. A rise in blood pressure increases GFR causing filtrate to flow through the nephron at a faster rate. As a result, the proximal convoluted tubule and loop of Henle have less time to reabsorb solutes such as sodium. Therefore, a higher concentration of sodium reaches the macula densa cells by the distal convoluted tubule. The macula densa cells detect this increase in concentration and *inhibit* the release of NO from the juxtaglomerular cells. This inhibits the dilation of the afferent arteriole by NO, reducing blood flow to the glomerulus [34]. One theory for hyperfiltration in T2DM, other than impaired autoregulation, is that hyperglycaemia and hyperinsulinaemia stimulate sodium reabsorption in the

proximal convoluted tubule (PCT) so less sodium reaches the macula densa and results in dilation of the afferent arteriole [22].

1.3.4 Tubular Damage through albumin reabsorption

Reabsorption of albumin occurs mainly in the first half of the PCT. The exact process is unknown however, there is strong evidence to suggest that albumin in endocytosed by way of two receptors called megalin and cubilin [32, 51, 52]. Albumin binds to cubilin at the brush border of the PCT. This binding is catalysed by megalin which also is thought to stimulate the internalisation of albumin into endosomes and fusion with hydrolysing lysosomes [52]. It is thought that there must also be alternative methods for reabsorption of albumin as in cubilin knockout mice urine albumin only increases around six-fold and does not enter the nephrotic range [29, 52].

In pathological states where more albumin is filtered through the glomerulus, it is thought that the normal reabsorption mechanism becomes saturated. This means excess albumin and other proteins pass into the urine. It is also thought that the continuous reabsorption of albumin and proteins in the PCT may lead to damage of the PCT epithelial cells leading to an inflammatory and fibrotic response in the tubular interstitium. This can lead to a progressive impairment of the reabsorption process and tubular damage [32].

When the capacity of the megalin mediated pathway leading to lysosomal digestion of proteins is exceeded this leads to the activation of alternative methods of protein digestion. These alternative methods may lead to improper digestion of albumin and important molecules [51].

Another study has shown that patients with type 1 and type 2 diabetes may shed megalin and cubilin into their urine. This could be a contributing factor towards albuminuria in diabetics [53. 54].

1.4 Hypertension and Type 2 Diabetes

Some individuals have pathologically high blood pressure (hypertension). Hypertension can also cause kidney damage and is linked with albuminuria. Most cases of hypertension are classed as 'essential hypertension' meaning the cause is undetermined although, we know of risk factors that can increase the chance of developing hypertension one of which is diabetes itself. Other risk factors include:

1) Increasing age
2) Gender (males generally at higher risk below the age of 65)
3) Family history
4) Ethnicity
5) Being overweight
6) Inactivity
7) Excess salt intake
8) Stress [55]

1.4.1 Epidemiology and complications of hypertension

Hypertension is increasingly prevalent likely due to increasing corresponding risk factors such as diabetes, obesity and poor diet. It is estimated that around 1 billion people are hypertensive worldwide [56]. The American Diabetes Association reported that between 2000 and 2012, 71% of adults with diabetes were hypertensive or were receiving treatment for hypertension [57].

Hypertension can lead to many different complications. These include ischaemic heart disease, peripheral artery disease and also kidney disease.

High pressure within the circulation leads to strain and increased oxygen demand of the heart muscle. The high pressure can also damage the blood vessels themselves. Over activation of smooth muscle can lead to thickening of the intima and media layers of the blood vessels and therefore narrowing of the lumen and further increase in pressure [58]. Damage to vessel wall endothelium from high blood pressure may also accelerate inflammation and the formation of atherosclerosis within the vessel wall [59, 60]. Atherosclerosis leads to coronary

and peripheral artery disease and predisposes individuals to myocardial infarction and cerebrovascular events.

Both thickening of the vessel wall and development of atherosclerosis stiffens the arteries, decreasing vessel compliance and exacerbating hypertension. This stiffening can be demonstrated by a wide pulse pressure (a large difference between the systolic and diastolic values).

1.4.2 Albuminuria in hypertension

Hypertension is the second largest cause of CKD after diabetes [1]. Together, diabetes and hypertension account for around two thirds of CKD cases [61]. Hypertension causes progressive hardening of the kidney's microvasculature (nephrosclerosis). It is also well established that there is a strong relationship between hypertension and albuminuria. A study found that moderately increased albuminuria was present in 40% of non-CKD hypertensive patients [62]. This may be due to hypertension itself leading to hyperfiltration of albumin or damage to the vascular endothelium as a result of high pressures.

1.4.3 Pathophysiology of blood pressure in hypertension and diabetes

There is much research into underlying causes of the association between essential hypertension and diabetes. However, the mechanisms are likely to be a complex interplay of many different factors. Common themes include salt handling, overstimulation of the sympathetic nervous system (SNS), increased peripheral resistance, endothelial dysfunction, renal dysfunction and disruption of circulating factors in obesity and diabetes. All of these themes are highly prevalent in the T2DM population which is likely why hypertension is so prevalent in this patient group.

The following sections will discuss these themes and normal blood pressure regulatory mechanisms which may be impaired in diabetes and hypertension.
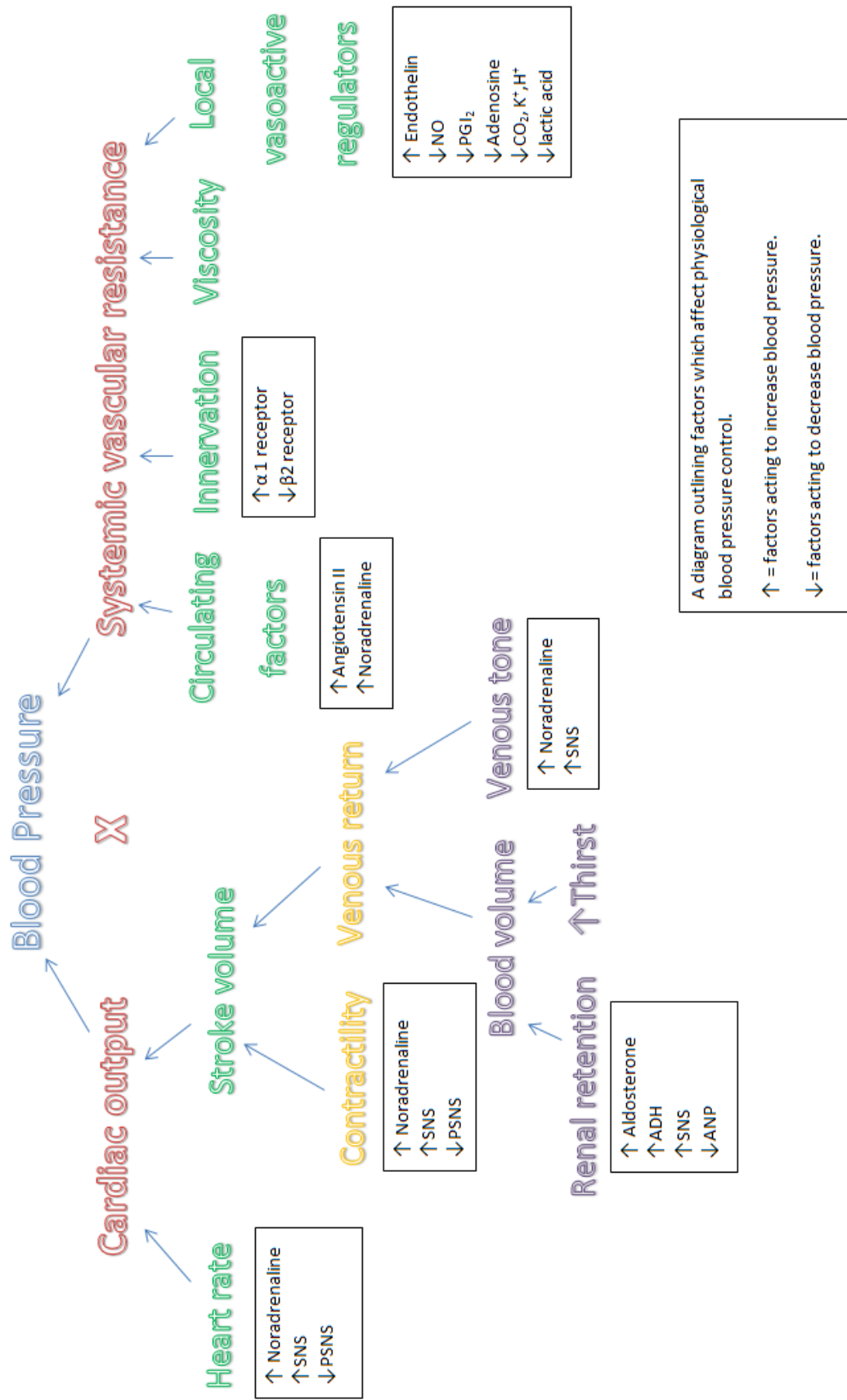
Figure 5 *Factors which affect physiological blood pressure control. Sympathetic nervous system (SNS), Parasympathetic nervous system (PSNS), Anti-diuretic hormone (ADH), Atrial natriuretic peptide (ANP), Nitric oxide (NO), Prostacyclin (PGI2), carbon dioxide (CO2), potassium (K), hydrogen (H).*

1.4.3.1 Intrinsic vasoactive substances

The vascular endothelium releases a number of factors that control local blood flow, locally. These factors include metabolites produced by tissue respiration and endothelial secretions.

1.4.3.1.1 Metabolites

Metabolites produced through tissue respiration such as carbon dioxide, potassium, hydrogen, lactic acid and adenosine have a vasodilatory effect on blood vessels. The effect of these metabolites can be demonstrated during active hyperaemia. For example, during exercise, tissues undergo active hyperaemia as increased metabolic activity leads to an increased generation of metabolites. These metabolites cause vasodilation leading to hyperaemia [63].

Reactive hyperaemia can occur when a vessel is occluded. The process is not as well understood as active hyperaemia however, it is thought that occlusions lasting several minutes cause an accumulation of vasodilatory metabolites leading to an episode of reactive hyperaemia once the occlusion is released [64]. The hyperaemia slowly declines as blood flow removes the metabolites and restores oxygen requirements. Shorter episodes of occlusion may result in hyperaemia through the myogenic auto-regulatory mechanism or inadequate oxygen to sustain smooth muscle contraction [63]. Active hyperaemia and reactive peak flow can be reduced in diabetic patients with microvascular damage [65, 66].

1.4.3.1.2 Endothelial secretions

The endothelium produces many paracrine vasoactive substances for example, NO, Prostacyclin (PGI_2) and endothelin [63, 67].

NO is an important vasodilator. NO is produced when the glycocalyx responds to the shear stress of blood flow through the capillaries. The glycocalyx causes increased expression of endothelial NO synthase enzyme which leads to the production of NO [68, 69]. Circulating insulin is also a stimulus for the release of NO and high blood glucose is known to decrease production of NO [70]. Damage to the endothelial glycocalyx, as witnessed in diabetes, may lead to a reduction in this vasodilatory mechanism.

PGI_2 has a similar vasodilatory role to NO. They are both stimulated by thrombin and also both contribute to the prevention of clot formation. If there is damage to the endothelium, decreased production of these factors can influence vasoconstriction and promotion of clot formation [63].

1.4.3.2 The Autonomic Nervous System

The autonomic nervous system (ANS) is an important regulator of blood pressure. Similarly to peripheral neuropathy, diabetes can lead to cardiac autonomic neuropathy (CAN) [71, 72]. As a result, the autonomic control of blood pressure is jeopardised and may contribute to hypertension amongst diabetic patients [71].

The ANS is responsible for acute regulation of blood pressure and globally controls many aspects of the cardiovascular system. The ANS can be activated by external stressors and the 'fight or flight' response but ANS blood pressure control is primarily activated through baroreceptors [34].

Baroreceptors are located within the carotid sinus and the aortic arch, responding to stretch within the arterial wall. When there is a decrease in stretch from baseline (lower blood pressure), baroreceptors send fewer signals to the hypothalamus via the sensory branch of the vagus nerve [34]. The hypothalamus registers the drop and sends messages to the adrenal medulla which releases noradrenaline.

Noradrenaline increases heart rate and contractility through β1 adrenergic receptors. β1 receptors are also present in the juxtaglomerular cells and so β1 agonism leads to increased renin secretion and activation of the blood pressure raising renin-angiotensin-aldosterone system (RAAS) [67]. Noradrenaline also activates α1 adrenergic receptors which lead to vasoconstriction which increases systemic vascular resistance, and in turn, blood pressure [67].

When baroreceptors detect an increase in stretch, more signals are sent, by the vagus to the hypothalamus. The hypothalamus activates stimulation of the parasympathetic vagal nerve fibres which release acetylcholine. Acetylcholine acts on muscarinic receptors. Muscarinic-2 receptors decrease atrial contractility and also decrease heart rate through suppression of the sinoatrial

node. Muscarinic-3 receptors increase nitric oxide release, causing vasodilation, reducing blood pressure. CAN may reduce the activation of this parasympathetic pathway leading to a sympathetic dominance, upregulating the RAAS and vasoconstriction [71, 72].

1.4.3.3 The Renin-angiotensin-aldosterone-system

Renin is an enzyme which is released from the juxtaglomerular cells in the kidney in response to low blood pressure or glomerular pressure. There are three principle promotors of renin release.

Firstly, the juxtaglomerular cells release renin when baroreceptors within the glomerular afferent arteriole sense a decrease in stretch. Secondly, renin is released in response to renal sympathetic nerve stimulation.

Thirdly, renin is released when the macula densa cells respond to the concentration of sodium within the distal convoluted tubule (DCT) urinary filtrate. When GFR increases due to a rise in blood pressure, filtrate flows through the nephron at a faster rate. As a result, the PCT and loop of Henle have less time to reabsorb solutes such as sodium. Therefore, a higher concentration of sodium reaches the macula densa cells by the distal convoluted tubule. The macula densa relay this message to the juxtaglomerular cells which *inhibit* release of renin into the blood stream [67].

When the opposite happens due to lower blood pressure, renin is inhibited to a lesser extent. The RAAS system is then activated and acts to increase blood pressure. The release of renin catalyses the formation of angiotensin II from angiotensinogen. Angiotensin II is a vasoconstrictor acting mainly via angiotensin 1 receptors [73]. Angiotensin constricts the arterioles of the glomerulus, but constricts the efferent arteriole to a greater extent which increases hydrostatic pressure inside the glomerulus and maintains GFR. This is the principle by which angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) decrease albumin excretion.

Angiotensin II also stimulates the release of the mineralocorticoid aldosterone from the adrenal glands which causes the principle cells within the DCT and collecting duct in the nephron to reabsorb more sodium, secreting potassium

into the tubule. Water then follows the sodium into the blood resulting in an increase in blood volume and pressure.

As well as the described classical RAAS pathway there are also tissue specific RAAS sites including local intrarenal RAAS activity [74]. Local intrarenal RAAS activity occurs when renin is secreted within the proximal tubules; this secretion is increased within DKD [75]. This could be a factor contributing to hypertension in this patient group. It is thought that hyperglycaemia itself increases angiotensin II production in the kidney, leading to constriction of the efferent arteriole and increased glomerular pressure [76].

1.4.3.4 Salt intake

One factor which is believed to be key in the mechanism of development of hypertension is dietary salt intake. It is recommended by the world health organisation that adults should consume less than 5 grams of salt per day. The World Health Organisation believes that reduction in salt intake to this level could prevent 2.5 million deaths a year through reduced cardiovascular risk [77].

Many studies have noted a positive correlation between increased salt intake and high blood pressure [78, 79]. It is not well understood why salt reduction would lead to a decrease in blood pressure. Low salt levels would normally act to increase activation of the RAAS and increase blood pressure. It is speculated that the effect may be due to the excessive salt intake within the modern day diet and inability of the kidney to excrete these large amounts of salt [78]. In response to the resultant salt loading, the extracellular space is expanded through passive movement of water leading to increased blood pressure [80]. However, without evidence of a specific mechanism it is difficult to confirm a causal relationship between salt intake and blood pressure. It could be that the rise in blood pressure may be due to other concurrent dietary habits that lead to high salt intake, such as obesity. One study showed no significant change in blood pressure as a result of high salt intake in obese patients compared to lean individuals [81]. Although all of the patients had an increase in plasma volume, they responded with a decrease in systemic vascular resistance and so BP was unchanged. This suggests that although salt intake may affect blood volume other pathological mechanisms must be present in order to lead to hypertension.

The Cochrane collaboration performed a meta-analysis of the impact of low versus high salt diet and blood pressure. The meta-analysis found that in normotensive people, reducing salt intake reduced blood pressure by less than 1%. In these normotensive patients there was also a significant increase in renin, angiotensin and noradrenaline production which may explain why blood pressure remained almost stable within this group. In hypertensive patients salt reduction led to a 3.5% decrease in blood pressure but a similar rise in renin, aldosterone and noradrenaline to the normotensive group [80]. These results suggest that reduction of salt intake may not be as vital to the pathophysiology of hypertension as once thought. In addition, these results included white participants only, the evidence for other ethnicities has not been rigorously meta-analysed.

Overall these findings seem to suggest that altering salt intake can influence BP. However, it seems unlikely to be a sole causative factor in the development of hypertension. Reduction of salt intake may only lead to a significant reduction in BP where it is already an initial contributing causative factor and excretion mechanisms are impaired.

1.4.3.5 Obesity related hypertension

The mechanism of high blood pressure seen in obese patients is also likely to be multifactorial. When excess body fat is produced, additional blood vessels are also produced to supply this tissue. As a result, total blood volume must increase to fill the circulation and systemic vascular resistance decreases. This increases the workload of the myocardium. In addition to this, inflammation and circulating lipids as a result of obesity predisposes the development of atherosclerosis which narrows vessel lumen and can increase systemic vascular resistance.

One of the leading theories for the mechanism which links obesity and hypertension is that of the hormone leptin. Leptin is the 'hunger hormone' which is released from adipose tissue to suppress the appetite [34]. Leptin usually induces endothelial dependent vasodilation and anti-coagulation through catalysis of NO production [82]. Leptin also stimulates the SNS which increases blood pressure. However, hyperleptinaemia can result from excessive amounts of adipose tissue [82]. Obese individuals seem to develop selective leptin

resistance as a result of this chronic hyperleptinaemia in which there is an impaired anorectic effect and leptin dependent NO production is reduced but the SNS is still stimulated [82].

Other theories suggest that SNS activity maybe upregulated in obesity as a result of impaired baroreceptor function, circulating free fatty acids (increased α-adrenergic activity), increased levels of angiotensin II or insulin [83]. Increased levels of angiotensinogen could be due to adipose tissue related release of angiotensinogen [83, 84]. Excess aldosterone is released as a result of adipose tissue released factors such as angiotensinogen. In healthy individuals, these adipose tissue factors usually exhibit a negative feedback response to higher levels of salt. However, obese patients appear to lack this negative feedback so excess aldosterone is released alongside excess salt intake. Together this leads to elevated blood pressure as well as kidney damage and cardiovascular disease [84].

Obesity related hypertension may also be a result of histological changes to the kidneys, primarily of increased pressure on the medulla from intrinsic or visceral fat [83]. It is thought that the increased pressure may reduce flow rate resulting in more time for sodium reabsorption within the tubule. Therefore less sodium reaches the DCT and the macula densa stimulate the RAAS, increasing blood pressure [83].

1.4.3.6 Insulin resistance and hyperglycaemia

Hyperglycaemia and insulin resistance particularly in diabetes may directly lead to hypertension. Insulin contributes to induction of NO production and so lack of insulin can result in loss of the vasodilatory and other previously discussed effects of endothelial nitric oxide [85]. Insulin also acts on the kidney to increase sodium reabsorption and raise blood pressure [86]. In addition, the low grade inflammation produced as a result of oxidative stress in hyperglycaemia contributes to endothelial dysfunction, stiffening of arteries and hence hypertension [87].

1.5 24-hour Blood Pressure Patterns

In addition to the previously discussed mechanisms of BP regulation, BP also has its own 24-hour pattern or circadian rhythm. This involves a drop in blood pressure during sleep and a surge in the morning on awakening. Others also report a second evening peak around 7pm [88]. It has been shown that a normal nocturnal dip is a 10-20% drop from an individual's daily baseline [89]. Those who drop more than 20% are considered extreme dippers and are also at increased risk of cardiovascular events unless the dip is caused by anti-hypertensive treatment [89]. It is thought that the morning surge is due to autonomic sympathetic activation on arousal. In a young healthy adult the morning surge should be around 20-25mmHg from the average nocturnal systolic blood pressure (SBP) however, in elderly patients with stiff arteries the rise can be around 40-60mmHg [88]. The morning surge in BP is thought to be responsible for the increased number of cardiovascular events, such as stroke and heart attack, which occur during the morning than other hours of the day.
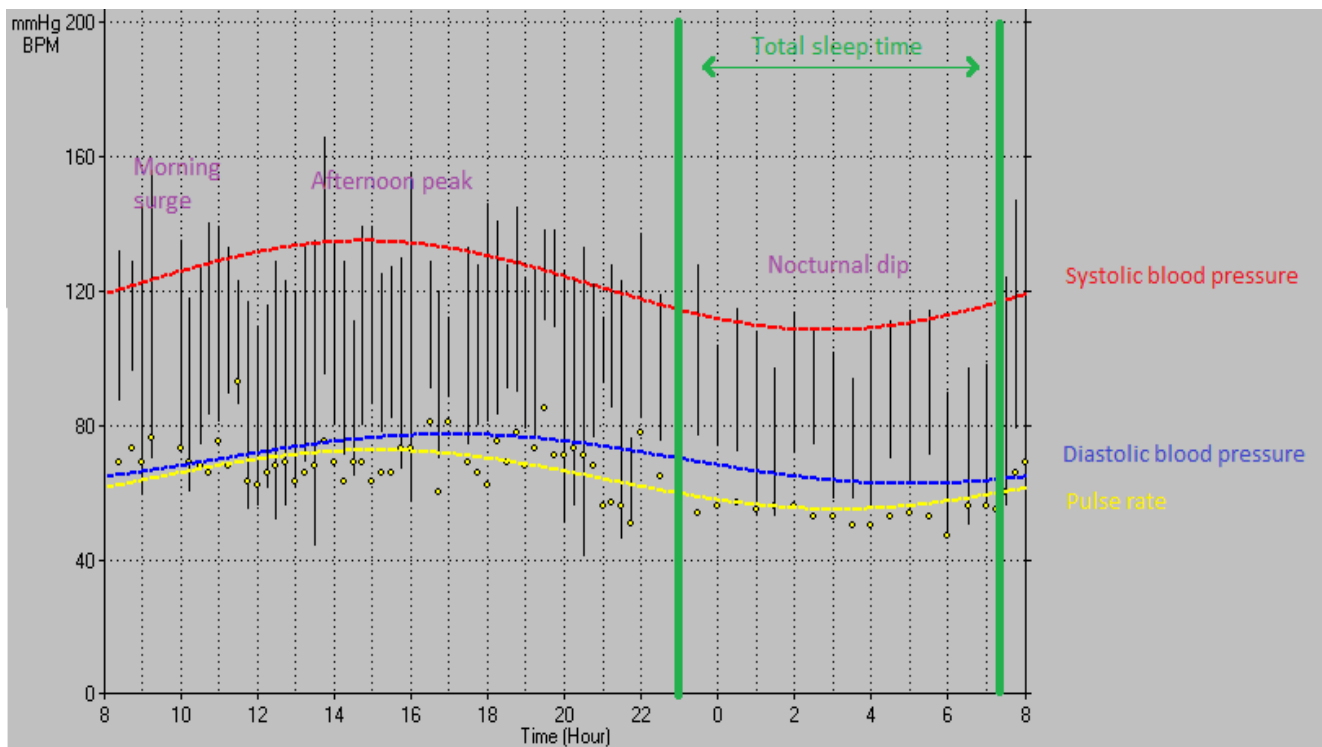
Figure 6 *A graph of a healthy individual's systolic, diastolic blood pressure and pulse rate against time (24-hour clock).*

1.5.1 Nocturnal Dipping

1.5.1.1 Prevalence of non-dipping in the diabetic population

Some individuals, particularly people with diabetes and hypertensive patients, display a loss of the nocturnal dip (non-dippers) or even a night time rise (risers). Both of these groups also show an increased risk of cardiovascular events [90-92]. An increase in 10mmHg of average nocturnal SBP may increase mortality by as much as 21% [93]. One study of 50 T2DM patients and 50 non-diabetic patients found that 74% of T2DM patients were non-dippers compared to 16% of non-diabetics [94]. It may be that some of the increased cardiovascular morbidity in people with diabetes is a result of loss of normal circadian BP rhythms. If this is correct, circadian BP rhythms could be an important treatment target in people with diabetes.

1.5.1.2 Pathophysiology of non-dipping

It is not well understood why people with diabetes show a loss of normal 24-hour BP patterns. A study in 1999 found loss of dipping in 73% of their hypertensive T2DM cohort [95]. This study found that non-dipping was associated with postprandial hyperglycaemia but not with fasting hyperglycaemia or HbA1c. They also found that non-dipping was related to duration of diabetes; non-dippers had diabetes on average for 4 years longer than dippers. This study hypothesised that postprandial hyperglycaemia leads to peaks of oxidative stress which lead to the production of ROS and endothelial damage [95]. This microvascular damage could perhaps lead to autonomic neuropathy (AN).

Over a decade later, a study was published examining the relationship of autonomic dysfunction to non-dipping [96]. They found that half of the T2DM cohort had AN and that those with AN had a significantly reduced nocturnal BP dip than those without (p<0.01). This study suggested that AN is an important factor in loss of the nocturnal BP dip and that AN may lead to a reduction of nocturnal vagal activity hence sympathetic prevalence.

The RAAS is also controlled by the sympathetic nervous system. It is thought that the RAAS could play a significant role in BP circadian rhythm as levels of renin, aldosterone and angiotensin II have been noted to be at their highest in the morning, falling throughout the day [97]. It is thought that intrarenal RAAS shows a circadian rhythm [98]. Some intrarenal RAAS may be due to angiotensinogen release from the liver which filters through the glomerulus. More angiotensinogen is filtered in response to increased glomerular permeability and hypertension. This may be another link in the relationship between kidney damage, hypertension and how they often progress in parallel[98].

Abnormal sympathetic activity could be an explanation for why people with hypertension also often exhibit loss of nocturnal dipping. A recent meta-analysis concluded that non-dipping in hypertension is a predictor of all cardiovascular outcomes, independent of average SBP [89]. However, some suggest that non-dipping is a sign of more advanced hypertension [99]. Perhaps non-dipping is independent of average SBP, but related to how established the underlying pathophysiological processes are.

Another factor that could contribute to loss of nocturnal dip is sleep apnoea. Sleep apnoea is a condition which is highly prevalent in the T2DM population likely due to common risk factors such as obesity [100]. Apnoeic episodes during the sleep period are thought to increase overall nocturnal blood pressure [88]. BP falls with the onset of the apnoea but then rises with termination of the episode. The influence of sleep apnoea on night time blood pressure in T2DM may be overlooked as sleep apnoea is often undiagnosed. It seems as though sleep apnoea could play a causative role towards loss of nocturnal dip but again, it may simply be a correlation due to similar risk factors.

1.5.2 Evidence for the effect of 24-hour Blood Pressure Patterns on Albuminuria

1.5.2.1 Non-Dipping

Several studies have specifically evaluated the relationship of nocturnal dipping among diabetic and hypertensive patients and albuminuria. There is an overarching theme that loss of nocturnal dipping correlates with increasing albuminuria. However, the specific parameters and strength of this relationship differ between studies. It is not clear whether nocturnal dipping patterns provide more valuable or additional information beyond average 24-hour SBP. The following sections will review the current literature on the effect of non-dipping blood pressure patterns on albuminuria.

1.5.2.1.1 Nocturnal Systolic BP and albuminuria

In 1997 a small study compared 24-hour blood pressure and albumin excretion rate (AER) in 20 T2DM patients with normal albuminuria and MIA [101]. They found that nocturnal SBP was significantly higher in the MIA group than the normal albuminuria group and that only nocturnal SBP showed a significant relationship with urine albumin excretion (p=0.05). The study found that the night time dip was reduced the most within the MIA group but this difference was not statistically significant, perhaps influenced by the small sample size.

A retrospective study of 16546 patients found that there were higher proportions of non-dippers and risers in patients with MIA and SIA than those with normal albuminuria. Thirty-eight percent of normal albuminuria patients, 43.3% of MIA patients and 43.8% of SIA patients were non-dippers. This study focussed on average nocturnal SBP levels rather than non-dipping and may involve selection bias as all patients were taken from an ambulatory blood pressure monitoring registry. The study found that high nocturnal SBP significantly correlated with increased albuminuria (p=0.001) [102]. However, the significance of the specifically nocturnal BP and albuminuria relationship may be as a result of the first morning void urine measurement. The urine measurement therefore, may correlate more strongly with night time blood pressure values. This study also combined people with and without diabetes within their analysis of blood pressure dipping and so may not be representative of the T2DM population.

Both of these studies commented primarily on average night time SBP rather than the patient's individual day to night BP ratio. Therefore, these studies are not particularly helpful in considering the importance of loss of dipping but they do provide evidence that SBP has a significant correlation with albuminuria. They seem to suggest that nocturnal SBP has a more significant relationship with albuminuria than daytime SBP. This relationship may be due to time of urine collection as night time urine may correlate more strongly with night time blood pressure values. Night time blood pressure measurements may also be more informative due to the lack of external stressors which are often present during the day and can affect BP variability.

1.5.2.1.2 Non-dipping and albuminuria in type 2 diabetics

One study followed 112 people with diabetes for 9.5 years. Individuals who went on to develop advanced nephropathy and albuminuria had significantly higher average 24-hour SBP and pulse pressure (PP) values. This study interestingly found that diastolic day to night BP ratio was an independent predictor of nephropathy progression (p=0.02) but that systolic day to night ratio was not (p=0.14) [103]. Strengths of this study were that patients were randomly selected and there was a long follow up period. It is worth noting, the night time BP was determined using a 'set night time period' rather than the patient's own sleep and wake times. This could mean the study's night time blood pressure values were not representative of the individuals actual sleep pattern and circadian rhythm. The study also used albumin creatinine ratio (ACR) rather than albumin excretion rate (AER) which is the gold standard measurement for albumin.

Other research supports the relationship between loss of nocturnal dip and increasing albuminuria and suggests that loss of circadian BP may be an indicator of poor prognosis [104, 105]. However, one of these studies only included male participants [104] and so is not relevant to the whole population. The second study found that high night-time SBP and loss of night time dipping both correlated with albuminuria but did not comment on the relationship between non-dipping and SBP, so it was not ascertained whether non-dipping independently contributed to albuminuria in this group [105].

Leitão et al performed a cross-sectional study of 270 T2DM patients to determine whether day to night ratio of BP was more or less strongly related to albuminuria than average 24-hour SBP [106]. The study used 24-hour urine collections and an immunoturbidimetric albumin assay alongside 24-hour ambulatory blood pressure monitoring. They found that both average SBP and day to night ratios significantly correlated with albuminuria. However, albuminuria had a more consistent and stronger correlation with average 24-hour SBP (r=0.418, p<0.001) than with day to night BP ratios (r=0.159, p=0.011). The significance of day to night BP ratios with albuminuria was not maintained after adjustment for age, diabetes duration and HbA1c. This could suggest that the observed relationship is a reflection of the severity of diabetes. This study had some limitations; the cohort was 77% white and so may not be entirely representative of any ethnicity, the patients had an average diabetes duration of 10 years and HbA1c of 8.2%. This may mean the patients had fairly advanced diabetes with overlap of microvascular complications. It would be beneficial to compare this group to a control group. All of the non-parametric data was log transformed before performing parametric statistical tests rather than using the raw data and non-parametric tests.

Another study of 663 patients with T2DM showed that non-dippers (n=230) had a significant correlation with albuminuria and also aortic pulse wave velocity showing that dipping may correlate with arterial stiffness [107]. This supports the theory that a common pathway of endothelial dysfunction may lead to abnormal 24-hour BP patterns and albuminuria. The main significant differences between these groups were increased ACR (5.0 ±17.1 vs 2.0 ±9.7, p=0.004) and reduced GFR (88.6 ±20.7 vs 100.2 ±25.7, p=0.001) in the non-dipping group compared to the normal dipper group. BMI (30.7 ±4.7 vs 29.9 ±4.6, p=0.036) and office SBP (139.0 ±16.4 vs 136.0 ±16.2, p=0.024) were increased in the non-dipping group but diabetes duration, HbA1c and age did not differ between groups. When this study removed participant data where 24-hour BP results were less than 60% successful, the relationship between non-dipping and ACR became borderline significant (p=0.051). The study also made no comment on the relationship between average SBP and ACR. The paper had no mention of exclusion criteria and did not mention any other diabetic complication patients may have had. This study used ACR instead of the gold standard AER.

1.5.2.1.3 Non-dipping and albuminuria in hypertension

As non-dipping is prevalent in hypertension as well as diabetes, it is important to understand the overlap between the two conditions in relation to albuminuria. Different studies have evaluated the relationship between albuminuria and 24-hour BP patterns in diabetes and hypertension in several different ways.

One study examined 141 newly diagnosed hypertensive patients without nephropathy or diabetes. This study found that patients with MIA had higher blood pressure values across the board than those with normal albuminuria. They also found that day to night ratio of diastolic blood pressure (DBP) was inversely associated with urine albumin excretion (UAE) independent of age and sex [108]. This study used a set night time period which, as previously mentioned, may not give an accurate representation of an individual's circadian BP rhythm. However, these results could suggest that day to night blood pressure ratio is relevant to albuminuria in the non-diabetic population. 24-hour AER was used in this study.

Another study compared non-dipping in non-diabetic hypertensive people (n=41) and people with T2DM and hypertension (n=72) across all ranges of albuminuria [109]. Average systolic BP was higher in the diabetic MIA and SIA groups. In the T2DM group, non-dipping was observed in 80%, 74%, 43% of the SIA, MIA and normal albuminuria groups respectively. Thirty-seven percent of the non-diabetic hypertensives were non-dippers. This suggests that although there is a relationship between hypertension and loss of circadian BP, this link may be stronger in the presence of diabetes. These results also suggest that there is a relationship between non-dipping and albuminuria. It remains to be determined whether this relationship is causative or whether these signs could be two products of a common causative pathway. It is worth noting that the participants were hospitalised for the purposes of the study and so BP may not be reflective of the values they experience in normal daily life. The study also used a set night time period rather than the patient's own sleep and wake times.

Inaba et al looked into day to night BP ratio specifically in normotensive people with diabetes [110]. Twenty-seven people with T2DM were compared to 10 age and body mass index (BMI) matched controls. The day to night ratio in patients

with MIA was significantly higher than healthy controls. There was also a correlation between AER and day to night BP ratio. DBP showed a slightly stronger correlation than SBP (r=0.589, p<0.05 and r=0.534, p<0.05 respectively). This suggests that diabetes has a relationship with non-dipping patterns independent of hypertension status. Interestingly, although there was no difference in HbA1c or fasting plasma glucose between groups the patients with MIA had longer duration of diabetes. The linear examination of variables was a strength of this study. Many of the other described studies may lose data by separating patients into discrete non-dipper/dipper, normal albuminuria/MIA/SIA groups. Linearly comparing day to night BP ratio and AER gives insight into the progressive relationship between the two variables. However, this study was small and examined already hospitalised in-patients which gives selection bias and could lead to misleading results as these patients were likely acutely unwell.

These results suggest that the potential relationship between non-dipping and albuminuria is not specific to the diabetic group but may be stronger in diabetes. Further study is needed to further clarify the relationships between these groups.

1.5.2.1.4 Non- dipping and albuminuria progression

Many studies do not evaluate the relationship between albuminuria and non-dipping in terms of progression of disease but simply take a snapshot of albuminuria and 24-hour BP. However, in one study, 957 T2DM patients were followed up for 3 years [111]. Although a higher proportion of non-dippers progressed to MIA than dippers, this study found that non-dipping patterns were not an independent predictor of albuminuria progression. However, night time rising was an independent predictor of albuminuria progression but ambulatory pulse pressure (PP) was the most informative predictor of albumin progression. Interestingly, there were a higher proportion of non-dippers in those with a PP over 65mmHg. However, this authors acknowledged itself that their dichotomous classification of BP patterns may have resulted in loss of information.

1.5.2.2 Other circadian blood pressure parameters and albuminuria

1.5.2.2.1 Pulse pressure

Increased PP is also associated with microalbuminuria in people with diabetes[112]. Knudsen et al evaluated the relationship between pulse pressure alongside circadian BP in T2DM [113]. This showed a significant relationship between average 24-hour BP and increasing albuminuria and also a significant relationship between loss of the nocturnal dip and increasing albuminuria. However, the study also showed that PP correlated with increasing albuminuria and that nocturnal PP was significantly higher in the SIA group. This could suggest that circadian variation in PP is also a contributor to albuminuria in diabetic patients.

1.5.2.2.2 Non-dipping heart rate

As well as non-dipping patterns in BP, heart rate (HR) should drop overnight. It seems that people with diabetes are also at risk of non-dipping heart rate as a result of AN. A study of 179 T2DM patients found that non-dipping heart rate was also significantly related to increasing albuminuria [114]. This suggests that AN may be an important factor in the mechanism behind a relationship between circadian BP rhythms and albuminuria.

1.5.2.2.3 Morning surge

Other studies have considered the relevance of other 24-hour BP patterns such as the morning surge in blood pressure (MBPS). One study followed 377 individuals recently diagnosed with T2DM for an average of 6.5 years [115]. They calculated the extent of the MBPS and evaluated its relationship with albuminuria throughout follow up. This found that an increase in MBPS over time is significantly associated with progression of albuminuria in normotensive T2DM patients. A patient whose MBPS increases by 25mmHg is at least 6 times more likely to go on to develop MIA [115]. Other studies support this finding that incremental increases in MBPS are associated with progression of kidney disease [116].

1.5.2.2.4 Afternoon peak

One group considered the relevance of a blood pressure peak in the afternoon (2pm-8pm) [117]. The study found that in those patients with a higher than average afternoon peak there was a higher prevalence of diabetic retinopathy and significant correlation with urine albumin excretion but this disappeared after adjustment for covariates including age, gender, HbA1c, duration of diabetes and 24-hour systolic BP. The afternoon peak perhaps needs further evaluation to determine whether it is a relevant measure in terms of progression of albumin.

Overall the evidence suggests that there is a relationship between abnormal 24-hour blood pressure patterns and progression of albuminuria in people with diabetes, irrespective of hypertension status. Particularly the rising or non-dipping BP, non-dipping HR and MBPS patterns. Non-dipping of PP may also be an informative measure. However, there is some discrepancy between whether diastolic dipping or systolic dipping has a stronger correlation with albuminuria. The mechanism linking dipping status and urine albumin excretion is still unknown and is likely complex. However, it seems that AN may play a significant role.

1.5.2.3 Circadian rhythms as a treatment target

If a relationship between loss of circadian rhythms and albuminuria exists, circadian BP rhythms could be an important treatment target for diabetic patients. Some studies have attempted to target these patterns. One study of 40 diabetic patients received 40mg of Olmesartan either on waking or at bedtime [118]. Nocturnal SBP and average 24-hour BP were significantly lowered in the bedtime dose group compared to the morning dose. The bedtime dose significantly increased the number of nocturnal dippers however, no significant differences were observed in albuminuria.

Another study showed similar results, bedtime administration of anti-hypertensives led to a significant decrease in nocturnal SBP but no significant difference in albumin excretion [119].

However, another study of 200 hypertensive patients randomised timings of medication administration and showed that bedtime administration of the angiotensin receptor blocker (ARB) valsartan led to a 41% reduction in 24-hour AER [120]. This was a significant reduction in albuminuria and highly correlated with a correction of nocturnal dipping independent of average 24-hour BP. Although there is some discrepancy between evidence of whether albuminuria can be targeted by bedtime administration of anti-hypertensives. This study had some important strengths including; large sample size, blood pressure was measured for 48 hours rather than 24 and all physical activity was monitored by an actigraph. However, the study excluded diabetic patients so the data may not be directly translated to the diabetic population and also described their method of urine albumin quantification as 'the routine automatic hospital techniques'. This is likely to be an assay which cannot measure albumin throughout the entire normal albuminuric range.

There is less evidence for how bedtime administration of anti-hypertensives may affect other BP patterns like MBPS. One study found that bedtime doxazosin seemed to diminish the MBPS and the reduction correlated with a reduction in urine albumin [121].

1.5.2.4 Urine collection and analysis

A common theme running through this literature review was the difference in choice of method of urine albumin collection and measurement, ACRs are prone to the variation of individual's creatinine excretion and may sometimes give an inaccurate result [122]. It is worth noting that, while AER is considered the gold standard collection method, timed collections are also prone to collection errors by the patient [123, 124]. Many of these studies either do not describe their urine albumin assay in detail or used the immunoturbidimetric method of albumin quantification which often cannot measure albumin values within the normal albuminuric range.

1.5.2.5 Conclusion

Overall there is evidence to suggest that non-dipping blood pressure patterns are more prevalent in diabetic patients with albuminuria than those without. However, the cause for this relationship is undetermined. As it is well established that higher average SBP leads to increased albuminuria and so it could be deduced that the lack of fall in BP adds to the overall 24-hour SBP level, hence increasing albuminuria. It could also be suggested that both albuminuria and non-dipping may result from a common pathway such as endothelial damage due to hypertension or hyperglycaemia. This is supported by the findings that non-dipping seems to be more prevalent in those with a longer duration of diabetes. No study analyses non-dipping and albuminuria in early diabetes or precisely into the normal albuminuric range, which is largely undetectable by conventional assays.

The loss of nocturnal dipping also occurs in people with hypertension in the absence of diabetes. However, there is less evidence for whether non-dipping affects albuminuria in this cohort.

If non-dipping does influence albuminuria, it is not clear whether it provides additional information to average SBP, pulse pressure or other circadian rhythms which have been shown to influence albuminuria levels.

Methods of urine collection and measurement differ between studies and it is still unclear which method is the optimum for quantification of urine albumin.

1.6 Thesis Aims and Objectives

1.6.1 Aims

- To further understanding of the relationships between 24-hour blood pressure patterns, primarily day to night blood pressure ratios, and urinary albumin excretion in untreated hypertension, treated hypertension and type 2 diabetes.

- Examine the effect of using different urine collection methods and albumin assays on the analysis of relationships between blood pressure and urine albumin excretion.

- Investigate whether integrity of the glycocalyx shows a relationship with albuminuria and BP patterns across hypertensive and type 2 diabetic populations. This may help to understand whether endothelial glycocalyx damage plays a role in development of pathological 24-hour BP patterns and albuminuria.

1.6.2 Objectives

1) I will perform a post-hoc analysis of the DASHER study, an 18 week interventional study in patients with grade II hypertension who were treatment naïve. DASHER used a strict protocolised treatment regime to maximise patients adherence to treatment and minimise side effects, treatment was changed every two weeks if the patient had not reached target blood pressure or they were experiencing side effects. I will explore the relationships between 24-hour blood pressure, albuminuria and glycocalyx integrity in these treatment naïve patients with hypertension before and after 18 weeks of blood pressure treatment. I will use both a commonly used albumin assay and highly sensitive albumin assay which will enable analysis of the entire normal albuminuric range. Analysis will be performed on both random spot urine samples and timed overnight urine samples.

2) I will prospectively study 34 type 2 diabetic participants from the BEAT-DKD study exploring the relationships between 24-hour blood pressure, albuminuria into the normal range and glycocalyx integrity in type 2 diabetic patients. I will use a commonly used albumin assay and highly sensitive albumin assay to enable analysis of the normal albuminuric range. Analysis will be performed on timed overnight urine samples and first morning void spot samples.

2. Chapter 2: Methods

2.1 Twenty-four-hour ambulatory blood pressure

2.1.1 Measurement

24-hour blood pressure was measured using an automated, oscillometric TM-2430 ambulatory blood pressure monitor (A&D, Draycott, Gloucestershire, UK).

2.1.1.1 Monitor set up

The monitor was set to record blood pressure every 15 minutes from 07:00 to 22:00, then every 30 minutes from 22:00 to 07:00. The patient wore the cuff for 24-hours. These timings were used as it is recommended that at least 30 measurements are taken during the awake period and 6 measurements are taken during sleep. This is due to the higher level of blood pressure variation during the day and less at night. Thus, more readings produce a better reflection of the daytime average blood pressure. In many 24-hour monitoring periods, patients may either not tolerate and remove the cuff or have episodes where the monitor cannot take a reading through error. As a result more frequent measurements maximises the chance of an adequate number of readings should these events take place. It has also been found that the more frequent measurements are not more disturbing to the patient than less frequent measurements [125].

2.1.1.2 Fitting the monitor

The cuff was placed on the patient's non-dominant arm unless there were any contraindications or objections (patient objection due to sleeping habits, musculoskeletal problem, wounds etc.). This occurred in 3 patients in the BEAT-DKD study. The correct size cuff was positioned correctly (see figure 7) as per the standard operating procedure, and connected to the monitor. A trial blood pressure measurement was taken to ensure patient comfort and that the monitor was working properly. The monitor was then set to the automatic programme.

The patient was asked to relax the arm down by their side and keep movement to a minimum during the readings if possible. The patient was also asked not to perform any strenuous exercise or get the blood pressure monitor wet. The patient was shown how to terminate the recordings should they not wish to continue with the 24-hour monitoring.

The monitor number, measured arm and time of recording was noted in the patient's clinical record form. The next day the cuff was removed; patient sleep and wake times were recorded. Data were retrieved using the A&D software and saved to the study drive. The cuff and monitor was then cleaned, ready for the next use.
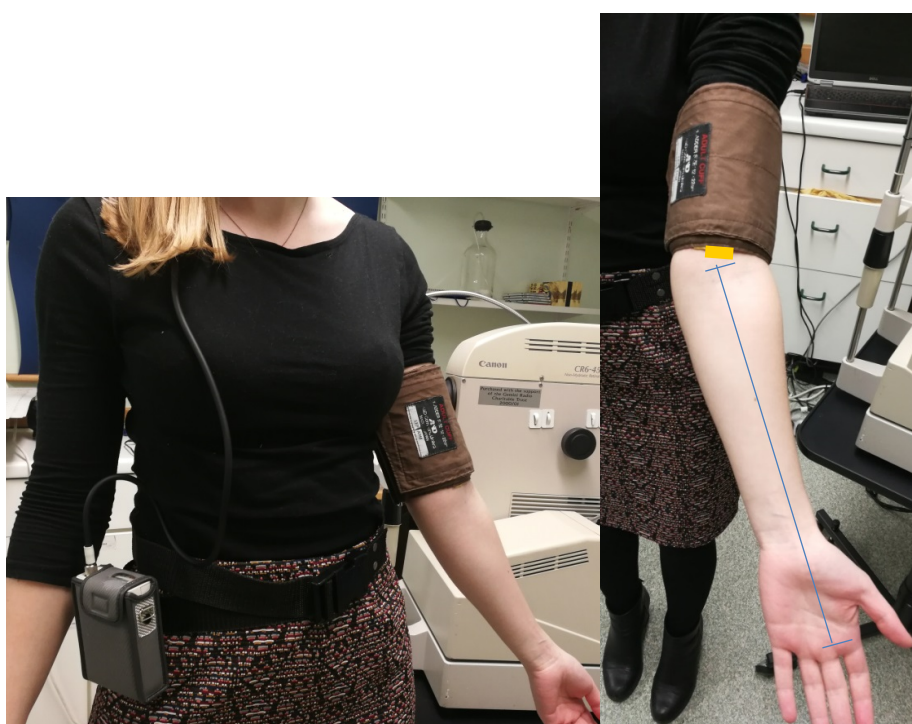
Figure 7 *Positioning of the 24-hour BP monitor and cuff.*

2.1.2 Validation and Quality control

The A&D TM-2430 Ambulatory blood pressure monitor has been validated by the British and Irish Hypertension Society across all age ranges, body mass indexes and sexes. It has also been shown to be accurate for those with high blood pressures [126, 127].

Quality control is carried out annually on all monitors used within this study by the Royal Devon and Exeter Hospital Medical Electronics Management. The most recent quality control check was carried out in April 2019.

2.1.3 Analysis

Data were retrieved and analysed by the A&D TM2430-13 (Doctor Pro) software. The software calculates various measurements from the full 24-hour period, the awake period and the sleep period (calculated from the patient's individual sleep and wake times). These include the mean and standard deviation of: systolic BP, diastolic BP, mean arterial pressure and pulse. If patients did not provide sleep and wake times then day and night values are missing and were not included in analysis.

The BP profile was further analysed resulting in several other measurements which are detailed below:

- **Pulse pressure** - calculated from each day period by subtracting the mean diastolic BP from the mean systolic BP.
- **Morning surge** (mmHg) - calculated using the *sleep-trough morning surge* method: (average systolic BP over the first 2 hours of waking) – (average of the three lowest systolic BP values during sleep)[128].
- **Double product (DP)** - calculated using the average systolic BP multiplied by the heart rate.
- **Pulse pressure product (PPP)** - calculated using the average pulse pressure multiplied by the heart rate.
- **Night time dipping** - calculated using the day/night ratios of BP parameters. Normal night time dipping of systolic BP is defined in the literature as a 10-20% drop compared to daytime average BP. All other BP parameters normal range will be determined based on their relationship to systolic BP day to night ratios [89].

2.2 GlycoCheck

2.2.1 Overview

The glycocalyx was measured using GlycoCheck (BV Microvascular health solutions software system, Maastricht, The Netherlands) and a side-stream dark-field camera (KK Technology, Honiton, England). The software generates a measurement of glycocalyx integrity called the perfused boundary region (PBR). The PBR is the difference between the width of the main blood cell column in a vessel and the outer edge of RBC deviation from the main column. This area is thought to include the most luminal part of the glycocalyx therefore, the higher the PBR, the lesser the glycocalyx integrity.

GlycoCheck uses a side stream dark field camera which uses a ring of green light emitting diodes surrounding a central lens. The green light is preferentially absorbed by red blood cells (RBCs) and so produces an image of RBC transit through vessels.
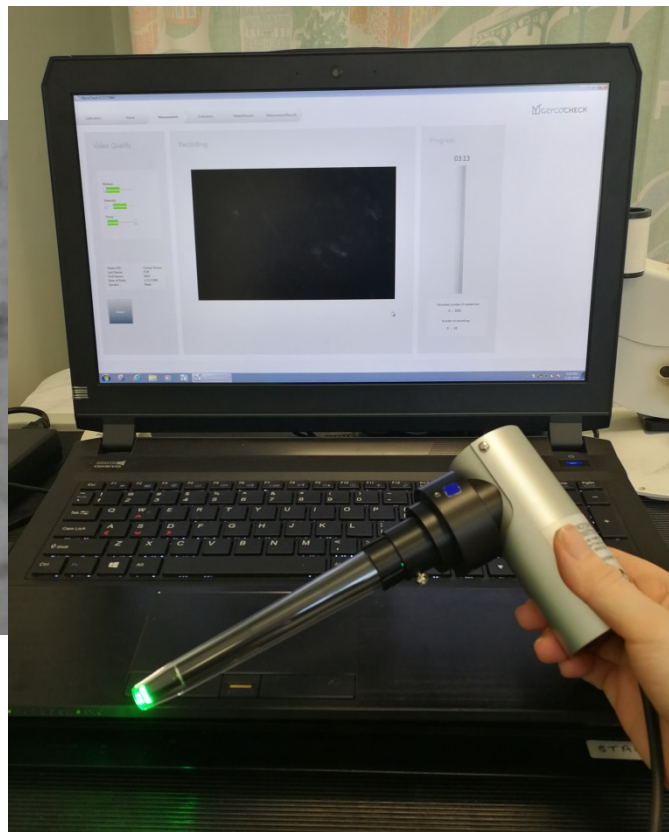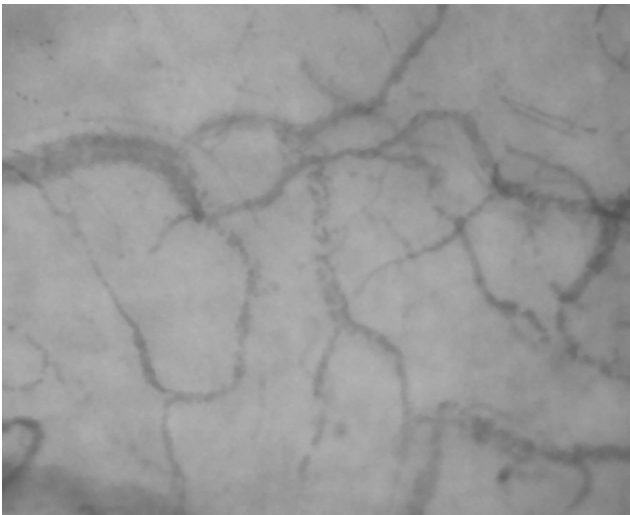
Figure 8 *Left: an example of a sublingual side stream dark field image as taken with GlycoCheck. Right: GlycoCheck computer and side stream dark field camera.*

2.2.2 Automated analysis

GlycoCheck automatically records video segments when adequacy is obtained. Video adequacy is based on tissue motion, illumination intensity and focus.

Recording continues until 3000 10μm vessel segments have been identified. Once recording is complete, GlycoCheck software automatically begins analysing each of the 3000 vascular segments. For each segment, 840 radial intensity profiles are obtained. From these profiles the median RBC column width (Median P50) and the outer edge of the RBC perfused region are calculated. The PBR is then calculated by the difference between the outer edge of RBC perfused region and the Median P50, divided by two. Each recording is then categorised by its individual MedianP50 giving different recordings for different vessel widths between 5 and 25 microns. One single PBR reading for 5-25 microns is also generated [129].
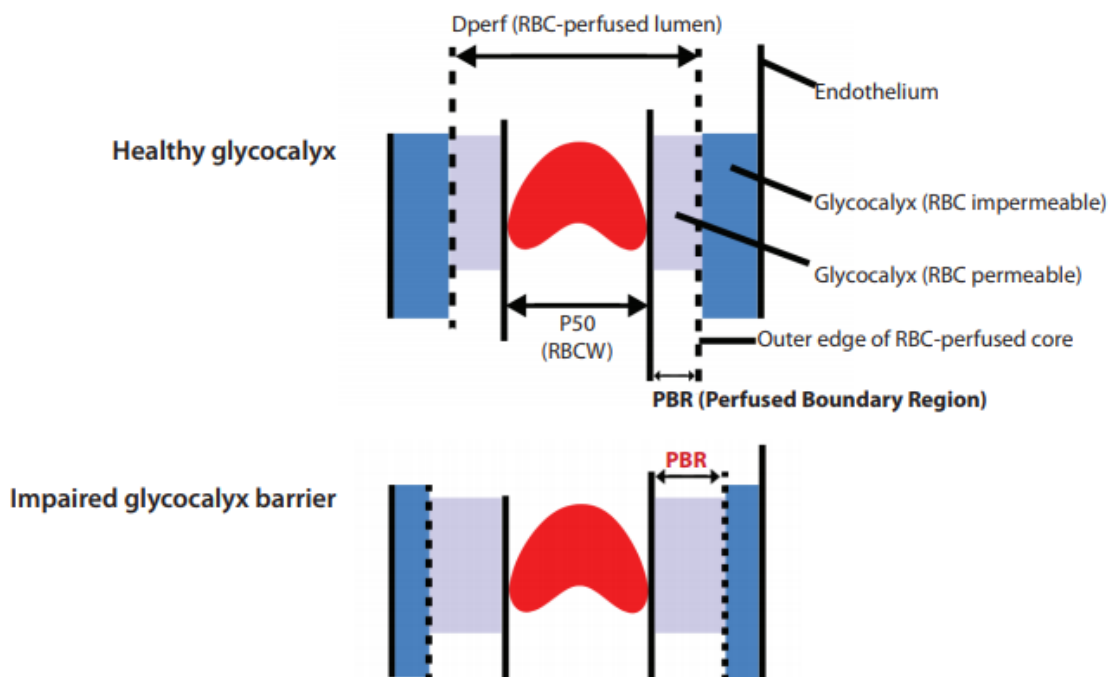
Figure 9 demonstrating PBR and Median P50. RBCW (red blood cell column width) [129].

2.2.3 Measurement

For the GlycoCheck measurement, patients were lying at 30 degrees in a supine position. Measurements were taken by a trained individual. A new disposable lens cap was used for each patient. The investigator sat behind the participant and the camera was positioned sublingually, perpendicular to the floor of the mouth, left or right of the frenulum. Excess pressure on the vessels was avoided and camera focus adjusted until the adequacy bar moved into the green. GlycoCheck automatically begins recording once adequacy is obtained. Areas of particularly looped capillaries were avoided. Three recordings were taken on each visit unless not possible due to participant compliance or time restraints. The software automatically generates a report and saves a copy to the hard drive.

Figure 10 *demonstrating the positioning of patient and GlycoCheck user during measurement.*

2.2.4 Reproducibility

2.2.4.1 External reproducibility

Reproducibility carried out by the GlycoCheck company (BV Microvascular health solutions software system, Maastricht, The Netherlands) found that in healthy adult males between the ages of 20 and 29 years, PBR 5-25 varied between 0.9 and 2.2. Variability between visits was around ± 0.1-0.2 in the same individual. Other studies which evaluated GlycoCheck software found that healthy controls (average age 44.1 years) had an average PBR of 3.3 (±0.4) which was significantly different to dialysis patients who had an average PBR of 3.6 (±0.5) [130].

A later study found healthy controls (average age 44.8 years) had an average PBR 5-25 of 1.82 (±0.16) and that ESRD patients had a significantly higher PBR 5-25 of 2.05. Those patients who were transplanted had a PBR that was not statistically significant from the healthy controls [131].

Both of these studies were conducted by the owners or manufacturers of GlycoCheck so requires independent verification. They state that the lower PBR of healthy controls in the more recent study is due to the newer method of calculating PBR with 3000 vessel segments [131]. They believe this is therefore more reliable.

2.2.4.2 Internal reproducibility

Reproducibility of the GlycoCheck technique was carried out on 2 participants at 5 separate visits. Three measurements were taken at each visit. Measurements were taken at the same time of day and participants were asked to abstain from caffeine and fast from food for 3 hours prior to the measurements.

Visit. Measurement	PBR 5-25	PBR high flow	PBR 5-9	PBR 10-19	PBR 20-25	Median P50
1.1	1.66	1.1	0.92	1.74	2.15	9.21
1.2	1.4	1.18	0.93	1.63	1.41	8.96
1.3	2.09	1.45	1.04	2.37	2.5	9.58
2.1	1.71	1.8	1.01	1.97	1.88	8.34
2.2	2.53	1.63	1.09	2.67	3.51	9.65
2.3	1.82	1.69	1.04	2.17	1.92	7.59
3.1	2.02	1.46	1.22	2.03	2.67	10.39
3.2	1.86	1.48	1.02	1.99	2.34	12.45
3.3	1.97	1.86	0.97	2.25	2.34	10.16
4.1	1.98	1.31	1.15	2.23	2.28	9.14
4.2	1.78	1.65	0.93	2.03	2.08	9.52
4.3	1.73	1.71	1.13	2.13	1.56	11.06
5.1	1.96	1.61	1.07	2.03	2.58	10.49
5.2	1.64	1.35	1.05	1.95	1.6	10.86
5.3	2.09	1.34	1.03	2.14	2.9	10.53
Min	1.4	1.1	0.92	1.63	1.41	7.59
Max	2.53	1.86	1.22	2.67	3.51	12.45
Mean 1 (±SD)	1.88 (±0.26)	1.51 (±0.22)	1.04 (±0.08)	2.09 (±0.25)	2.25 (±0.55)	9.86 (±1.19)
95% CI	1.74-2.03		0.99-1.08	1.95-2.23		
Visit to Visit variability						
Mean 2 (±SD)	1.88 (±0.12)	1.51 (±0.17)	1.04 (±0.04)	2.09 (±0.13)	2.25 (±0.23)	9.86 (±1.00)
Coefficient of variation	6.17	11.77	4.25	6.23	10.35	10.19

Table 1 Patient 1 GlycoCheck reproducibility results. Visit.measurement numbers ie. 1.1 correlated to visit number one and measurement number taken. Mean 1 is the average of all readings from all visits. Mean 2 is the mean of each visits average PBR. Standard deviation and coefficient of variation of Mean 2 shows visit to visit variability of PBR.

Visit. Measurement	PBR 5-25	PBR high flow	PBR 5-9	PBR 10-19	PBR 20-25	Median P50
1.1	1.93	1.83	1.11	2.11	2.31	8.35
1.2	2.09	0.94	1	2.23	2.75	10.18
1.3	1.85	1.43	1	2.1	2.14	11.43
2.1	1.98	1.24	1.04	2.32	2.19	10.05
2.2	1.78	1.34	0.96	2.08	1.94	9.19
2.3	1.95	1.2	1.03	2.07	2.5	8.81
3.1	1.93	1.1	1.14	2.48	1.68	8.97
3.2	1.7	1.45	0.97	1.9	1.97	8.59
3.3	2.22	2.42	1.12	2.41	2.81	10.03
4.1	2.16	1.15	1.13	2.46	2.51	9.4
4.2	2.14	1.34	1.08	2.21	2.9	9.85
4.3	2.04	1.59	1.09	2.28	2.41	9.14
5.1	1.97	1.03	1.16	2.19	2.33	7.46
5.2	2.46	1.45	1.31	2.6	3.17	7.55
5.3	2.26	0.68	1.26	2.33	2.98	9.26
Min	1.7	0.68	0.96	1.9	1.68	7.46
Max	2.46	2.42	1.31	2.6	3.17	11.43
Mean 1 (±SD)	2.03 (±0.20)	1.35 (±0.41)	1.09 (±0.10)	2.25 (±0.19)	2.44 (±0.42)	9.22 (±1.03)
95% CI	1.92-2.13		1.04-1.15	2.15-2.35		
Visit to visit variability						
Mean 2 (±SD)	2.03 (±0.14)	1.35 (±0.22)	1.09 (±0.09)	2.25 (±0.10)	2.44 (±0.28)	9.22 (±0.70)
Coefficient of variation	6.73	16.31	8.30	4.40	11.49	7.56

Table 2 *Patient 2 GlycoCheck reproducibility results. Visit. Measurement numbers ie. 1.1 correlated to visit number one and measurement number one taken. Mean 1 is the average of all readings from all visits. Mean 2 is the mean of each visits average PBR. Standard deviation and coefficient of variation of Mean 2 shows visit to visit variability of PBR.*
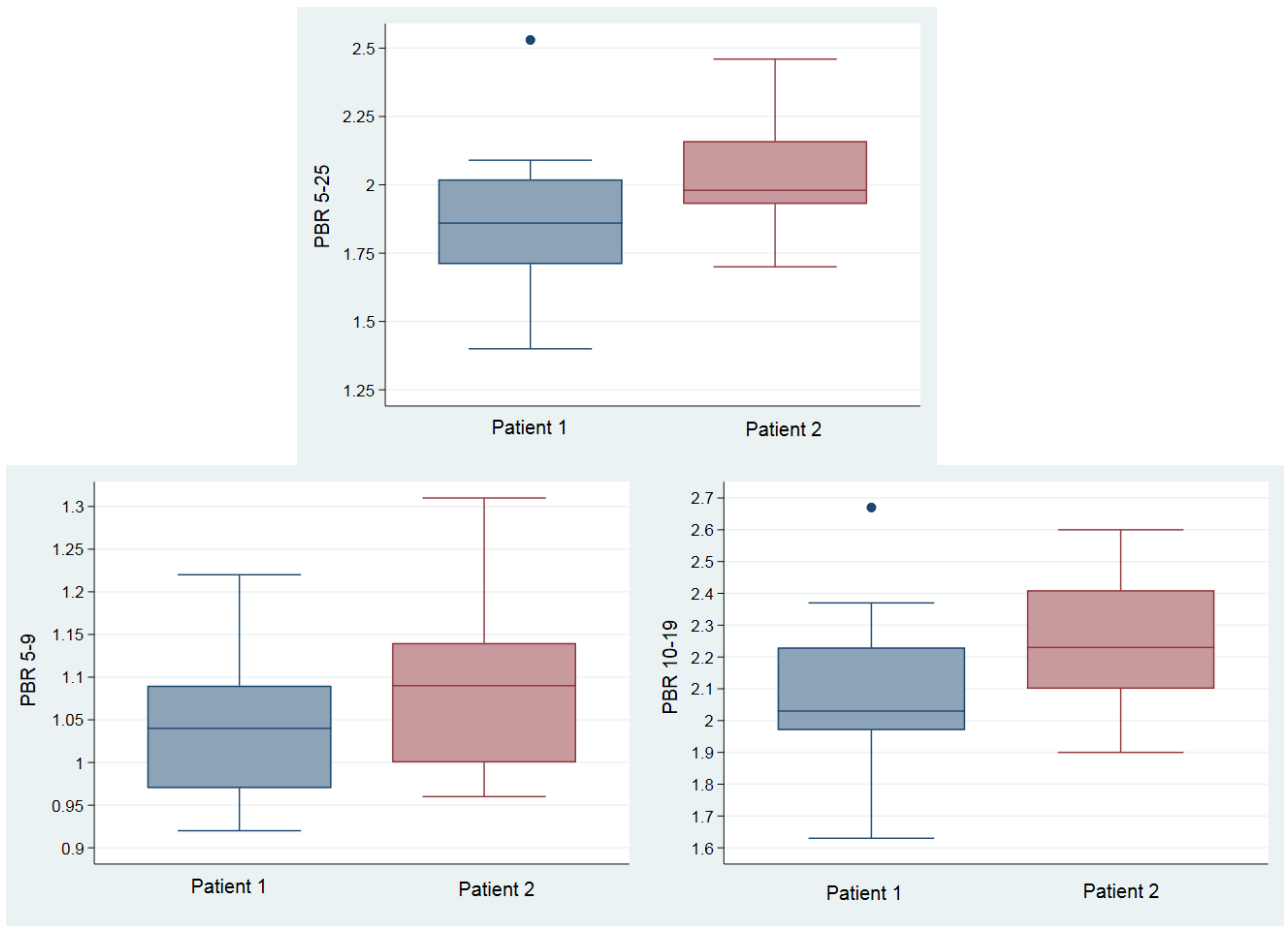
Figure 11 *The spread of data from patient one and patient two over the 5 visits.*
Each patient was healthy and aged 23-25.

For this study the overall PBR 5-25 measurement will be used. Individual focus
will also be given to the PBR 5-9 and PBR 10-19 as these measurements are of
smaller vessels, which more accurately reflect the size of the capillaries within
the glomerulus.

It is yet to be determined what may be a clinically significant change in PBR,
however it has been suggested that PBR is a reliable method of measuring the
glycocalyx through its relationship with serum marks of glycocalyx shedding [130]
[131]. As a result, for this study, PBR will be used as a marker of glycocalyx
integrity to ascertain whether there is a relationship between the glycocalyx,
blood pressure and albuminuria.

2.3 Urine Albumin Quantification

2.3.1 Collection

For all urine collections, participants were asked to avoid any strenuous activity or sexual activity 24-hours prior to the collection. Females were also advised not to collect urine during their menstrual period as this can lead to false readings.

2.3.1.1 Overnight urine

The participant was asked to pass urine into the toilet before they go to bed and record the time. After this point any urine passed was collected into a 2.5 litre bottle. The first morning urine was also collected into the bottle and the time recorded. The collection is then complete. The bottle was kept in a cool place until the appointment that day. Women may use a sterile bowl to decant urine into the bottle.

2.3.1.2 Spot samples

Spot samples were either first or second morning voids or random spot test on arrival to the research centre.

For first and second morning void participants were given two urine containers. The participant was asked to fill each container to 50ml if possible and note the time of the void. The containers were then stored in a cool place until the appointment that day.

For the random spot test patients were asked to pass urine into a container within the patient toilet cubicle and hand back to the research nurse during their appointment.

2.3.2 Analysis

2.3.2.1 Cobas Immunoturbidimetry

Urines were initially analysed using the Cobas immunoturbidimetric assay (Roche Diagnostics, Rotkreuz, Switzerland) which is a common clinical laboratory method for quantifying urine albumin. Turbidimetry uses antibodies to bind to albumin which results in precipitation of immune complexes. The more precipitation of albumin immune complexes, the more turbid the sample becomes. The albumin concentration is then calculated from the amount of light able to pass through the sample. Cobas immunoturbidimetry of albumin has a lower limit of detection of 3μg/ml and so cannot be used to quantify albuminuria throughout the normal range [132]. The Cobas company test repeatability using human samples and controls (n=21) and show a coefficient of variation between 0.5-1.9%.

2.3.2.2 Fitzgerald ELISA

Overnight and spot urine samples were re-tested using the Fitzgerald MicroAlbumin ELISA kit (Fitzgerald industries international, North Acton, MA, USA), which is a highly sensitive immunoassay designed to detect levels of albumin into the normal albuminuric range.

The assay kit contains microwells to which purified human albumin is bound. Urine samples are pipetted into the wells along with anti-human-albumin-peroxidase conjugate. Urine albumin will compete with the bound albumin for the anti-human-albumin-peroxidase conjugate, the more urine albumin present the fewer complexes will form with the bound albumin. The microwells are then washed with sodium azide leaving behind only the bound complexes. Addition of an acid results in the bound complexes turning yellow. The intensity of the yellow colour is measured photometrically at 450nm. The amount of colour is therefore inversely proportional to the concentration of albumin.

The Fitzgerald company state the assay has an analytical range between 0.15 to 400mg/L with a lower limit of detection of 0.5mg/L. Repeatability show inter-assay variation between 2.9-5.2% and intra-assay variation between 3.3-5.3%.

A different, independent validation of the assay found a lower limit of detection of 0.39mg/L with a limit of quantitation of 0.78mg/L. and when repeatability was analysed found a coefficient of variation between 6.44-18.42% [132].

2.4 Sudoscan measurement

Sudoscan (Impeto Medical, San Diego, CA, USA) is a non-invasive test that was used to measure sudomotor function of the hands and feet as an indicator of small nerve neuropathy within the BEAT-DKD group. Sudomotor function is measured through electrochemical skin conductance. Hands and feet are placed on metal plates (electrodes) which emit a low voltage current which extracts chloride from sweat glands. The glands which produce this sweat are supplied by small nerve fibres which are prone to damage even at the early stages of diabetes and so a reduced sweat response correlates to small nerve damage.

The electrochemical skin conductance is measured in microseimens. A result of less than 60 microseimens represents reduced sudomotor function. Sudoscan has a sensitivity of 0.75, specificity of 0.98 and <0.05 error [133].

2.5 HbA1c

HbA1C was analysed on the TOSOH G8 automated HPLC analyser (Tosoh Bioscience, Inc. South San Francisco, CA, USA) by ion-exchange chromatography. This method has a coefficient of variation below 2%.

2.6 Fasting glucose

Fasting glucose was analysed on the 702 photometric module of the Cobas 8000 automated platform (Roche Diagnostics, Rotkreuz, Switzerland). This method has a coefficient of variation below 2%.

2.7 BMI

BMI was calculated by dividing the individual's weight in kilograms by the square of their height in metres and displayed to one decimal place:

Weight (kg) ÷ Height^2 (m)= BMI.

2.8 Body fat percentage

Body fat percentage was measured using a Tanita BF-350 body composition analyser (Tanita corporation of America, Inc., Arlington Heights, IL, USA). The body composition analyser uses four metal plates on which the individual stands with bare feet. A low frequency electrical current is passed through the feet. The signal passes more quickly through the water in hydrated muscle than body fat. This resistance is known as impedance. The analyser determines the percentage body fat by how quickly the electrical signal passes through the body and back to the analyser.

2.9 Statistical analysis

Data were analysed using STATA 15. The distribution of all variables was assessed using histograms and q-q plots. Where data were non-normally distributed data were summarized using median and interquartile ranges.

Correlation was initially assessed using two way scatter plots. *Pearson correlation* was used to correlate parametric variables and *Spearman's rank* was used to correlate non-parametric variables.

Paired and un-paired Student's t tests were used to compare matched and unmatched parametric variables from week 1 and week 18 whereas *Wilcoxon signed-rank test* was used to compare matched non-parametric variables. *Mann Whitney U test* was used to compare unmatched non-parametric variables.

3. Chapter 3: The DASHER Study

3.1 The DASHER Study Overview

Data for this analysis were taken from the DASHER study conducted between 2015 and 2016 (ISRCTN57475376).

The DASHER study (Describing And treating Severe HypERtension) was a single centre, non-blinded, cohort study of 54 patients in the South West of England. As described in chapter 1, hypertension is a significant healthcare burden and can lead to serious complications if not managed quickly and effectively. The DASHER study aimed to determine the proportion of patients with a new diagnosis of severe hypertension who reached the target blood pressure of <140/90 after 18 weeks of protocolised treatment. Participants underwent 24-hour ambulatory blood pressure monitoring and urinalysis at week 1 and week 18. Therefore, the DASHER study provides a useful data set to observe the relationship between 24-hour blood pressure patterns and urine albumin in hypertensive patients.

Patients were screened to assess their suitability for the study. Patients were then examined and monitored over an 18 week period during which they were started on a nurse-led antihypertensive regime.

3.2 Aim

The overall aim of this chapter is to examine the relationship between 24-hour ambulatory blood pressure parameters, particularly night time dipping patterns, and albuminuria into the normal albuminuric range and glycocalyx integrity in hypertensive patients, before and after protocolised treatment.

3.3 Objectives

1. Assess whether the protocolised hypertension treatment changes blood pressure parameters, albuminuria levels or glycocalyx integrity.
2. Assess how albuminuria relates to the following different aspects of 24-hour blood pressure and circadian blood pressure rhythms:

   - 24-hour, day and night parameters *(systolic BP, diastolic BP, heart rate, pulse pressure, mean arterial pressure (MAP), double product, pulse pressure product)*

   - Blood pressure and heart rate variability

   - Circadian rhythms *(day to night blood pressure ratio and morning surge)*

3. Assess whether the different aspects of 24-hour blood pressure relate to glycocalyx integrity.
4. Assess whether glycocalyx integrity is associated with albuminuria levels.

3.4 Methods

3.4.1 Participants:

Patients with newly diagnosed, untreated hypertension were referred from either primary care or secondary care (including the emergency department) following a single clinic systolic blood pressure reading of ≥170mmHg. The study team then performed screening to determine whether patients met the inclusion and exclusion criteria.

3.4.1.1 Inclusion criteria

- New diagnosis of severe hypertension with systolic BP ≥170mmHg by their usual medical care provider and ambulatory blood pressure daytime average systolic BP ≥150mmHg when performed by the study team starting at the screening visit.
- Aged 18 +

3.4.1.1 Exclusion criteria

- Known eGFR <60 ml/min/1.73m^2 by Modification of Diet in Renal Disease Study (MDRD) formula.
- Previous or current prescription of any medication used in the study protocol.
- Previous renal artery intervention.
- Bleeding diathesis.
- Haemaglobin <10 g/dl.
- Platelet count <100 x10^9/l.
- Inability to provide informed consent.
- Pregnancy or breast-feeding.
- Hypertension-related event (including stroke or acute kidney injury) within the preceding 3 months.
- Any condition, including hypertensive urgency, which requires more immediate BP lowering or tailored anti-hypertensive strategy at enrolment.

3.4.2 Study Protocol

The DASHER study protocol was approved by the South West – Cornwall and Plymouth Research Ethics Committee (REC ref. 15/SW/0077) and fully conformed to the principles of the Declaration of Helsinki and the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

Written, informed consent was taken for participation and for storage and retesting of samples. Patient anonymity was maintained throughout and documents stored securely.

3.4.3 Study design

For detailed methods of data collection techniques, see Chapter 2.

<u>Screening visit</u>

Preliminary informed consent
Clinic blood pressure measurements
Simple blood tests
24-hour blood pressure monitor reading.

<u>Visit 2</u>

Full informed consent	Fasting blood tests
Drug review	***Overnight and spot urine***
Physical examination	ECG
Lifestyle questionnaire	BMI
Microvascular functions	Epworth score

The patient then received a clinical consultation and started on anti-hypertensive medication protocol.

<u>Visits 3-9</u>

Review of treatment and drug titration.

<u>Visit 10</u>

Final 24-hour ambulatory blood pressure monitoring

BMI, microvascular function tests, blood samples, ***overnight urine and spot urine.***

3.4.4 Anti-hypertensive treatment protocol

The patients were initially started on *Amlodipine 5mg once daily* and *Candesartan 8mg once daily*. Ingestion of the drugs was initially separated by 12 hours but then patients were allowed to adjust the timings to suit their lifestyle. Patient BP was then monitored every two weeks, if target BP was not achieved then treatment was titrated or altered as follows:

Week 2: Candesartan increased to 16mg once daily.
Week 4: Addition of drug 3 - Indapamide 2.5mg once daily.
Week 6: Renal function check and secondary hypertension screen.
Week 8: Add spironolactone 25mg once daily (if normal potassium) OR switch indapamide to bendroflumethiazide 10mg once daily (reviewed dependent on potassium level)
Week 10: Directly observed therapy, renal check and renal denervation decision.
Week 12: Renal denervation.
Week 14: Add Doxazosin 4mg at night, increased to 8mg daily after 2 weeks.
Week 18: End point follow up.

3.4.5 Measurement

24-hour ambulatory blood pressure monitoring was undertaken and analysed as described in chapter 2.1. GlycoCheck measurements were taken and analysed as described in chapter 2.2. Urine albumin was collected by timed overnight and spot urine samples and measured by both the Cobas and Fitzgerald assays as described in Chapter 2.3.

3.4.6 Statistical analysis

Statistical analysis was performed using STATA 15 as described in Chapter 2.4.

A cohort of 54 patients allowed detection of detection of correlation coefficients ≥0.40, where $p \leq 0.05$ with a statistical power of 80%.

3.5 Results

3.5.1 Patient demographics

The table below shows the patient demographics before and after treatment. The cohort was made up of 54 individuals with an average age of 59 (±10) so represents the older population. There was a slight male predominance (59%). The vast majority of the cohort was of white-British ethnicity. All other parameters remained the same, statistically, between week 1 and week 18 with the exception of clinic BP and number of anti-hypertensive medications.

Variable	Week 1 (n=54)	Week 18 (n=54)	P value
Age (years)	59.13 (±10.78)	-	-
Sex - Female	22 (41%)	-	-
Ethnicity	98% White British	-	-
BMI (kg/m^2)	29.17 (25.64 – 33.46)	29.09 (25.6 – 34.06)	0.9325
Clinic SBP/DBP (mmHg)	170/102 (±15/12)	130/79 (±11/8)	<0.0001/<0.0001
Creatinine (mmol/l)	74.69 (±13.21)	77.48 (±14.12)	0.1956
HbA1c (mmol/mol)	38.22 (±3.42)	38.29 (±3.65)	0.4745
Alcohol (units/week)	10.98 (±14.28)	7.45 (±10.16)	0.1520
Smoking status Present, past	6 (11%), 23(42%)	-	-
Epworth score	5.69 (±3.42)	-	-
Exercise (hours/week)	3.5 (1 - 7)	5 (2 - 8)	0.2998
No of Antihypertensive medications	0	2.75 (±0.77)	<0.0001
Antihypertensive treatment at week 18 (n (%)) CCB/ARB/ Indapamide/ Spironolactone	53(98%)/ 46(85%)/ 30 (56%) /10(19%)		

Table 3 Patient demographics at week 1 and week 18. Data are presented as either mean (± standard deviation) or median (Interquartile range), Number (percentage of cohort %).

3.5.2 Objective 1: Assess whether the protocolised hypertension treatment changes blood pressure parameters, albuminuria levels or glycocalyx integrity.

3.5.2.1 Does the protocolised hypertension treatment alter blood pressure?

The DASHER protocolised hypertension treatment significantly reduced many aspects of blood pressure as detailed in tables 4, 5 and 6. The treatment reduced average 24-hour blood pressure from 156 to 130 **(p<0.0001)** and 24-hour diastolic blood pressure from 90 to 75 **(p<0.0001)**. The treatment also significantly decreased clinic systolic and diastolic blood pressure (**p<0.0001** and **p<0.0001** respectively). The treatment regime did not alter pulse rate, morning surge or any of the day to night ratios. The treatment regime reduced blood pressure variability but increased heart rate variability.
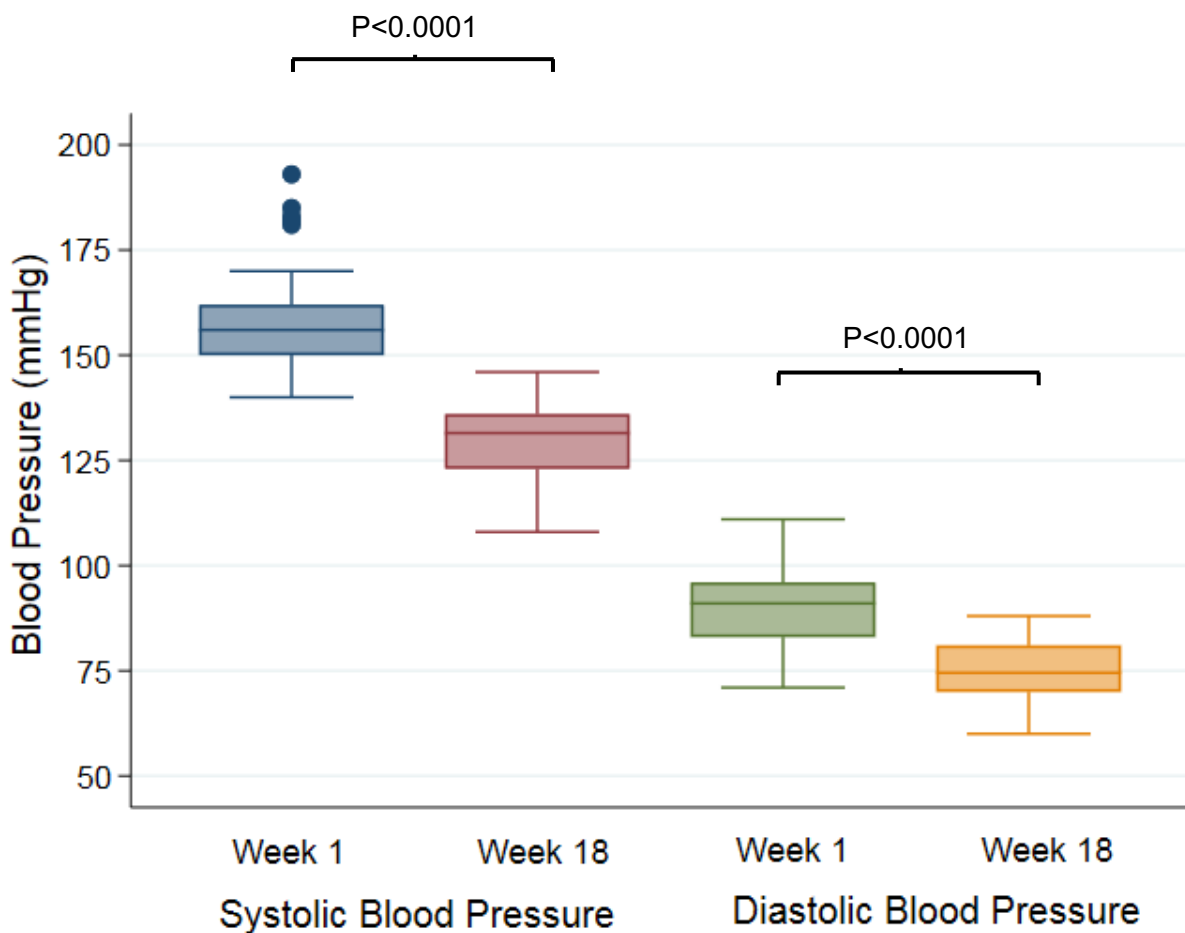
Figure 12 The spread of the cohort's average 24-hour systolic and diastolic blood pressures at week 1 (pre-treatment) and week 18 (post-treatment).

Variable	Week 1	Week 18	P value
24-hour SBP (mmHg)	156 (150-162)	129.78 (±9.48)	p<0.0001
Daytime SBP (mmHg)	163.74 (±11.37)	134.8 (±10.07)	p<0.0001
Night SBP (mmHg)	138.6 (±15.34)	112.94 (±12.35)	p<0.0001
24-hour DBP (mmHg)	89.61 (±9.02)	74.72 (±6.23)	p<0.0001
Daytime DBP (mmHg)	92.74 (±9.46)	77.83 (±6.65)	p<0.0001
Night DBP (mmHg)	78.23 (±10.18)	64.08 (±7.79)	p<0.0001
24-hour PP (mmHg)	69.93 (±14.38)	55.06 (±7.05)	p<0.0001
Daytime PP (mmHg)	71 (±9.09)	56.96 (±7.29)	p<0.0001
Night PP (mmHg)	60.38 (±10.46)	48.87 (±9.34)	p<0.0001
24-hour MAP (mmHg)	112.73 (±9.84)	92.7 (±6.76)	p<0.0001
Daytime MAP (mmHg)	116.07 (±9.08)	96.58 (±7.21)	p<0.0001
Night MAP (mmHg)	98 (±11.07)	79.96 (±8.51)	p<0.0001
24-hour pulse (mmHg)	73.02 (±8.14)	73.37 (±7.72)	p=0.5413
Daytime pulse (mmHg)	75.55 (±8.14)	76.08 (±7.72)	p=0.2281
Night pulse (mmHg)	64.17 (±9.39)	63.6 (±8.18)	p=0.9203
24-hour DP (mmHg)	115.28 (±14.85)	95.35 (±13.21)	p<0.0001
Daytime DP (mmHg)	123.66 (±15.91)	102.98 (±14.08)	p<0.0001
Night DP (mmHg)	88.69 (±14.98)	71.93 (±12.77)	p<0.0001
24-hour PPP (mmHg)	50.84 (±11.05)	40.37 (±6.56)	p<0.0001
Daytime PPP (mmHg)	53.39 (±7.42)	43.49 (±7.06)	p<0.0001
Night PPP (mmHg)	38.37 (±6.98)	31 (±6.91)	p<0.0001
Morning surge (mmHg)	45.5 (31.5-54.3)	37.3 (28.1-48.75)	p=0.0918
Clinic Systolic BP (mmHg)	168 (160-178)	130 (123-137)	p<0.0001
Clinic Diastolic BP (mmHg)	102 (95-108)	79 (74-87)	p<0.0001

Table 4 *showing the mean (±standard deviation) or median (interquartile range) for 24-hour, day and night average BP parameters at week 1 and week 18. P values from a student's t test or Wilcoxon signed rank test are shown. Those parameters which showed a significant difference between week 1 and week 18 are highlighted.*

Variable	Week 1	Week 18	P value
Standard deviation of 24-hour SBP	22.59 (±4.51)	20.94 (17.71-23.76)	p=0.0364
Standard deviation of daytime SBP	19.95 (±4.47)	20 (±3.87)	p=0.9685
Standard deviation of night SBP	16.29 (±5.51)	14.28 (±5.46)	p=0.0075
Standard deviation of 24-hour DBP	18.38 (±3.4)	17.68 (±4.02)	p=0.2405
Standard deviation of daytime DBP	17.95 (±3.65)	17.76 (±4.56)	p=0.7906
Standard deviation of night DBP	12.49 (±5.14)	10.55 (±5.24)	p=0.0393
Standard deviation of 24-hour pulse	10.87 (9.82-12.84)	13.07 (10.51-15.44)	p=0.0175
Standard deviation of daytime pulse	11.21 (±2.67)	13.17 (±4.19)	p=0.0034
Standard deviation of night pulse	6.01 (±2.88)	6.8 (±2.91)	p=0.2148

Table 5 Mean (±standard deviation) or median (interquartile range) for the standard deviation of individual patients' 24-hour, day and night BP parameters at week 1 and week 18. P values from a paired t test or Wilcoxon signed rank test. Those parameters which showed a significant difference between week 1 and week 18 are highlighted.
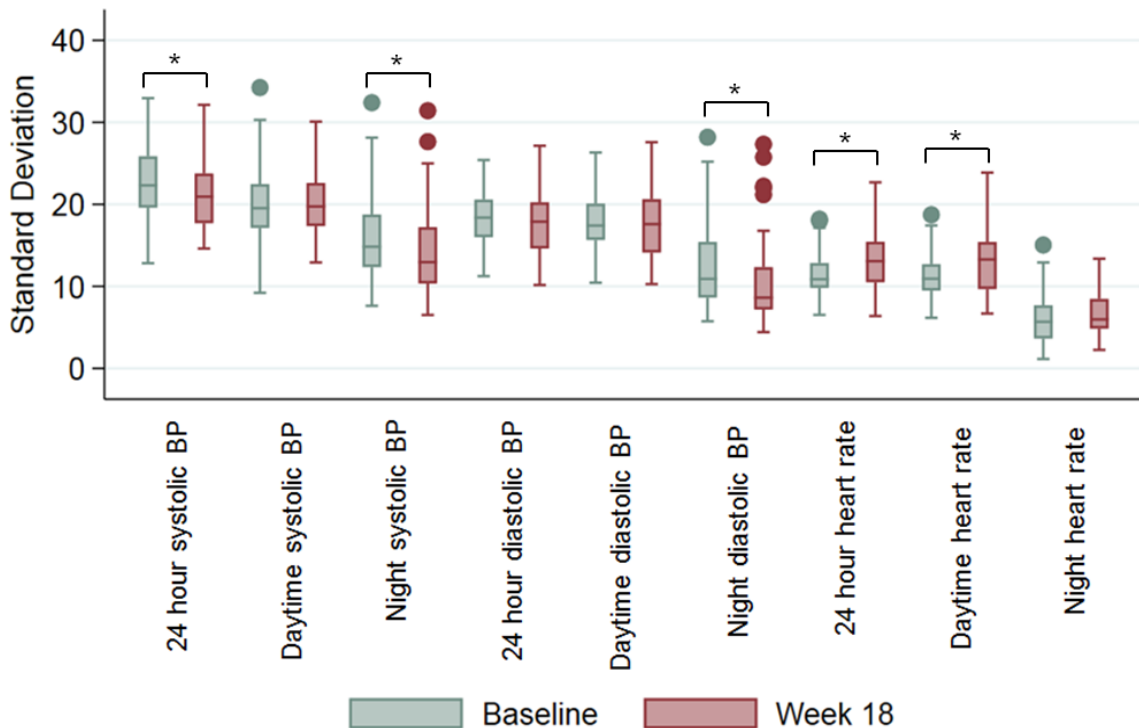
Figure 13 The spread of the standard deviation of individual patient's blood pressure parameters at week 1 and week 18. * denotes a significant difference with p value<0.05.

Variable	Week 1	Week 18	P value
Day to night SBP ratio	1.2 (±0.12)	1.21 (±0.12)	p=0.4028
Day to night DBP ratio	1.2 (±0.13)	1.23 (±0.12)	p=0.0697
Day to night HR ratio	1.19 (±0.11)	1.2 (±0.1)	p=0.3723
Day to night PP ratio	1.2 (1.08-1.29)	1.15 (1.07-1.3)	p=0.6800
Day to night PPP ratio	1.42 (±0.24)	1.44 (±0.29)	p=0.4375
Day to night DP ratio	1.42 (±0.19)	1.45 (±0.21)	p=0.1570

Table 6 showing the mean (±standard deviation) or median (interquartile range) for the day to night BP ratio at week 1 and week 18. P values from a paired t-test or Wilcoxon signed rank test.
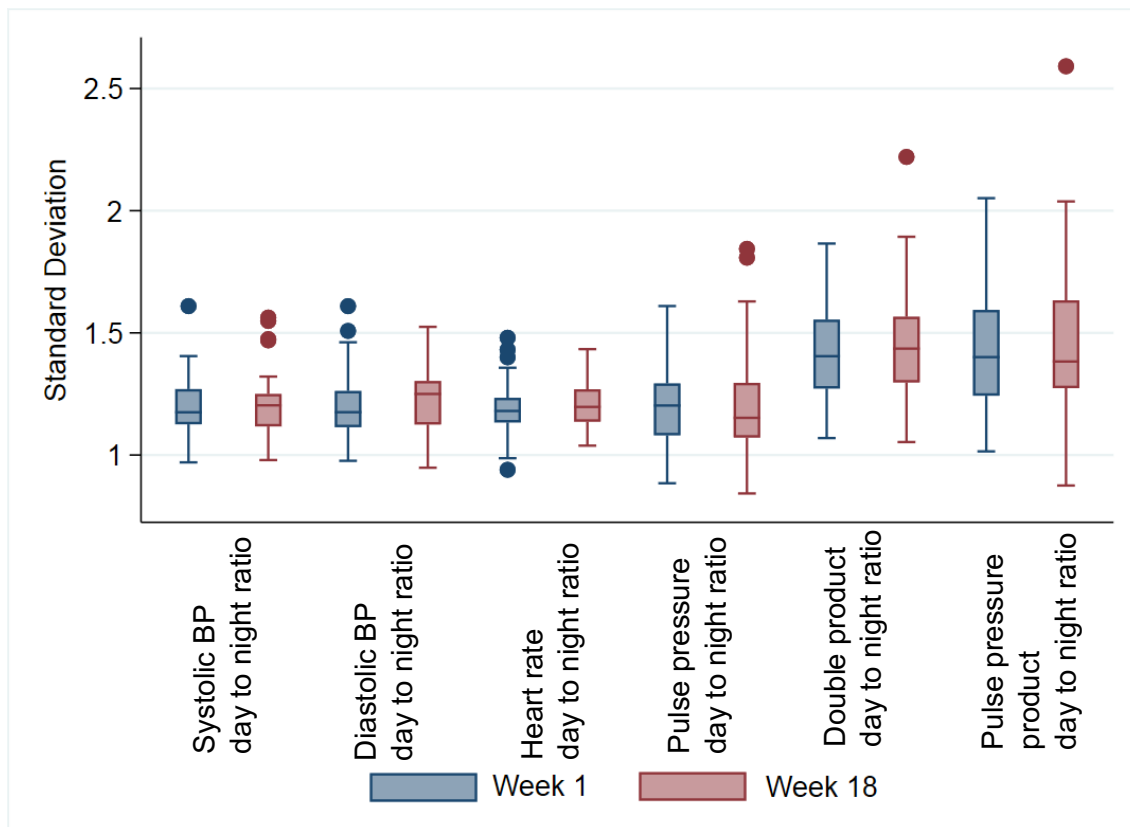
Figure 14 The spread of day to night ratios for different blood pressure parameters at week 1 and week 18.

3.5.2.2 Interpretation of urinary albumin excretion using assays with different ranges of detection

First we must look at the difference between each assay's quantification of albumin. As described in 2.3.2, two different assays were used to quantify albumin excretion. Cobas is a commonly used assay, in the clinical setting, to measure albumin but cannot detect concentrations below 3µg/ml and, as a result, often cannot quantify albumin excretion in the lower range. The Fitzgerald assay can detect concentrations down to 0.5µg/ml. In this cohort, the Cobas assay was able to detect 37% and 20% of urinary albumin concentrations at week 1 and week 18 respectively. The Fitzgerald assay was able to detect urinary albumin concentrations in the entire cohort.

	(n)	Min	Lower quartile	Median	Upper quartile	Max
Cobas ON ACR week 1	20 (37% detectable)	0.42	0.78	1.40	3.34	10.01
Cobas ON ACR week 18	11 (20% detectable)	0.34	0.53	1.26	1.47	3.42
Cobas ON AER week1	20 (37% detectable)	2.97	6.88	11.41	41.87	144.57
Cobas ON AER week 18	11 (20% detectable)	3.71	4.83	7.13	16.97	32.70
Fitz ON ACR week 1	54	0.27	0.64	1.04	1.81	8.46
Fitz ON ACR week 18	53	0.33	0.60	0.78	1.29	4.81
Fitz ON AER week 1	54	1.81	6.2	8.57	13.56	122.60
Fitz ON AER week 18	53	2.60	5.5	7.5	11.11	32.56
Fitz Spot ON ACR week 1	54	0.20	0.93	1.32	2.64	28.75
Fitz Spot ON ACR week 18	54	0.02	0.70	0.91	1.3	6.79

Table 7 showing the median, interquartile range, minimum and maximum values for patient Overnight (ON) ACR (µg/mg), ON AER (µg/minute) and Spot sample ACR using Cobas and Fitzgerald ELISA urine albumin measurements.

As demonstrated in the figure 15, a Mann-Whitney U test shows that the Cobas and Fitzgerald analysis of the same urine sample is not significantly different. The Cobas method is still able to identify albumin excretion rates into the normal albuminuric range as the Cobas method is able to detect lower AERs in more concentrated urine samples.

For example, if one individual excreted 4µg of albumin in a 24-hour period but was hydrated and also excreted 1.5L of water they would have a urinary albumin concentration of 2.67µg/ml and the Cobas assay would fail to detect this. If the same individual excreted 4µg of albumin in 24-hours, but was dehydrated and only excreted 0.5L of water they would have a urinary albumin concentration of 8µg/ml which would be readily detected by the Cobas assay.

Therefore, the Cobas assay is able to detect low albumin excretion rates, but only under certain circumstances. In this cohort, the Cobas assay was able to quantify AER in 100% of individuals with moderately increased albuminuria but only 28% and 14% of AERs in those with normal albuminuria at week 1 and week 18 respectively.
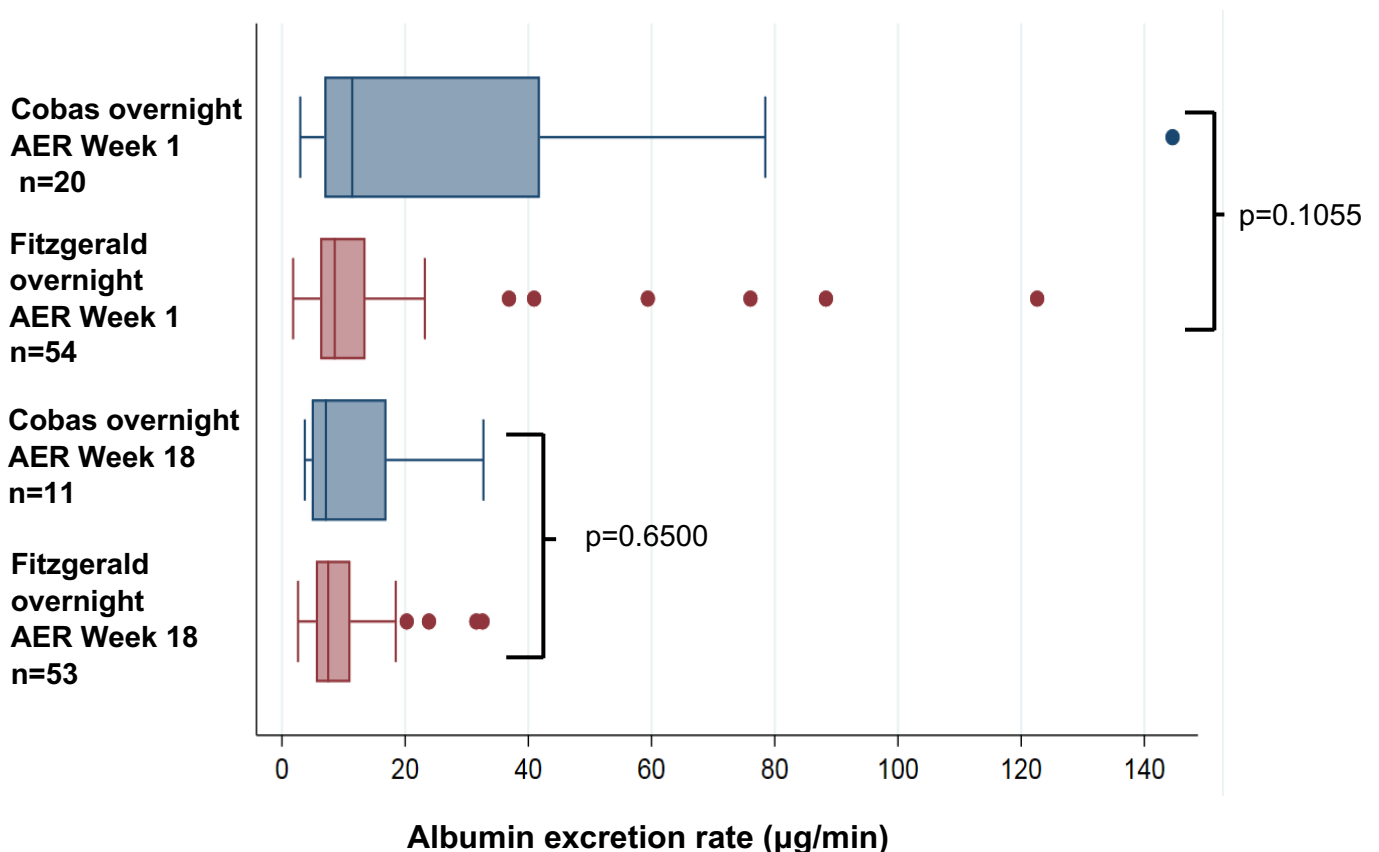
Figure 15 *The spread of albumin excretion rate values from both Cobas and Fitzgerald analysis of overnight urine samples.*

Often, when using the Cobas assay, a value of 2.9µg/ml is substituted for undetectable values in order to calculate an albumin excretion rate. Caution should be exercised when using this means of approximating AERs for undetectable albumin values as it is significantly different to the Fitzgerald AER; Week1 **(p=0.0006, n=54),** Week 18 **(p=<0.0001, n=53)** (see figure 16)**.**
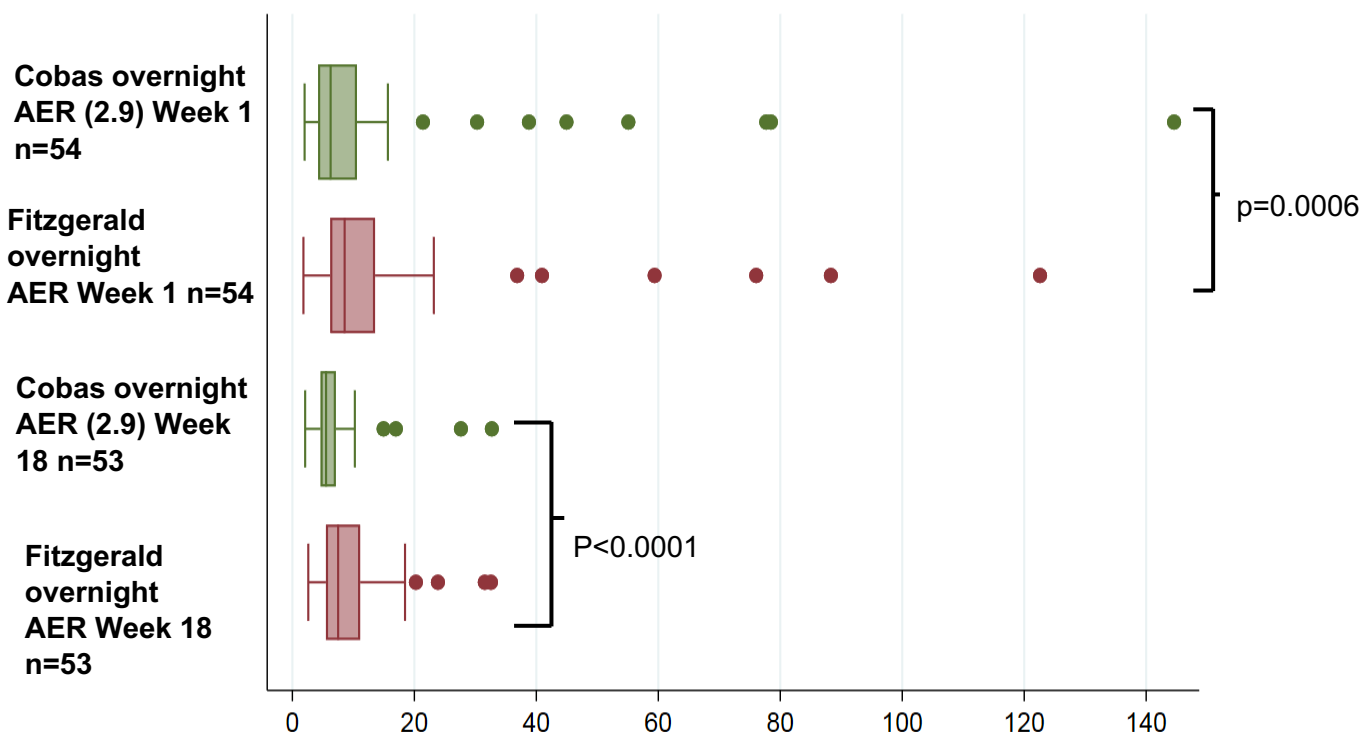
Figure 16 *The spread of albumin excretion rate values from both Cobas and Fitzgerald analysis of overnight urine samples. Cobas undetectable albumin values were substituted with 2.9µg/ml to calculate AER for entire cohort.*

Albumin creatinine ratio was also analysed. Mann-Whitney U test showed that Cobas ACR and Fitzgerald ACR of the same overnight sample at week 1 were not significantly different **(p=0.1505)**. Cobas ACR and Fitzgerald ACR of the same overnight week 18 sample were also not significantly different **(p=0.2162)**.

The Cobas assay was able to quantify ACR in 80% and 100% of moderately increased albuminuria patients at week 1 and week 18 respectively. Cobas quantified ACR in 32% and 19% of individuals with normal albuminuria at week 1 and 18 respectively. Use of the Fitzgerald assay allowed quantification of ACR for all patients at both weeks.
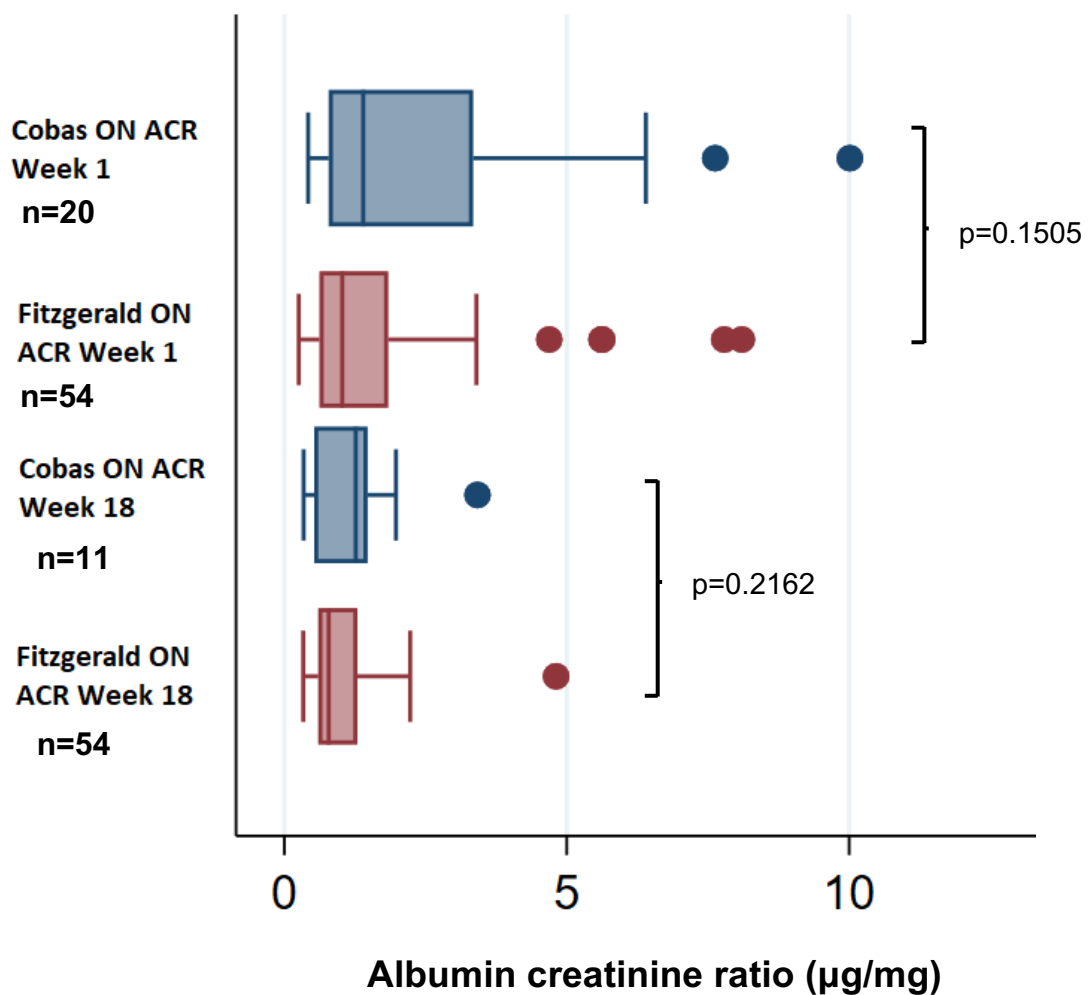
Figure 17 *The spread of albumin creatinine ratio values from both Cobas and Fitzgerald analysis of overnight urine.*

When Fitzgerald ACR is compared to Fitzgerald AER of the same sample (figure 18) AER generally shows a higher level of albuminuria based on the definition for moderately increased albuminuria when using ACR (3.5µg/mg) and AER (20µg/min).
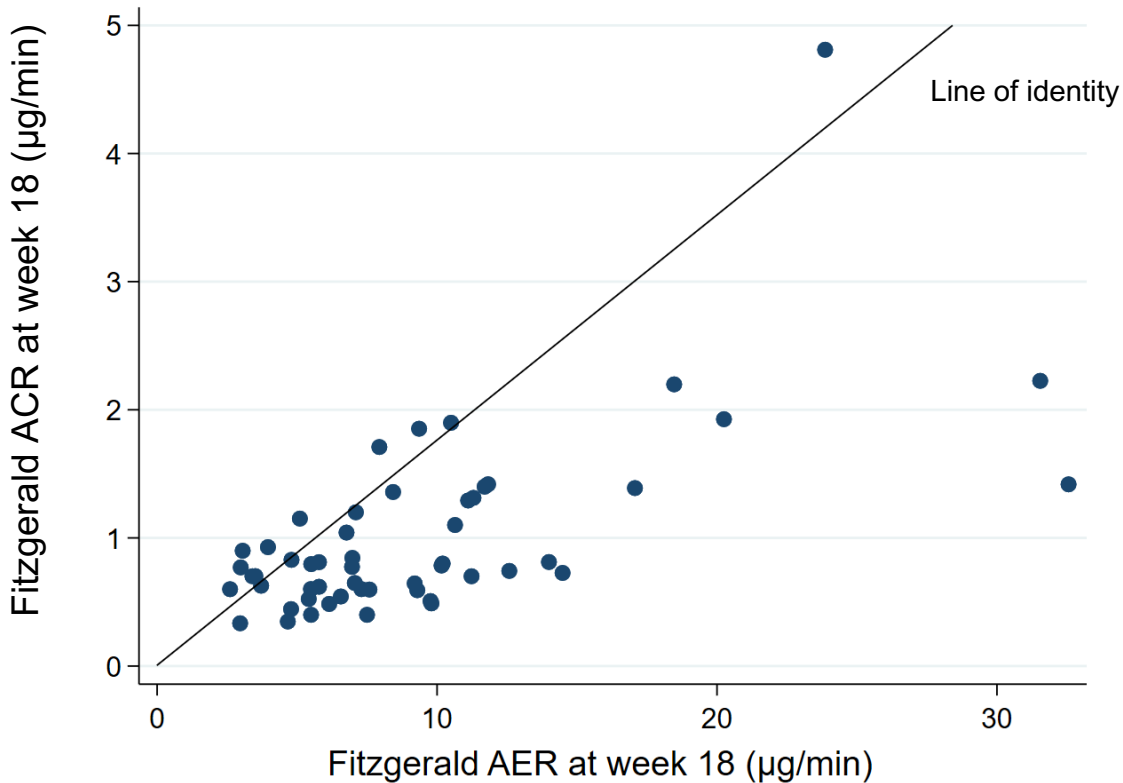
Figure 18 *a scatter graph showing AER against ACR (Fitzgerald assay) of the same sample with a line of identity based on the point at which an individual would be classed as having moderately increased albuminuria (3.5y=20x), n=53.*

Spot urine sample ACRs were compared to ACRs from the overnight samples at both week 1 and week 18. Spot urine samples showed significantly higher ACR values than the overnight samples (see figure 19). This may be due to the influence of urinary albumin excretion variability during the day. Overnight urine samples may be more representative of basal urinary albumin excretion but do not take daytime albumin excretion variability into consideration.
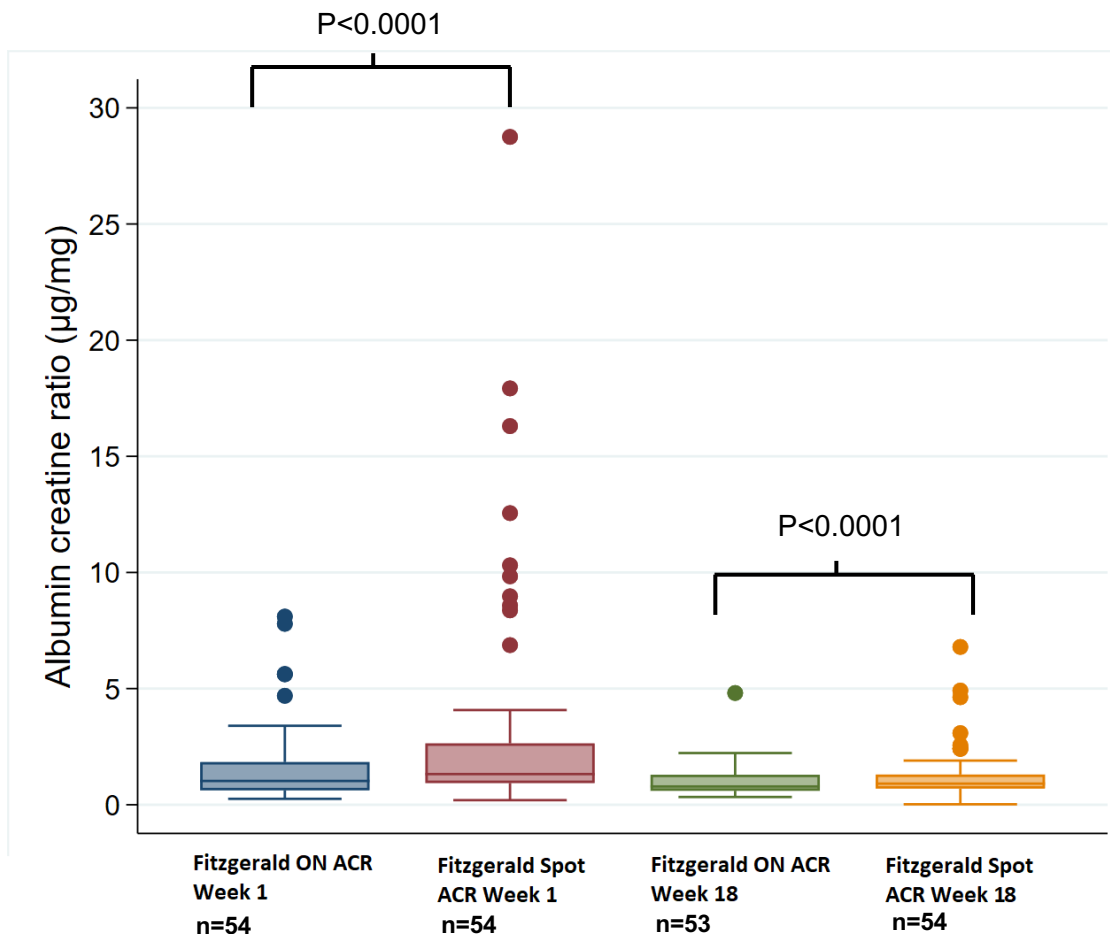
Figure 19 *The spread of albumin creatinine ratio values from both Cobas and Fitzgerald analysis of overnight urine and spot urine samples.*

Due to this analysis, for the purposes of this research, most emphasis will be placed on analysing Fitzgerald overnight AER urine samples as it gives accurate measurements for the whole cohort's urine albumin excretion. As well as this, the overnight urine collection sample matches the overnight blood pressure measurement period and may be subject to less variability.

3.5.2.3 Does the hypertension treatment protocol alter albuminuria?

3.5.2.3.1 Change in the cohorts' albumin excretion rate from week 1 to week 18

Initially the standard Cobas turbidimetry assay was used to determine whether albumin levels changed between week 1 and week 18. There were 8 individuals on whom a Wilcoxon signed-rank test could be performed. Wilcoxon signed-rank test of patients' AER gave a borderline significant result with only 8 matched observations **(p=0.0499).**

When all urinary albumin levels were detectable using the Fitzgerald albumin assay, analysis showed a significant difference between overnight AER at week 1 and week 18 **(p=0.0275)** (figure 20). There was also a significant difference between ACR of the overnight sample at week 1 and week 18 **(p=0.0027).** There was a significant difference between week 1 and week 18 spot urine sample ACR **(p=<0.0001)** (figure 21).
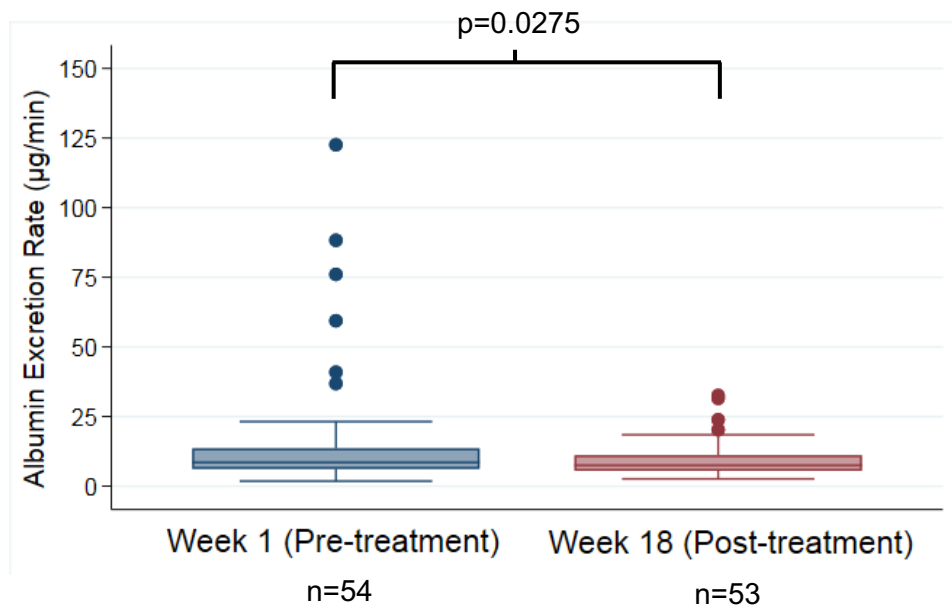
Figure 20 The spread of Fitzgerald AER results at week 1 and week 18. One patient's overnight urine sample was missing at week 18.
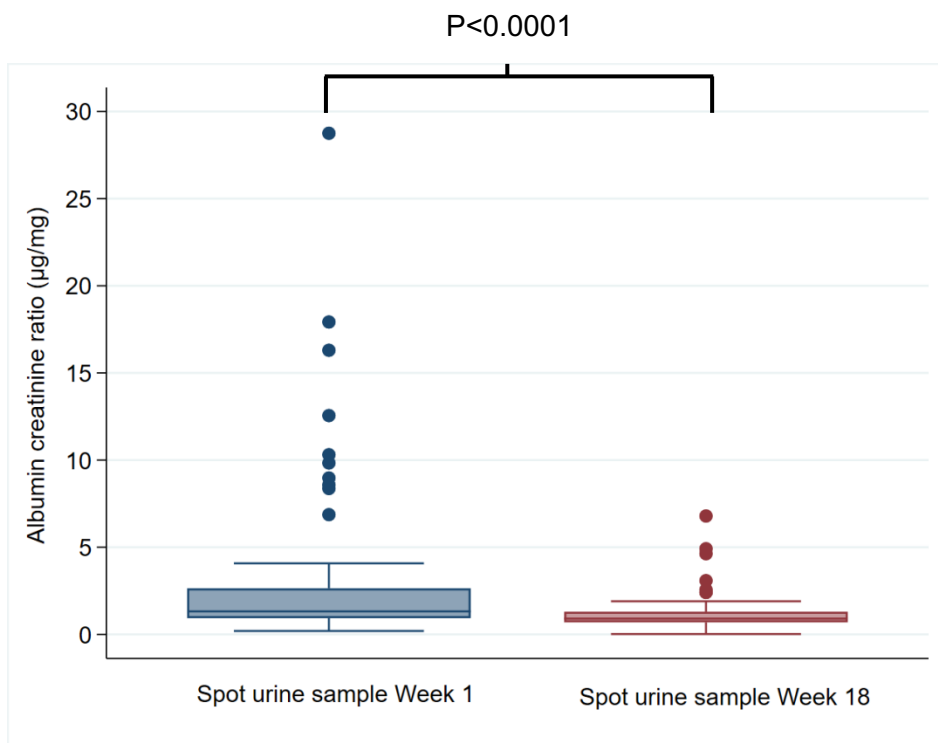
Figure 21 The spread of patient Spot urine ACR (Fitzgerald assay) results at week 1 (n=54) and week 18 (n=54).

As all patients urinary albumin was detectable, the Fitzgerald assay allowed separation and analysis of the normal and moderately increased albuminuria groups. Wilcoxon test showed that AER was significantly lower after treatment amongst patients who had moderately increased albuminuria at baseline **(p=0.0180).** AER was not significantly lower after treatment in those who had normal albuminuria at baseline **(p=0.4196).**

Previous work has shown that cardiovascular risk is associated with albumin excretion even within the normal range [134]. This data was explored further to investigate whether the relationship between treatment and reduction in AER was exclusive to those with moderately increased albuminuria (>20µg/min) or whether there was some crossover into the normal albuminuria group.

Comparison of absolute change in AER from week 1 to week 18 was compared to baseline AER in the whole cohort. There was significant correlation between reduction in albumin excretion with treatment and baseline albumin excretion rate **(Rs=0.6714, p=<0.0001**) (see figure 22).
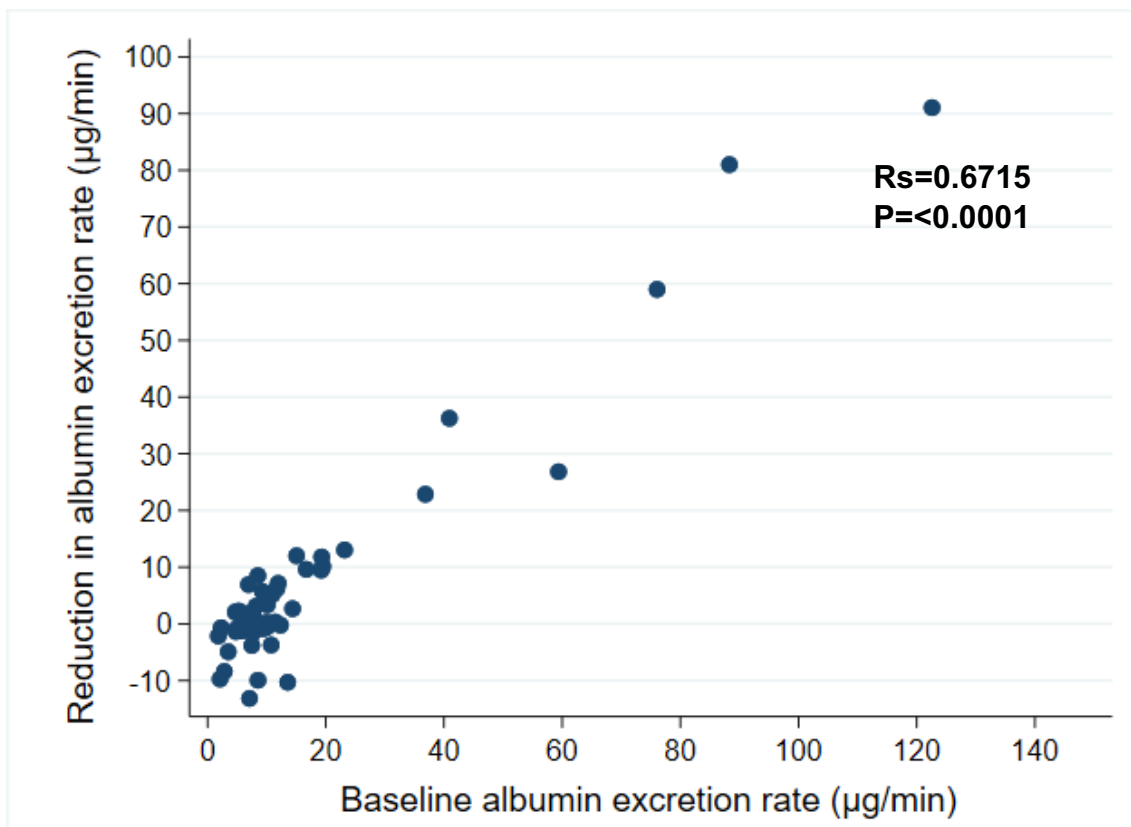
Figure 22 *A scatter graph of absolute change in Fitzgerald AER from week 1 to week 18 against baseline AER, n=54.*

When figure 22 is examined in more detail at the lower ranges of albumin excretion (see figure 23 for expanded figure), above 15µg/min all individuals reduced AER with treatment by 10µg/min or more. Below 15µg/min all individuals slightly increased or decreased their albumin levels a similar amount after treatment. This could be accounted for by inter-assay variation (coefficient of variation=18%)[132] and natural variation of trace amounts of urinary albumin (see figure 23). This reference point of 15µg/min was included in further analysis as it seems to more accurately describe the group of individuals in whom urine albumin excretion was affected by BP level and treatment.
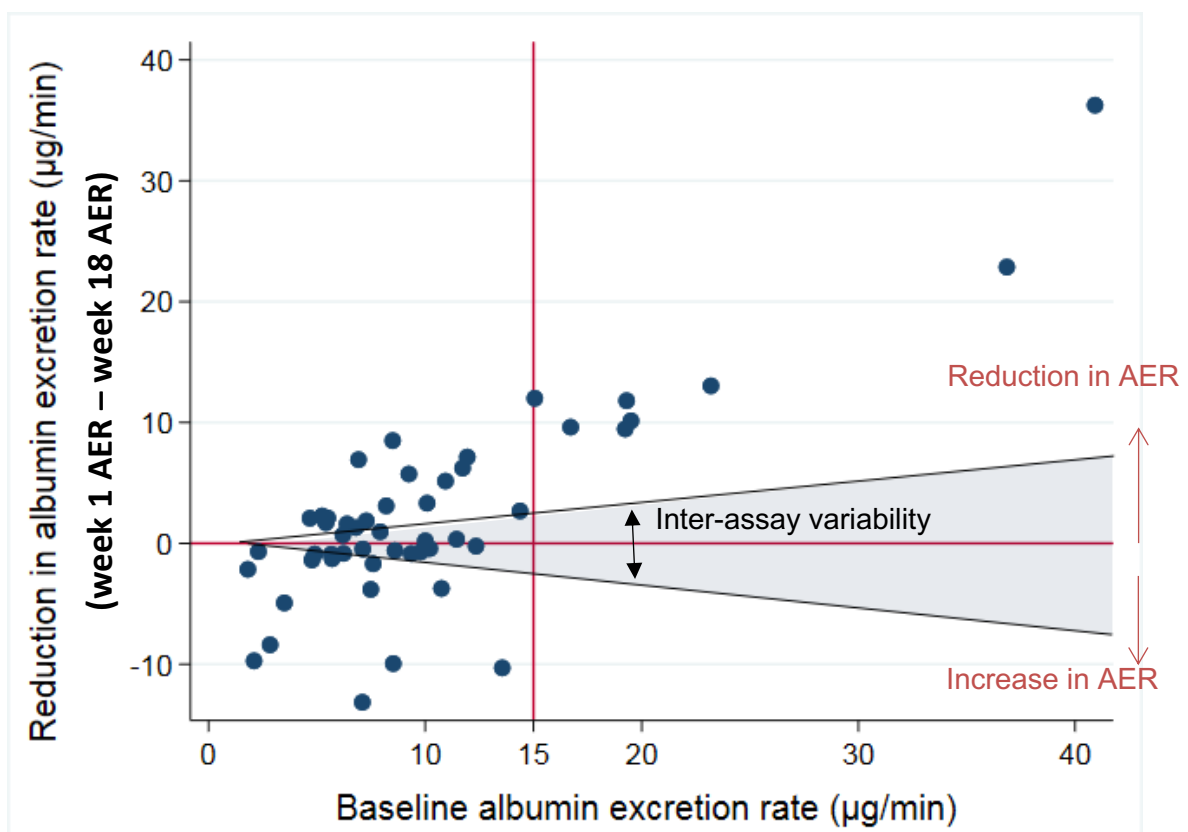
Figure 23 *A section of figure 22 – a scatter graph of absolute change in Fitzgerald AER from week 1 to week 18 against baseline AER in patients with a baseline AER<50. The shaded area accounts for inter-assay variability (18%). Horizontal reference line indicates 0 above which patients reduced AER, below which patients increased AER after treatment. Vertical reference line shows AER=15µg/min above which all patients reduced AER with treatment.*

3.5.2.3.2 How does reduction of different components of blood pressure relate to reduction in urinary albumin excretion?

To further explore the effect of BP treatment on AER, percentage reduction of different BP components and percentage reduction of albumin excretion were compared. These blood pressure components include 24-hour, day and night values for systolic, diastolic, mean arterial pressure, pulse pressure, double product and pulse pressure product. Reduction in albumin excretion is expressed as percentage change, [(week 18 AER – Week 1 AER) ÷ week 1 AER] x 100. Within the entire cohort, there was significant correlation between percentage change in AER and percentage reduction of MAP but no other significant correlations.
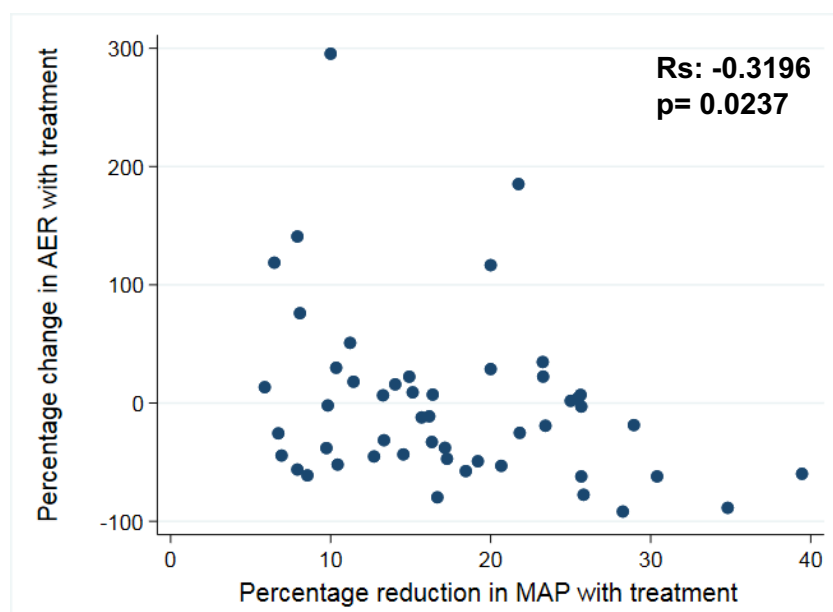
Figure 24 *A scatter diagram showing percentage change in Fitzgerald AER against percentage reduction in mean arterial pressure (n=50).*

When the cohort was split into those who had moderately increased albuminuria (n=7) and normal albuminuria (n=47) at baseline there were no observable correlations between percentage reduction in AER and percentage reduction in different BP parameters. The cohort was split into those with a baseline AER above 15µg/min (n=12) and below 15µg/min (n= 42) for reasons as previously described. This analysis showed some borderline significant and significant relationships in the baseline AER>15µg/min group but no significant relationships in the baseline AER<15µg/min group (see figure 25 and 26).
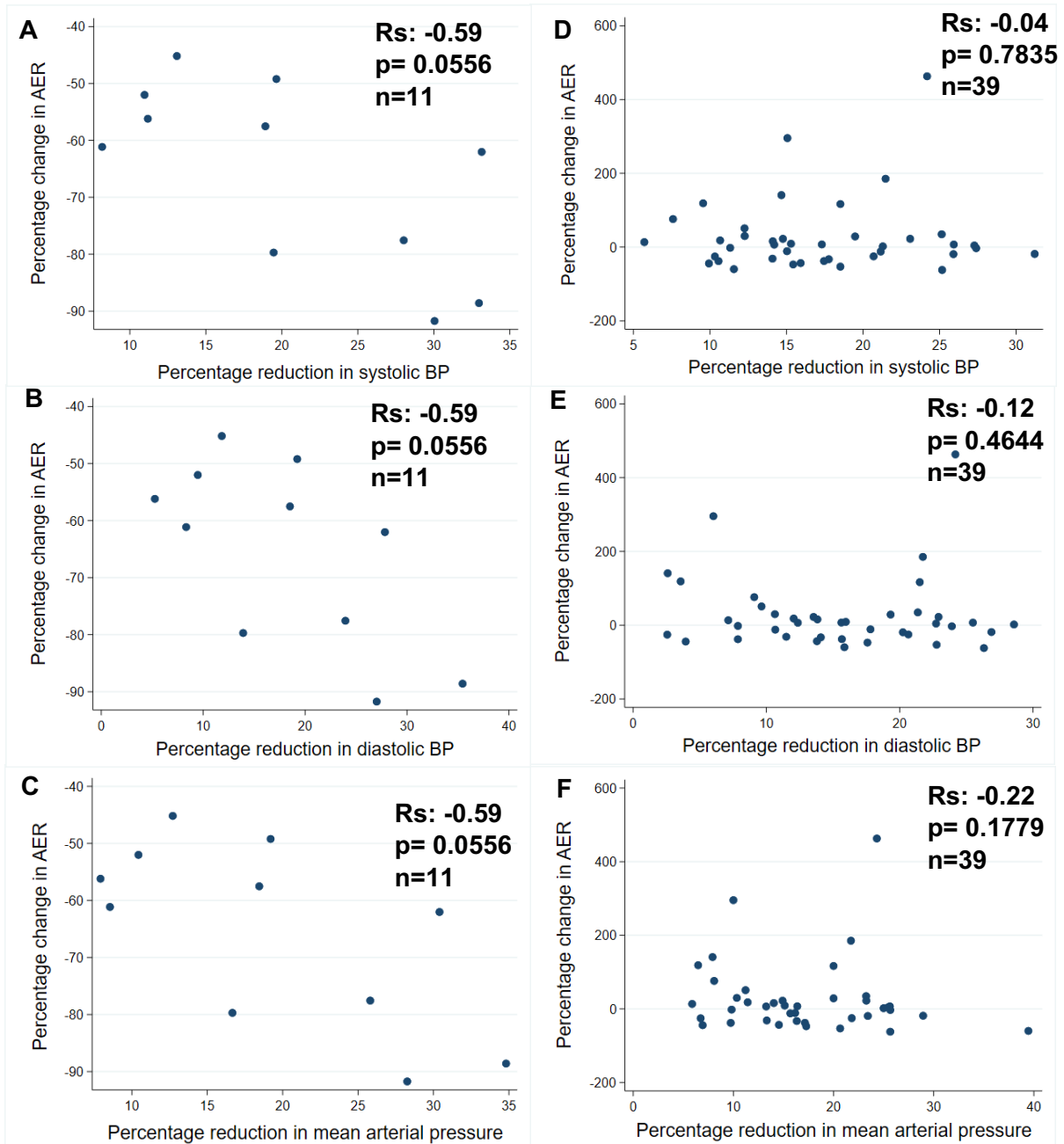
Figure 25 *scatter diagrams showing percentage change in Fitzgerald AER against percentage reduction in systolic BP, diastolic BP and mean arterial pressure in those with a baseline AER>15µg/min (A, B and C) and AER<15µg/min (D, E and F). Correlation coefficients and p values are shown by each graph. N values are lower than previously described groups due to missing AER values at week 18, hence reductions could not be calculated.*
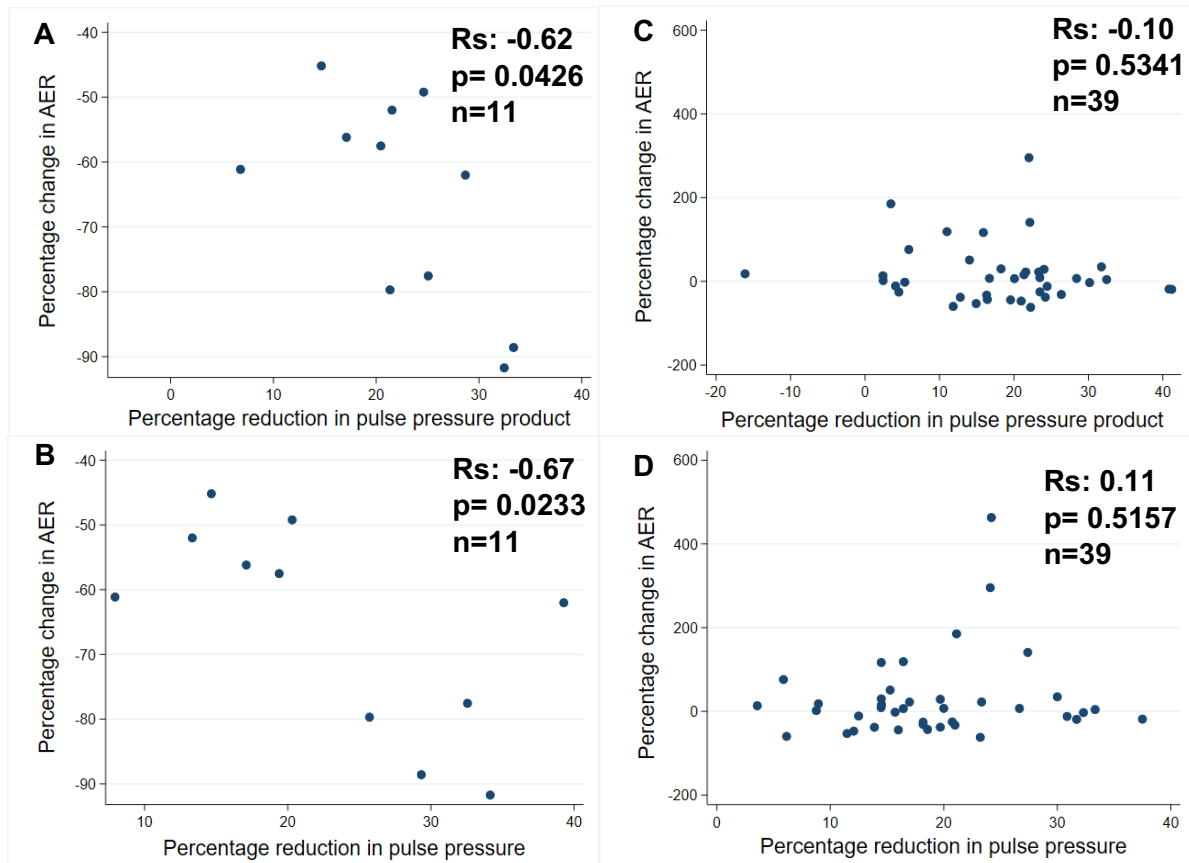
Figure 26 *scatter diagrams showing percentage change in Fitzgerald AER against percentage reduction in pulse pressure and pulse pressure product in those with a baseline AER>15µg/min (A and B) and AER<15µg/min (C and D). Correlation coefficient and p values are shown. N values are lower than previously described groups due to missing AER values at week 18, hence reductions could not be calculated.*

3.5.2.4 Does the protocolised hypertension treatment alter glycocalyx integrity?

Glycocalyx integrity, as determined by the GlycoCheck method, did not show a significant difference between week 1 and week 18 as outlined in table 8 and figure 27 below.

Variable	Week 1	Week 18	P value
PBR 5-25	1.63 (±0.19)	1.65 (±0.17)	p=0.3707
PBR 5-9	0.99 (0.91-1.09)	0.99 (0.93-1.04)	p=0.7498
PBR 10-19	1.69 (±0.21)	1.69 (±0.19)	p=0.5996

Table 8 showing the mean (±standard deviation) or median (interquartile range) for glycocalyx integrity parameters at week 1 and week 18. P values shown are from paired t test or Wilcoxon signed rank test.
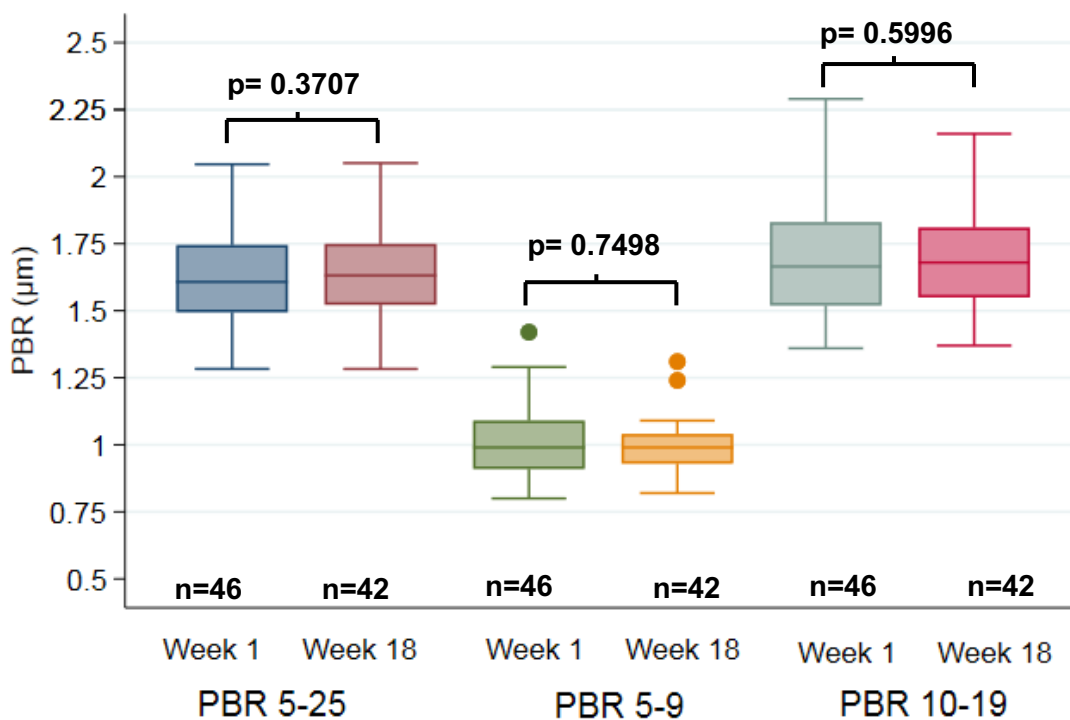
Figure 27 The spread of patient PBR results at week 1 and week 18.

We explored the relationship between percentage change of glycocalyx integrity and percentage change in different blood pressure parameters after treatment. Reduction in systolic BP, diastolic BP and MAP significantly correlated with an increase in PBR 5-25, 5-9 and 10-19 (increase in glycocalyx integrity) (see figure 28-30).

Legend:
- Percentage change in PBR 5-25 — (Rs: 0.32, p=0.0393) n=42
- Percentage change in PBR 5-9 — (Rs: 0.33, p=0.0308) n=42
- Percentage change in PBR 10-19 — (Rs: 0.36 p=0.0200) n=42
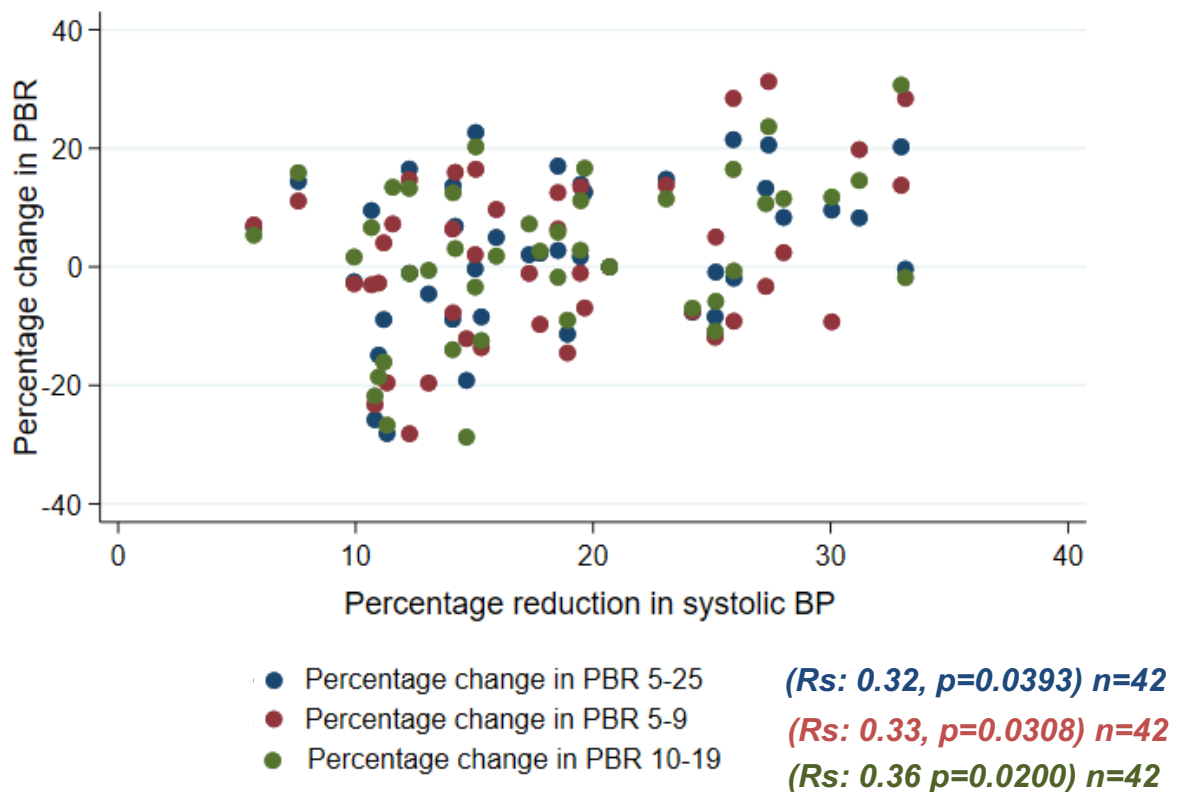
Figure 28 *Percentage change in glycocalyx integrity (PBR 5-25, PBR 5-9 and PBR 10-19) against percentage reduction in systolic BP from baseline to week 18.*
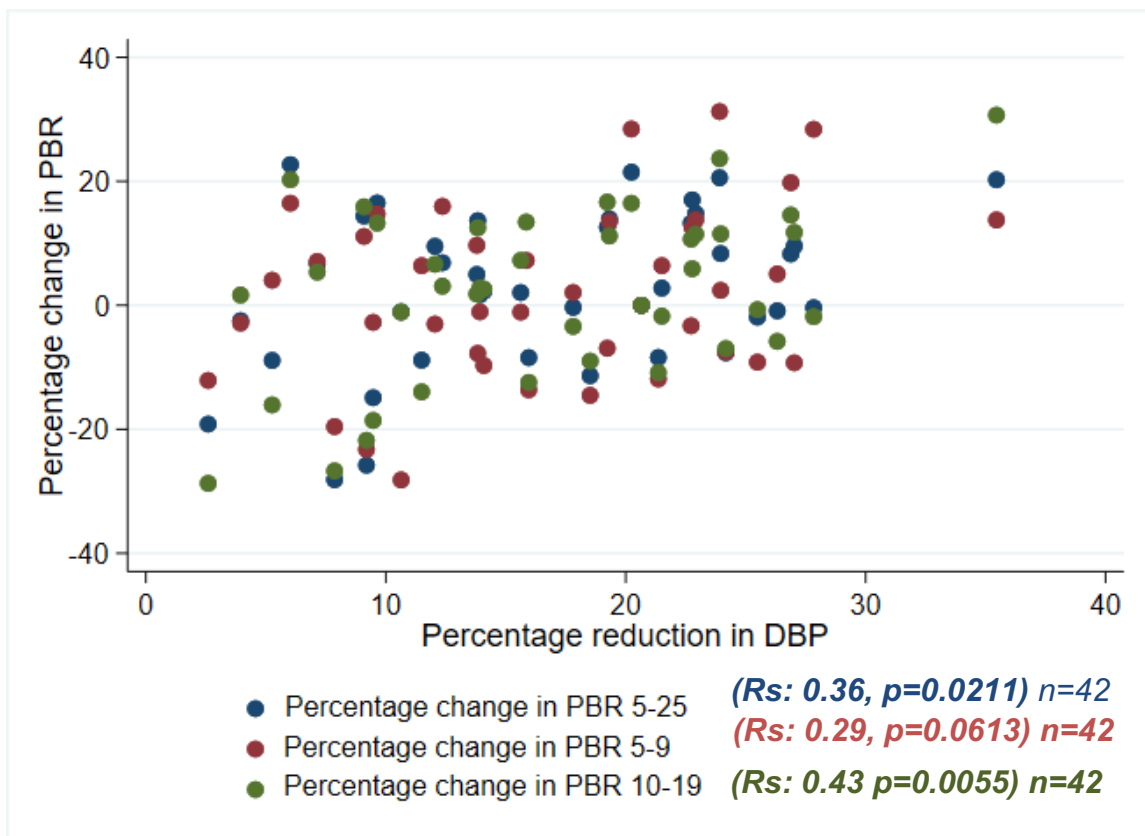
Figure 29 *Percentage change in glycocalyx integrity (PBR 5-25, PBR 5-9 and PBR 10-19) against percentage reduction in diastolic BP from baseline to week 18.*
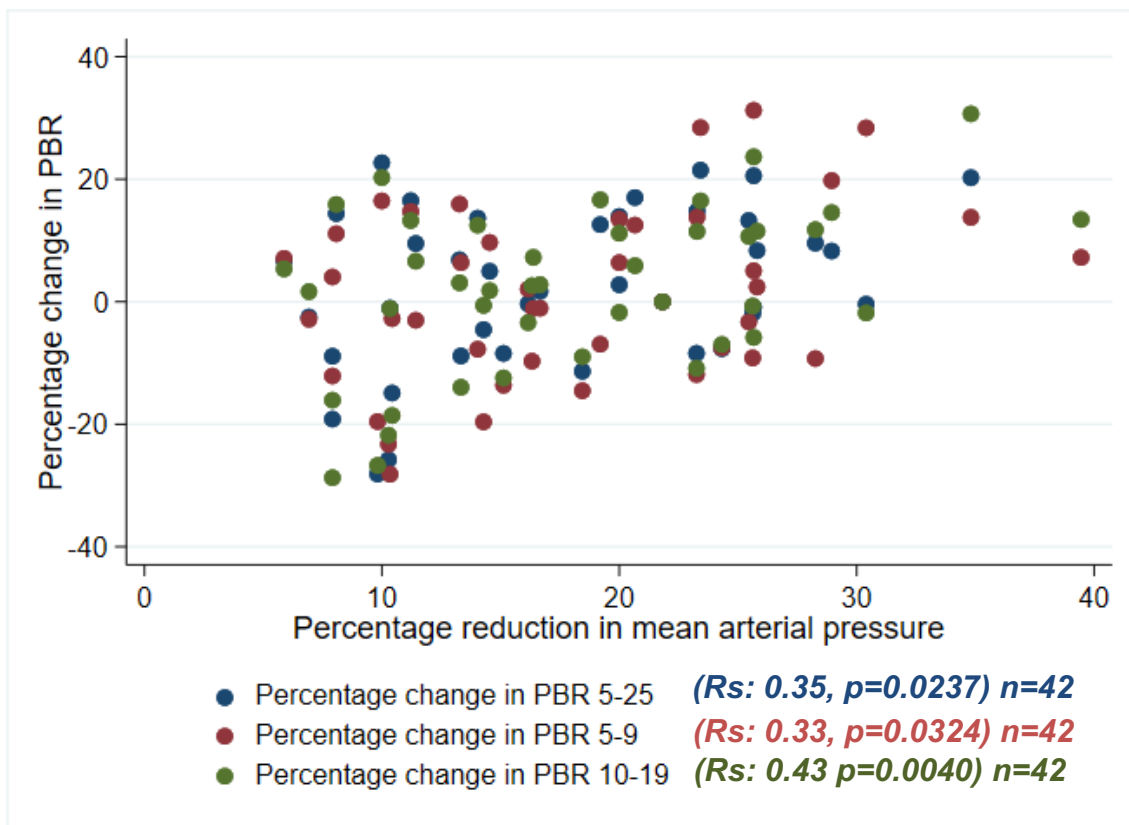
Figure 30 *Percentage change in glycocalyx integrity (PBR 5-25, PBR 5-9 and PBR 10-19) against percentage reduction in MAP from baseline to week 18.*

3.5.3 Objective 2: Assess how albuminuria relates to different aspects of 24-hour blood pressure and circadian blood pressure rhythms

3.5.3.1 Albumin excretion and 24-hour, day and night blood pressure parameters

When the Cobas assay was used to assess the relationship between urine albumin excretion and 24-hour blood pressure many patients had undetectable albumin values. Therefore there were 20 observations at week 1 and 11 observations at week 20. One patient did not provide sleep and wake times so their day and night BP could not be calculated. Comparison of Cobas AER and blood pressure parameters showed correlation with systolic BP, pulse pressure, mean arterial pressure and pulse pressure product. Results are detailed in table 9.

	Week 1			Week 18		
	Rs/r	p value	n=	Rs/r	p value	n=
24-hour SBP	0.6712	0.0012	20	0.7091	0.0146	11
day SBP	0.6658	0.0019	19	0.6256	0.0395	11
night SBP	0.6509	0.0025	19	0.5786	0.0622	11
24-hour DBP	0.4239	0.0625	20	0.2374	0.4820	11
day DBP	0.4839	0.0358	19	0.2273	0.5015	11
night DBP	0.4917	0.0325	19	0.3196	0.3380	11
24-hour PP	0.6757	0.0011	20	0.6834	0.0204	11
day PP	0.5973	0.0069	19	0.7260	0.0114	11
night PP	0.4890	0.0336	19	0.1735	0.6099	11
24-hour MAP	0.3892	0.0899	20	0.4806	0.1346	11
day MAP	0.5777	0.0096	19	0.4404	0.1752	11
night MAP	0.5880	0.0081	19	0.4155	0.2037	11
24-hour PPP	0.4030	0.0781	20	0.7818	0.0045	11
day PPP	0.3158	0.1878	19	0.5545	0.0767	11
night PPP	0.3519	0.1395	19	0.5273	0.0956	11

Table 9 *Correlation coefficients (Rs/r), p values and observations of some blood pressure parameters against Cobas immunoturbidimetry albumin excretion rate at week 1 and week 18. Statistically significant results are highlighted.*

To explore whether the loss of correlation at week 18 was due to the effect of treatment or reduction in sample size due to increased number of undetectable albumins, the relationships in the same 20 individuals as previously analysed with the Cobas assay, were compared using the Fitzgerald assay results. This analysis showed complete loss of correlations at week 18 and supports that the loss of correlations shown previously may be due to the effects of the anti-hypertensive treatment.

	Week 1			Week 18		
	Rs/r	p value	n=	Rs/r	p value	n=
24-hour SBP	0.5476	0.0124	20	0.2057	0.3842	20
day SBP	0.5559	0.0135	19	0.1669	0.4947	19
night SBP	0.5296	0.0197	19	0.3418	0.1520	19
24-hour DBP	0.4187	0.0662	20	0.2318	0.3255	20
day DBP	0.4831	0.0362	19	0.1056	0.6670	19
night DBP	0.3854	0.1032	19	0.3203	0.1813	19
24-hour PP	0.5288	0.0165	20	0.2151	0.3624	20
day PP	0.5086	0.0262	19	0.1310	0.5930	19
night PP	0.3210	0.1802	19	0.0941	0.7014	19
24-hour MAP	0.4132	0.0701	20	0.2190	0.3535	20
day MAP	0.5417	0.0166	19	0.1358	0.5793	19
night MAP	0.4806	0.0372	19	0.3147	0.1894	19
24-hour PPP	0.3684	0.1100	20	0.3474	0.1334	20
day PPP	0.3158	0.1878	19	0.2509	0.3002	19
night PPP	0.2606	0.2811	19	0.2982	0.2149	19

Table 10 *Correlation between blood pressure parameters and Fitzgerald albumin excretion rate at week 1 and week 18 of the group of 18 individuals previously analysed with the Cobas albumin assay. Statistically significant results are highlighted.*

Comparison of the whole cohort using the more sensitive Fitzgerald albumin assay gave a higher number of observations. When correlating albumin and blood pressure using the entire cohort, many of the correlations observed at baseline in the Cobas analysis were weakened or lost, and all correlations were lost at week 18 see table 11. One participant did not give their sleep and wake times and so their day and night BP values were not included in analysis.

	Week 1			Week 18		
	Rs/r	p value	n=	Rs/r	p value	n=
24-hour SBP	0.2780	0.0418	54	0.0716	0.6105	53
day SBP	0.2653	0.0549	53	0.1438	0.3091	52
night SBP	0.2708	0.0499	53	0.1149	0.4175	52
24-hour DBP	0.3959	0.0030	54	0.0660	0.6388	53
day DBP	0.3722	0.0061	53	0.0444	0.7545	52
night DBP	0.2315	0.0954	53	0.1757	0.2129	52
24-hour PP	0.0917	0.5097	54	0.0178	0.8995	53
day PP	0.0860	0.5402	53	0.1132	0.4244	52
night PP	0.1702	0.2230	53	-0.1330	0.3471	52
24-hour MAP	0.4432	0.0008	54	0.0765	0.5859	53
day MAP	0.3739	0.0058	53	0.1020	0.4716	52
night MAP	0.2522	0.0685	53	0.1403	0.3213	52
24-hour PPP	0.0240	0.8632	54	0.0906	0.5231	53
day PPP	0.0153	0.9135	53	0.1108	0.4341	52
night PPP	0.0163	0.9078	53	-0.0060	0.9665	52

Table 11 showing correlation coefficients (Rs/r), p values and observations of some blood pressure parameters against Fitzgerald albumin excretion rate at week 1 and week 18. Statistically significant results are highlighted.
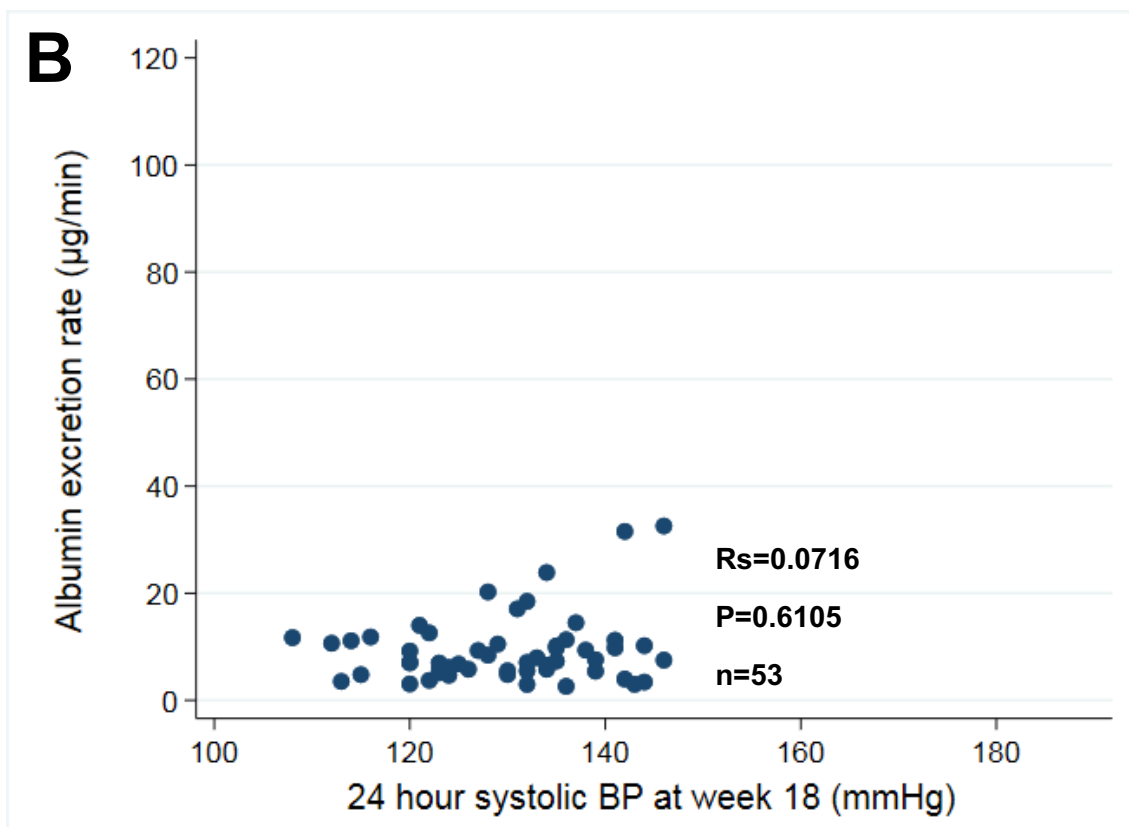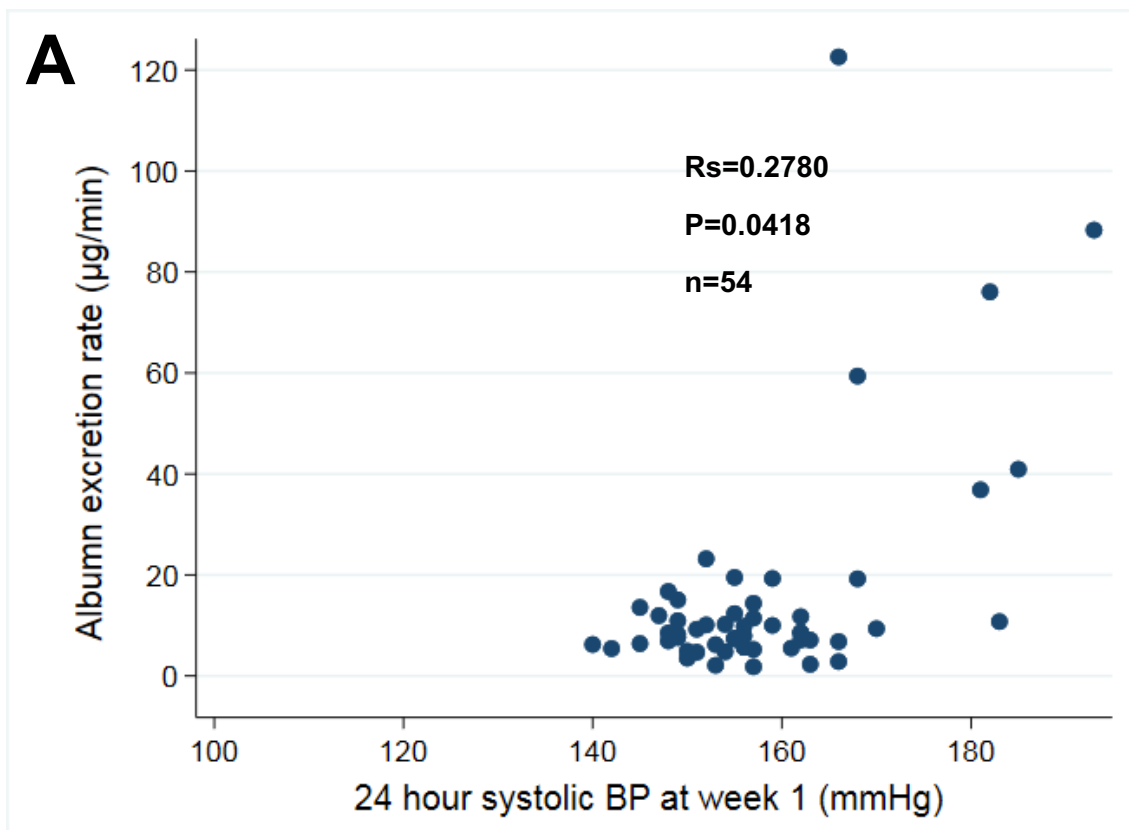
Figure 31 *Scatter diagrams showing Fitzgerald albumin excretion rate against 24-hour systolic BP at week 1 (A) and week 18 (B). Graphs are set to the same scale for comparison.*

To further analyse the relationship between albumin excretion and blood pressure the cohort was split into those with moderately increased albuminuria and normal albuminuria at baseline. At week 1 there were no significant correlations between BP parameters and Fitzgerald AER in either the moderately increased albuminuria (n=7) or normal albuminuria (n=47) group. At week 18 there were also no significant correlations between BP parameters and Fitzgerald AER in either the moderately increased albuminuria (n=8) or normal albuminuria (n=45) group.

The correlations in the moderately increased albuminuria group may have been influenced by the small sample size. These correlations were then observed within the previously described groups of patients with baseline AER above and below 15µg/min. Within the group of patients with baseline AER above 15µg/min there were 12 observations at week 1. Observations showed a significant relationship with systolic BP (24-hour systolic BP **(Rs: 0.7005, p=0.0112)** see figure 32) and borderline significant relationships with pulse pressure **(Rs: 0.54, p=0.0682)** and mean arterial pressure **(Rs: 0.56, p=0.0581).** At week 1 there were 39 observations within the group with a baseline AER below 15 µg/min and no significant correlations were observed between AER and any BP parameters.

At week 18 there were 13 observations within the baseline AER>15 µg/min group and 39 observations within the group with a baseline AER<15µg/min. There were no significant correlations observed between AER and BP parameters in either group at week 18.

When comparing BP parameters to the Spot urine ACR of the whole cohort there were also no significant correlations at week 1 or week 18.

When comparing BP parameters to the Spot urine ACR of those with normal albuminuria at baseline (n=46) there were also no significant correlations at week 1 or week 18. In those with moderately increased albuminuria at baseline (n=8) the following correlations were observed. Twenty-four-hour systolic BP **(Rs=0.8095, p=0.0149)** (see figure 33)**,** 24-hour diastolic BP **(Rs=0.7785, p=0.0229),** MAP **(Rs=0.8333, p=0.0102)**. Borderline correlation with morning surge of systolic BP **(Rs=0.7500, p=0.0522)**. All correlations were lost at week 18.
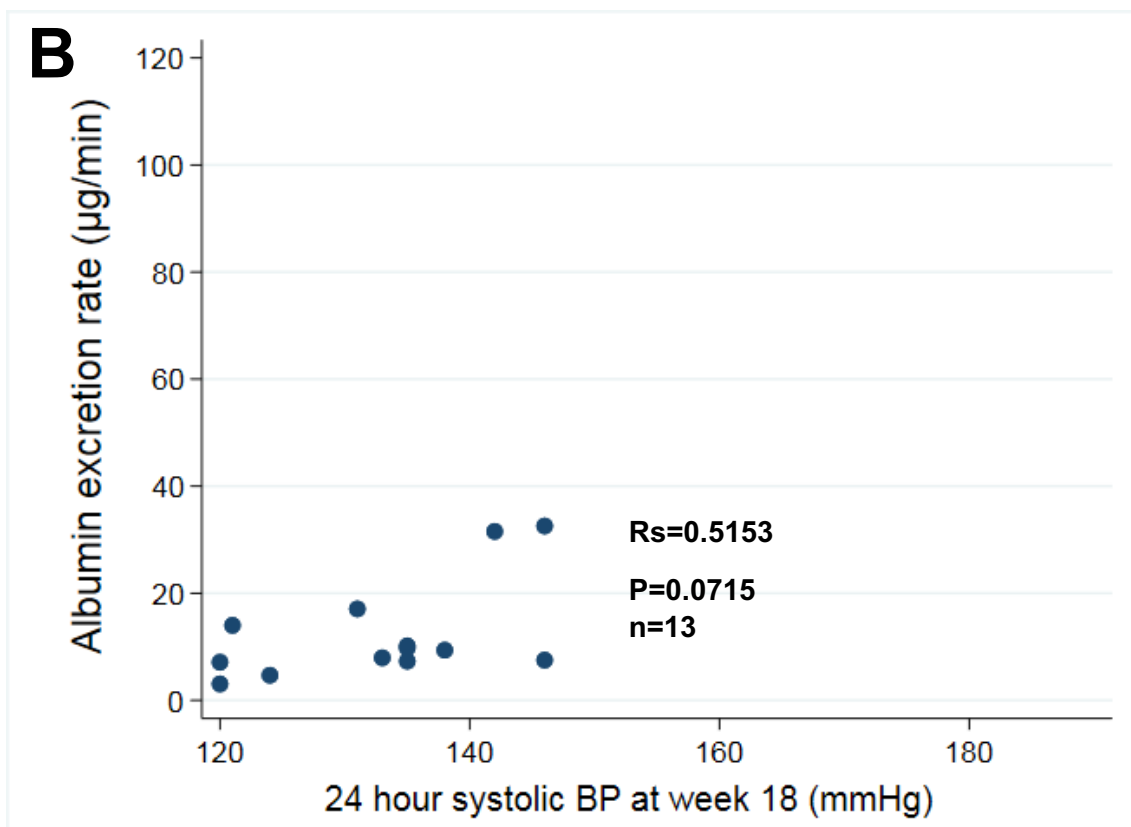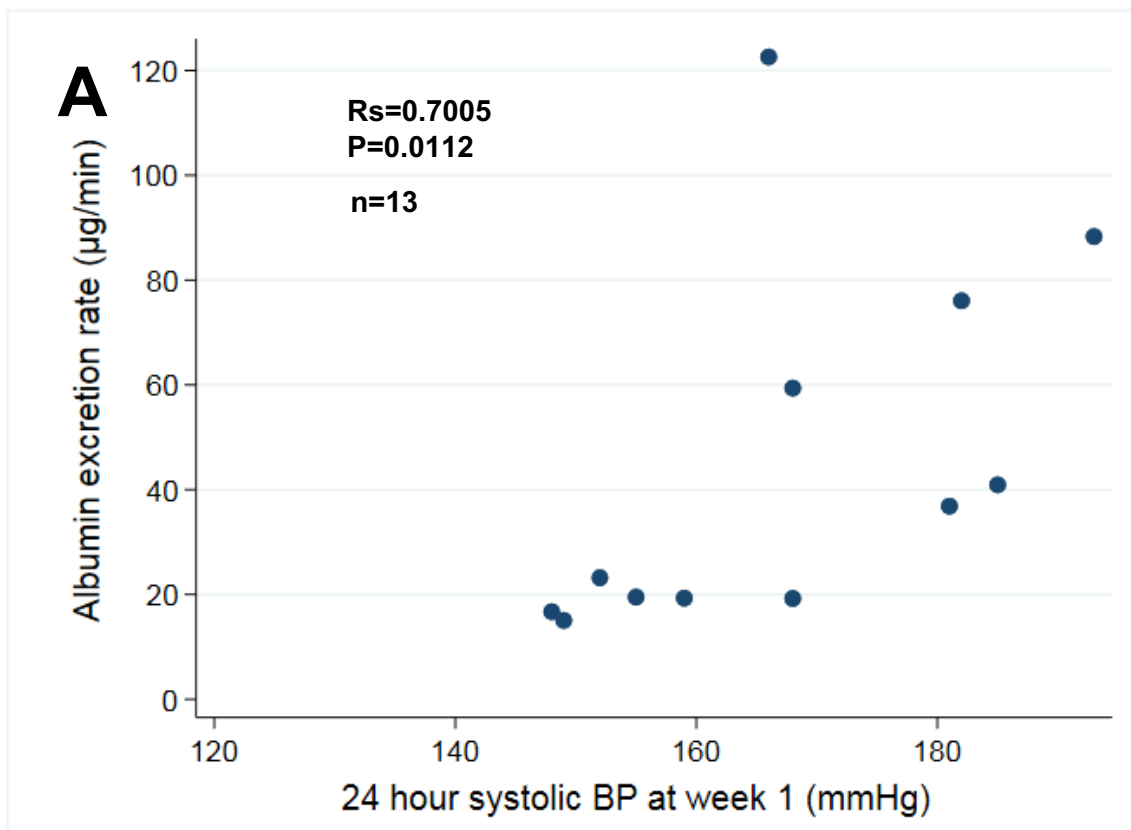
Figure 32 *Scatter diagrams showing Fitzgerald albumin excretion rate against 24-hour systolic BP at week 1 (A) and week 18 (B) in individuals with a baseline AER>15µg/min. Graphs are set to the same scale for comparison.*
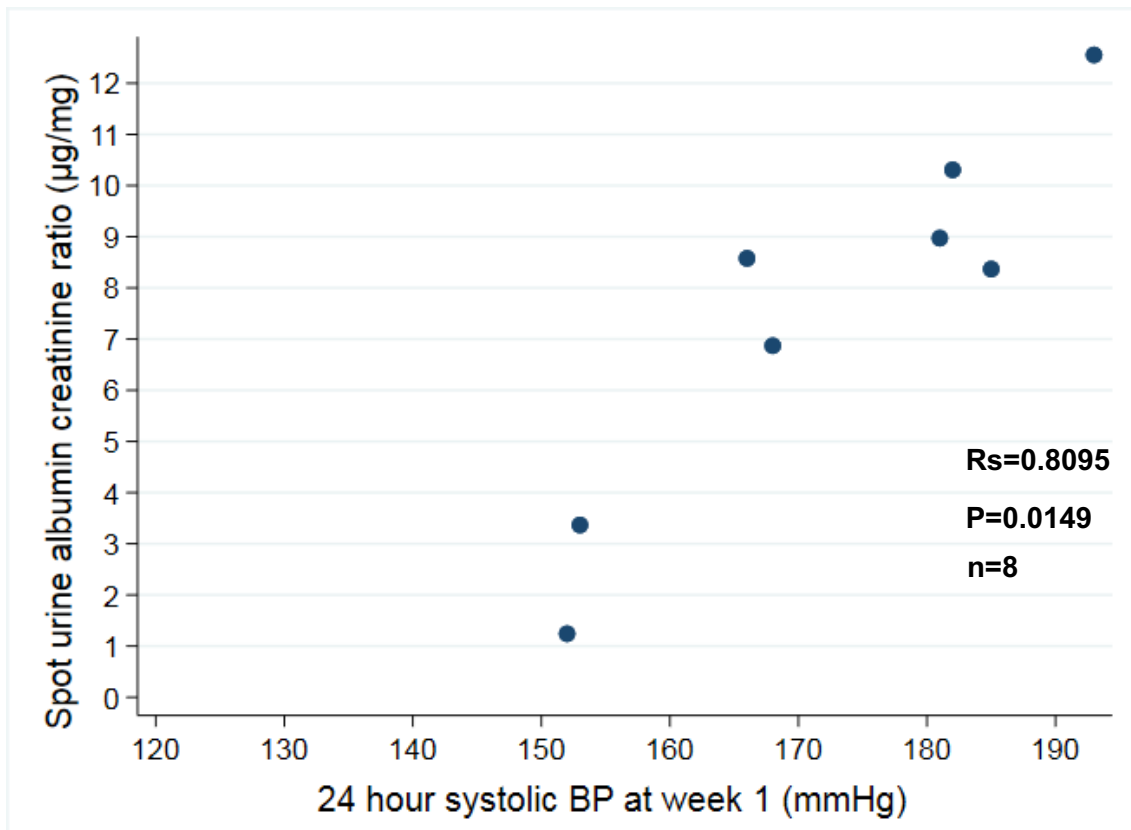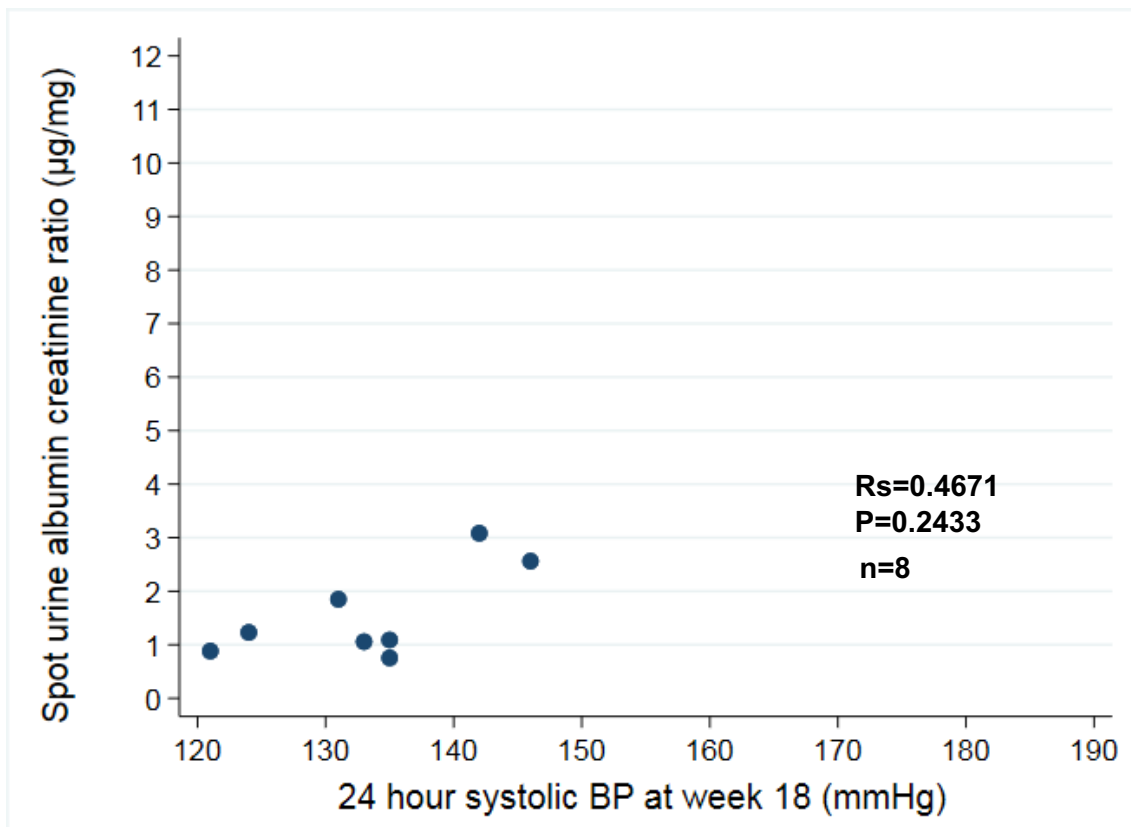
Figure 33 *scatter diagrams showing Fitzgerald albumin creatinine ratio against 24-hour systolic BP at week 1 (A) and week 18 (B) in individuals with moderately increased albuminuria at baseline. Graphs are set to the same scale for comparison.*

3.5.3.2 Albumin excretion and blood pressure and heart rate variability

No correlation was observed amongst any of the blood pressure variability parameters (systolic, diastolic and heart rate standard deviation) and Fitzgerald albumin excretion rate or Fitzgerald Spot urine sample ACR at week 1 or week 18. See figure 34 and 35. These are additional hypothesis generating analyses.
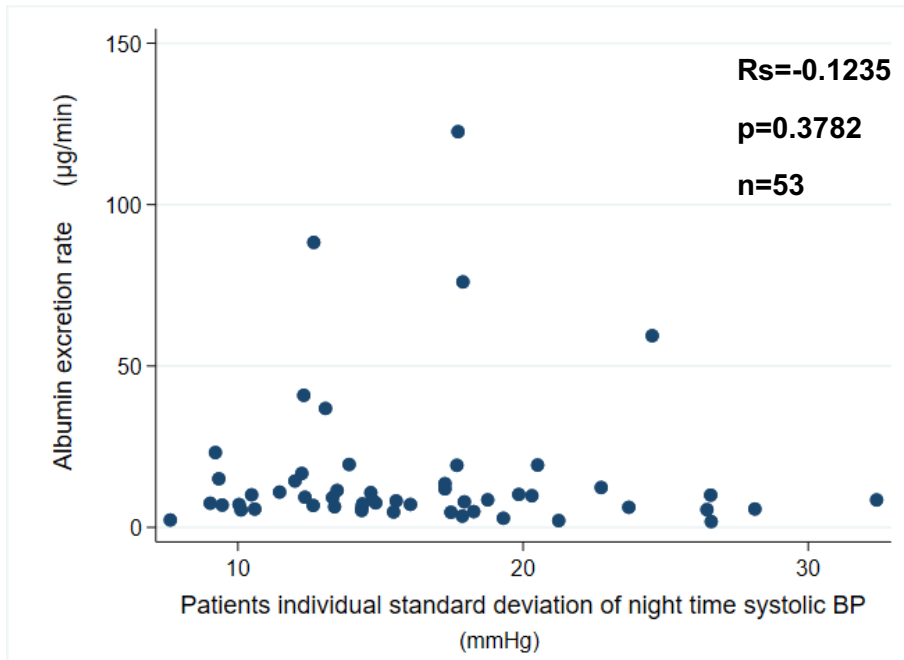
Figure 34 *Fitzgerald albumin excretion rate at week 1 against individual patient's standard deviation of night time systolic BP at week 1.*
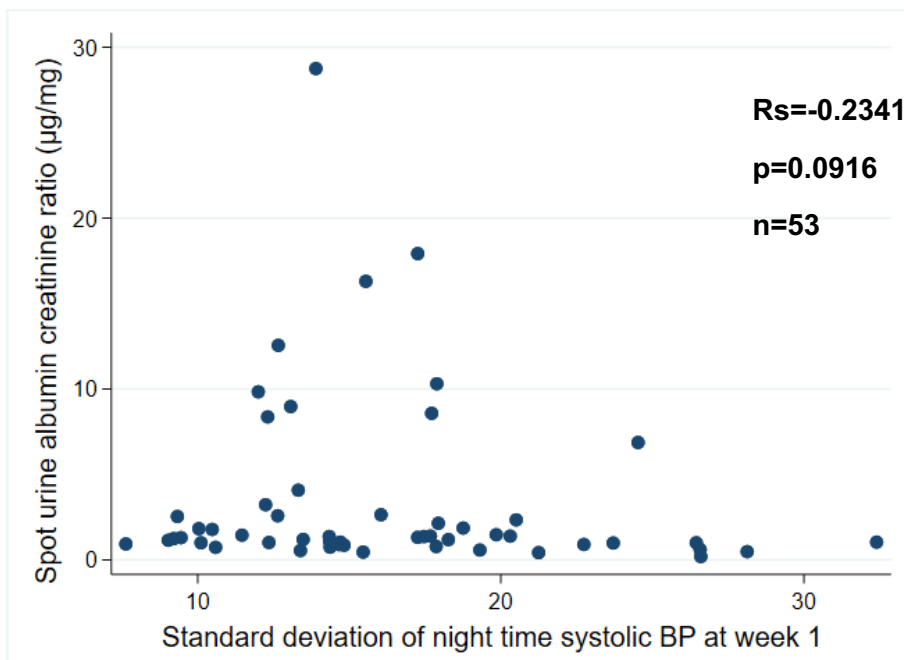
Figure 35 *Fitzgerald spot urine sample ACR at week 1 against individual patient's standard deviation of night time systolic BP at week 1.*

3.5.3.3 Urinary albumin excretion and blood pressure circadian rhythms

There was no association between day to night ratio of any of the BP variables and AER or ACR in the whole group at week 1 (day to night ratio of systolic BP **(p=0.7196)**). In the moderately increased albuminuria group there was also no association (day to night ratio of systolic BP **(p=0.8192)**), although it is worth noting that there was only 1 patient with extreme dipping of systolic BP and 1 patient with non-dipping of systolic BP in the moderately increased albuminuria group. The scatter graph resembles a bell shaped curve rather than a U-shaped curve which would be expected should the extremes of day to night BP ratios relate to worsening albuminuria (see figure 36).
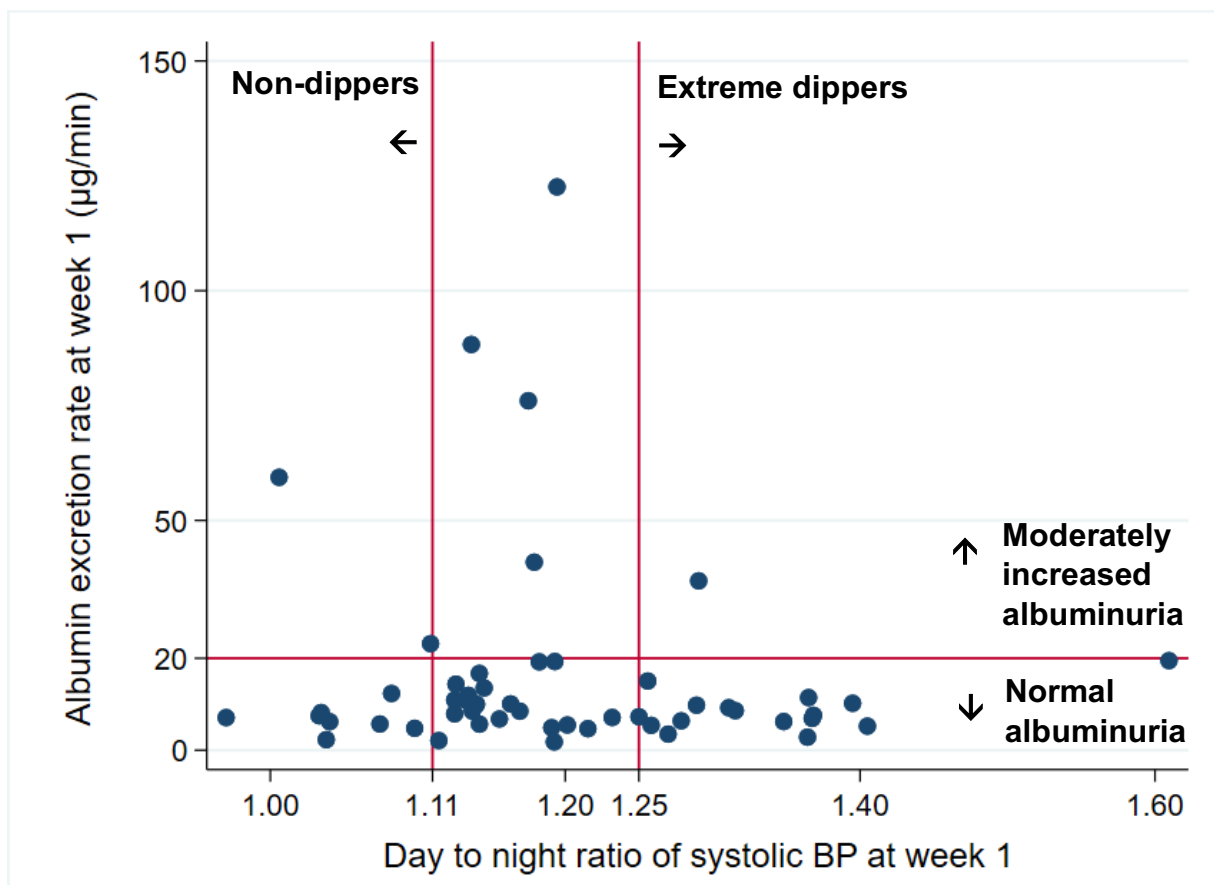
Figure 36 *Fitzgerald albumin excretion rate at week 1 against day to night ratio of systolic BP at week 1. Day to night ratio of 1.11 is equal to a night time BP of 90% of daytime BP, 1.25 is a night time BP equal to 80% of daytime BP.*

	Week 1			Week 18		
	Rs	p value	n=	Rs	p value	n=
SBP D:N	-0.0498	0.7233	53	0.0166	0.9067	52
DBP D:N	0.0394	0.7795	53	-0.1480	0.2950	52
MAP D:N	-0.0072	0.9591	53	-0.0990	0.4850	52
PP D:N	-0.1204	0.3907	53	0.1857	0.1857	52
HR D:N	0.1463	0.2960	53	-0.1124	0.4277	52
DP D:N	0.0846	0.5471	53	-0.0319	0.8222	52
PPP D:N	-0.0036	0.9794	53	0.1165	0.4107	52
Morning surge	-0.3201	0.0220	51	0.0410	0.7752	51

Table 12 *Spearman correlation coefficients, p values and observations of day to night blood pressure ratios and morning surge against Fitzgerald albumin excretion rate at week 1 and week 18. Statistically significant results are highlighted.*

Morning surge of systolic BP showed a negative correlation with albumin excretion suggesting that the greater the morning surge the lesser the albumin excretion, (see figure 37). Here this significant finding could due to chance as a result of the multiple negative analyses.
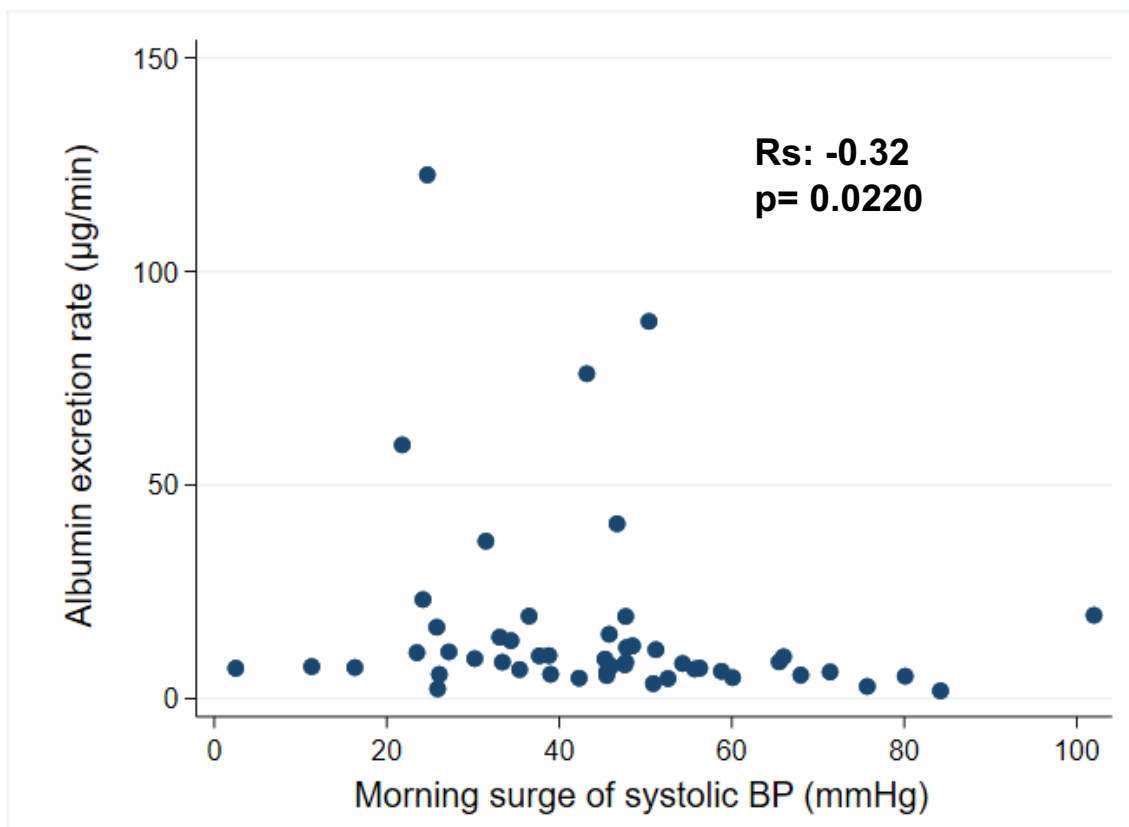
Figure 37 *scatter diagram showing Fitzgerald albumin excretion rate at week 1 against morning surge at week 1 (n=51).*

The relationship between dipping patterns and urinary albumin excretion was also assessed by splitting the cohort into groups of non-dippers, normal-dippers and extreme-dippers. In the literature, normal-dipping of systolic BP is defined as 10-20% of daytime average based on circadian rhythm studies. It has since been found that individuals who lay outside this normal range have an increased cardiovascular risk [135]. Studies that consider dipping of diastolic blood pressure and other BP parameters are rare. When diastolic BP dipping is considered, a normal range of 10-20% is often quoted. This is not likely to represent the true level of normal night time diastolic dipping. In healthy, normotensive individuals diastolic BP drops by a larger proportion than systolic BP overnight [136, 137]. To calculate and define the normal range within this thesis, dipping of other BP parameters has been compared to systolic dipping. Figure 38 and 39 show the calculation of dipping parameters for diastolic BP and mean arterial BP at week 1.
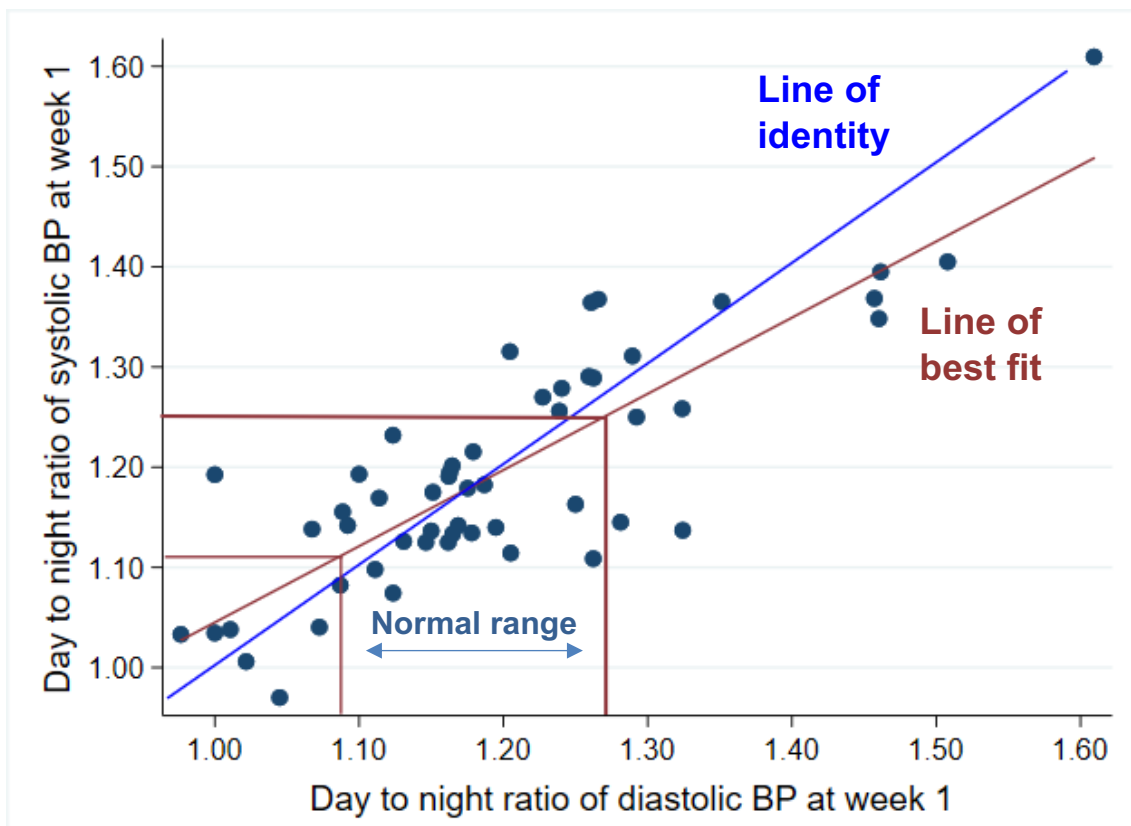
Figure 38 *a scatter graph demonstrating the method to calculate the range of normal night time dipping of diastolic BP (n=53).*
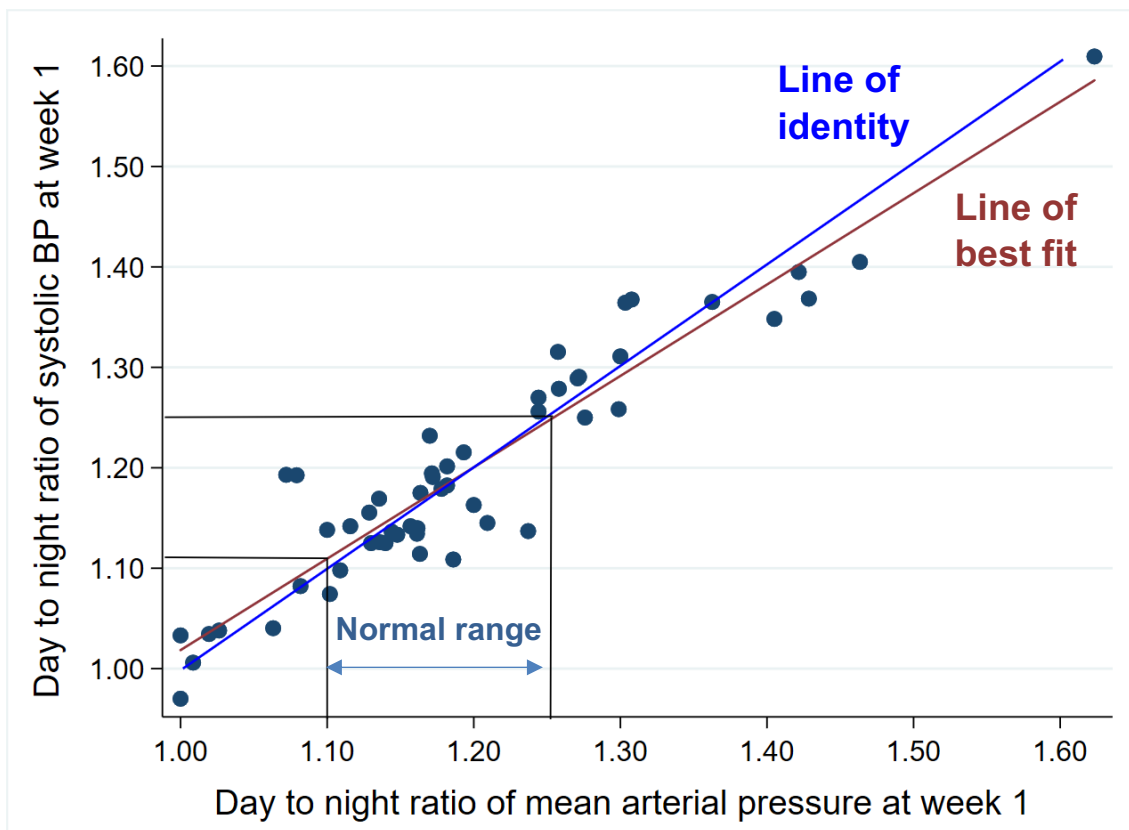
Figure 39 a scatter graph demonstrating the method to calculate the range of normal dipping within mean arterial pressure (n=53).

Using this method I have defined the normal ranges of BP dipping for all day to night ratios of BP within this cohort. Diastolic BP normal dipping is 1.09-1.27 as shown in figure 38 and mean arterial pressure normal dipping is 1.10-1.25 as shown in figure 39.

Using these definitions, no significant relationships were observed between albumin excretion and blood pressure dipping when the cohort was split into non-dippers, normal-dippers and extreme-dippers for any blood pressure parameter at week 1 or week 18. Figures 40-43 show some examples of this. This analysis focussed on Fitzgerald overnight AERs, but dipping was also analysed against Fitzgerald spot urine ACR which showed no significant relationships.
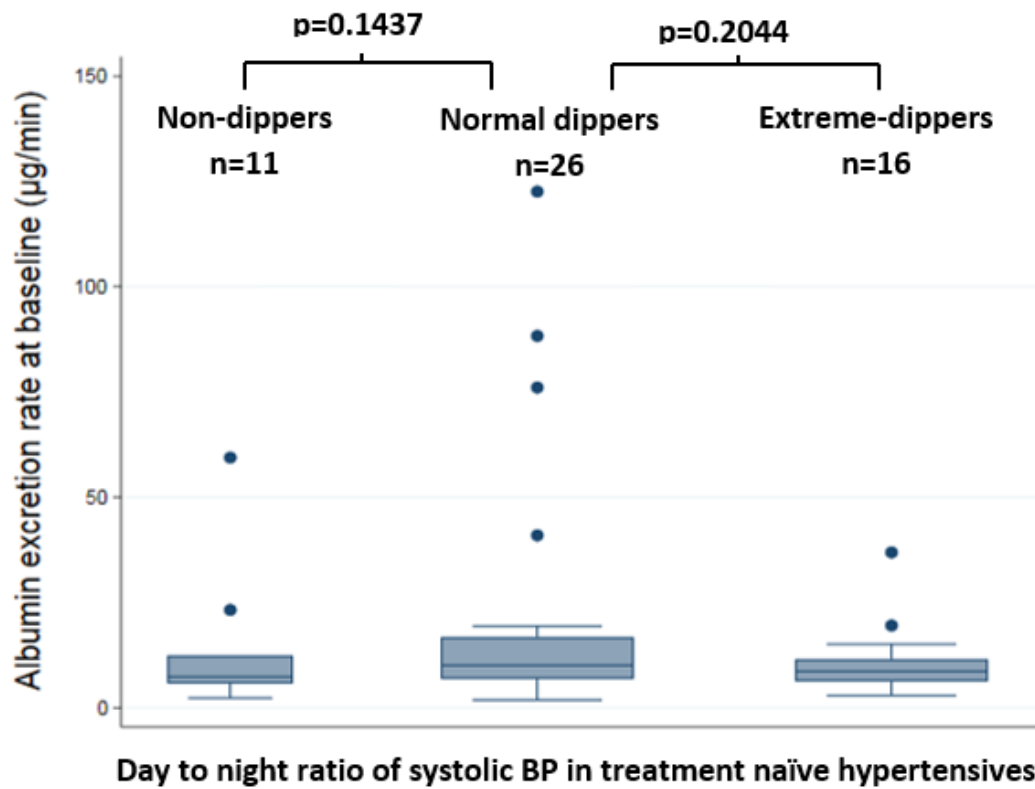
Figure 40 *The spread of Fitzgerald albumin excretion rates for non-dippers (<1.11), normal-dippers (1.11-1.25) and extreme-dippers (>1.25) of systolic BP at week 1.*
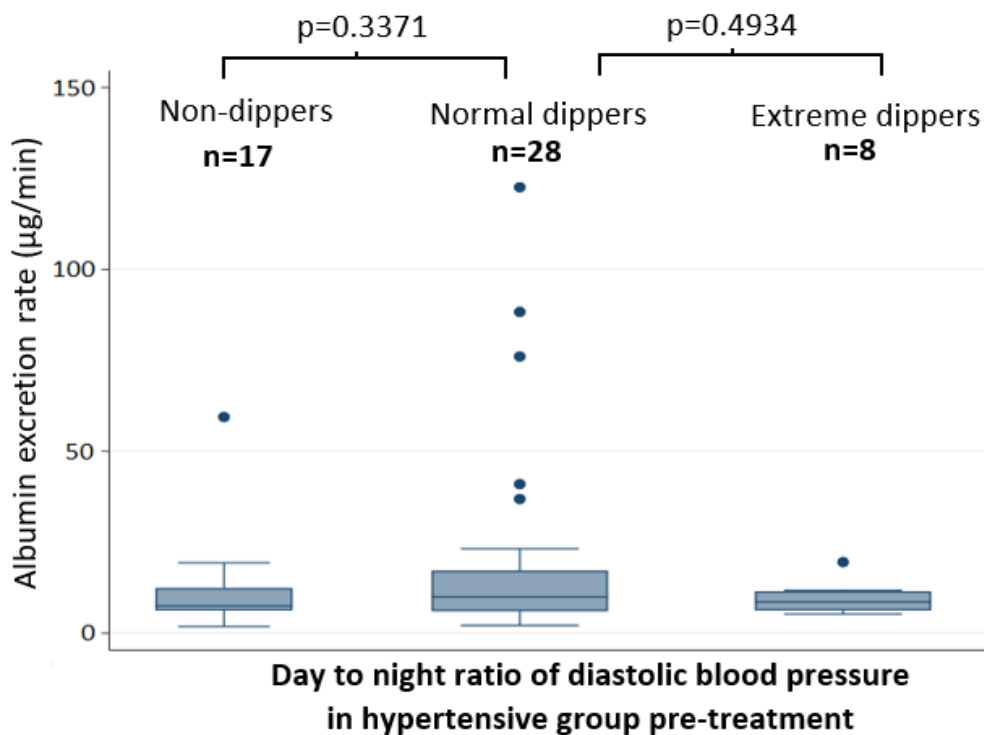
Figure 41 *The spread of Fitzgerald albumin excretion rates for non-dippers (<1.09), normal-dippers (1.09-1.29) and extreme-dippers (>1.29) of diastolic BP at week 1.*
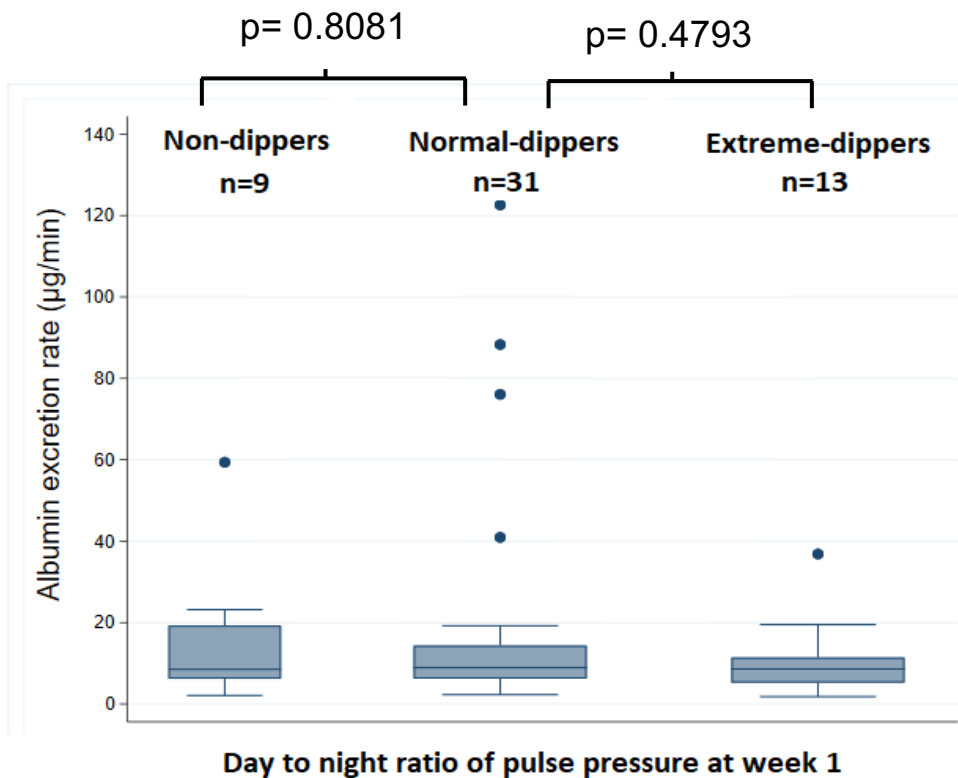
Figure 42 *The spread of Fitzgerald albumin excretion rates for non-dippers (<1.04), normal-dippers (1.04-1.29) and extreme-dippers (>1.29) of pulse pressure at week 1.*
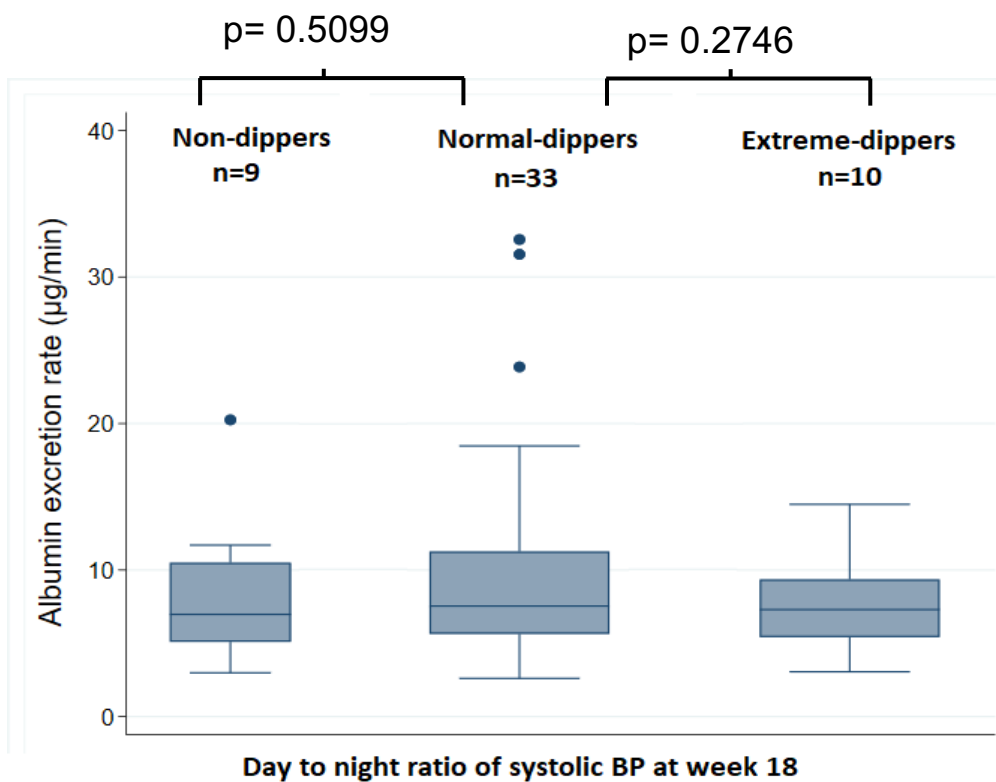
Figure 43 *The spread of Fitzgerald albumin excretion rates for non-dippers (<1.11), normal-dippers (1.11-1.25) and extreme-dippers (>1.25) of systolic BP at week 18.*

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At week 18 several individuals who presented as non-dippers and extreme dippers became normal dippers. As an additional hypothesis generating analysis, these individuals were examined to assess whether normalisation of these abnormal dipping patterns related to reduction in albumin excretion rate. This analysis showed no significant results although the group sizes were small.
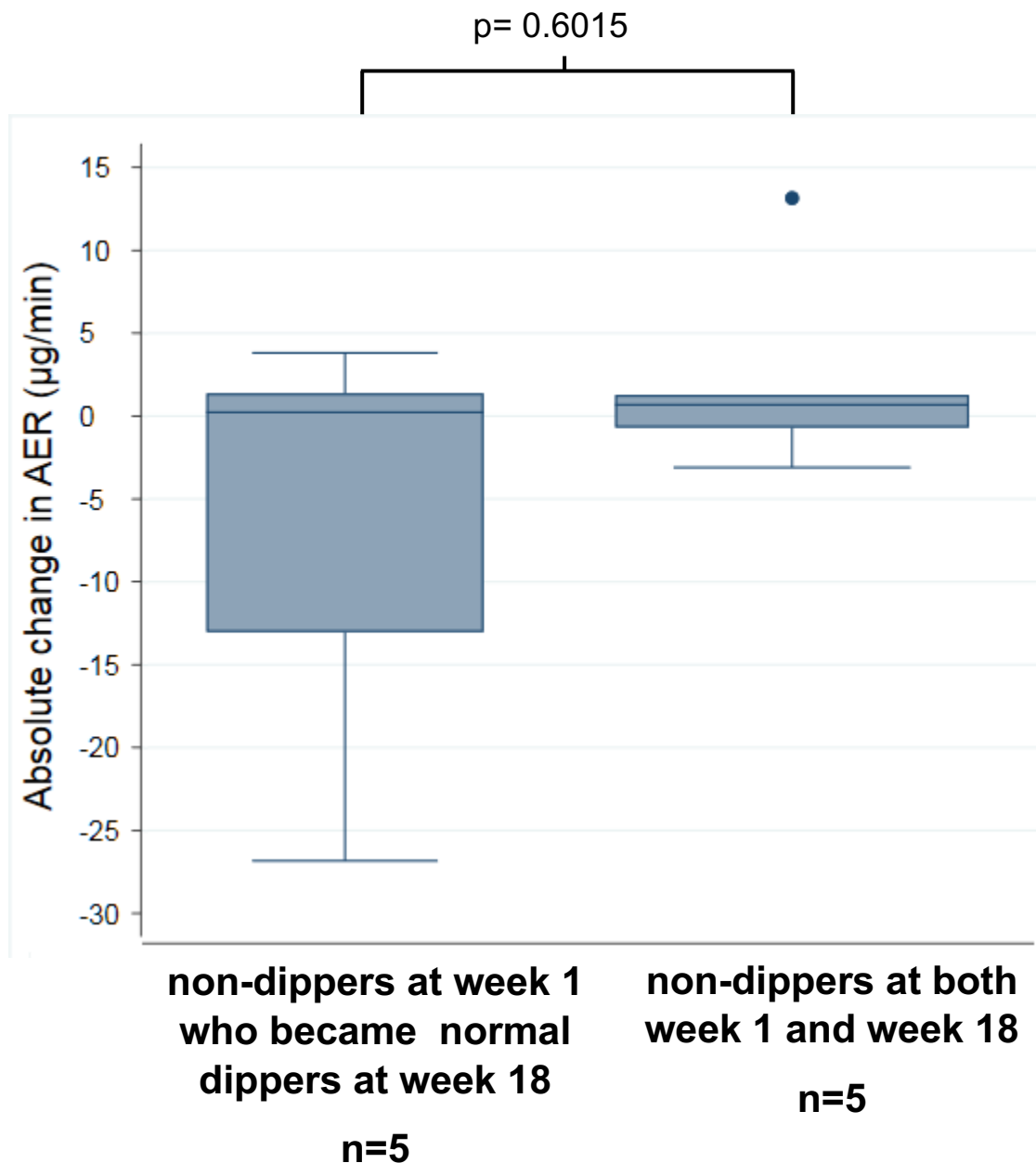
Figure 44 *The spread of change in Fitzgerald AER from week 1 to week 18 in those individuals who showed non dipping patters at week 1 but normal dipping at week 18, and those who remained non-dippers at both week 1 and week 18.*

3.5.4 Objective 3: Assess whether the different aspects of 24-hour blood pressure relate to glycocalyx integrity

In this cohort, glycocalyx integrity was associated with morning surge of systolic BP at week 1 (PBR 5-25 and PBR 10-19 **Rs:=-0.4506, p=0.0028** and **Rs:=-0.4291, p=0.0046** respectively). However, these correlations were not held at week 18. There were no other observed associations between glycocalyx and blood pressure parameters.
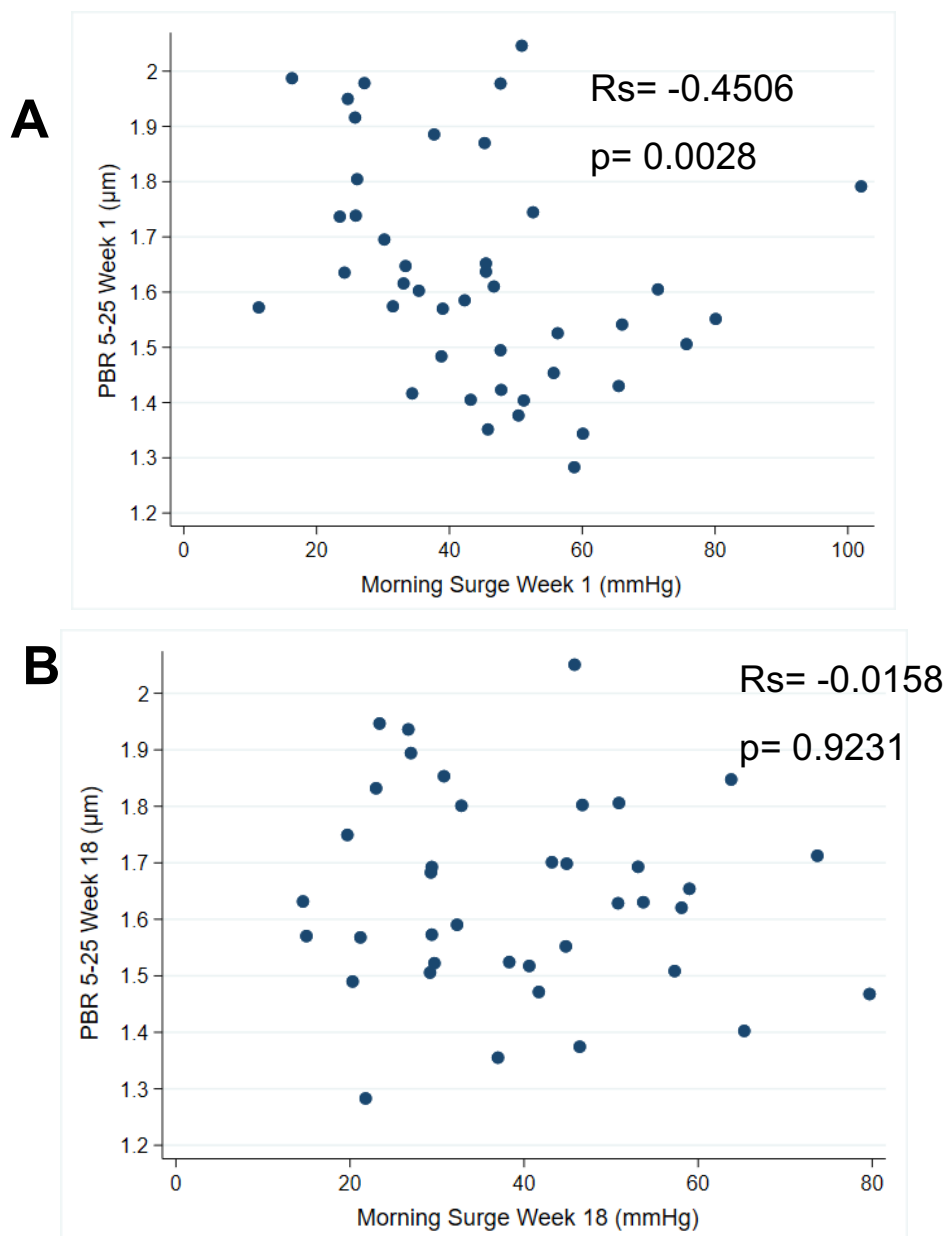
Figure 45 (A) showing the significant correlation between glycocalyx integrity (PBR 5-25) and morning surge at week 1(n=42). (B) showing the insignificant correlation between glycocalyx integrity (PBR 5-25) and morning surge at week 18 (n=39).

Systolic blood pressure reduction was the main outcome of the DASHER study. As shown in the graph below, 24-hour average systolic blood pressure and PBR 5-25 show no significant correlation (**Rs:= -0.0643, p=0.6710** at week 1 and **Rs:= -0.1062, p=0.5088** at week 18). Day to night blood pressure ratios also show no correlation with PBR.
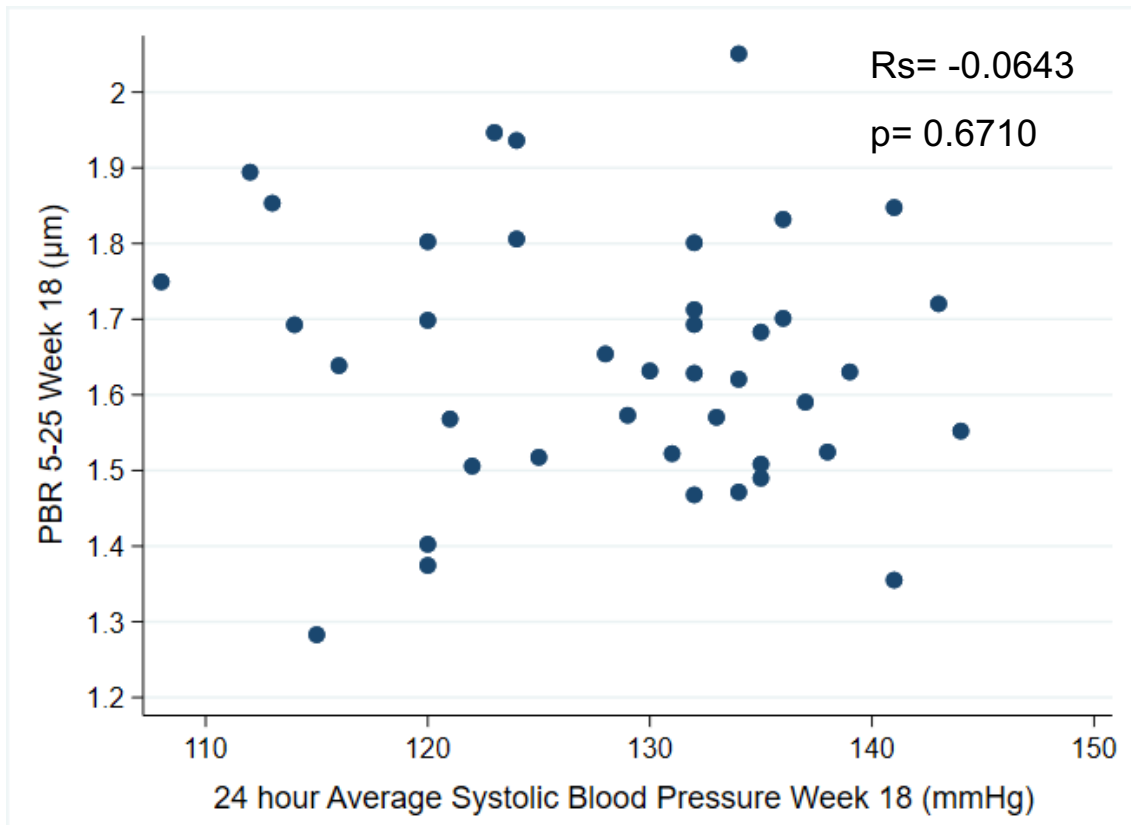
Figure 46 *A scatter diagram showing the insignificant correlation between glycocalyx integrity (PBR 5-25) and 24-hour average systolic blood pressure at week 18 (n=41).*

3.5.5 Objective 4: Assess whether glycocalyx integrity is associated with albuminuria levels

Glycocalyx integrity was not observed to be related to any of the measures of urine albumin (table 13). There was no relationship within the moderately increased albuminuria, normal albuminuria, AER>15µg/min or AER<15µg/min groups. There was also no observed relationship between percentage change in AER and percentage change in PBR (see figure 48).

	n=	PBR 5-25	PBR 5-9	PBR 10-19
Cobas ACR week 1	16	Rs:=-0.0941 p=0.7288	Rs:=0.0752 p=0.7819	Rs:=-0.1104 p=0.6841
Cobas ACR week 18	6	Rs:=0.4286 P=0.3965	Rs:=-0.4058 p=0.4247	Rs:=0.6000 p=0.2080
Cobas AER week 1	16	Rs:=-0.1971 p=0.4645	Rs:=0.0708 p=0.7944	Rs:=-0.2046 p=0.4473
Cobas AER week 18	6	Rs:=0.2571 P=0.6228	Rs:=-0.7247 p=0.0.1032	Rs:=0.6000 p=0.2080
Fitzgerald AER week 1	40	Rs:=-0.1414 p=0.3485	Rs:=-0.0354 p=0.8154	Rs:=-0.1429 p=0.3433
Fitzgerald AER week 18	40	Rs:=-0.0861 p=0.5972	Rs:=-0.1691 p=0.2906	Rs:=-0.1236 p=0.4412
Fitzgerald Spot week 1	46	Rs:= -0.1457 p=0.3339	Rs:=-0.0472 p=0.7554	Rs:=-0.1613 p=0.2841
Fitzgerald Spot week 18	41	Rs:=0.0226 p=0.8882	Rs;=-0.0766 p=0.6298	Rs:=0.0300 p=0.8503

Table 13 *Correlation coefficients, p values and observation numbers for Urine albumin measurements against PBR 5-25, 5-9 and 10-19.*
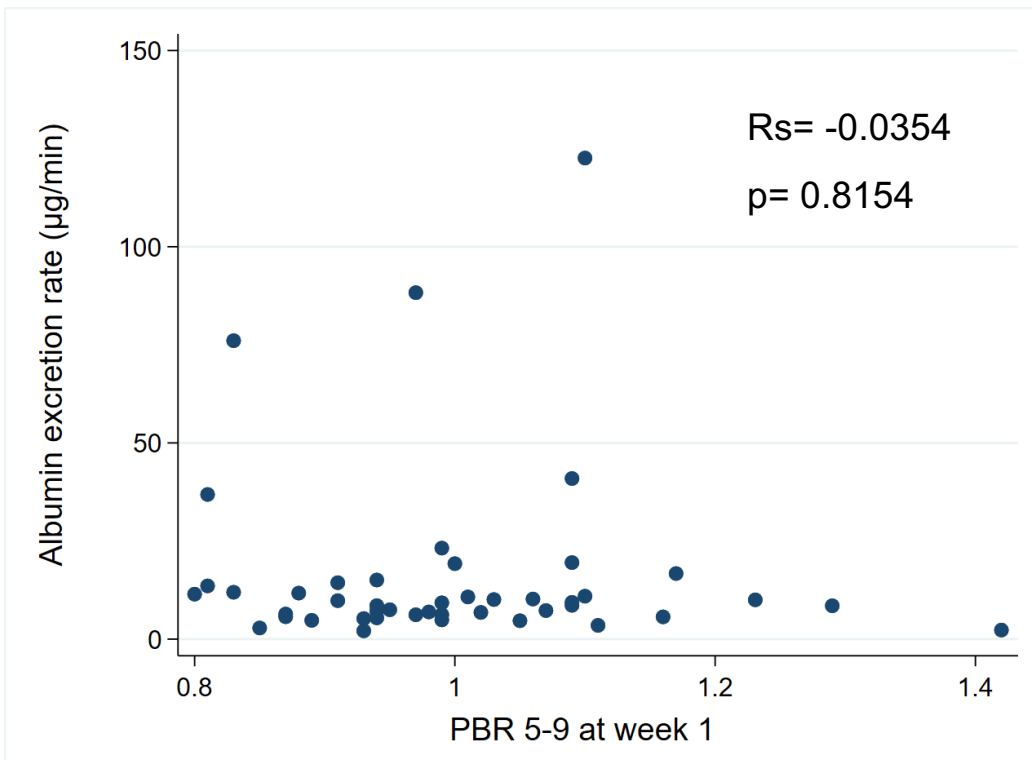
Figure 47 *A scatter graph showing the relationship between Fitzgerald albumin excretion rate and PBR 5-9 at week 1 (n=45).*
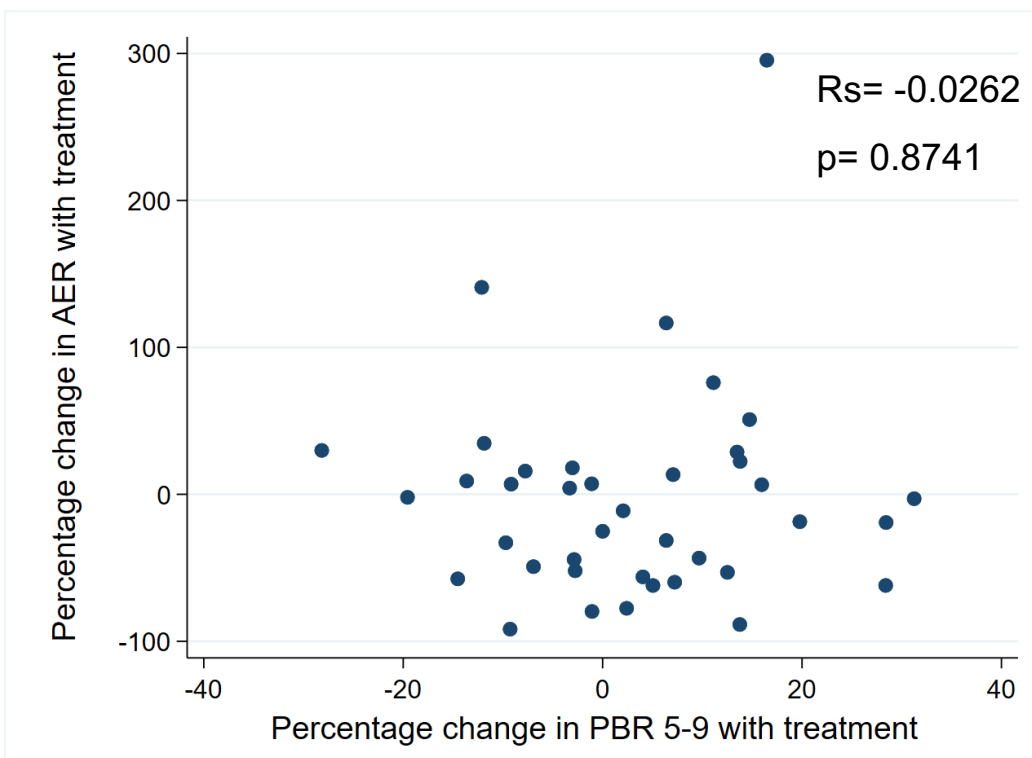
Figure 48 *A scatter graph showing the relationship between percentage change in Fitzgerald albumin excretion rate and percentage change in PBR 5-9 with treatment (n=42).*

3.6 Discussion of the DASHER study

3.6.1 Discussion

This study showed that the anti-hypertensive treatment regime significantly reduced both blood pressure and albuminuria in a group of treatment naïve, grade II hypertensive patients within 18 weeks. However, there was no relationship between the change in BP from day to night (day to night ratio) and albumin excretion rate within this group. It is estimated that 30-40% of treated hypertensives' blood pressure remains outside the target range [138]. As such, the reduction of systolic blood pressure to target in 80% of the patients is an achievement in itself. The management of hypertension is not the main aim of this thesis and will not be discussed in further detail.

3.6.1.1 Quantification of urinary albumin excretion

There is ongoing debate on the best way to quantify an individual's true urinary albumin excretion. Firstly, there is the decision of how best to collect a urine sample and secondly, which assay to analyse it with. This study used both overnight urine collections from which albumin excretion rates and albumin creatinine ratios were calculated, and also spot sample urines from which albumin creatinine ratios were calculated. This study used the Cobas assay which is a commonly used albumin assay, both clinically and in research, and the Fitzgerald assay which has a much lower limit of detection than the Cobas. The results of this study show that the mean urinary albumin excretion values of the same urine samples were not significantly different when analysed with the Cobas and the Fitzgerald assay. This held true for both AER and ACR measurements. Therefore, the Cobas assay remains a valid means of quantifying albumin, particularly clinically where the importance of albumin excretion measurements rests mainly within the moderately increased and severely increased albuminuric ranges.

However, this study has shown that the Cobas assay cannot measure all of the patient's urinary albumin levels when they fall within the normal range. The Cobas assay was unable to detect albumin concentrations in 72% of normal albuminuric participants' samples. The loss of these numbers is of the utmost importance within clinical research as demonstrated within this study. The inability to detect urine albumin values meant that, even with a sample size of 54, there were only 8 matched observations to determine whether the blood pressure treatment protocol significantly reduced albumin excretion and this test only gave a borderline significant result. Using the Cobas assay within research leads to the need for very large sample sizes to detect profound changes in urinary albumin excretion. It also means the findings may not be truly generalisable to the healthy population as they are based on a biased population. This study also showed that the Cobas assay gave correlations between albumin and blood pressure at week 18 that were not present when examining the entire cohort which could have led to false interpretation and conclusions around the strength of relationships to AER in the healthy population.

Considering further the implications of the inability to measure albumin in a complete cohort, I investigated one common approach to dealing with missing values; that is to assign a normal value at the detection limit of the assay in order to calculate an AER or ACR. When a value of 2.9μg/ml was substituted for the undetectable albumin concentrations in this analysis, Cobas AER and ACR results differed significantly from the Fitzgerald AER and ACR results. This shows that substitution of undetectable albumin values is not a valid means of approximating albumin excretion in research cohorts as it is significantly different from the Fitzgerald analysis which was able to detect all albumin levels in this cohort. Consequently, the Fitzgerald or other highly sensitive albumin assay should be used preferentially to Cobas within clinical research.

The second question is whether timed urine samples (AERs) are preferable to spot sample ACRs. There has been much debate as to which is the best collection method [123, 124, 139, 140]. Urinary albumin concentration is influenced by the dilution of urine. This is usually accounted for by calculating an albumin excretion rate (AER) or relating albumin excretion to another solute filtered by the kidneys such as creatinine. Twenty-four hour or overnight timed urine

collections are usually considered the gold standard as the accuracy of albumin creatinine ratios is subject to the considerable variability of creatinine excretion due to muscle mass, diet, activity and normal variation [122, 123, 140]. It is well know that timed urine collections also have their fall downs and are prone to collection errors [123]. This thesis' compared Fitzgerald AER to Fitzgerald ACR of the same overnight urine sample. As the two are not directly comparable, this comparison used a line of identity based on the point at which an individual is classed as having moderately increased albuminuria with ACR and AER calculations (3.5y=20x). This showed that AER gave what would be classed as consistently higher albumin excretion levels relative to ACR. The most common collection errors of an overnight urine sample (as used in this study) are likely to be as follows; Emptying the bladder into the collection bottle before bed rather than, correctly, into the toilet which would give a falsely high AER; Emptying the bladder into the toilet during the night which would give a falsely low AER; Incorrectly writing down sleep times, either shorter time interval which would give a higher AER or longer time interval giving a lower AER. It is impossible to know which of these errors occur more commonly. It is more likely that within a large group, the cumulative effect of these errors would balance each other out to some extent rather than give a consistently higher AER reading. The most likely reason for the difference between AER and ACR in this group is the variability of urinary creatinine excretion. More creatinine excreted relative to albumin will lead to a lower ACR. This could be relevant within this group as creatinine excretion strongly correlates with increased body mass [140] and 75% of individuals in this cohort were classed as overweight or obese. However, creatinine excretion correlates more with muscle mass than fat mass and there was no measure within this study to approximate body fat percentage. Therefore, it was not possible to accurately assess the effect of variation of muscle mass on creatinine variability in this study.

The spot sample ACR may be less reliable than the overnight AER due to creatinine variation. As well as this, the spot samples at both week 1 and week 18 show a significantly higher ACR than ACR of the overnight sample. This is may be due to the substantial daytime variation of urinary albumin excretion which is likely primarily related to activity [132, 141]. This means that the overnight urine sample may not represent an individuals' total 24-hour albumin excretion

and perhaps underestimates cardiovascular and renal risk in cohorts in whom overnight samples are used [141]. The focus of this research is mainly on the effect of night time blood pressure patterns and correlations with blood pressure that could be weakened by excess variability. Overnight urine samples were therefore deemed more useful within this analysis as they remove most of the influence of activity related urinary albumin variability.

3.6.1.2 Reduction of urinary albumin excretion with the anti-hypertensive treatment protocol

This study showed that the 18 week anti-hypertensive treatment protocol significantly reduced albuminuria within the cohort. This result was examined in more detail as it is known that renal risk and cardiovascular risk differ with different levels of albuminuria [27, 134]. Results in this thesis showed that albuminuria was significantly reduced by blood pressure treatment in those with moderately increased albuminuria at baseline but not in those who had normal albuminuria at baseline. The study also found that the correlation between reduction of albuminuria with treatment and baseline AER appears to continue into the normal albuminuric range, perhaps to the level of 15µg/min after which the correlation becomes less distinct (figure 22 and 23). This is interesting as 15µg/min has been used in the past to mark the threshold between normal and moderately increased albuminuria [134, 142]. A review of screening and monitoring albuminuria suggests that it could be wise to lower the threshold for abnormal albuminuria to 15µg/min rather than the currently used 20µg/min due to the continuation of cardiovascular risk into this range [143].

Percentage reduction of systolic, diastolic, mean arterial pressure showed borderline significant correlations and pulse pressure showed significant correlation with percentage reduction in AER in the >15µg/min group but no significant correlations were observed in the moderately increased albuminuria group. However, this is likely due to the small sample size of the moderately increased albuminuria group. Within the >15µg/min group, reduction in pulse pressure showed a stronger correlation with reduction in AER than reduction of systolic BP, diastolic BP and MAP. While the influence of systolic BP on

albuminuria is well described, some studies have suggested that pulse pressure relates to albumin excretion as strongly, if not more than systolic BP [112, 144]. In this cohort, the sample size is too small to comment on whether this is a true difference (n=11).

This poses the question of why the anti-hypertensive treatment regime would only significantly affect those who already have increased albumin excretion at baseline. It could be that the effect is simply regression towards the mean, or that there is a difference in pathophysiology between the groups. There are two main ways in which the anti-hypertensive treatment would reduce urinary albumin excretion; through reduction of blood pressure and transmission of this to hydrostatic pressure within the glomerulus, and through the effect of the Angiotensin receptor blocker on the glomerular arterioles (relative dilation of the efferent glomerular arteriole). This data shows that not all individuals with severe hypertension have pathological albuminuria. It may be that individuals who presented with high end normal albuminuria or moderately increased albuminuria already had some dysfunction of their glomerular blood flow regulatory mechanisms as described in chapter 1. This dysfunction would make them prone to albuminuria through increased hydrostatic pressure that comes alongside severe hypertension. As a result when the treatment reduced hydrostatic pressure and dilated the efferent glomerular arteriole, in these individuals, there may have been a significant difference in glomerular pressure resulting in a decrease in urinary albumin excretion. In the lower normal albuminuria group whose regulatory mechanisms may have been working normally (maintaining glomerular pressure in spite of severe hypertension), the reduction in blood pressure may not have had a significant effect on urinary albumin excretion. In these individuals, variation from week 1 to week 18 may simply be due to natural activity related variation or variation of the assay. To my knowledge this theory has not been researched and may be complex to assess in vivo, in humans.

Another theory for the relationship between increased albuminuria and hypertension at week one in this study involves the bi-directional relationship of albuminuria and cardiovascular disease. As described in chapter 1.3.1, albuminuria is an independent risk factor for cardiovascular disease. Others have also found that albuminuria can predict progression of hypertension, even

within the normal range of albuminuria [145-147]. This could mean that the relationship observed in this study is not as simple as high blood pressure forcing more albumin through the glomerulus, but that those who begin to increase their urinary albumin, damage their kidneys and exacerbate their hypertension. The higher blood pressure then exacerbates the albuminuria and the vicious cycle continues. The treatment protocol would successfully halt this cycle by targeting both the systemic blood pressure and the intra-glomerular pressure.

3.6.1.3 Which aspects of blood pressure are related to urinary albumin excretion?

As hypertensive treatment led to reduction of many different components of blood pressure alongside urinary albumin (systolic, diastolic, mean arterial pressure, pulse pressure, BP variation, double product and pulse pressure product), these components were analysed to gain understanding of which aspects of blood pressure most strongly related to urinary albumin excretion. Although all correlations were lost between blood pressure and urinary albumin excretion at week 18, the results show that this may be as a result of a change in range of blood pressure values: At week 1 blood pressures primarily ranged from 140-190mmHg, whereas at week 18 80% of the cohorts' blood pressures were between 120 and 140mmHg. The loss of correlation at week 18 may mean that blood pressure within the target range does not influence urinary albumin excretion or that the relationship within lower blood pressures is too weak to detect within this sample size. The higher levels of blood pressure show a much more striking relationship within which it is easier to detect a correlation within this group size.

The blood pressure parameters that showed the strongest relationships with urinary albumin excretion in this cohort were systolic, diastolic and mean arterial pressure at week 1, although the correlations within the entire cohort were weak. This is likely due to the previously described discrepancy between the strength of relationships to blood pressure in the normal albuminuria and moderately increased albuminuria group. However, there were no significant

correlations observed between different blood pressure parameters and urinary albumin excretion within the separated normal albuminuria or moderately increased albuminuria groups. The lack of correlation within the moderately increased albuminuria group was likely influenced by the small sample size (n=7). When the AER >15μg/min group were analysed (n=12) there was a significant correlation between Fitzgerald AER and 24-hour systolic blood pressure (p=0.0112) and borderline relationships with pulse pressure (p=0.0682) and mean arterial pressure (p=0.0581). Significant relationships were also observed between the spot sample ACR and 24-hour systolic, 24-hour diastolic and mean arterial pressure in those with moderately increased albuminuria at baseline (n=8). The sample sizes of these groups limit the conclusions that can be drawn from these data. However, the fact that systolic, diastolic, mean arterial pressure and pulse pressure related to urinary albumin excretion in several different forms of analysis within this cohort is in line with current literature on the topic [146, 148].

In this cohort there were no associations between blood pressure variability and urinary albumin excretion which is contrary to emerging literature suggesting that short term variability of blood pressure is potentially the strongest predictor of moderately increased albuminuria in untreated hypertensives [149, 150]. There are multiple factors which may explain these apparently discordant results. The nature of the present cohort's severe hypertension at week 1 may have had such a large effect on albumin excretion that it masked the relationship between BP variability and AER. The effect of anti-hypertensive treatment, particularly CCBs which have been shown to reduce BP variability [151], may have also influenced the relationship between BP variability and AER at week 18.

In terms of variability of blood pressure, the morning surge in systolic BP also showed some borderline correlation with the spot sample ACR (p=0.0522). While not significant, this could suggest that the spot sample (usually taken in the morning) is influenced by the magnitude of a person's systolic morning surge. These readings could lead to false impressions of an individual's overall daily albumin excretion. However, due to the concurrent multiple analyses which were all negative, this finding could be due to chance and should be investigated further.

3.6.1.4 Urinary albumin excretion and day to night blood pressure ratios

The main aim of this thesis was to examine the relationship between day to night blood pressure ratios and urinary albumin excretion and to compare this relationship with the relationship of AER to other standardly measured BP parameters as previously discussed in 3.6.1.3. In this cohort there were no significant relationships between day to night ratios and urinary albumin excretion.

Day to night ratios were not altered by the anti-hypertensive treatment protocol. Previous research has attempted to enhance the night time blood pressure dip, as discussed in 1.5.2.3, by bedtime administration of anti-hypertensive medication [118, 121]. Within the DASHER study time of medication ingestion was not controlled or noted. However, there were 5 non-dipper individuals who became normal dippers with treatment and these individuals seemed to have a larger reduction in AER than the non-dippers who remained non-dippers. This result was not statistically significant although with a sample size of 5 a type 2 error cannot be ruled out. As discussed in 1.5.2.3, evidence for targeting night time dipping patterns to reduce albuminuria is inconclusive and warrants further investigation.

It could be argued that the effect of the severe hypertension on urinary albumin excretion could be masking a more subtle relationship between day to night BP ratios and AER. If this was the case, a relationship may have become observable at week 18 when the vast majority of patients' blood pressures were controlled to target. As no relationship was observed at week 18 this is less likely to be true, although at week 18 the cohorts AER was also significantly lower making it less likely that a relationship would be observed.

If the theory of both non-dipping and extreme dipping contributing to albuminuria is correct, one would expect to see a U-shaped relationship between day to night ratios and AER. In this cohort, the relationship resembled a bell shaped curve. Contrary to evidence discussed in 1.5.2, the likely conclusion from these results is that within this cohort, day to night BP ratio has no observable effect on urinary albumin excretion. Day to night ratio is likely not independently associated with level of albuminuria and is as such would not be a suitable target for reno-protective strategies directed at reducing albuminuria.

3.6.1.5 Relationship of glycocalyx integrity to urinary albumin excretion and blood pressure

Analysis of relationships to glycocalyx integrity showed no observable relationship between PBR values and AER. This is contrary to earlier work discussed in 1.3.2.3 [26, 36, 41, 42] It may be that glycocalyx integrity is less influential in the pathophysiology of albuminuria in hypertension as it is in diabetes as the vast majority of glycocalyx related albuminuria research focus on diabetes.

PBR also showed no correlation with BP parameters. However, a reduction with treatment in diastolic, systolic and mean arterial pressure all consistently correlated with an increase in PBR. This is surprising as a higher PBR implies worse glycocalyx integrity. As well as this, results showed that a greater morning surge resulted in a lower PBR value. It should follow that higher blood pressure values increase shear stress on the glycocalyx, damage the integrity and increase the PBR. These results suggest the opposite is true. These results may mean that the glycocalyx does not respond to changes in blood pressure in the way we would expect. Acutely, the integrity of the glycocalyx may be damaged by increased blood pressure but the long-term response of the glycocalyx to high blood pressure may not be so simple. There is some research to further understanding of glycocalyx function under shear stress [152] but this does not shed light on the long term response and so the subject requires further research.

3.6.2 Limitations of the research

This is a post hoc analysis of the DASHER study. As such, the study was not purposefully designed to answer the present question. Firstly, the sample size was not calculated for the purpose of this research but to enable the detection of clinically significant change in BP with treatment. Although the cohort size was initially adequate to detect correlations above 0.40 to 80% power, when the cohort was split into separate groups of non-dippers, extreme-dippers and different levels of albuminuria there was inadequate power to detect correlations. Therefore, correlations found in these smaller groups are only suggestive of a relationship, not definitive. Spot urines were random samples not first morning voids which may have influenced albumin excretion variability. The timings of drug ingestion were not noted; ideally we would have known which patients took the drugs in the evening as this could affect night time dipping and morning surge. The ethnicity of the cohort reflects the population of Devon from which it is drawn. Consequently, there is substantially less variation in ethnicity compared with the UK population as a whole which does reduce the wider generalisability of these findings. There were no measures of cardiac autonomic neuropathy which would have added to the critical analysis.

Statistical analysis often involved multiple analyses and so one off significant findings such as the association of morning surge with spot urine albumin should be interpreted with caution.

It is also worth noting that the duration of which each participant had been experiencing high blood pressure prior to treatment is likely to have varied. This duration could have had an effect on markers such as albuminuria and glycocalyx integrity, an even longer follow up period may have shed light on this effect.

Inter-arm difference in BP, which has been shown to be related to cardiovascular risk[153], was not taken into account when deciding on placement of the ABPM cuff. This may have had an effect on the analysis of overall BP measurements between patients, but there is little evidence to suggest whether lack of consideration of inter-arm difference would have altered the observed night-time dipping of blood pressure.

3.6.3 Implications for future work

Future work should strongly consider the use of a highly sensitive albumin assay for research concerning urinary albumin excretion in order to truly assess relationships to albumin excretion in the population. Substitutions for undetectable values are not a valid means of approximating low AERs and could cause incorrect interpretation of population data. Judgement should be used, considering the relative merits of AER or ACR and the aims of the research in question, when deciding which type of measurement to use. There is still no perfect method of quantifying albuminuria and future work should focus on reducing the effect of variability and collection error in urine sampling.

This research also supports other findings that the threshold for moderately increased albuminuria should perhaps be lowered into the normal albuminuric range to 15µg/min [143].

In terms of blood pressure, future work should focus on larger studies of hypertensive patients with adequate power when split into groups of non-dippers, normal-dippers and extreme dippers to observe relationships. Future work should also aim to determine whether short term blood pressure variability really is a strong marker of albuminuria, particularly within the hypertensive population, and if so could this be a worthwhile treatment target.

Future work on the glycocalyx must focus on the normal acute and long term physiological changes in response to different stimuli such as high blood pressure to determine how treatment of blood pressure may or may not adversely affect glycocalyx. Other work could aim to validate the GlycoCheck method of glycocalyx analysis with a sufficient sample size and validation using biological markers of glycocalyx health to further our understanding of these results.

3.7 Conclusion

Overall, this study demonstrated no relationship between day to night blood pressure ratios and urine albumin excretion in hypertensive patients before and after treatment. Relationships of similar strength were observed between urine albumin excretion and systolic, diastolic, mean arterial pressure and pulse pressure. However, these relationships were only present at week 1 and in those with AER above 15µg/min. This suggests that blood pressure most strongly influences albuminuria at higher blood pressure values within those who already have some underlying damage to their glomerular regulatory mechanisms. It could also support the theory that albuminuria itself exacerbates hypertension.

The results support earlier work by others suggesting that the threshold for moderately increased albuminuria could be lowered as the relationship between cardiovascular risk and morbidity continues into the current normal albuminuric range [143].

The study showed no significant relationship between albuminuria and glycocalyx integrity and so the GlycoCheck measure of glycocalyx was not informative of albuminuria. Glycocalyx integrity showed an interesting response to blood pressure treatment and the physiology of glycocalyx response to long term changes in blood pressure needs further research.

This research also recommends that for future clinical research involving albuminuria, a highly sensitive assay should be used, rather than the Cobas assay as it cannot describe the entire normal albuminuric population and substitution for undetectable values is not a valid method to approximate AER or ACR. However, the best method of correcting for urine dilution is still unclear and further work should be put into perfecting AER, ACR or a new method entirely.

Chapter 4: The BEAT-DKD Study

4.1 BEAT-DKD Study Overview

BEAT-DKD is an EU IMI funded study of 31 European partner institutions. The imaging work package uses MRI, renal ultrasound, Pet-CT, urine and blood testing. Additionally, in Exeter, 24-hour ambulatory blood pressure monitoring and microvascular imaging are being undertaken.

The data used in the present chapter are derived from the BEAT-DKD study. I have been responsible for the collection, quality control, data entry and analysis of the ambulatory blood pressure monitoring within this study.

As described in Chapter 1, diabetic kidney disease is a significant and ever increasing healthcare burden. The BEAT-DKD work package, undertaken in Exeter, has the primary aim of identifying novel microvascular imaging biomarkers of early disease in patients with diabetes and an eGFR>30ml/min/1.73m^2. This aims to increase understanding of the early stages of DKD development and enable early identification of those with risk factors of faster disease progression. Further understanding of the pathogenesis and epidemiology of DKD may lead to better prevention of end stage renal disease in this patient group.

4.2 Aim

The overall aim of this chapter is to examine the relationship between 24-hour ambulatory blood pressure parameters, urinary albumin excretion into the normal albuminuric range and glycocalyx integrity in people with type 2 diabetes.

4.3 Objectives

1. Assess how urinary albumin excretion relates to the following different aspects of 24-hour blood pressure and circadian blood pressure rhythms:

   - 24-hour, day and night parameters *(systolic BP, diastolic BP, heart rate, pulse pressure, mean arterial pressure, double product, pulse pressure product)*

   - Blood pressure and heart rate variability

   - Circadian rhythms *(day to night blood pressure ratio and morning surge).*

2. Assess whether the different measures of 24-hour blood pressure, day and night parameters relate to glycocalyx integrity.

3. Assess whether glycocalyx integrity is associated with albuminuria.

4. Assess how day to night blood pressure ratios relate to different markers of diabetes:
   - *HbA1c, Fasting blood glucose*
   - *Duration of diabetes*
   - *Peripheral neuropathy*
   - *BMI and body fat percentage*

4.4 Methods

4.4.1 Participants:

Participants were identified and recruited into BEAT-DKD from several sources. This includes databases of patients in Exeter who were interested in participating in research studies such as the Exeter 10,000 and DARE cohorts. Patients were also identified and recruited from clinics. Patients will be followed up for 4 years.

4.4.1.1 Inclusion criteria

- Aged 18-80 years inclusive.
- Unchanged anti-diabetic and anti-hypertensive medication in the last 3 months.

Patients were divided into 3 different groups:

Group 1: Control, non-diabetic individuals with normal albuminuria and eGFR>60ml/min.

Group 2: Diabetic individuals with normal albuminuria and an eGFR ≥30ml/min.

Group 3: Diabetic individuals with moderately increased albuminuria with an eGFR ≥30ml/min.

4.4.1.1 Exclusion criteria:

Group 1 (Control)

Albuminuria; eGFR ≤ 60ml/min; presence of diabetes (HbA1c >48mmol/mol); known cardiovascular, cerebrovascular or peripheral artery disease.

Group 1- 3

 eGFR < 30 ml/min; Significant comorbidities with life expectancy of < 1 year; Use of investigational drug within 1 month prior to screening; Renal or hepatic pathology; Metastatic malignancy; Malignancy with expected survival <4 years; Any other significant disease or disorder which, through participation, may either adversely affect the patient or the study.

In addition to exclusion criteria above, if participants in group 3 were not receiving ACE inhibitors or ARBs at screening, they were referred to their GP with recommendations by a physician on the Exeter BEAT-DKD research team. These participants were invited back to be re-consented and screened for the study > 3 months after their original screening visit.

4.4.2 Study Protocol

The BEAT-DKD study protocol was approved by the NHS Research Ethics Committee, Health Authority and local R&D, Royal Devon and Exeter NHS Foundation Trust. (REC ref. 18/SW/0061).

Written, informed consent was obtain from all patients prior to entering the study including for the storage and retesting of urine samples. Patient anonymity was maintained throughout and documents stored securely. The study was carried out according to the declaration of Helsinki.

4.4.3 Study Design

For detailed methods of data collection see Chapter 2.

> **Screening visit**
>
> **Standard clinical examination (BMI)**   **Sudoscan assessment**
> **HbA1c and Fasting glucose**   **Medical history**

> **Baseline assessments (2-3 visits)**
>
> **Microvascular assessments including:** *GlycoCheck, Overnight urine collection, Spot urine, 24-hour ambulatory blood pressure monitoring.*

4.4.4 Measurements

24-hour ambulatory blood pressure monitoring was undertaken and analysed as described in chapter 2.1. GlycoCheck measurements were taken and analysed as described in chapter 2.2. Urine albumin was collected by timed overnight and spot urine samples and measured by both the Cobas and Fitzgerald assays as described in Chapter 2.3. Sudoscan, HbA1c, Fasting glucose, BMI and body fat percentage measurement methods are described in detail in 2.4-2.8.

4.4.5 Statistical analysis

Statistical analysis was performed using STATA 15 as described in Chapter 2.4.

A cohort of 34 patients allowed detection of correlation coefficients ≥0.50, where $p \leq 0.05$ with a statistical power of 80%.

4.5 Results

4.5.1 Patient demographics

During my research period, BEAT-DKD was recruiting the first baseline cohort of patients. According to projected recruitment it was expected that 80 patients would be recruited. The study was however delayed in starting so the number of patients recruited was smaller than originally anticipated (n=34).

This patient cohort showed an average age of 69 (±6.8) so represents the older population. The cohort was predominantly male (65%). The patients had an above average body fat percentage and the average BMI was within the 'obese' BMI bracket. Fifty-nine percent of patients were diagnosed hypertensive and on varying antihypertensive treatment. The characteristics of the 34 individuals recruited are shown in table 14.

Variable	Week 1 (n=34)
Age (years)	69.0 (±6.8)
Sex – Female	12 (35%)
BMI (kg/m^2)	31.3 (±5.4)
Waist: hip ratio	0.98 (±0.1)
Body fat (%)	33.5 (29.3-40.5)
Clinic systolic BP/diastolic BP (mmHg)	148/76 (136/70-156/82)
HbA1c (mmol/mol)	60.8 (±11.9)
Diabetes duration (years)	13.33 (±8.5)
Fasting glucose (mmol/L)	8.86 (±2.74)
Peripheral neuropathy history	4 (12%)
eGFR (mL/min per 1.73 m^2)	80.0 (64.5-90.0)
Diagnosed Hypertensive (n(%))	20 (59%)
Moderately increased albuminuria (n (%))	19 (55%)
Anti-hypertensive treatment (n(%)) None/ACE/ARB/CCB/β-blocker/ diuretic	11(32%)/ 21(62%)/ 5(15%)/ 8(24%)/ 11(32%)/ 15(44%)

Table 14 *Patient demographics for the T2DM cohort in BEAT-DKD expressed as mean (±standard deviation) or median (lower quartile-upper quartile) or number (percentage of total. Percentage of patients receiving different types of anti-hypertensive treatment is also shown.*

4.5.2 Objective 1: Assess how urinary albumin relates to different aspects of 24-hour blood pressure and circadian blood pressure rhythms

4.5.2.1 Interpretation of urinary albumin excretion using assays with different ranges of detection

When using the Cobas albumin assay, only 20 (59%) of the patients had detectable urinary albumin values. In contrast, when urinary albumin was measured using the Fitzgerald assay, all patients had detectable urinary albumin levels (n=34). The following table 15 and figure 49 and 50 show the spread of results from each assay and urine sample. There was no observable significant difference between any of the different measures of urinary albumin in the urine samples collected as shown in figures 49 and 50.

	Detectable albumins (n(%))	Min	Lower quartile	Median	Upper quartile	Max
Cobas ON ACR (µg/mg)	20 (59%)	0.72	1.47	3.68	14.53	27.04
Cobas ON AER (µg/min)	20 (59%)	2.78	11.02	27.55	98.97	360.61
Fitz ON ACR (µg/mg)	34	0.55	1.46	4.07	17.08	41.6
Fitz ON AER (µg/min)	34	2.99	10.02	25.47	99.74	554.67
Fitz Spot ACR (µg/mg)	34	0.72	1.65	3.70	19.71	47.40

Table 15 Cobas and Fitzgerald assay results of overnight urine sample and a spot urine sample (AER and ACR values).
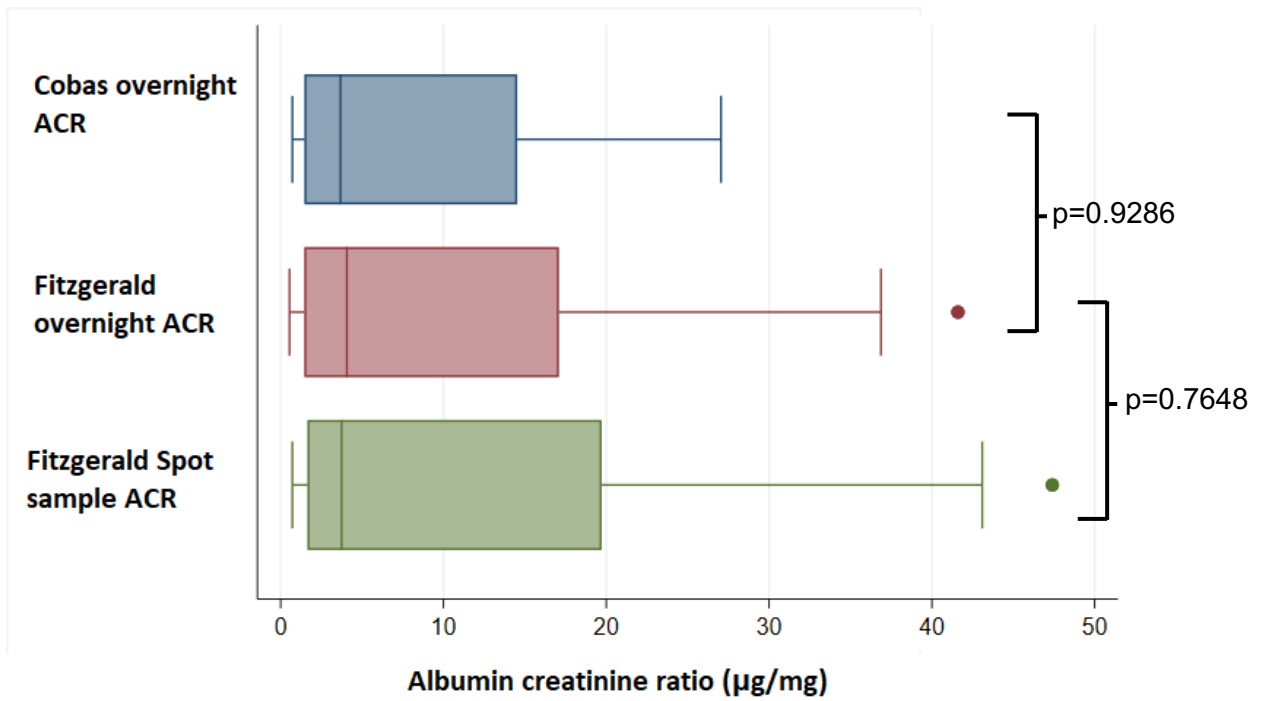
Figure 49 The spread of Cobas overnight (n=20), Fitzgerald overnight (n=34) and Fitzgerald spot urine sample (n=34) albumin creatinine ratios.
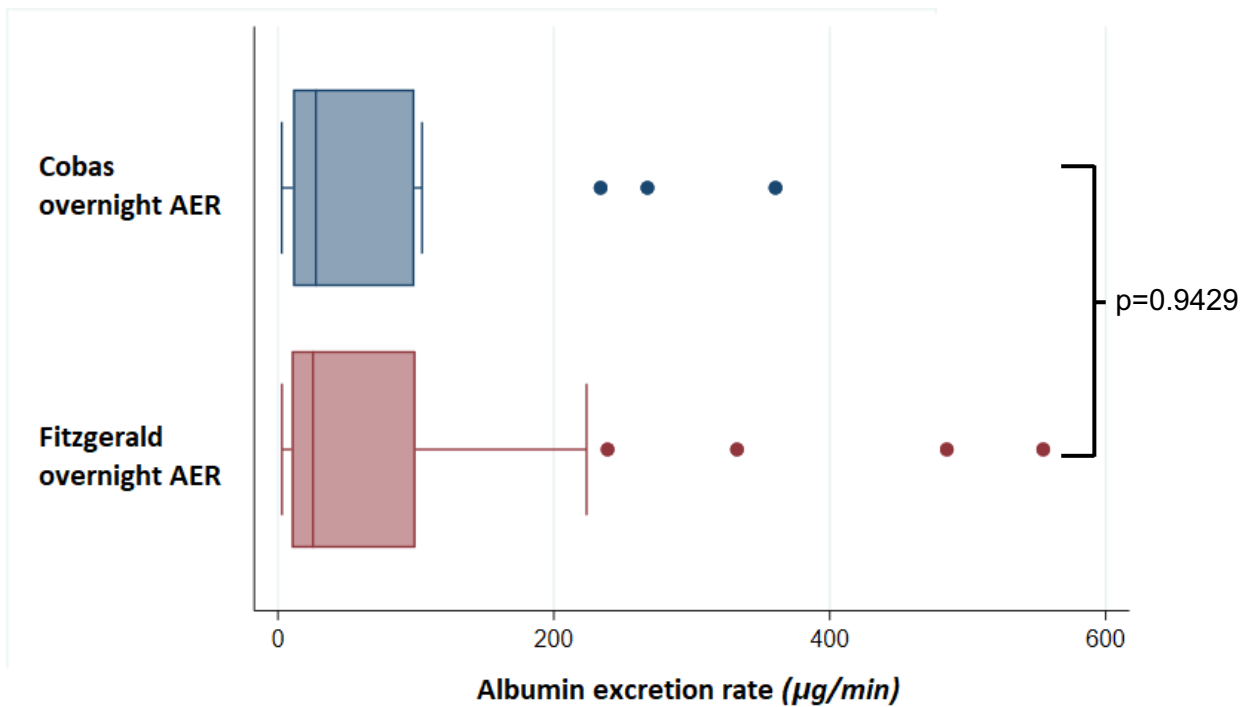
Figure 50 The spread of Cobas overnight (n=20) and Fitzgerald overnight (n=34) albumin excretion rates.

4.5.2.2 Urinary albumin excretion and 24-hour, day and night blood pressure parameters

Firstly, blood pressure parameters were compared to urinary albumin using the Cobas albumin excretion rate (see table 16). Only 20 (59%) patients had detectable albumin levels using the Cobas assay. This analysis showed no evidence of a significant relationship between blood pressure and urinary albumin level. The Cobas assay was only able to quantify urine albumin concentration in 2 of 15 patients with normal albuminuria (13%).

	Cobas albumin excretion rate (µg/min)		
	Rs	p value	n=
24-hour SBP (mmHg)	0.1054	0.6582	20
day SBP (mmHg)	0.1036	0.6730	19
night SBP (mmHg)	-0.0360	0.8836	19
24-hour DBP (mmHg)	-0.0603	0.8006	20
day DBP (mmHg)	0.0088	0.9715	19
night DBP (mmHg)	0.0492	0.8414	19
24-hour PP (mmHg)	0.0196	0.9346	20
day PP (mmHg)	0.0079	0.9743	19
night PP (mmHg)	-0.0694	0.7777	19
24-hour MAP (mmHg)	0.0256	0.9146	20
day MAP (mmHg)	0.0880	0.7202	19
night MAP (mmHg)	0.1097	0.6547	19
24-hour PPP (mmHg*bpm)	-0.0120	0.9599	20
day PPP (mmHg*bpm)	0.0070	0.9773	19
night PPP (mmHg*bpm)	-0.0702	0.7753	19

Table 16 *Spearman correlation coefficients (Rs), p values and number of blood pressure parameters against Cobas albumin excretion rate in patients with type 2 diabetes. Units for PPP are mmHg times by beats per minute (bpm).*
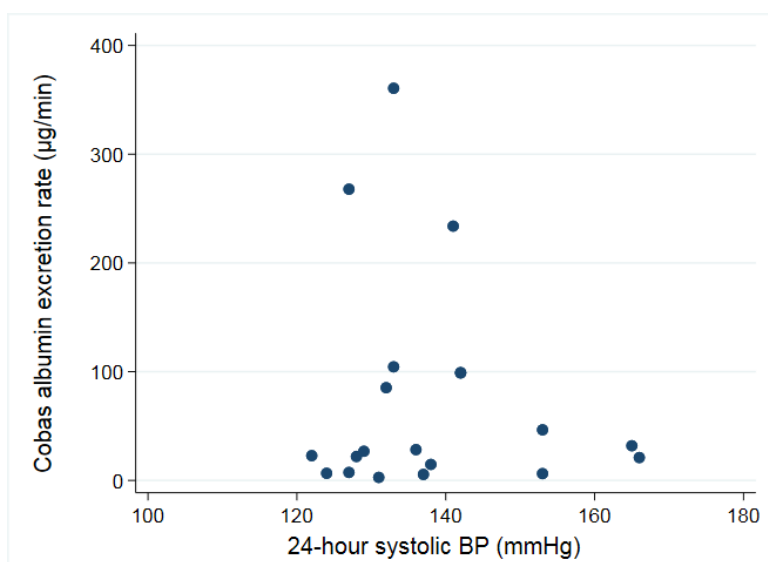
Figure 51 *A scatter graph showing the distribution of Cobas AER values by 24-hour systolic blood pressure in patients with type 2 diabetes n=20.*

When all patients' urinary albumin levels were detectable using the Fitzgerald assay, no correlations were observed between different BP parameters and albumin excretion rate or spot urine samples with the exception of 24 hour pulse pressure product (see table 17 and figure 52).

	Fitzgerald AER			Fitzgerald Spot urine ACR		
	Rs	p value	n=	Rs	p value	n=
24-hour SBP (mmHg)	0.2072	0.2397	34	0.0869	0.6250	34
day SBP (mmHg)	0.1755	0.3286	33	0.0246	0.8919	33
night SBP(mmHg)	0.1720	0.3384	33	0.1272	0.4806	33
24-hour DBP(mmHg)	0.0346	0.8458	34	-0.1277	0.4717	34
day DBP(mmHg)	0.0040	0.9823	33	-0.1536	0.3933	33
night DBP(mmHg)	0.0909	0.6149	33	-0.0082	0.9639	33
24-hour PP(mmHg)	0.2398	0.1719	34	0.1861	0.2920	34
day PP(mmHg)	0.2916	0.0997	33	0.2082	0.2449	33
night PP(mmHg)	0.1966	0.2728	33	0.1896	0.2906	33
24-hour MAP(mmHg)	0.1184	0.5049	34	-0.0303	0.8648	34
day MAP(mmHg)	0.0773	0.6688	33	-0.0862	0.6334	33
night MAP(mmHg)	0.1981	0.2692	33	0.1490	0.4078	33
24-hour PPP (mmHg*bpm)	0.2905	0.0956	34	0.3665	0.0330	34
day PPP (mmHg*bpm)	0.2717	0.1261	33	0.3189	0.0705	33
night PPP (mmHg*bpm)	0.2066	0.2488	33	0.2689	0.1302	33

Table 17 Spearman correlation coefficients (Rs), p values and number of blood pressure parameters versus Fitzgerald albumin excretion rate and Fitzgerald Spot urine ACR in patients with type 2 diabetes.
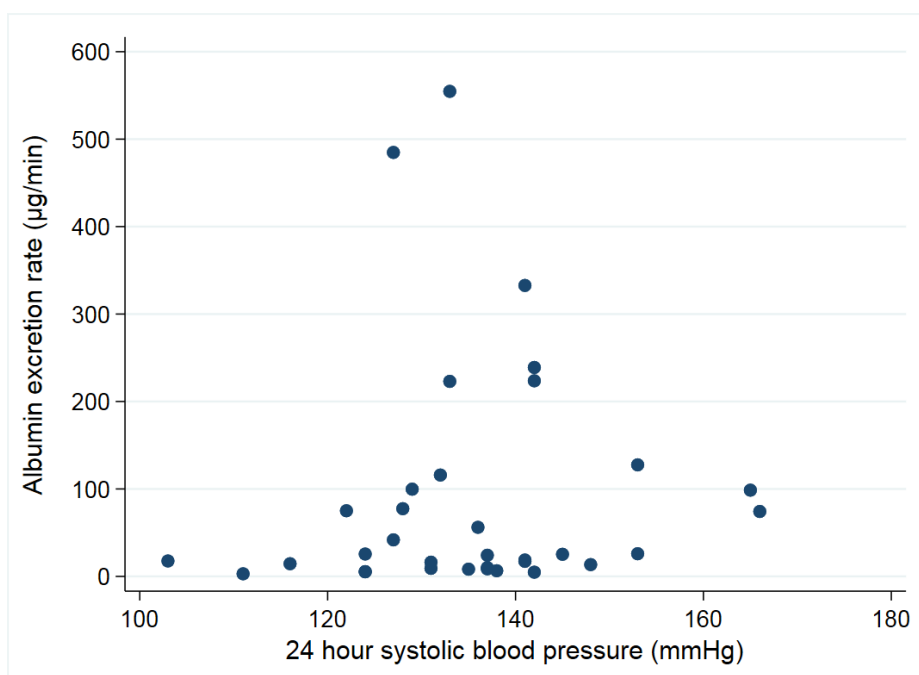
Figure 52 A scatter graph showing the distribution of Fitzgerald AER values by 24-hour systolic blood pressure in patients with type 2 diabetes, n=34.

As cardiovascular risk differs with level of albuminuria, to further analyse these relationships the cohort was split into groups of normal albuminuria and moderately increased albuminuria. This analysis showed no significant relationships (see table 18).

	Normal albuminuria			Moderately increased albuminuria		
	Rs/r	p value	n=	Rs/r	p value	n=
24-hour SBP (mmHg)	0.1201	0.6699	15	-0.0228	0.9260	19
day SBP (mmHg)	0.0877	0.7559	15	0.0145	0.9546	18
night SBP (mmHg)	0.0877	0.7561	15	-0.1302	0.6067	18
24-hour DBP (mmHg)	0.2617	03462	15	-0.1319	0.5903	19
day DBP (mmHg)	0.2417	0.3855	15	0.0010	0.9967	18
night DBP (mmHg)	0.1614	0.5654	15	-0.1539	0.5420	18
24-hour PP (mmHg)	0.0734	0.7949	15	-0.0079	0.9743	19
day PP (mmHg)	0.0750	0.7905	15	-0.0559	0.8256	18
night PP (mmHg)	0.0682	0.8093	15	-0.0589	0.8165	18
24-hour MAP (mmHg)	0.1930	0.4908	15	-0.0896	0.7152	19
day MAP (mmHg)	0.1614	0.5654	15	0.0031	0.9903	18
night MAP (mmHg)	0.1199	0.6705	15	-0.1094	0.6655	18
24-hour PPP (mmHg*bpm)	-0.1964	0.4829	15	-0.1088	0.6576	19
day PPP (mmHg*bpm)	-0.0964	0.7325	15	-0.0898	0.7231	18
night PPP (mmHg*bpm)	-0.2964	0.2834	15	-0.1249	0.6215	18

Table 18 Correlation coefficients (Rs/r), p values and number of blood pressure parameters against Fitzgerald albumin excretion in patients with type 2 diabetes with normal albuminuria (<20µg/min) and moderately increased albuminuria (>20µg/min).

4.5.2.3 Urinary albumin excretion and blood pressure variability

Blood pressure variability (as assessed by standard deviation of BP) showed some relationship with the T2DM group. Night time diastolic BP variability was also associated with Cobas ACR (p=0.0458) (figure 53). Blood pressure variability was not significantly associated with Fitzgerald albumin excretion rate in the entire cohort (figure 54 and 56). Blood pressure variability was significantly associated with Fitzgerald AER for both night time systolic and diastolic blood pressure in those with an AER above 20 µg/min (moderately increased albuminuria) (figure 55 and 57). There was no relationship within the normal albuminuria group. There was no evidence of correlation between other BP variability parameters and Cobas ACR or AER.
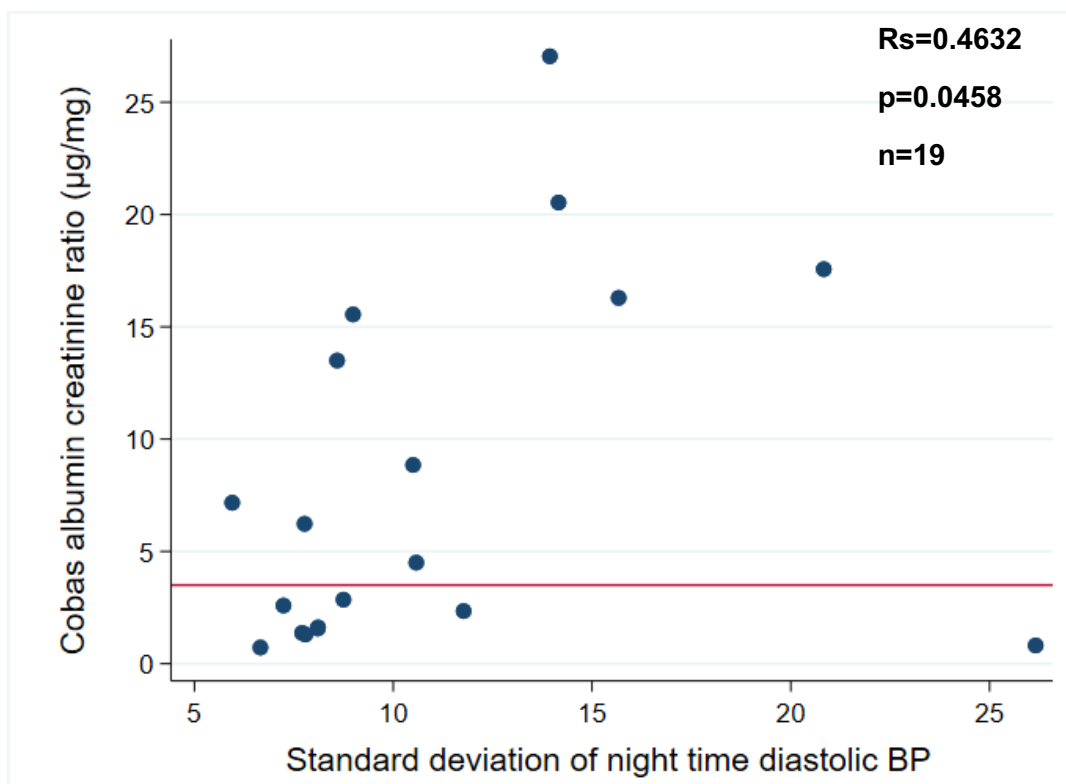
Figure 53 *The relationship between Cobas ACR and night time diastolic variability in patients with type 2 diabetes (n=19). Red line represents the threshold for moderately increased albuminuria (>3.5µg/mg).*

Entire cohort
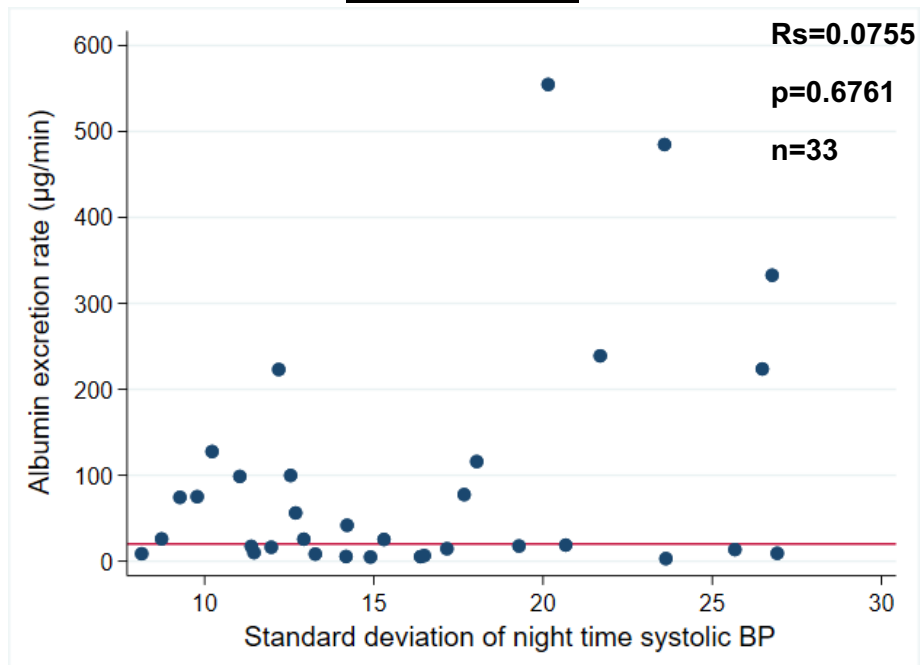
Figure 54 *Scatter diagram showing the standard deviation of night time systolic BP against Fitzgerald albumin excretion rate in patients with type 2 diabetes (n=33). Red line represents the threshold between normal albuminuria and moderately increased albuminuria (20μg/min).*

Moderately increased albuminuric patients
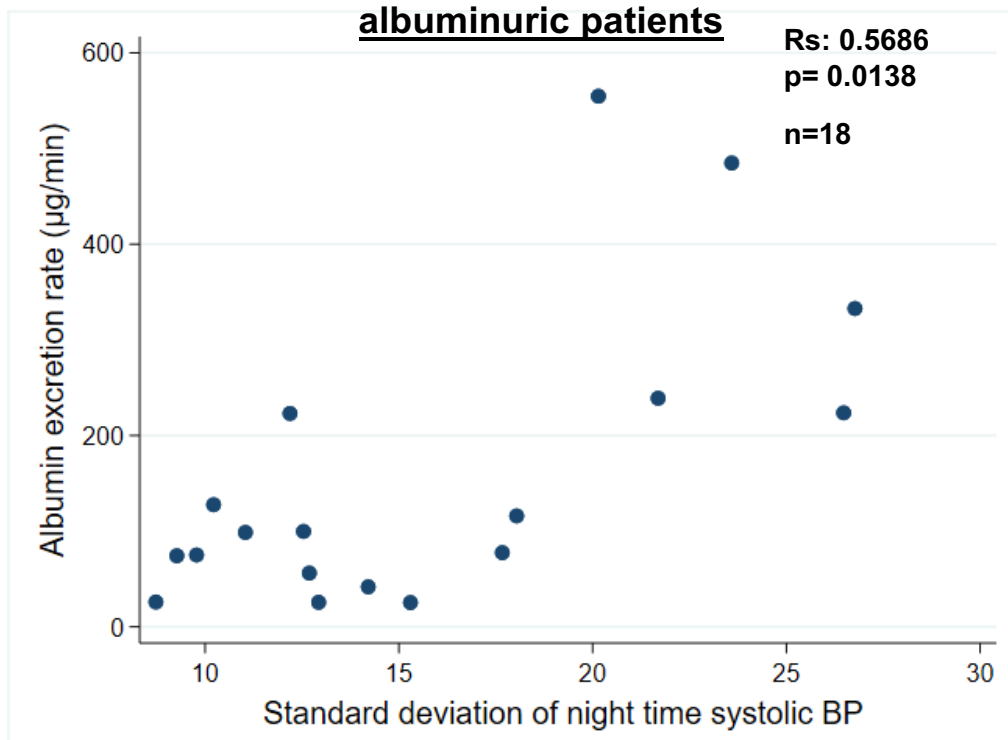
Figure 55 *The relationship between Fitzgerald AER and night time systolic variability in patients with type 2 diabetes with moderately increased albuminuria (>20μg/min) (n=18).*
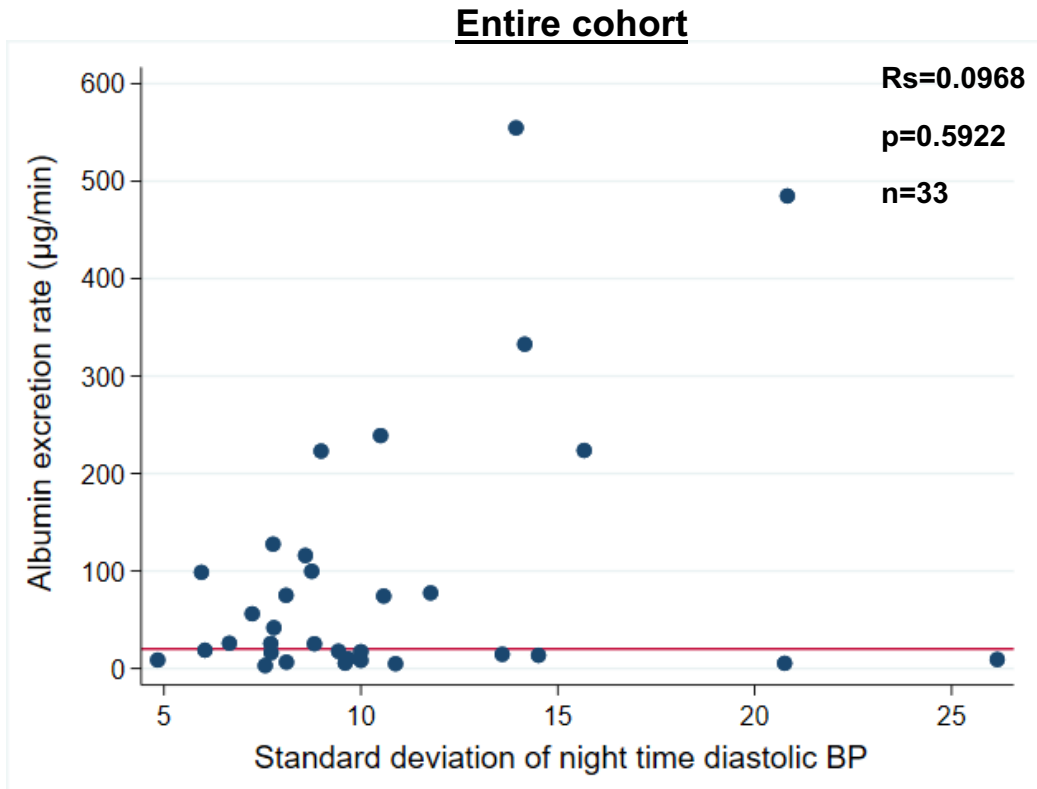
Figure 56 *Standard deviation of night time diastolic BP against Fitzgerald albumin excretion rate in patients with type 2 diabetes (n=33). Red line represents the threshold between normal albuminuria and moderately increased albuminuria (20µg/min).*
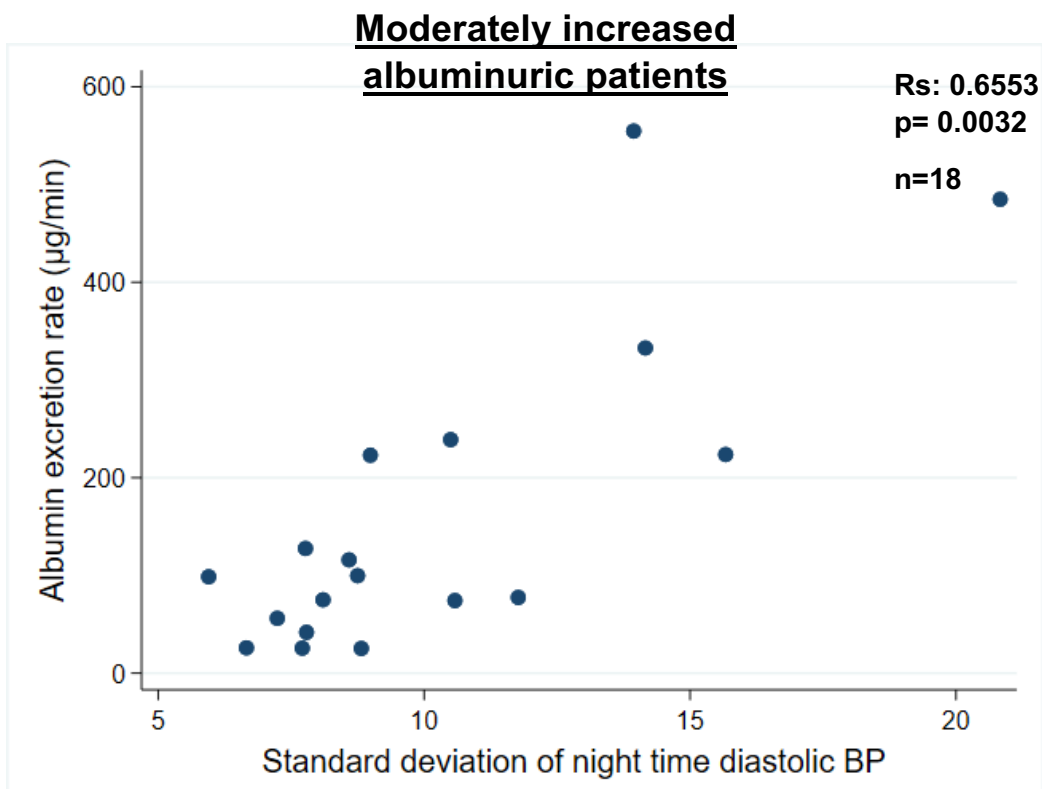
Figure 57 *The relationship between Fitzgerald AER and night time diastolic variability in patients with type 2 diabetes with MIA (>20µg/min) (n=18).*

4.5.2.4 Urinary albumin excretion and blood pressure circadian rhythms

No significant correlation was observed with either Cobas or Fitzgerald measures of albumin and non-dipping of different BP parameters (see table 19). An example relationship of Fitzgerald albumin excretion rate and day to night ratio of systolic BP is shown below in figure 58.
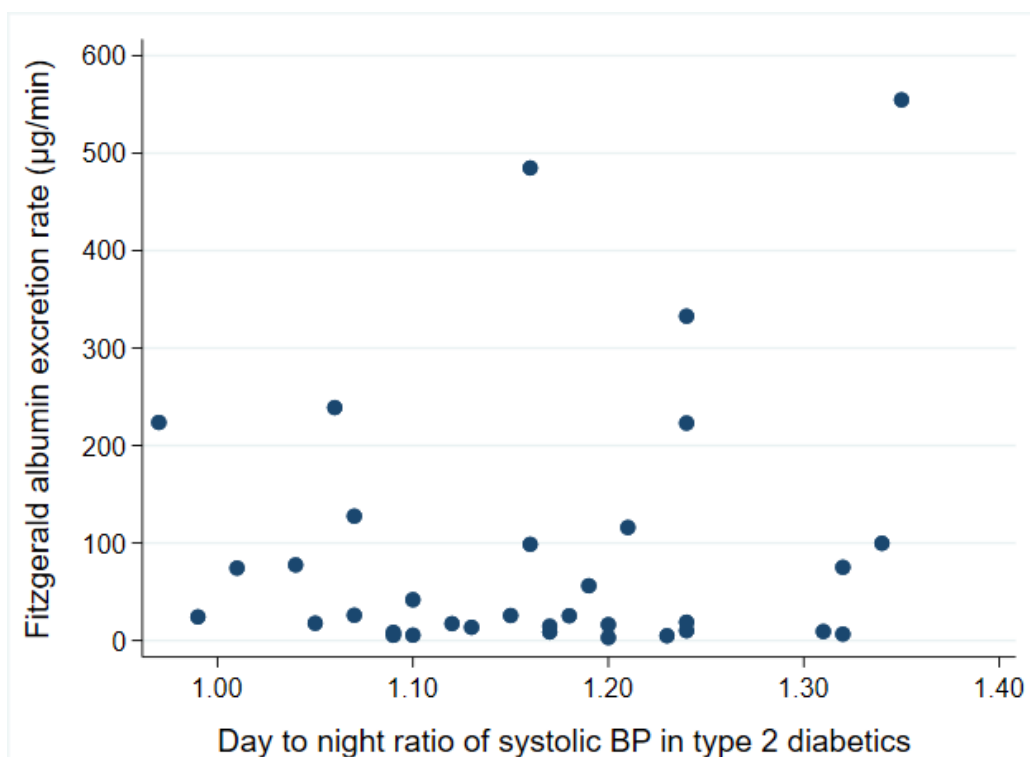
Figure 58 *The relationship between day to night ratio of systolic BP and Fitzgerald AER in patients with type 2 diabetes (n=34).*

	Fitzgerald AER			Spot urine Fitzgerald ACR		
	Rs	p value	n=	Rs	p value	n=
SBP D:N	-0.0320	0.8575	34	-0.0751	0.6729	34
DBP D:N	-0.1351	0.4462	34	-0.1409	0.4267	34
MAP D:N	-0.0946	0.6006	34	-0.1213	0.5012	34
PP D:N	0.0699	0.6991	34	0.0381	0.8332	34
HR D:N	-0.1407	0.4348	34	-0.0702	0.6979	34
DP D:N	-0.1223	0.4977	34	-0.1237	0.4929	34
PPP D:N	-0.0187	0.9177	34	-0.0003	0.9985	34
Morning surge (mmHg)	-0.1629	0.3812	31	-0.1222	0.5126	31

Table 19 *Spearman correlation coefficients of Fitzgerald AER and Spot urine Fitzgerald ACR and day to night ratios of blood pressure in patients with type 2 diabetes.*

The relationships were also analysed by splitting the cohort into groups of non-dippers, normal dippers and extreme dippers. As also described in Chapter 3.5.3.3 normal dipping values for diastolic BP and other BP parameters were calculated by comparison to systolic BP day to night ratio (see figure 59 below). When split into non-dipper, normal dipper and extreme dipper categories, the pathological non-dipper and extreme dipper categories showed no statistically significant differences (see figure 60 and 61). Statistical analysis was only performed to assess the difference between normal dippers and the abnormal non-dippers and extreme dippers. The non-dipper and extreme dipper groups were not statistically compared as this was not a component of the original research question.
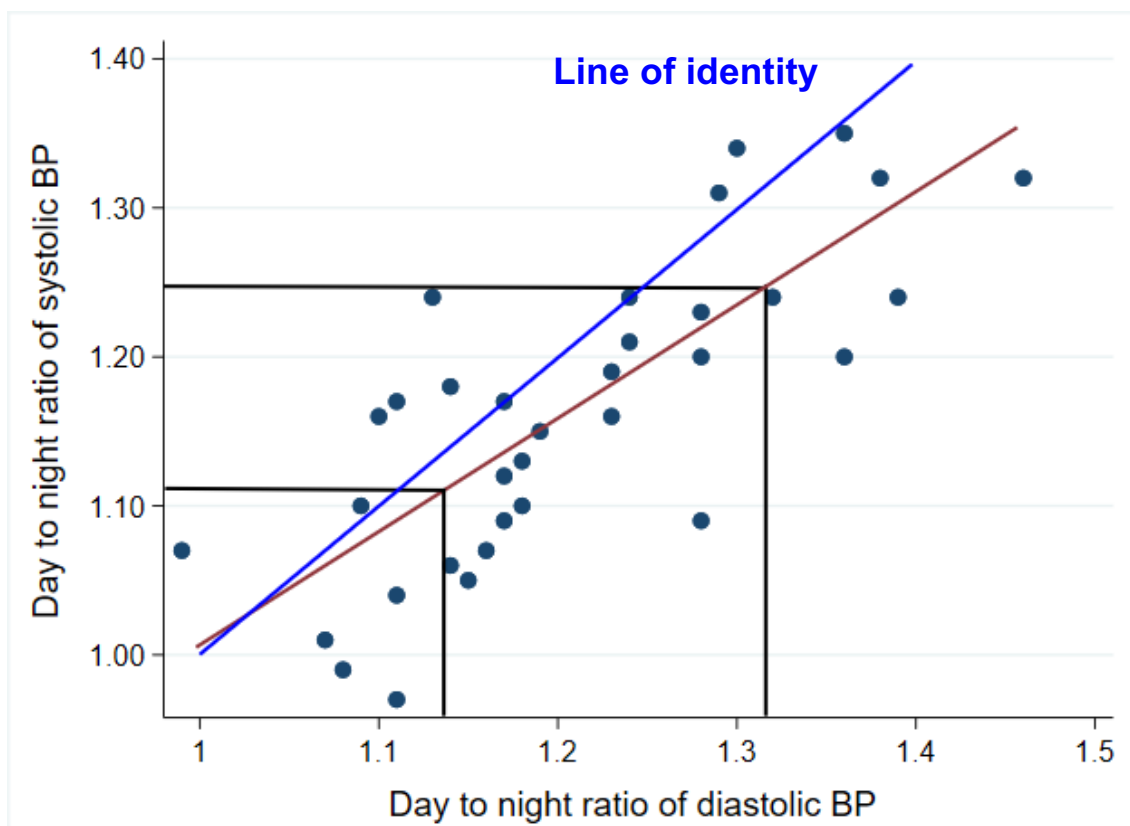
Figure 59 *The method of calculating the normal range for diastolic night time dipping (1.14-1.32) through comparison to systolic day to night ratio (normal range 1.11-1.25) in the type 2 diabetic cohort. Red line represents the linear regression of each variable, the blue line represents the line of identity)*

Day to night ratio of systolic BP
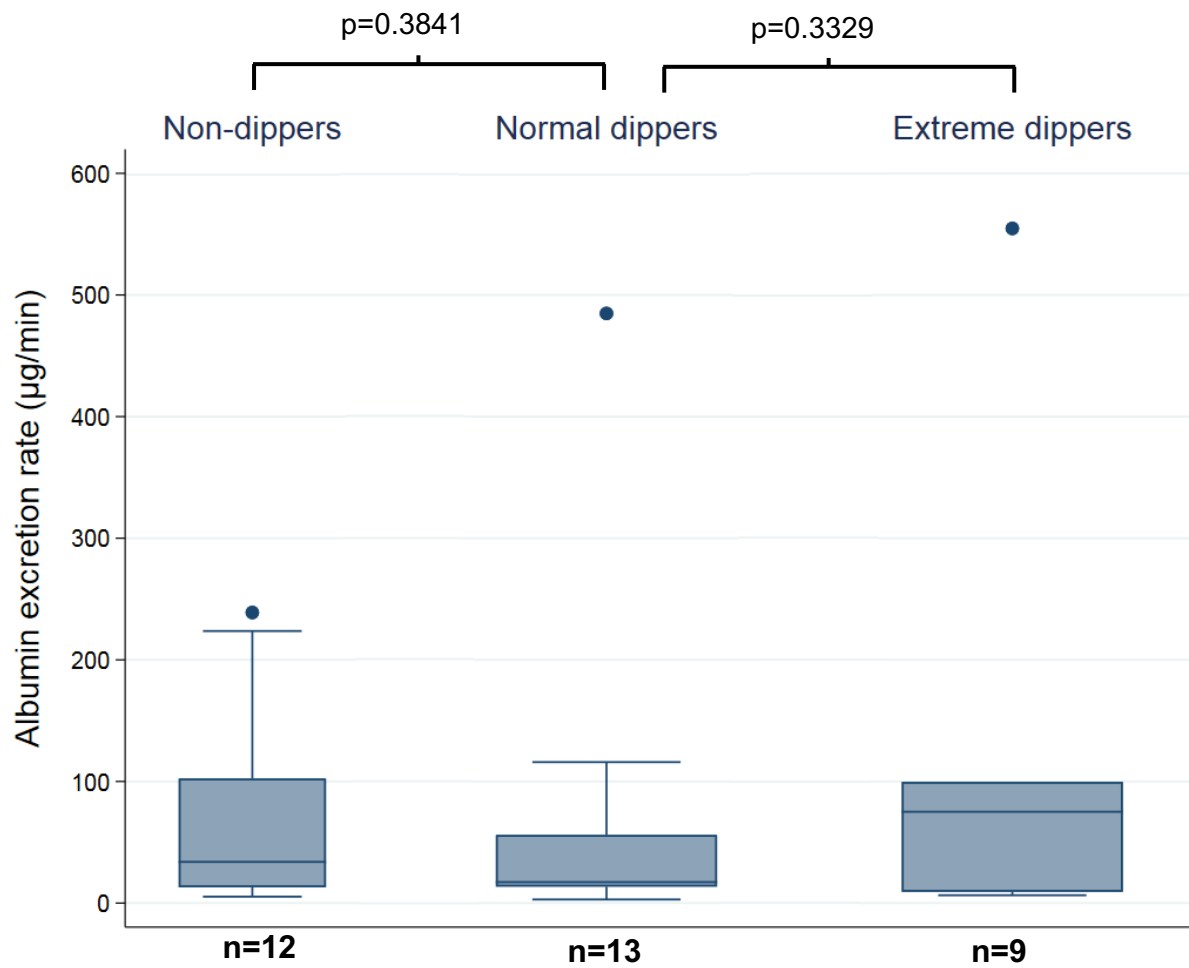
Figure 60 *The spread of Fitzgerald AER values of patients who displayed non-dipping (median: 33.9, IQR: 13-102.5µg/min), normal dipping (median: 17.3, IQR 13.5-56.2µg/min) and extreme dipping (median:75.1, IQR:9.2-99.7µg/min) of systolic BP.*

Day to night ratio of diastolic BP
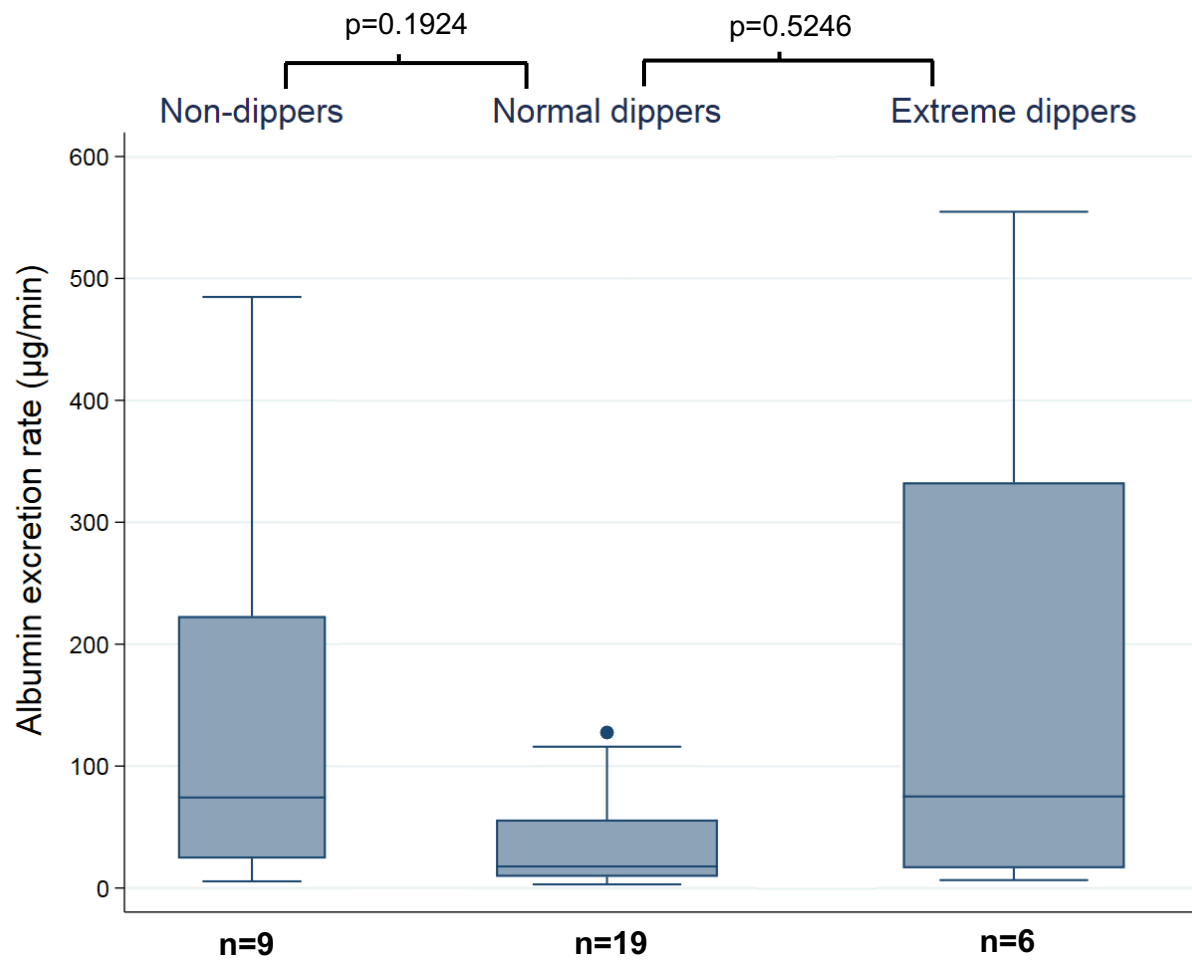
Figure 61 *The spread of Fitzgerald AER values of patients who displayed non-dipping (median: 74.3, IQR: 24.2-223µg/min), normal dipping (median: 17.7, IQR: 9.2-56.2µg/min) and extreme dipping (median: 75.1, IQR: 16.2-332.7µg/min) of diastolic BP.*

Morning surge of systolic BP also showed no relationship with AER **(p=0.3812)** as shown in the graph below (figure 62).
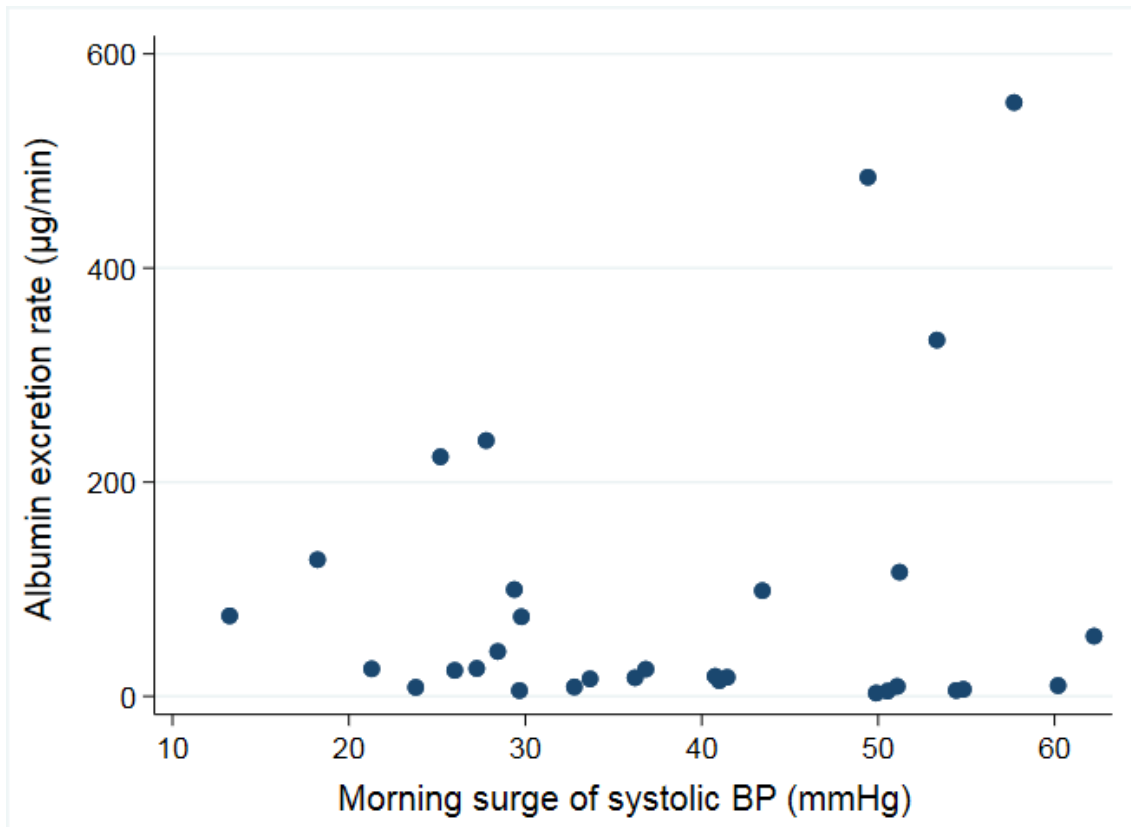
Figure 62 *A scatter graph of the relationship between morning surge and Fitzgerald albumin excretion rate in patients with type 2 diabetes (n=31).*

4.5.3 Objective 2: Assess whether the different aspects of 24-hour blood pressure relate to glycocalyx integrity.

There were no statistically significant correlations with different aspects of 24-hour blood pressure and glycocalyx integrity. The T2DM group had an **average PBR 5-25 of 1.91 (±0.17)** and an **average PBR 5-9 of 1.05 (±0.05)**. Glycocalyx integrity was also compared to clinic BP measurements taken the same morning as the GlycoCheck measurement. Clinic BP showed a borderline significant relationship between PBR 5-25 and diastolic BP (see figure 63), however when only the smallest vessels were examined the relationship was lost (see figure 64). Clinic systolic and mean arterial pressure did not relate to PBR 5-25 (see table 20).

	PBR 5-25	n=	PBR 5-9	n=
24-hour systolic BP (mmHg)	r=-0.209, p=0.9161	29	r=0.0620, p=0.7540	29
Day systolic BP (mmHg)	r=-0.0358, p=0.8563	28	r=0.0593, p=0.7643	28
Night systolic BP (mmHg)	r=-0.0441, p=0.8238	28	r=0.0591, p=0.7651	28
24-hour diastolic BP (mmHg)	r=0.2049, p=0.2956	29	r=0.0398, p=0.8407	29
Day diastolic BP (mmHg)	r=0.1562, p=0.4274	28	r=0.0597, p=0.7629	28
Night diastolic BP (mmHg)	r=0.2085, p=0.2869	28	r=-0.0241, p=0.9032	28
24-hour pulse pressure (mmHg)	r=-0.1797, p=0.3601	29	r=0.0617, p=0.7552	29
Day pulse pressure (mmHg)	r=-0.1803, p=0.3586	28	r=0.0439, p=0.8246	28
Night pulse pressure (mmHg)	r=-0.1912, p=0.3297	28	r=0.0957, p=0.6281	28
Night systolic BP variability (mmHg)	r=-0.3316, p=0.0847	28	r=0.0146, p=0.9413	28
Night diastolic BP variability (mmHg)	r=-0.2157, p=0.2702	28	r=0.0807, p=0.6830	28
Systolic BP Day:Night ratio	r=0.0635, p=0.7484	28	r=0.0177, p=0.9287	28
Diastolic BP Day:night ratio	r=-0.0821, p=0.6777	28	r=0.0913, p=0.6440	28
Clinic systolic BP (mmHg)	r=0.0737, p=0.7039	29	r=0.0035, p=0.9857	29
Clinic diastolic BP (mmHg)	r=0.3524, p=0.0608	29	r=0.0193, p=0.9209	29
Clinic mean arterial pressure (mmHg)	r=0.2767, p=0.1462	29	r=0.0148, p=0.9392	29

Table 20 *Pearson correlation coefficients between blood pressure parameters and PBR 5-25 and PBR 5-9.*
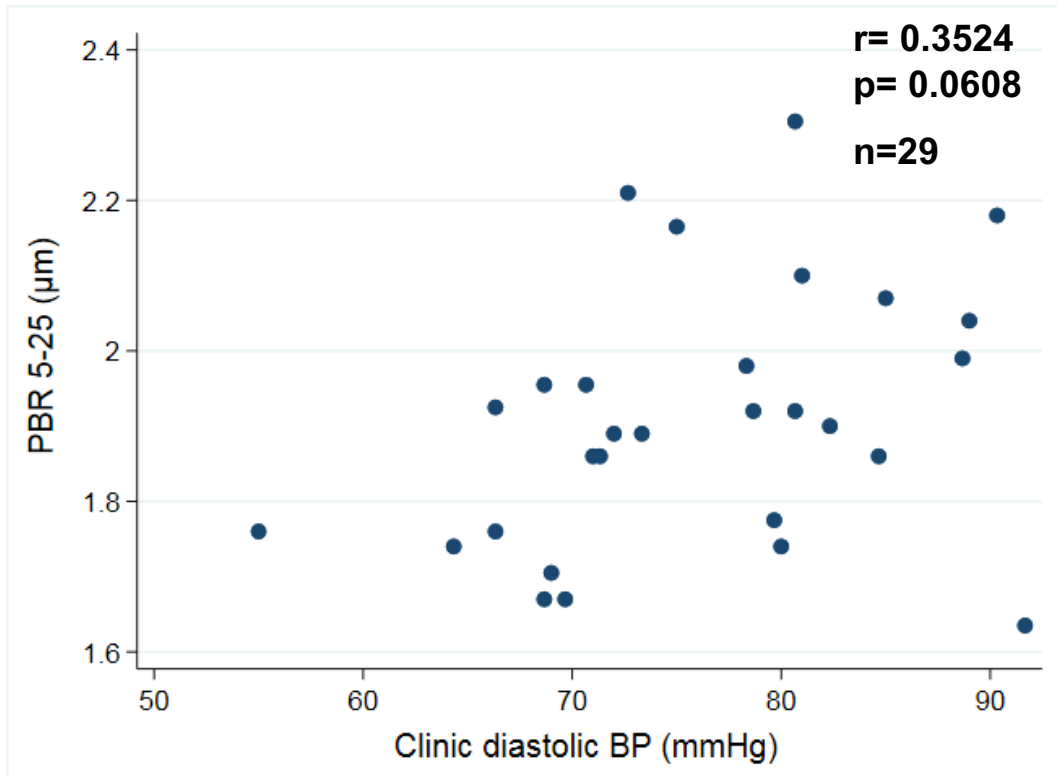
Figure 63 Scatter graph showing clinic diastolic blood pressure against PBR 5-25.
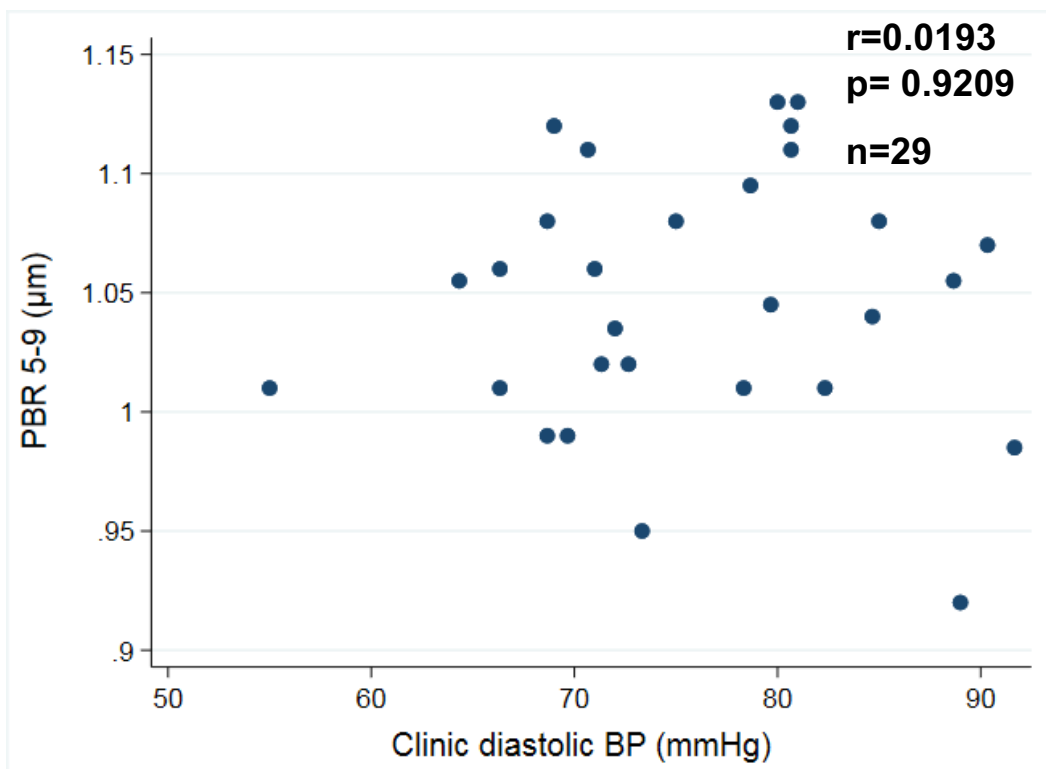
Figure 64 Scatter graph showing clinic diastolic blood pressure against PBR 5-9.

In terms of the variability of blood pressure, negative associations, which just failed to reach significance, were present for diastolic and systolic BP variability within the larger blood vessels (PBR 5-25) (see figures 65 and 66).
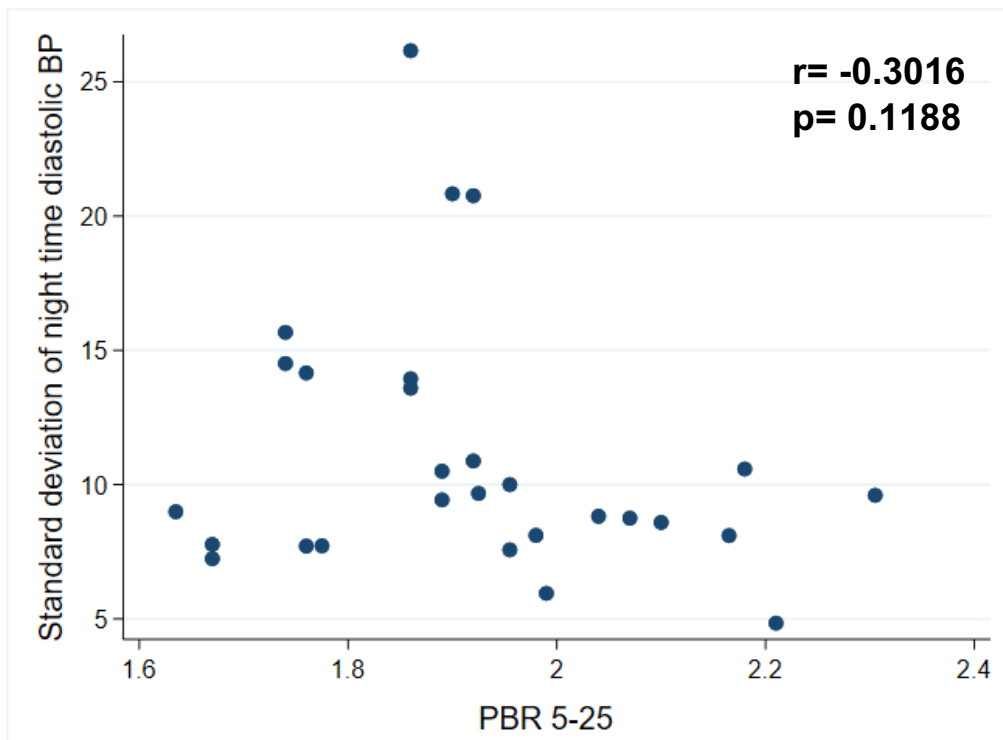
Figure 65 *Scatter graph showing standard deviation of night time diastolic blood pressure against PBR 5-25, n=28.*
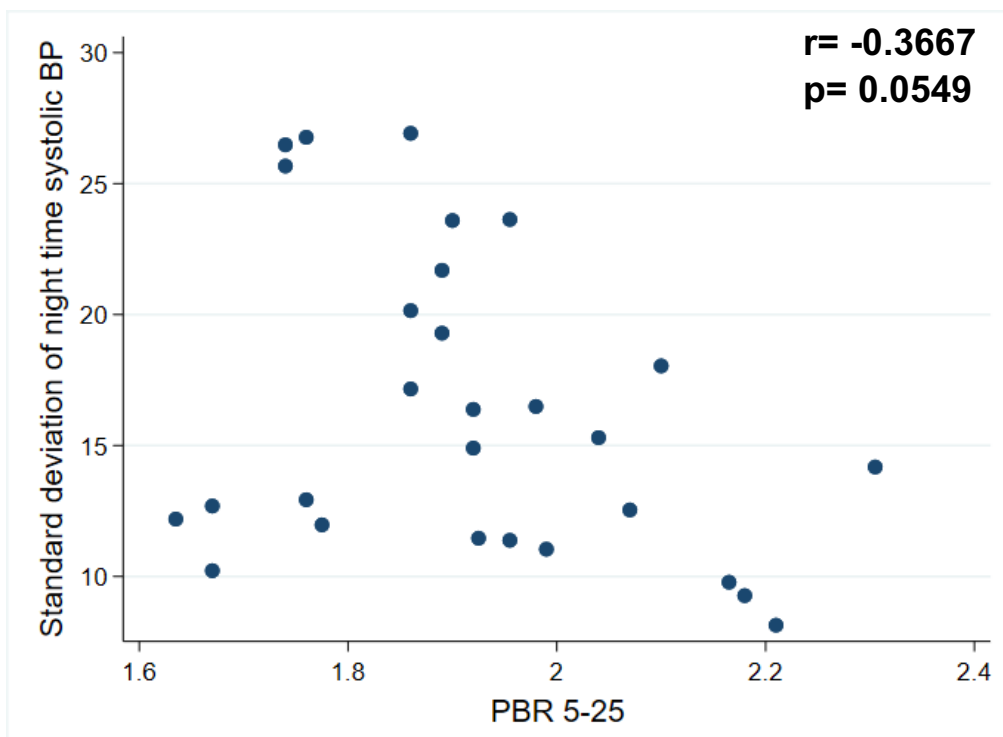
Figure 66 *Scatter graphs showing standard deviation of night time systolic blood pressure against PBR 5-25, n=28.*

4.5.4 Objective 3: Assess whether glycocalyx integrity is associated with albuminuria

Glycocalyx integrity showed no statistically significant correlation with any measure of urinary albumin excretion (see table 21, figure 67 and figure 68).

	PBR 5-25	n=	PBR 5-9	n=
Cobas ON ACR	Rs= -0.1767, p=0.5126	15	Rs=-0.0074 p=0.9782	15
Cobas ON AER	Rs= -0.2809, p=0.3106	15	Rs=-0.1209, p=0.6677	15
Fitzgerald Spot ACR	Rs=-0.2799, p=0.1491	28	Rs=-0.1320, p=0.5033	28
Fitzgerald ON ACR	Rs=-0.3369, p=0.0796	28	Rs=-0.3018, p=0.1186	28
Fitzgerald ON AER	Rs=-0.2996, p=0.1214	28	Rs=-0.3111, p=0.1071	28

Table 21 showing correlation coefficients and p values for different urine albumin measures against PBR 5-25 and PBR 5-9.
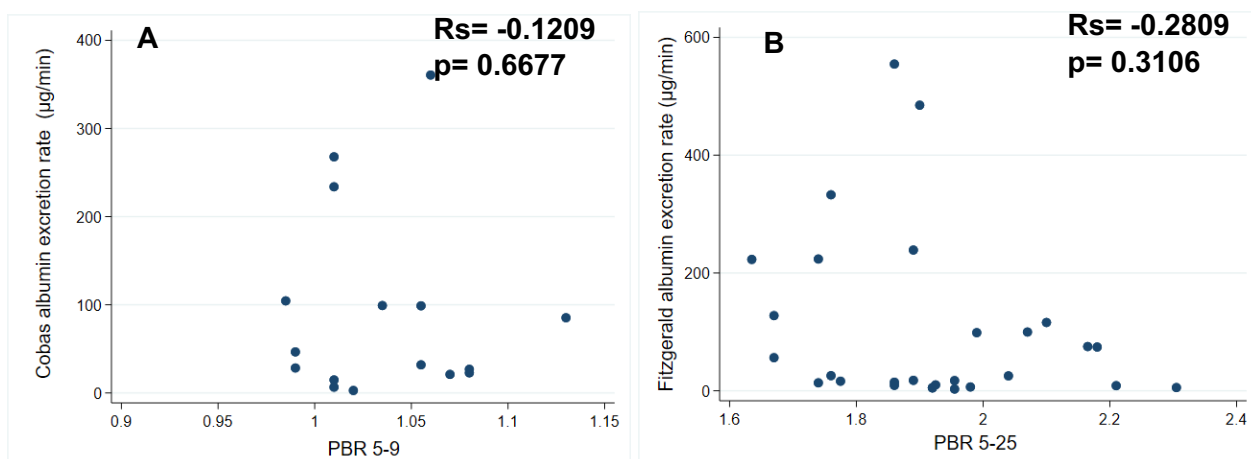
Figure 67 Scatter graphs showing Cobas albumin excretion rate against PBR 5-9 (A, n=15) and PBR 5-25 (B, n=15).
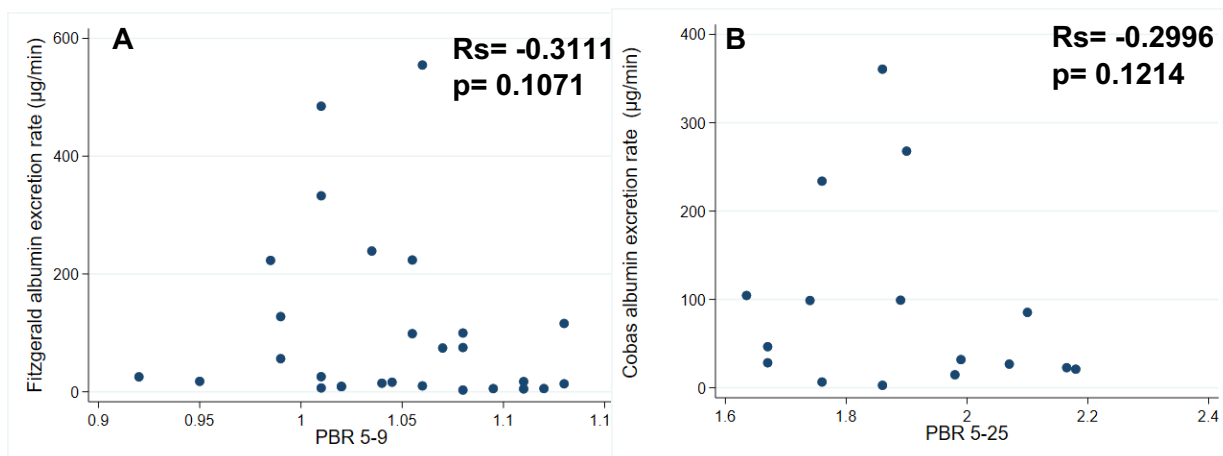
Figure 68 Scatter graphs showing Fitzgerald albumin excretion rate against PBR 5-9 (A, n=28) and PBR 5-25 (B, n=28).

4.5.5 Objective 4: Assess how day to night blood pressure ratios relate to characteristics of diabetes

No relationships were observed between non-dipping BP patterns and diabetic markers with the exception of a borderline significant relationship between day to night ratio of heart rate and diabetes duration as shown in table 22 and figure 69. This was an additional hypothesis generating analysis and it is likely that this finding is chance due to multiple analyses.

	BMI (n=34)	HbA1c (n=32)	Diabetes duration (n=30)	Fasting glucose (n=30)	Body fat (%) (n=34)	Sudoscan (n=32)
DN systolic BP	Rs=0.1832 p=0.2996	Rs=-0.0402 p=0.8269	Rs=-0.1737 P=0.3586	Rs=-0.0211 p=0.9120	Rs=-0.0631 p=0.7229	Rs=0.1781 p=0.3295
DN diastolic BP	Rs=0.0455 p=0.7982	Rs=-0.0034 p=0.9853	Rs=-0.1129 p=0.5524	Rs=-0.0664 p=0.7273	Rs=-0.0654 p=-0.7132	Rs=0.0836 p=0.6492
DN heart rate	Rs=0.0772 p=0.6644	Rs=-0.2046 p=0.2614	Rs=0.3513 P=0.0570	Rs=-0.2152 p=0.2535	Rs=-0.0999 p=0.5739	Rs=-0.0959 p=0.6015
DN pulse pressure	Rs=0.2214 p=0.2082	Rs=0.0138 p=0.9404	Rs=-0.0412 p=0.8288	Rs=0.0102 p=0.9572	Rs=-0.0612 p=0.7312	Rs=0.1658 p=0.3645
DN double product	Rs=0.1779 p=0.3142	Rs=-0.1552 p=0.3963	Rs=0.1199 p=0.5279	Rs=-0.1869 p=0.3226	Rs=-0.0698 p=0.6947	Rs=0.0398 p=0.8288
DN pulse pressure product	Rs=0.2210 p=0.2091	Rs=-0.1383 p=0.4502	Rs=0.1208 p=0.5248	Rs=-0.1411 p=0.4571	Rs=-0.0533 p=0.7645	Rs=0.0820 p=0.6555
Morning surge (mmHg)	Rs=0.0863 p=0.6443	Rs=-0.2002 p=0.2977	Rs=-0.0478 p=0.8127	Rs=-0.2748 p=0.1654	Rs=-0.0520 p=0.7811	Rs=0.1333 p=0.4905

Table 22 Correlation coefficients and p values for day to night blood pressure and heart rate ratios and morning surge of systolic blood pressure against diabetic markers.
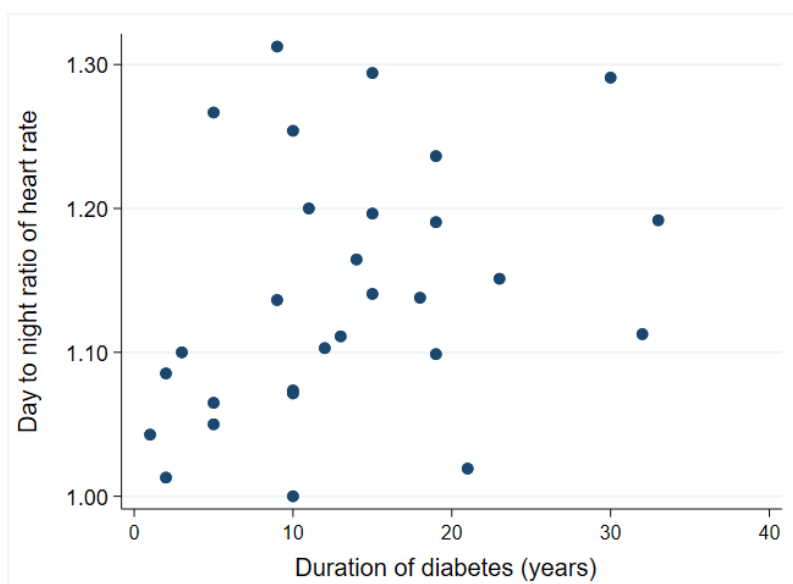
Rs= 0.3513
p= 0.0570

Figure 69 A scatter graph showing day to night ratio of heart rate against duration of diabetes, n=30.

4.6 Discussion of the BEAT-DKD Study

4.6.1 Discussion

4.6.1.1 Quantification of urinary albumin excretion

The analysis of different collection methods and assays used to measure urinary albumin excretion in this cohort showed similar results to the DASHER cohort of patients, the results of which are discussed in detail in chapter 3.6.1. The Cobas assay results did not differ significantly from the Fitzgerald assay results in this cohort but again, the Cobas assay was unable to quantify urinary albumin in a large proportion of patients with normal albuminuria (87%) and so cannot be used to meaningfully investigate albuminuria in this group of patients or the general healthy population. In this cohort, the spot urine sample results were not statistically different from the overnight sample. This may be as the spot sample was a first morning void. First morning void is likely to be similar to the overnight urine samples as it is produced during the asleep period and will be subject to much of the same conditions as the overnight urine sample. The first morning void is also less subject to activity related variation of albumin excretion. For these reasons and as demonstrated by these results and other studies, first morning voids are preferable to random spot samples unless the question one is seeking to address requires the effect of activity on urinary albumin to be taken into account [154, 155].

4.6.1.2 Urinary albumin excretion and 24-hour blood pressure parameters

Before examining the relationship between circadian blood pressure rhythms and albuminuria, this report will consider how standard blood pressure parameters affected albumin excretion. This cohort showed no correlation between urinary albumin excretion and systolic or diastolic blood pressure, mean arterial pressure or pulse pressure which is contrary to the literature. It may be that blood pressure is not the largest factor influencing albuminuria within this cohort and so the relationship is masked due to other diabetic

pathophysiology. For example, a relationship between blood pressure and AER in this cohort may be unobservable due to the small sample size (n=34) and a lower range of blood pressure values (100-165mmHg). Another factor which could be affecting this relationship in this cohort is that 23 (68%) patients, were receiving anti-hypertensive treatment. Of these individuals receiving anti-hypertensive treatment, the vast majority of patients were receiving either ACE inhibitors or ARBs as part of their treatment. This treatment is likely to affect the relationship between blood pressure and albumin excretion due to their mechanism of action on the glomerulus, as described in chapter 1. This relationship of blood pressure parameters to AER in this cohort will be discussed further in chapter 5, in comparison to the DASHER study results.

Night time variability of blood pressure did show correlation with albumin excretion in the diabetic cohort, but only within those with moderately increased albuminuria. This relationship may only be observable during the night time period due to the lack of external influencers which are usually present during waking hours. Night time BP variability may therefore be more representative of an individual's basal BP variation and allow easier visualisation of relationships to variability in smaller sample sizes. This finding is supported by several other studies which have shown the importance of blood pressure variability in albumin excretion and renal damage [149, 156].

It is likely that the pathophysiology of increased blood pressure variability may involve increased fasting blood glucose, cardiac autonomic neuropathy and arterial stiffness and that some night time sympathetic arousal may be increased in diabetic patients with increased BP variability [156]. BP variability has also been shown in other studies to be informative of renal disease progression, independent of average BP [149]. However, it is uncertain whether BP variability directly leads to increased albumin excretion or whether BP variability and albuminuria are both concomitant signs of a worsening disease progression.

Higher night time BP variability has also been shown to indicate risk of cardiovascular events [156] and increasing renal atherosclerotic damage [157]. Interestingly, in this cohort, glycocalyx integrity (a marker of vessel endothelium health) showed a trend of negative correlation with night time diastolic BP variability and 24-hour systolic variability. This could suggest that as night time variability increases, glycocalyx integrity improves. This could indicate an

interesting relationship between blood pressure variability and glycocalyx regeneration. It may be that there is a physiological compensation in glycocalyx regeneration in response to increased blood pressure variability and hence shear stress on the glycocalyx. However these results did not reach statistical significance and the correlation may well be due to chance, this is supported by there being no observable relationship between glycocalyx integrity and other BP variability parameters. The relationship was also only observable within the entire range of blood vessels including larger blood vessels (PBR 5-25).

4.6.1.3 Day to night blood pressure ratios and urinary albumin excretion

When considering day to night variation of blood pressure (dipping, non-dipping and extreme-dipping) the data showed no linear correlations. When day to night ratio was split into the three groups there were also no statistically significant differences between groups. Non-dippers and extreme dippers seemed to have higher average albumin excretion than 'normal' dippers across all parameters, demonstrating the U-shaped curve which you would expect to see should non-dipping and extreme dipping relate to worsening albuminuria. Again, these results did not reach statistical significance and do not provide strong support for the theory that non-dipping and extreme dipping demonstrate relationships with increased albumin excretion in type 2 diabetes. The lack of statistical significance could be due to under powering as a result of splitting the cohort in three.

In an attempt to gain insight into the why these BP patterns may develop in type 2 diabetes, day to night ratios were compared to different markers of diabetes. If loss of normal day to night ratios are a complication of type 2 diabetes such as autonomic neuropathy it is likely to be more prevalent in those who have been subjected to hyperglycaemia for a longer period or with evidence of other diabetic complications. In this cohort, day to night ratios showed no relationship with Sudoscan results (a measure of small peripheral nerve damage). It would be expected that if patients had cardiac autonomic neuropathy manifesting as an abnormal day to night BP ratio, they may also show signs of peripheral neuropathy [71].

4.6.1.4 Glycocalyx integrity, blood pressure and urinary albumin excretion

Beyond the previously discussed relationship with BP variability, glycocalyx integrity showed no significant relationship with albumin excretion or any blood pressure parameter including day to night blood pressure ratios. This suggests that the glycocalyx integrity is not involved in the mechanism behind the relationship between increased albumin excretion and blood pressure. One study which focussed on mathematically modelling the glycocalyx suggested that the porosity of the glycocalyx, rather than thickness, exerted more of an effect on the vessel wall permeability and therefore albumin leakage [158]. This could be an explanation for the lack of relationship shown in this study. The lack of correlation between ambulatory blood pressure and glycocalyx integrity may be influenced by the different time courses of the two measurements. This is supported by the borderline significant relationship between glycocalyx integrity and clinic diastolic blood pressure measurement of the same morning. As the glycocalyx is constantly degraded and reformed as it is sheared by blood flow, to properly assess the relationship between glycocalyx and blood pressure, the two would need to be measured simultaneously. Simultaneous measurement of markers of glycocalyx shedding would provide further insight.

4.6.2 Limitations

This study had several limitations. Firstly, this cohort is representative of older diabetic patients and so the results may not be relevant to younger people with diabetes. The cohort also had a male predominance so may not represent the general population. Due to time constraints of this project only 34 patients were recruited into this study which meant more modest correlations may not have been identified. This was particularly relevant when the cohort was split into groups as became necessary through analysis. This project used data from the BEAT-DKD study and so was not specifically designed for the purpose of this research. The overnight urine samples used to calculate AER with the highly sensitive albumin assay were taken on a different day to the overnight ambulatory monitoring potentially weakening the association between the two measures. Simultaneous urine and blood pressure measurements would have been a more robust design. No tests were undertaken as a measure of cardiac autonomic neuropathy. In addition to this there was no control group to compare this data to as the control group recruitment to BEAT-DKD had only 4 subjects at the deadline for data analysis within this thesis.

Due to the multiple statistical analyses performed, one off significant findings should be interpreted with caution, such as the relationship between duration of diabetes and day to night heart rate ratio are likely due to chance

4.6.3 Implications for future work

These results suggest the need for a larger study of people with diabetes to increase the power across each dipping group. Although several studies have looked at BP dipping in diabetes, they have not used a highly sensitive albumin assay and so can only make assumptions about the normal albuminuria group of patients. These assumptions are confounded by assigning a common "undetectable" value to values which cannot be measured, which I have shown to give a significantly different data set to the Fitzgerald assay where all values are detectable. These data were inconclusive as to whether non-dipping patterns relate to increased urine albumin levels in the diabetic population. The main area for future work should be to further understand the mechanism behind the development of non-dipping patterns. This would involve detailed analysis of diabetic markers and autonomic function alongside several ambulatory blood pressure measurements. If there is a relationship between abnormal dipping and increased AER but it is due to both being concomitant signs of diabetic pathology and not a direct causal relationship, then BP dipping may not be a beneficial treatment target. This may explain why studies aiming to reverse abnormal dipping have not yet found substantial effects on AER.

4.7 Conclusion

This type 2 diabetic cohort showed no statistically significant relationship between albumin excretion and systolic, diastolic or other standard components of BP. This is contrary to the literature and could be influenced by the narrow range of blood pressure values and ACE or ARB treatment of the majority of patients in the cohort. Night time BP variability in those with moderately increased albuminuria showed the strongest relationship with AER. This cohort showed no statistically significant relationship between dipping patterns and AER. However, this could be due to limited statistical power of each group when the cohort is split into three. Abnormal dipping patterns showed little relationship with diabetic markers apart from diabetes duration and so little can be drawn from these results about the pathophysiology of loss of normal dipping. Glycocalyx integrity was not related to average BP, dipping patterns or AER and is unlikely to be involved in the pathophysiology behind abnormal dipping or increased AER in this group. Further research is needed to understand the mechanism behind abnormal dipping and whether it is a relevant treatment target.

Chapter 5: Comparison of results from the DASHER and BEAT-DKD studies

5.1 Discussion

This chapter will discuss the similarities and differences between the results from both the hypertensive cohort and the T2DM cohort to explore the relationships between albuminuria, blood pressure and glycocalyx in these closely linked conditions.

5.1.1 Comparison of 24-hour blood pressure and AER in the T2DM and hypertensive cohorts

The two cohorts showed striking differences in how aspects of the patients' blood pressure related to albuminuria. Untreated hypertensives showed a correlation between urine albumin excretion and average systolic, diastolic, mean arterial blood pressure and pulse pressure but no other correlations and most strongly in those with higher levels of urine albumin. However all of these correlations were lost when the hypertensive patients were treated. The diabetic group (68% of whom were already receiving anti-hypertensive treatment) showed no relationship with any of these parameters, even within the moderately increased albuminuria group. These results were examined further to determine whether there are true differences in these relationships within the hypertensive and diabetic groups or whether the differences observed are due to sample size, range of albumin values, range of blood pressure values or anti-hypertensive treatment.

Firstly we will consider the spread of data within the untreated hypertensive, treated hypertensive and the T2DM groups. As illustrated by figures 70-72, which are taken from chapter 3 and 4, the range of average systolic blood pressure values of the diabetic group (100-165mmHg) spans the entire range of the treated hypertensives but does not quite reach the highest value of the untreated hypertensives (190mmHg).

It could be argued that the lack of relationship between urine albumin and blood pressure in the T2DM group is as a result of a narrower and lower range of blood pressure values. Fesler et al also showed that urine albumin excretion is unchanged by blood pressures below 140mmHg [159]. In this case, the T2DM cohort might have shown a similar spread of data to the treated hypertensive group. The treated hypertensive and T2DM groups have similar demographics. As well as this, 68% of the diabetic group were receiving anti-hypertensive treatment, the vast majority of which included an ACE inhibitor or ARB. Eighty-five percent of the treated hypertensive group were also taking an ARB. Despite the similar demographics, similar range of blood pressures and lack of correlation between AER and blood pressure, these two groups showed very different ranges of urine albumin. The highest urine AER observed in the treated hypertensive group was 32µg/min, whereas 56% of the T2DM group had an AER higher than 32µg/min the highest of which being 554µg/min. There was also no difference in AER between those receiving anti-hypertensive medication and those not receiving anti-hypertensive medication in the T2DM group (p=0.3643). Despite 50% of patients achieving target blood pressure, the T2DM group still exhibited very high levels of urinary albumin, this suggests that there are different factors contributing to albuminuria within the T2DM group that are not present within the treated hypertensive group.

The mechanism behind these high levels of urinary albumin in the T2DM group are likely to involve aspects of diabetic pathophysiology as described in chapter 1.3. Glomerular hyperfiltration was targeted by the use of ACE inhibitors and ARBs in many of the T2DM patients, therefore pathology of the glomerular filtration barrier may play a larger role in the increased albuminuria in this group. The measurement of glycocalyx showed no relationship to urine albumin excretion, suggesting that damage to the glycocalyx is not involved in the mechanism despite other studies suggesting its importance [36, 41, 160, 161]. This could mean that damage to other aspects of the glomerular filtration barrier such as the basement membrane, podocytes and slit diaphragm have a larger role in development of albuminuria in this group.

Hypertensive group pre-treatment
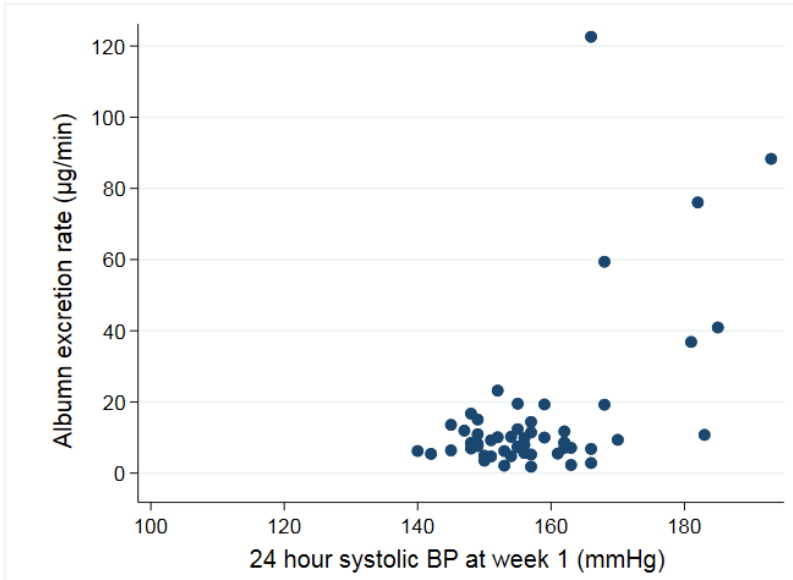
Figure 70 The spread of Fitzgerald albumin excretion rates compared to 24-hour average systolic BP in the hypertensive group before treatment.

Hypertensive group post-treatment
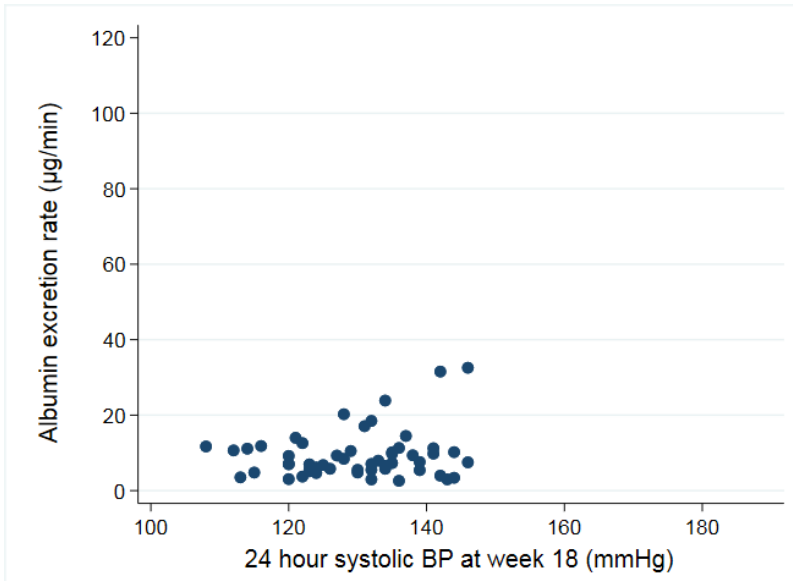
Figure 71 The spread of Fitzgerald albumin excretion rates compared to 24-hour average systolic BP in the hypertensive group after treatment.

Type 2 diabetes group
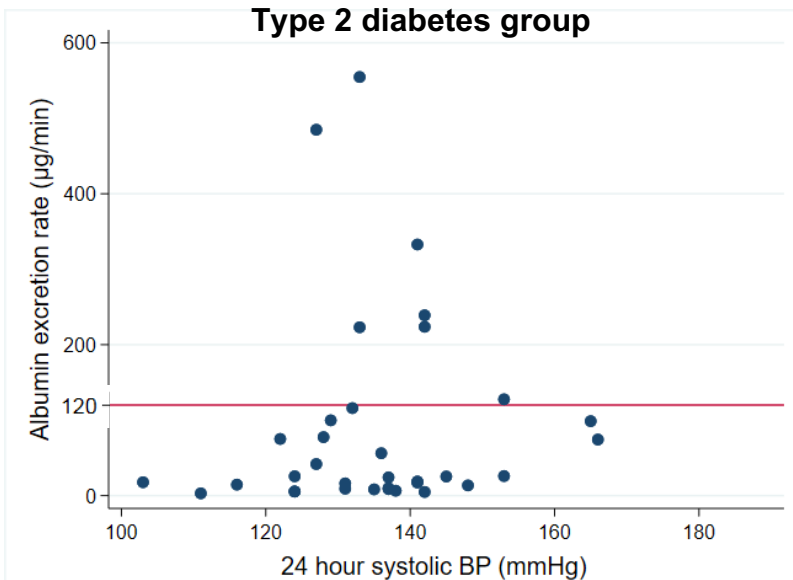
Figure 72 The spread of Fitzgerald albumin excretion rates compared to 24-hour systolic BP in the T2DM group. The red line indicates 120µg/min, the upper limit of the y axis of figure 70 and 71, for comparison of albumin excretion rates across the 3 groups.

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5.1.2 Comparison of day to night ratio of blood pressure and AER in the T2DM and hypertensive cohorts

Neither the T2DM group nor the hypertensive group showed a relationship between urinary albumin and day to night blood pressure ratios. There were also no observable significant relationships when the cohorts were split into normal dippers, non-dippers and extreme dippers. The lack of relationship in both groups may indicate that dipping status has no effect on urinary albumin. If dipping status does affect urine albumin, it is likely that the sample sizes of these studies would have been too small to detect these relationships.

It is worth noting that the relationship between AER and day to night ratio showed slightly different distributions in the hypertensive group and T2DM group. In the hypertensive group the relationship resembled a bell shaped curve whereas in the T2DM group the relationship resembled a U shaped curve. This is particularly evident within the day to night ratio of diastolic blood pressure, which, interestingly, several others found to have a stronger relationship with AER than day to night ratio of systolic BP [103, 109, 110]. However, as illustrated in figure 73, this U shaped relationship is dependent on the 6 individuals in the T2DM group with an AER above the range of the untreated hypertensives. Below 200µg/min, both groups display a similar distribution.
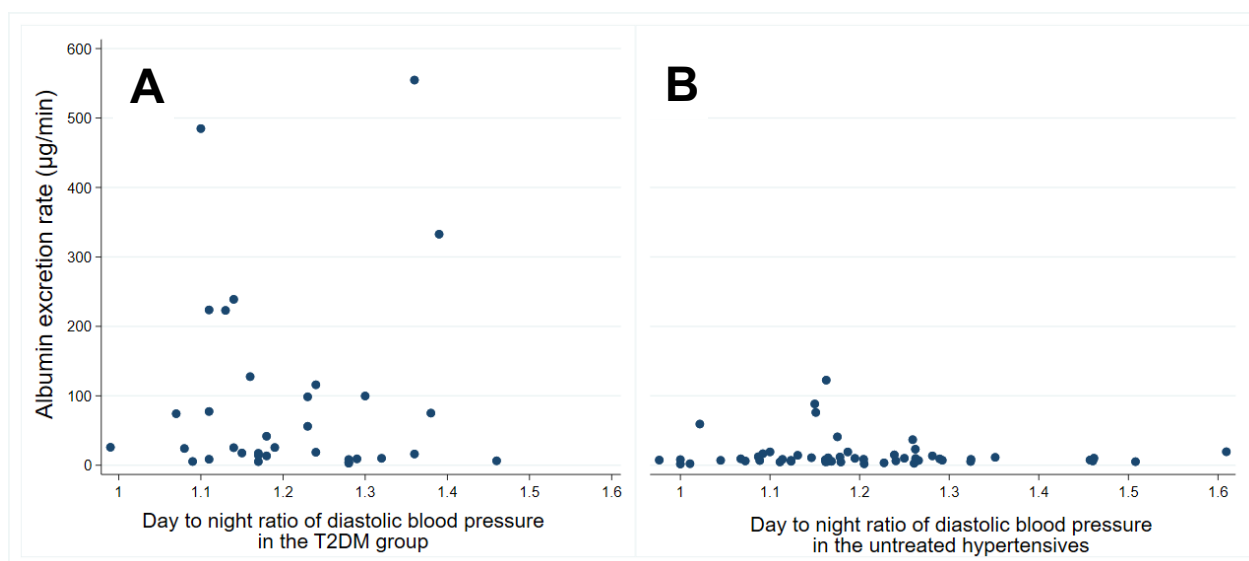
Figure 73 *The relationship between day to night ratio of diastolic blood pressure and Fitzgerald albumin excretion rate in (A) the type 2 diabetic group, n=34 and (B) the hypertensive group before treatment, n=54.*

While it is possible that this finding is due to chance, it could suggest that those with advanced diabetic pathology, in this case high AER, are also likely to display abnormal blood pressure dipping patterns. This could support the theory that the mechanism behind non-dipping patterns, while linked to cardiovascular risk, is not directly related to urinary albumin excretion but often goes hand in hand with certain disease states as has been observed in the diabetic and hypertensive population. This theory is further supported by the previously discussed reports that extreme dipping of blood pressure does not give rise to increased cardiovascular risk when induced by anti-hypertensive treatment [89], suggesting that the abnormal day to night ratio itself is not the determinant of increased cardiovascular risk. It may be that the underlying mechanism of the extreme dipping, which may involve cardiac autonomic neuropathy is associated with increased cardiovascular risk.

Following this, one limitation of these studies was that there was no control over, or notation of anti-hypertensive medication ingestion time. Therefore, it is not possible to know whether medication administration time influenced dipping status in either group.

It is also worth considering that there is some debate on the reproducibility of blood pressure dipping[162-164] and it seems likely that sleep quality may impact night-time dipping. For this reason with only one 24-hour BP measurement individuals may be misclassified as dippers or non-dippers. Future research should aim to identify and observe individuals with true loss of night time dipping rather than those in whom dipping is merely observed to be absent on occasion, perhaps with repeated ABPM measurements and monitoring of sleep quality.

5.1.3 Comparison of short-term blood pressure variability and AER in the T2DM and hypertensive cohorts

Short-term blood pressure variability, as assessed in this work by the standard deviation of blood pressure, is often discussed in the same category as day to night ratios of blood pressure. In this thesis, the T2DM and hypertensive groups showed a difference in the relationship of blood pressure variability (standard deviation of blood pressure) to urinary albumin. In the hypertensive group there was no observed relationship between blood pressure variability and AER. Individuals with moderately increased albuminuria or above within the T2DM group showed a stronger relationship between AER and night time BP variability than with any other aspect of 24-hour ambulatory blood pressure. Some studies and meta-analyses have shown a link between blood pressure variability and increased cardiovascular risk and mortality in both diabetes and hypertension [165-168] These studies vary greatly in their size, cohort demographic and protocol, so direct comparison between them and this research is difficult. However the core conclusions drawn from this research are useful to consider. In a meta-analysis by Stevens et al.[166] the results are comparable to this research as the cohort of patients were older adult of European decent, and people with diabetes were included. Another strength of this study is that it included three measurements of blood pressure variability independent of the mean. Additionally ABPM measurements were over 24 hours with at least 14 day time readings. This meta-analysis showed that blood pressure variability of all kinds was linked to mortality. Their blood pressure variability measurement was independent of the mean similar to the work of Rothwell et al.[168], which shows that visit to visit and short-term blood pressure variability, independently of the mean blood pressure, predicts vascular outcomes such as stroke. These studies are of considerable size increasing the robustness of their conclusions, and the demographics are comparable to this study. The weaknesses of these pieces of research are that due to their size the exact method of blood pressure measurement may have been variable and difficult to standardise. These studies while different in their approach to this study, provide good evidence that BP variability is a significant component of blood pressure and likely contributes to mortality independently of average systolic BP, which is often the sole focus of BP measurement. Other work has found associations between

short-term blood pressure variability and urinary albumin excretion [149, 169-171]. However the difficulty in separating blood pressure variability from the mean systolic blood pressure hinders this work and should be assessed in more detail. If a true link exists between short term blood pressure variability and albumin excretion, the mechanism is unclear. It could be that dramatic fluctuations in blood pressure over a short period of time damage vessel health and influence cardiovascular risk and urinary albumin filtration. However, sympathetic drive is likely one of the major determinants of blood pressure variability [156]. It could be that the relationship between blood pressure variability and increased urine albumin excretion as seen within the individuals with moderately increased albuminuria in the T2DM group of this research, is similar to the relationship proposed between day to night ratio BP and AER. That is that increased blood pressure variability, including day to night ratio, is a sign of underlying pathophysiology that is also related to increased albumin excretion and cardiovascular risk, such as diabetic pathophysiology leading to cardiac autonomic neuropathy and diabetic kidney disease.

These relationships need further exploration as standard deviation of blood pressure may not be the most robust method of determining blood pressure variability [172]. Standard deviation of blood pressure may be related to mean blood pressure, however within this thesis there was no observed relationship between blood pressure variability and mean blood pressure in either cohort. Despite this, average real variability may be a more reliable measure of blood pressure variability and should be used in future analysis [172].

5.1.4 Use of GlycoCheck as a measure of glycocalyx integrity

The GlycoCheck measure of glycocalyx integrity showed no relationship with blood pressure or albuminuria with the exception of the previously discussed percentage change in blood pressure and change in PBR before and after treatment in the hypertensive group.

The average measurements of PBR 5-25 in healthy individuals as stated by the GlycoCheck reproducibility studies (described in 2.2.4) suggests a healthy range of PBR 5-25 is 1.86 (±0.16). The hypertensive group both pre and post treatment showed an average PBR 5-25 of 1.63 (±0.19) and 1.65 (±0.17), indicating a similar PBR or in fact, healthier PBR than healthy individuals. The T2DM cohort had an average PBR 5-25 of 1.91 (±0.17) which is again, not dissimilar from the healthy individuals of GlycoCheck reproducibility studies.

While the external reproducibility of GlycoCheck conducted in this thesis showed that the measurement technique gives reproducible results for individuals from visit to visit (coefficient of variation between 6.17 and 6.73), the relevance of this technique to determine actual differences between individuals seems questionable.

The results of this thesis show the need for extensive validation of the GlycoCheck method of measuring glycocalyx integrity and its relevance in research and clinical practice.

5.1.5 Measurement of urinary albumin excretion

The measurement of urinary albumin within this thesis has already been discussed extensively within chapters 3 and 4, but will be discussed again briefly here. Measurement of urinary albumin within both the DASHER and BEAT studies showed the Cobas albumin assay was able to detect albumin in less than 28% of normal albuminuric urine samples. With the ever increasing interest in the continuation of cardiovascular risk into the normal range of albuminuria, it is vital that an assay with a lower limit of detection such as the Fitzgerald assay be used in future research. The Cobas assay may give false conclusions when used to examine relationships into the normal albuminuric range.

This thesis has also shown that substitution of a normal albumin value in place of those that are undetectable with the Cobas assay is not an accurate measure of approximating these missing values and should not be used in future research.

Urinary albumin creatinine ratio of first morning void spot samples did not differ significantly from overnight urine samples, whereas the random spot ACR were significantly different from the overnight samples. This shows two things: random spot samples may be influenced by activity related variability of albumin excretion in the daytime and also overnight urine samples may not be representative of an individual's total 24-hour urinary albumin excretion. These results emphasise the need for consideration of research aims when choosing a method of urine collection. For example, for a study aiming to explore the effect of albumin toxicity on the kidneys, a 24-hour urine collection may be more suitable than overnight or spot sample.

5.1.6 Is reduction of albuminuria truly associated with reduction of cardiovascular risk?

Interesting work is emerging debating whether the reduction of albuminuria can be used as a surrogate for decreased cardiovascular mortality. The effect of increased urine albumin levels on the increased risk of cardiovascular mortality is well described and discussed throughout this thesis. Although, the significance of whether decreasing albuminuria through treatment truly lowers cardiovascular risk, independent of other factors is still under scrutiny. Two studies in 2011 and 2016 retrospectively analysed data and concluded that reduction of albumin did indeed lower cardiovascular risk independent of systolic blood pressure[173, 174]. In 2019 Harrison et al conducted a meta-analysis of randomised controlled trials concluding that reduction of albumin shows inconsistent effects on cardiovascular mortality[175], however a response to this article by Panahi et al questioned the robustness of Harrison et al's complex statistical analysis and suggested that this may have influenced the results of the analysis[176]. A randomised controlled trial of 8494 participants using blood pressure treatment, blood glucose treatment and placebos analysed whether changes in urine albumin predicted changes in mortality risk[177]. They found that reduction in urine albumin did significantly predict reduction in mortality, particularly macrovascular mortality, but only when regression to the mean was accounted for which they claim no other study has done previously.

In light of this evidence it seems that the questionable strength of the relationship between reduction of albuminuria and cardiovascular risk could be due to lack of consideration of regression to the mean in previous work which may have skewed the data in both directions. Future work should consider the true strength of this association and consequently whether urine albumin excretion is an appropriate treatment target.

5.3 Implications for future work

Future research should use a highly sensitive albumin assay such as the Fitzgerald, where possible, and take care when choosing a urine collection method in relation to the aims of the study.

Future work should also aim to determine whether the relationship between BP variability and target organ damage is due to correlation or causation and should use a robust method of determining short-term BP variability.

The GlycoCheck method of measuring glycocalyx integrity requires further validation to determine if it has a role in the clinical setting.

5.4 Conclusion

In conclusion, this work has shown that a highly sensitive albumin assay should be used within clinical research but that the Cobas assay may still be relevant in clinical practice. This work has also shown that there is need for research into a better method of adjusting for urine dilution when quantifying albumin as both AER and ACR have limitations. When conducting research care should be taken to choose an appropriate method of urine collection, considering their benefits and limitations.

This research also supports the lowering of the threshold of moderately increased albuminuria from 20µg/min to 15µg/min as relationships between blood pressure and AER continue to this level.

This work has also shown no relationship between day to night ratio of blood pressure and urine albumin excretion and speculates that any previous observed relationship is due to concomitant signs of underlying pathophysiology.

The T2DM group show different relationships between AER and aspects of 24-hour blood pressure than untreated hypertensives, perhaps due to a lower range of blood pressures and other factors more strongly influencing AER.

In the untreated hypertensives there were strong correlations with systolic, diastolic, mean arterial pressure and pulse pressure but only in those with an AER above 15µg/min. This may mean that higher levels of albuminuria contribute to hypertension or that hypertension mainly exacerbates albuminuria in those with underlying glomerular damage.

In the diabetic group the strongest association was between AER and night time blood pressure variability in those with moderately increased AER. More research is needed into the relevance of BP variability on target organ damage to determine whether there is a causal relationship or if it is a correlation due to underlying pathophysiology.

The GlycoCheck measure of glycocalyx integrity showed no statistically significant relationships to AER or BP. GlycoCheck shows visit to visit reproducibility in individuals but requires further validation to show its relevance in determining differences between individuals.

References

1. Webster AC, Nagler EV, Morton RL, Masson P. Chronic Kidney Disease. Lancet 2017;389(10075):1238-52.

2. NHS Kidney Care - Insight Health Economics. Chronic Kidney Disease in England: The Human and Financial Cost, 2012.

3. UK Renal Registry. 20th Annual Report of the Renal Association. Bristol, UK, 2018.

4. Diabetes UK. Number of people living with diabetes doubles in twenty years. Secondary Number of people living with diabetes doubles in twenty years 2018. https://www.diabetes.org.uk/about_us/news/diabetes-prevalence-statistics.

5. Kidney Disease Improving Clinical Outcomes. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney Int Suppl 2013;3(1):63-72.

6. Horowitz B, Miskulin D, Zager P. Epidemiology of hypertension in CKD. Adv Chronic Kidney Dis 2015;22(2):88-95.

7. The World Health Organisation. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus Geneva, Switzerland: WHO Press, 2011.

8. The World Health Organisation. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia. Geneva, Switzerland, 2006.

9. BMJ Best Practice. Type 2 diabetes in adults. Secondary Type 2 diabetes in adults 2018. https://bestpractice.bmj.com/topics/en-gb/24/pdf/24.pdf.

10. National Institute for Health and Care Excellence. Diabetes - type 2 Secondary Diabetes - type 2 2017. https://cks.nice.org.uk/diabetes-type-2.

11. UpToDate. Pathogenesis of type 2 diabetes mellitus. Secondary Pathogenesis of type 2 diabetes mellitus 2016. https://www.uptodate.com/contents/pathogenesis-of-type-2-diabetes-mellitus?csi=82b5ab6b-2db9-4b17-957d-c89e93c61c4b&source=contentShare - H5.

12. Mintz ML. Role of the Kidney in Type 2 Diabetes and Mechanism of Action of Sodium Glucose Cotransporter-2 Inhibitors. J Fam Pract 2016;65(12 Suppl).

13. Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of plaque formation and rupture. Circ Res 2014;114(12):1852-66.

14. Schmidt AM, Hori O, Brett J, Yan SD, Wautier JL, Stern D. Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. Arterioscler Thromb 1994;14(10):1521-8.

15. Rojas A, Morales MA. Advanced glycation and endothelial functions: a link towards vascular complications in diabetes. Life Sci 2004;76(7):715-30.

16. Garay-Sevilla ME, Regalado JC, Malacara JM, Nava LE, Wróbel-Zasada K, Castro-Rivas A, et al. Advanced glycosylation end products in skin, serum, saliva and urine and its association with complications of patients with type 2 diabetes mellitus. J Endocrinol Invest 2005;28(3):223-30.

17. Wautier JL, Wautier MP, Schmidt AM, Anderson GM, Hori O, Zoukourian C, et al. Advanced glycation end products (AGEs) on the surface of diabetic erythrocytes bind to the vessel wall via a specific receptor inducing oxidant stress in the vasculature: a link between surface-associated AGEs and diabetic complications. Proc Natl Acad Sci U S A 1994;91(16):7742-6.

18. Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products: sparking the development of diabetic vascular injury. Circulation 2006;114(6):597-605.

19. Chawla A, Chawla R, Jaggi S. Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? Indian J Endocrinol Metab 2016;20(4):546-51.

20. Tousoulis D, Kampoli AM, Tentolouris C, Papageorgiou N, Stefanadis C. The role of nitric oxide on endothelial function. Curr Vasc Pharmacol 2012;10(1):4-18.

21. Cohen RA. Role of nitric oxide in diabetic complications. Am J Ther 2005;12(6):499-502.

22. Bakris G L. Overview of diabetic nephropathy. Secondary Overview of diabetic nephropathy 2018.
https://www.uptodate.com/contents/overview-of-diabetic-nephropathy.

23. Gnudi L. Molecular mechanisms of proteinuria in diabetes. Biochem Soc Trans 2008;36(Pt 5):946-9.

24. Fioretto P, Mauer M. Histopathology of diabetic nephropathy. Semin Nephrol 2007;27(2):195-207.

25. Marshall CB. Rethinking glomerular basement membrane thickening in diabetic nephropathy: adaptive or pathogenic? Am J Physiol Renal Physiol 2016;311(5):F831-f43.

26. Satchell SC, Tooke JE. What is the mechanism of microalbuminuria in diabetes: a role for the glomerular endothelium? Diabetologia 2008;51(5):714-25.

27. Hillege HL, Fidler V, Diercks GF, van Gilst WH, de Zeeuw D, van Veldhuisen DJ, et al. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. Circulation 2002;106(14):1777-82.

28. Ninomiya T, Perkovic V, de Galan BE, Zoungas S, Pillai A, Jardine M, et al. Albuminuria and kidney function independently predict cardiovascular and renal outcomes in diabetes. J Am Soc Nephrol 2009;20(8):1813-21.

29. Amsellem S, Gburek J, Hamard G, Nielsen R, Willnow TE, Devuyst O, et al. Cubilin is essential for albumin reabsorption in the renal proximal tubule. J Am Soc Nephrol 2010;21(11):1859-67.

30. Smink PA, Lambers Heerspink HJ, Gansevoort RT, de Jong PE, Hillege HL, Bakker SJ, et al. Albuminuria, estimated GFR, traditional risk factors, and incident cardiovascular disease: the PREVEND (Prevention of Renal and Vascular Endstage Disease) study. Am J Kidney Dis 2012;60(5):804-11.

31. Imai E, Horio M, Yamagata K, Iseki K, Hara S, Ura N, et al. Slower decline of glomerular filtration rate in the Japanese general population: a longitudinal 10-year follow-up study. Hypertens Res 2008;31(3):433-41.

32. D'Amico G, Bazzi C. Pathophysiology of proteinuria. Kidney International 2003;63(3):809-25.

33. Miner JH. The Glomerular Basement Membrane. Experimental Cell Research 2012;318(9):973-78.

34. Tortora GJ. Tortora's principles of anatomy & physiology / Gerard J. Tortora, Bryan Derrickson, 2017:0-52.

35. Rostgaard J, Qvortrup K. Electron microscopic demonstrations of filamentous molecular sieve plugs in capillary fenestrae. Microvasc Res 1997;53(1):1-13.

36. Rabelink TJ, de Zeeuw D. The glycocalyx--linking albuminuria with renal and cardiovascular disease. Nat Rev Nephrol 2015;11(11):667-76.

37. Alphonsus CS, Rodseth RN. The endothelial glycocalyx: a review of the vascular barrier. Anaesthesia 2014;69(7):777-84.

38. Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. The endothelial glycocalyx: composition, functions, and visualization. Pflugers Arch 2007;454(3):345-59.

39. Qiu HY, Fan WR, Huang SM, Liu F, Tang WZ, Zuo C. [Effect of high concentration of glucose on thickness of glycocalyx and expression of syndecan-1 and glypican-1 in cultured human renal glomerular endothelial cells]. Sichuan Da Xue Xue Bao Yi Xue Ban 2010;41(6):980-5.

40. Tabit CE, Chung WB, Hamburg NM, Vita JA. Endothelial dysfunction in diabetes mellitus: molecular mechanisms and clinical implications. Rev Endocr Metab Disord 2010;11(1):61-74.

41. Salmon AH, Satchell SC. Endothelial glycocalyx dysfunction in disease: albuminuria and increased microvascular permeability. J Pathol 2012;226(4):562-74.

42. Levick JR, Smaje LH. An analysis of the permeability of a fenestra. Microvasc Res 1987;33(2):233-56.

43. Padberg JS, Wiesinger A, di Marco GS, Reuter S, Grabner A, Kentrup D, et al. Damage of the endothelial glycocalyx in chronic kidney disease. Atherosclerosis 2014;234(2):335-43.

44. van den Berg BM, Vink H, Spaan JA. The endothelial glycocalyx protects against myocardial edema. Circ Res 2003;92(6):592-4.

45. Harvey SJ, Jarad G, Cunningham J, Rops AL, van der Vlag J, Berden JH, et al. Disruption of glomerular basement membrane charge through podocyte-specific mutation of agrin does not alter glomerular permselectivity. Am J Pathol 2007;171(1):139-52.

46. Goldberg S, Harvey SJ, Cunningham J, Tryggvason K, Miner JH. Glomerular filtration is normal in the absence of both agrin and perlecan-heparan sulfate from the glomerular basement membrane. Nephrol Dial Transplant 2009;24(7):2044-51.

47. van den Hoven MJ, Wijnhoven TJ, Li JP, Zcharia E, Dijkman HB, Wismans RG, et al. Reduction of anionic sites in the glomerular basement

membrane by heparanase does not lead to proteinuria. Kidney Int 2008;73(3):278-87.

48. Cao Z, Cooper ME. Pathogenesis of diabetic nephropathy. J Diabetes Investig 2011;2(4):243-7.

49. Gambara V, Mecca G, Remuzzi G, Bertani T. Heterogeneous nature of renal lesions in type II diabetes. J Am Soc Nephrol 1993;3(8):1458-66.

50. Hayashi K, Kanda T, Homma K, Tokuyama H, Okubo K, Takamatsu I, et al. Altered renal microvascular response in Zucker obese rats. Metabolism 2002;51(12):1553-61.

51. Christensen EI, Gburek J. Protein reabsorption in renal proximal tubule-function and dysfunction in kidney pathophysiology. Pediatr Nephrol 2004;19(7):714-21.

52. Dickson LE, Wagner MC, Sandoval RM, Molitoris BA. The proximal tubule and albuminuria: really! J Am Soc Nephrol 2014;25(3):443-53.

53. Thrailkill KM, Nimmo T, Bunn RC, Cockrell GE, Moreau CS, Mackintosh S, et al. Microalbuminuria in type 1 diabetes is associated with enhanced excretion of the endocytic multiligand receptors megalin and cubilin. Diabetes Care 2009;32(7):1266-8.

54. Ogasawara S, Hosojima M, Kaseda R, Kabasawa H, Yamamoto-Kabasawa K, Kurosawa H, et al. Significance of urinary full-length and ectodomain forms of megalin in patients with type 2 diabetes. Diabetes Care 2012;35(5):1112-8.

55. UpToDate. Overview of hypertension in adults. Secondary Overview of hypertension in adults 2019. https://www.uptodate.com/contents/overview-of-hypertension-in-adults.

56. Best practice BMJ. Essential hypertension. Secondary Essential hypertension 2018. https://bestpractice.bmj.com/topics/en-gb/26.

57. Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2017. Atlanta, GA, 2017.

58. Hollander W. Role of hypertension in atherosclerosis and cardiovascular disease. The American Journal of Cardiology 1976;38(6):786-800.

59. Frostegård J. Immunity, atherosclerosis and cardiovascular disease. BMC Med 2013;11:117.

60. Alexander RW. Theodore Cooper Memorial Lecture. Hypertension and the pathogenesis of atherosclerosis. Oxidative stress and the mediation of

arterial inflammatory response: a new perspective. Hypertension 1995;25(2):155-61.

61. Federation. NK. About Chronic Kidney Disease. Secondary About Chronic Kidney Disease. https://www.kidney.org/atoz/content/about-chronic-kidney-disease - causes.

62. Mihajlov R, Stoeva D, Pencheva B, Bogusheva E, Ruseva A, Gencheva-Angelova I. Albuminuria and Glomerular Filtration in Patients with Essential Hypertension. Clin Lab 2015;61(7):677-85.

63. Paterson DJ. *Levick's Introduction to Cardiovascular Physiology*: Taylor & Francis Limited, 2018.

64. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39(2):257-65.

65. Lanting SM, Barwick AL, Twigg SM, Johnson NA, Baker MK, Chiu SK, et al. Post-occlusive reactive hyperaemia of skin microvasculature and foot complications in type 2 diabetes. J Diabetes Complications 2017;31(8):1305-10.

66. Vogelberg KH, Mayer P, König M. Decrease of macrovascular hyperemic response of blood flow in diabetics with arterial neuropathy. Klin Wochenschr 1990;68(12):588-93.

67. Mulroney SE. Netter's essential physiology / Susan E. Mulroney, Adam K. Myers ; illustrations by Frank H. Netter ; contributing illustrators, Carlos A.G. Machado, John A. Craig, James A. Perkins, 2009:0-3.

68. Yen W, Cai B, Yang J, Zhang L, Zeng M, Tarbell JM, et al. Endothelial surface glycocalyx can regulate flow-induced nitric oxide production in microvessels in vivo. PLoS One 2015;10(1):e0117133.

69. Pahakis MY, Kosky JR, Dull RO, Tarbell JM. The role of endothelial glycocalyx components in mechanotransduction of fluid shear stress. Biochem Biophys Res Commun 2007;355(1):228-33.

70. Kolluru GK, Bir SC, Kevil CG. Endothelial Dysfunction and Diabetes: Effects on Angiogenesis, Vascular Remodeling, and Wound Healing. Int J Vasc Med 2012;2012.

71. Serhiyenko VA, Serhiyenko AA. Cardiac autonomic neuropathy: Risk factors, diagnosis and treatment. World J Diabetes 2018;9(1):1-24.

72. Agashe S, Petak S. Cardiac Autonomic Neuropathy in Diabetes Mellitus. Methodist Debakey Cardiovasc J 2018;14(4):251-56.

73. Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. American Journal of Physiology-Cell Physiology 2007;292(1):C82-C97.

74. Siragy HM, Carey RM. Role of the intrarenal renin-angiotensin-aldosterone system in chronic kidney disease. Am J Nephrol 2010;31(6):541-50.

75. Lu X, Roksnoer LCW, Danser AHJ. The intrarenal renin–angiotensin system: does it exist? Implications from a recent study in renal angiotensin-converting enzyme knockout mice. Nephrology Dialysis Transplantation 2013;28(12):2977-82.

76. Leehey DJ, Singh AK, Alavi N, Singh R. Role of angiotensin II in diabetic nephropathy. Kidney Int Suppl 2000;77:S93-8.

77. World Health Organization. Salt reduction. Secondary Salt reduction 2015. http://www.who.int/news-room/fact-sheets/detail/salt-reduction.

78. Ha SK. Dietary Salt Intake and Hypertension. Electrolytes & Blood Pressure : E & BP 2014;12(1):7-18.

79. Meneton P, Jeunemaitre X, de Wardener HE, MacGregor GA. Links between dietary salt intake, renal salt handling, blood pressure, and cardiovascular diseases. Physiol Rev 2005;85(2):679-715.

80. Graudal NA, Hubeck-Graudal T, Jurgens G. Effects of low sodium diet versus high sodium diet on blood pressure, renin, aldosterone, catecholamines, cholesterol, and triglyceride. Cochrane Database of Systematic Reviews 2017(4).

81. Bonfils PK, Taskiran M, Damgaard M, Goetze JP, Floyd AK, Funch-Jensen P, et al. The influence of high versus low sodium intake on blood pressure and haemodynamics in patients with morbid obesity. J Hypertens 2013;31(11):2220-9; discussion 29.

82. Beltowski J. Leptin and the regulation of endothelial function in physiological and pathological conditions. Clin Exp Pharmacol Physiol 2012;39(2):168-78.

83. Kotsis V, Stabouli S, Papakatsika S, Rizos Z, Parati G. Mechanisms of obesity-induced hypertension. Hypertens Res 2010;33(5):386-93.

84. Kawarazaki W, Fujita T. The Role of Aldosterone in Obesity-Related Hypertension. Am J Hypertens 2016;29(4):415-23.

85. Kida T, Oku H, Horie T, Matsuo J, Kobayashi T, Fukumoto M, et al. NADPH Oxidase-Mediated ROS Production Determines Insulin's Action on the Retinal Microvasculature. Invest Ophthalmol Vis Sci 2015;56(11):6754-61.

86. DeFronzo RA. The effect of insulin on renal sodium metabolism. A review with clinical implications. Diabetologia 1981;21(3):165-71.

87. Cheung BM, Li C. Diabetes and hypertension: is there a common metabolic pathway? Curr Atheroscler Rep 2012;14(2):160-6.

88. Smolensky MH, Hermida RC, Castriotta RJ, Portaluppi F. Role of sleep-wake cycle on blood pressure circadian rhythms and hypertension. Sleep Med 2007;8(6):668-80.

89. Salles GF, Reboldi G, Fagard RH, Cardoso CR, Pierdomenico SD, Verdecchia P, et al. Prognostic effect of the nocturnal blood pressure fall in hypertensive patients: the ambulatory blood pressure collaboration in patients with hypertension (ABC-H) meta-analysis. Hypertension 2016;67(4):693-700.

90. Ayala DE, Moya A, Crespo JJ, Castineira C, Dominguez-Sardina M, Gomara S, et al. Circadian pattern of ambulatory blood pressure in hypertensive patients with and without type 2 diabetes. Chronobiol Int 2013;30(1-2):99-115.

91. Giles TD. Circadian rhythm of blood pressure and the relation to cardiovascular events. J Hypertens Suppl 2006;24(2):S11-6.

92. Cuspidi C, Meani S, Salerno M, Valerio C, Fusi V, Severgnini B, et al. Cardiovascular target organ damage in essential hypertensives with or without reproducible nocturnal fall in blood pressure. J Hypertens 2004;22(2):273-80.

93. O'Brien E, Kario K, Staessen JA, de la Sierra A, Ohkubo T. Patterns of ambulatory blood pressure: clinical relevance and application. The Journal of Clinical Hypertension 2018;20(7):1112-15.

94. Bhardwaj S, Verma N, Anjum B, Bhardwaj K. Variations in 7-day/24-h circadian pattern of ambulatory blood pressure and heart rate of type 2 diabetes patients. J Diabetes Investig 2014;5(6):728-33.

95. Przewlocka-Kosmala M, Bancarz A, Kosmala W. [Diurnal variation of blood pressure in type II diabetic patients]. Pol Merkur Lekarski 1999;7(42):253-5.

96. Spallone V, Maiello MR, Cicconetti E, Pannone A, Barini A, Gambardella S, et al. Factors determining the 24-h blood pressure profile in normotensive patients with type 1 and type 2 diabetes. J Hum Hypertens 2001;15(4):239-46.

97. White WB. Importance of blood pressure control over a 24-hour period. J Manag Care Pharm 2007;13(8 Suppl B):34-9.

98. Ohashi N, Isobe S, Ishigaki S, Yasuda H. Circadian rhythm of blood pressure and the renin–angiotensin system in the kidney. Hypertension Research 2016;40:413.

99. de la Sierra A, Gorostidi M, Banegas JR, Segura J, de la Cruz JJ, Ruilope LM. Nocturnal hypertension or nondipping: which is better associated with the cardiovascular risk profile? Am J Hypertens 2014;27(5):680-7.

100. West SD, Nicoll DJ, Stradling JR. Prevalence of obstructive sleep apnoea in men with type 2 diabetes. Thorax 2006;61(11):945-50.

101. Mitchell TH, Nolan B, Henry M, Cronin C, Baker H, Greely G. Microalbuminuria in patients with non-insulin-dependent diabetes mellitus relates to nocturnal systolic blood pressure. Am J Med 1997;102(6):531-5.

102. Ruiz-Hurtado G, Ruilope LM, de la Sierra A, Sarafidis P, de la Cruz JJ, Gorostidi M, et al. Association Between High and Very High Albuminuria and Nighttime Blood Pressure: Influence of Diabetes and Chronic Kidney Disease. Diabetes Care 2016;39(10):1729-37.

103. Knudsen ST, Laugesen E, Hansen KW, Bek T, Mogensen CE, Poulsen PL. Ambulatory pulse pressure, decreased nocturnal blood pressure reduction and progression of nephropathy in type 2 diabetic patients. Diabetologia 2009;52(4):698-704.

104. Fogari R, Zoppi A, Malamani GD, Lazzari P, Albonico B, Corradi L. Urinary albumin excretion and nocturnal blood pressure in hypertensive patients with type II diabetes mellitus. Am J Hypertens 1994;7(9 Pt 1):808-13.

105. Lindsay RS, Stewart MJ, Nairn IM, Baird JD, Padfield PL. Reduced diurnal variation of blood pressure in non-insulin-dependent diabetic patients with microalbuminuria. J Hum Hypertens 1995;9(4):223-7.

106. Leitao CB, Canani LH, Kramer CK, Moehlecke M, Pinto LC, Ricardo ED, et al. Blood pressure means rather than nocturnal dipping pattern are related to complications in Type 2 diabetic patients. Diabet Med 2008;25(3):308-13.

107. Jennersjo PE, Wijkman M, Wirehn AB, Lanne T, Engvall J, Nystrom FH, et al. Circadian blood pressure variation in patients with type 2 diabetes--relationship to macro- and microvascular subclinical organ damage. Prim Care Diabetes 2011;5(3):167-73.

108. Redon J, Liao Y, Lozano JV, Miralles A, Pascual JM, Cooper RS. Ambulatory blood pressure and microalbuminuria in essential hypertension: role of circadian variability. J Hypertens 1994;12(8):947-53.

109. Equiluz-Bruck S, Schnack C, Kopp HP, Schernthaner G. Nondipping of nocturnal blood pressure is related to urinary albumin excretion rate in patients with type 2 diabetes mellitus. Am J Hypertens 1996;9(11):1139-43.

110. Inaba M, Negishi K, Takahashi M, Serizawa N, Maruno Y, Takahashi K, et al. Increased night:day blood pressure ratio in microalbuminuric normotensive NIDDM subjects. Diabetes Res Clin Pract 1998;40(3):161-6.

111. Palmas W, Pickering T, Teresi J, Schwartz JE, Eguchi K, Field L, et al. Nocturnal blood pressure elevation predicts progression of albuminuria in elderly people with type 2 diabetes. J Clin Hypertens (Greenwich) 2008;10(1):12-20.

112. Cirillo M, Stellato D, Laurenzi M, Panarelli W, Zanchetti A, De Santo NG. Pulse pressure and isolated systolic hypertension: association with microalbuminuria. The GUBBIO Study Collaborative Research Group. Kidney Int 2000;58(3):1211-8.

113. Knudsen ST, Poulsen PL, Hansen KW, Ebbehoj E, Bek T, Mogensen CE. Pulse pressure and diurnal blood pressure variation: association with micro- and macrovascular complications in type 2 diabetes. Am J Hypertens 2002;15(3):244-50.

114. Magri CJ, Xuereb RG, Fava S. Non-dipping heart rate and microalbuminuria in type 2 diabetes mellitus. Cardiology 2014;129(1):28-35.

115. Barbieri M, Rizzo MR, Fava I, Sardu C, Angelico N, Paolisso P, et al. Awaking Blood Pressure Surge and Progression to Microalbuminuria in Type 2 Normotensive Diabetic Patients. J Diabetes Res 2016;2016:5876792.

116. Turak O, Afsar B, Siriopol D, Ozcan F, Cagli K, Yayla C, et al. Morning Blood Pressure Surge as a Predictor of Development of Chronic Kidney Disease. J Clin Hypertens (Greenwich) 2016;18(5):444-8.

117. Kramer CK, Leitao CB, Canani LH, Gross JL. Afternoon blood pressure increase: a blood pressure pattern associated with microvascular complications in type 2 diabetes mellitus. Am J Hypertens 2011;24(1):64-9.

118. Tofe Povedano S, Garcia De La Villa B. 24-hour and nighttime blood pressures in type 2 diabetic hypertensive patients following morning or evening administration of olmesartan. J Clin Hypertens (Greenwich) 2009;11(8):426-31.

119. Rossen NB, Knudsen ST, Fleischer J, Hvas AM, Ebbehoj E, Poulsen PL, et al. Targeting nocturnal hypertension in type 2 diabetes mellitus. Hypertension 2014;64(5):1080-7.

120. Hermida RC, Calvo C, Ayala DE, López JE. Decrease in urinary albumin excretion associated with the normalization of nocturnal blood pressure in hypertensive subjects. Hypertension (dallas, tex : 1979) 2005;46(4):960-68.

121. Kamoi K, Ikarashi T. The bedtime administration of doxazosin controls morning hypertension and albuminuria in patients with type-2 diabetes: evaluation using home-based blood pressure measurements. Clin Exp Hypertens 2005;27(4):369-76.

122. Gansevoort RT, Brinkman J, Bakker SJL, De Jong PE, de Zeeuw D. Evaluation of Measures of Urinary Albumin Excretion. American Journal of Epidemiology 2006;164(8):725-27.

123. Abdelmalek JA, Gansevoort RT, Lambers Heerspink HJ, Ix JH, Rifkin DE. Estimated albumin excretion rate versus urine albumin-creatinine ratio for the assessment of albuminuria: a diagnostic test study from the Prevention of Renal and Vascular Endstage Disease (PREVEND) Study. Am J Kidney Dis 2014;63(3):415-21.

124. Miller WG, Bruns DE, Hortin GL, Sandberg S, Aakre KM, McQueen MJ, et al. Current Issues in Measurement and Reporting of Urinary Albumin Excretion. Clinical Chemistry 2009;55(1):24.

125. Pickering TG. *Ambulatory Monitoring and Blood Pressure Variability*. London: Science Press Ltd., 1990.

126. Palatini P, Frigo G, Bertolo O, Roman E, Da Corta R, Winnicki M. Validation of the A&D TM-2430 device for ambulatory blood pressure monitoring and evaluation of performance according to subjects' characteristics. Blood Press Monit 1998;3(4):255-60.

127. Yip GW-K, So H-K, Li AM, Tomlinson B, Wong S-N, Sung RY-T. Validation of A&D TM-2430 upper-arm blood pressure monitor for ambulatory blood pressure monitoring in children and adolescents, according to the British Hypertension Society protocol. Blood Press Monit 2012;17(2):76-79.

128. Kario K. Morning surge in blood pressure and cardiovascular risk: evidence and perspectives. Hypertension 2010;56(5):765-73.

129. Glycocheck. Clinical assessment of vascular health with GlycoCheck Glycocalyx Measurement Software. Secondary Clinical assessment of vascular health with GlycoCheck Glycocalyx Measurement Software 2012. https://pdfs.semanticscholar.org/a7ac/01c6d2059e4f297a09584fa770c80 24a945c.pdf.

130. Vlahu CA, Lemkes BA, Struijk DG, Koopman MG, Krediet RT, Vink H. Damage of the endothelial glycocalyx in dialysis patients. J Am Soc Nephrol 2012;23(11):1900-8.

131. Dane MJ, Khairoun M, Lee DH, van den Berg BM, Eskens BJ, Boels MG, et al. Association of kidney function with changes in the endothelial surface layer. Clin J Am Soc Nephrol 2014;9(4):698-704.

132. Chapman D. The Relationship between Low Levels of Urinary Albumin Excretion and Microvascular Function. University of Exeter, 2017.

133. Eh Schwarz P, Brunswick P, Calvet J-H. EZSCAN™ a new technology to detect diabetes risk. The British Journal of Diabetes & Vascular Disease 2011;11(4):204-09.

134. Klausen K, Borch-Johnsen K, Feldt-Rasmussen B, Jensen G, Clausen P, Scharling H, et al. Very Low Levels of Microalbuminuria Are Associated With Increased Risk of Coronary Heart Disease and Death

Independently of Renal Function, Hypertension, and Diabetes. Circulation 2004;110(1):32-35.

135. Liu M, Takahashi H, Morita Y, Maruyama S, Mizuno M, Yuzawa Y, et al. Non-dipping is a potent predictor of cardiovascular mortality and is associated with autonomic dysfunction in haemodialysis patients. Nephrology Dialysis Transplantation 2003;18(3):563-69.

136. Koroboki E, Manios E, Psaltopoulou T, Vemmos K, Michas F, Alexaki E, et al. Circadian variation of blood pressure and heart rate in normotensives, white-coat, masked, treated and untreated hypertensives. Hellenic J Cardiol 2012;53(6):432-8.

137. Musameh MD, Nelson CP, Gracey J, Tobin M, Tomaszewski M, Samani NJ. Determinants of day-night difference in blood pressure, a comparison with determinants of daytime and night-time blood pressure. J Hum Hypertens 2017;31(1):43-48.

138. Rossi GP. The Challenges of Arterial Hypertension. Frontiers in cardiovascular medicine 2015;2:2-2.

139. Younes N, Cleary PA, Steffes MW, de Boer IH, Molitch ME, Rutledge BN, et al. Comparison of Urinary Albumin-Creatinine Ratio and Albumin Excretion Rate in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study. Clinical Journal of the American Society of Nephrology 2010;5(7):1235.

140. Kalantari K, Bolton WK. A Good Reason to Measure 24-Hour Urine Creatinine Excretion, but Not to Assess Kidney Function. Clinical Journal of the American Society of Nephrology 2013;8(11):1847.

141. Fagerstrom P, Sallsten G, Akerstrom M, Haraldsson B, Barregard L. Urinary albumin excretion in healthy adults: a cross sectional study of 24-hour versus timed overnight samples and impact of GFR and other personal characteristics. BMC nephrology 2015;16:8-8.

142. Mogensen CE, Chachati A, Christensen CK, Close CF, Deckert T, Hommel E, et al. Microalbuminuria: An Early Marker of Renal Involvement in Diabetes. Uremia Investigation 1985;9(2):85-95.

143. de Jong PE, Curhan GC. Screening, Monitoring, and Treatment of Albuminuria: Public Health Perspectives. Journal of the American Society of Nephrology 2006;17(8):2120.

144. Palmas W, Moran A, Pickering T, Eimicke JP, Teresi J, Schwartz JE, et al. Ambulatory pulse pressure and progression of urinary albumin excretion in older patients with type 2 diabetes mellitus. Hypertension 2006;48(2):301-8.

145. Viazzi F, Pontremoli R. Blood pressure, albuminuria and renal dysfunction: the 'chicken or egg' dilemma. Nephrology Dialysis Transplantation 2014;29(8):1453-55.

146. Xu H, Huang X, Risérus U, Cederholm T, Lindholm B, Ärnlöv J, et al. Urinary albumin excretion, blood pressure changes and hypertension incidence in the community: effect modification by kidney function. Nephrology Dialysis Transplantation 2014;29(8):1538-45.

147. Hirayama A, Konta T, Hozawa A, Kawasaki R, Watanabe T, Shibata Y, et al. Slight increase in urinary albumin excretion within the normal range predicts incident hypertension in a community-based Japanese population: the Takahata study. Hypertens Res 2015;38(1):56-60.

148. Shibata M, Sato KK, Uehara S, Koh H, Kinuhata S, Oue K, et al. Blood pressure components and the risk for proteinuria in Japanese men: The Kansai Healthcare Study. Journal of epidemiology 2017;27(11):505-10.

149. Farrag HMA, Amin AS, Abdel-Rheim AR. Relation of short-term blood pressure variability to early renal effects in hypertensive patients with controlled blood pressure. Blood Press Monit 2019.

150. Mule G, Calcaterra I, Costanzo M, Geraci G, Guarino L, Foraci AC, et al. Relationship Between Short-Term Blood Pressure Variability and Subclinical Renal Damage in Essential Hypertensive Patients. J Clin Hypertens (Greenwich) 2015;17(6):473-80.

151. Zhang L, Yang J, Li L, Liu D, Xie X, Dong P, et al. Comparison of amlodipine versus other calcium channel blockers on blood pressure variability in hypertensive patients in China: a retrospective propensity score-matched analysis. J Comp Eff Res 2018;7(7):651-60.

152. Jiang XZ, Lu Y, Luo KH, Ventikos Y. Understanding endothelial glycocalyx function under flow shear stress from a molecular perspective. Biorheology 2019.

153. Clark CE, Taylor RS, Butcher I, Stewart MC, Price J, Fowkes FG, et al. Inter-arm blood pressure difference and mortality: a cohort study in an

asymptomatic primary care population at elevated cardiovascular risk. Br J Gen Pract 2016;66(646):e297-308.

154. Witte EC, Lambers Heerspink HJ, de Zeeuw D, Bakker SJ, de Jong PE, Gansevoort R. First morning voids are more reliable than spot urine samples to assess microalbuminuria. J Am Soc Nephrol 2009;20(2):436-43.

155. Saydah SH, Pavkov ME, Zhang C, Lacher DA, Eberhardt MS, Burrows NR, et al. Albuminuria prevalence in first morning void compared with previous random urine from adults in the National Health and Nutrition Examination Survey, 2009-2010. Clin Chem 2013;59(4):675-83.

156. Parati G, Ochoa JE, Salvi P, Lombardi C, Bilo G. Prognostic Value of Blood Pressure Variability and Average Blood Pressure Levels in Patients With Hypertension and Diabetes. Diabetes Care 2013;36(Supplement 2):S312.

157. Kawai T, Ohishi M, Kamide K, Nakama C, Onishi M, Ito N, et al. Differences between daytime and nighttime blood pressure variability regarding systemic atherosclerotic change and renal function. Hypertension Research 2012;36:232.

158. Facchini L, Bellin A, Toro EF. Modeling Loss of Microvascular Wall Homeostasis during Glycocalyx Deterioration and Hypertension that Impacts Plasma Filtration and Solute Exchange. Curr Neurovasc Res 2016;13(2):147-55.

159. Fesler P, du Cailar G, Ribstein J, Mimran A. Heterogeneity of cardiorenal characteristics in normotensive subjects. Hypertension 2004;43(2):219-23.

160. Nieuwdorp M, Mooij HL, Kroon J, Atasever B, Spaan JA, Ince C, et al. Endothelial glycocalyx damage coincides with microalbuminuria in type 1 diabetes. Diabetes 2006;55(4):1127-32.

161. Fridén V, Oveland E, Tenstad O, Ebefors K, Nyström J, Nilsson UA, et al. The glomerular endothelial cell coat is essential for glomerular filtration. Kidney Int 2011;79(12):1322-30.

162. Abdalla M, Goldsmith J, Muntner P, Diaz KM, Reynolds K, Schwartz JE, et al. Is Isolated Nocturnal Hypertension A Reproducible Phenotype? Am J Hypertens 2016;29(1):33-8.

163. Hinderliter AL, Routledge FS, Blumenthal JA, Koch G, Hussey MA, Wohlgemuth WK, et al. Reproducibility of blood pressure dipping: relation to day-to-day variability in sleep quality. J Am Soc Hypertens 2013;7(6):432-9.

164. Ben-Dov IZ, Ben-Arieh L, Mekler J, Bursztyn M. Blood pressure dipping is reproducible in clinical practice. Blood Press Monit 2005;10(2):79-84.

165. Pierdomenico SD, Di Nicola M, Esposito AL, Di Mascio R, Ballone E, Lapenna D, et al. Prognostic value of different indices of blood pressure variability in hypertensive patients. Am J Hypertens 2009;22(8):842-7.

166. Stevens SL, Wood S, Koshiaris C, Law K, Glasziou P, Stevens RJ, et al. Blood pressure variability and cardiovascular disease: systematic review and meta-analysis. BMJ 2016;354:i4098.

167. Rothwell PM. Does blood pressure variability modulate cardiovascular risk? Curr Hypertens Rep 2011;13(3):177-86.

168. Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlöf B, et al. Prognostic significance of visit-to-visit variability, maximum systolic blood pressure, and episodic hypertension. Lancet 2010;375(9718):895-905.

169. Yin LH, Yan WJ, Guo ZX, Zhou FZ, Zhang HY. Relation between blood pressure variability and early renal damage in hypertensive patients. Eur Rev Med Pharmacol Sci 2017;21(9):2226-31.

170. Madden JM, O'Flynn AM, Dolan E, Fitzgerald AP, Kearney PM. Short-term blood pressure variability over 24 h and target organ damage in middle-aged men and women. J Hum Hypertens 2015;29(12):719-25.

171. Mulè G, Calcaterra I, Costanzo M, Morreale M, D'Ignoto F, Castiglia A, et al. Average real variability of 24-h systolic blood pressure is associated with microalbuminuria in patients with primary hypertension. J Hum Hypertens 2016;30(3):164-70.

172. Mena L, Pintos S, Queipo NV, Aizpúrua JA, Maestre G, Sulbarán T. A reliable index for the prognostic significance of blood pressure variability. J Hypertens 2005;23(3):505-11.

173. Holtkamp FA, de Zeeuw D, de Graeff PA, Laverman GD, Berl T, Remuzzi G, et al. Albuminuria and blood pressure, independent targets for cardioprotective therapy in patients with diabetes and nephropathy: a post hoc analysis of the combined RENAAL and IDNT trials. Eur Heart J 2011;32(12):1493-9.

174. Viazzi F, Muiesan ML, Schillaci G, Salvetti M, Pucci G, Bonino B, et al. Changes in albuminuria and cardiovascular risk under antihypertensive treatment: a systematic review and meta-regression analysis. J Hypertens 2016;34(9):1689-97.

175. Harrison TG, Tam-Tham H, Hemmelgarn BR, Elliott M, James MT, Ronksley PE, et al. Change in Proteinuria or Albuminuria as a Surrogate for Cardiovascular and Other Major Clinical Outcomes: A Systematic Review and Meta-analysis. Can J Cardiol 2019;35(1):77-91.

176. Panahi MH, Bidhendi Yarandi R. Controversy Over the Surrogacy of Proteinuria or Albuminuria for Cardiovascular Outcomes. Can J Cardiol 2019;35(9):1256.e5-56.e6.

177. Jun M, Ohkuma T, Zoungas S, Colagiuri S, Mancia G, Marre M, et al. Changes in Albuminuria and the Risk of Major Clinical Outcomes in Diabetes: Results From ADVANCE-ON. Diabetes Care 2018;41(1):163-70.