Sleep in patients with Type 2 Diabetes: The impact of sleep apnoea, sleep duration, and sleep quality on clinical outcomes

by

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Abstract

Introduction

Type 2 Diabetes (T2DM) and sleep-related disorders share common risk factors such as obesity; but the inter-relationships between T2DM and sleep disorders are not well examined.

Aims

In this thesis I aimed to assess:

1. The longitudinal impact of obstructive sleep apnoea (OSA) on microvascular complications in patient with T2DM.
2. The relationship between sleep quality, sleep duration and adiposity in patients with T2DM

Methods

To examine the first aim, I utilised the data collected from a previous project that examined the cross-sectional associations between OSA and microvascular complications in patients with T2DM and followed up the study participants longitudinally using 1-2-1 interviews and electronic health records. For aim 2, I conducted a cross-sectional study in patients with young-onset T2DM who were recruited from Heart of England NHS Foundation Trust.

Result

For Aim 1: Depending on the microvascular outcome examined, we had approximately 200 patients in the analysis. Patients were followed up for 2.5 years for renal outcomes, and 4-4.5 years for retinopathy and neuropathy outcomes. The prevalence of OSA was 63%. I found that baseline OSA was significantly associated with greater decline of eGFR and

greater progression to pre-proliferative and proliferative retinopathy. I also found that OSA was associated with progression to a combined outcome of foot insensitivity or diabetic foot ulceration but this was a non-significant trend (p=0.06). In addition, I found that patients who received and were compliant with continuous positive airway pressure (CPAP) treatment (delivered during routine care) had improvements in heart rate variability parameters by study end.

For Aim 2: Poor sleep quality and shorter sleep duration were associated with increased total body fat% after adjustment for potential confounders.

Conclusion

I found that OSA plays an important role in the progression of microvascular complications in patients with T2DM. Whether treatment with CPAP has a favourable impact on microvascular complications is currently being examined in a randomised controlled trial.

I also found that sleep duration and quality are associated with increased adiposity. The direction of this relationship need to be examined in longitudinal studies and interventional trials.

Acknowledgement

I am indebted to a large number of people, without whom, it would not be possible to complete this work. First and foremost, I would like to say a big thank you to my husband and 2 young children who stood by me throughout all the long hours and lost weekends. Secondly, I would like to thank Dr Abd Tahrani who helped me through thick and thin and was a constant source of support and inspiration throughout this journey. I would also like to thank my research team at Birmingham Heartlands Hospital especially Safia Begum, who became a friend.

I am thankful to Dr Asad Ali and Mr Nicholls in their help in analysing the sleep studies. I would also like to thank Dr Neil Raymonds, University of Warwick; for helping me with statistical analysis.

In the end, I would like to thank all the patients who devoted their time and support in lifting this project up and carrying it to the finishing line. This project would not have been completed without them. I hope that this piece of research forms the basis of many other trials to come and translate into excellent clinical practice.

Outputs

This project has been translated into following data papers, reviews and oral presentations.

Data Papers

Foot insensitivity is associated with renal function decline in patients with type 2 diabetes: a cohort study. **Quratul A. Altaf**, Hamed Sadiqi, Milan K. Piya and Abd A. Tahrani. BMC Endocr Disord. 2016 Nov 22; 16(1):64.

The relationship between obstructive sleep apnea and intra-epidermal nerve fibre density, PARP activation and foot ulceration in patients with type 2 diabetes. **Quratul A. Altaf**, Asad Ali, Milan K. Piya, Neil T.Raymond, Abd A.Tahrani. J Diabetes Complications. 2016 Sep-Oct; 30(7):1315-20.

Cardiac autonomic neuropathy predicts renal function decline in patients with type 2 diabetes: a cohort study. Abd A. Tahrani, Kiran Dubb, Neil T. Raymond, Safia Begum, **Quratul A. Altaf**, Hamed Sadiqi, Milan K. Piya, Martin J. Steven. Diabetologia. 2014 Jun; 57(6):1249-56.

Obstructive sleep apnea and diabetic nephropathy: a cohort study. Abd A. Tahrani, Asad Ali, Neil T. Raymond**,** Safia Begum**,** Kiran Dubb, **Quratul A. Altaf**, Milan K. Piya, Anthony H. Barnett, Martin J. Stevens. Diabetes Care. 2013 Nov; 36 (11):3718-25.

Obstructive Sleep Apnoea and retinopathy in patients with Type 2 Diabetes: A longitudinal Study. **Quratul A. Altaf**, Paul Dodson, Asad Ali, Neil T. Raymond, Helen Wharton, Hannah Fellows, Rachel Hampshire-Bancroft, Mirriam Shah, Emma Shepherd, Jamili Miah, Anthony H. Barnett, Abd A Tahrani. American Journal of Respiratory and Critical Care Medicine Vol 196, No. 7, 2017.

Review Article

Novel therapeutics for type 2 diabetes: insulin resistance. Quratul A. Altaf, AH Barnett, AA Tahrani. Diabetes Obes Metab. 2015 Apr; 17 (4):319-34.

Oral Presentation

Shorter sleep duration and poor sleep quality are associated with increased adiposity in patients with young type 2 diabetes: a cross-sectional study
An oral presentation in DUK 2017. Also presented as a poster.

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Abbreviation Index

AASM: American Academy of Sleep Medicine

ACR: urinary albumin/creatinine ratio

AGE: advanced glycation end-products

AHI: apnoea hypopnea index

AKT: protein kinase B

ALE: advanced lipoxidation end-product

AR: aldose Reductase

AT-II: angiotensin II

BDNF: brain derived neurotrophic factor

bFGF: basic fibroblast growth factor

BHH: Birmingham Heartlands Hospital

BMI: body mass index

BP: blood pressure

CAN: cardiac Autonomic Neuropathy

CHD: coronary heart disease

CKD: chronic kidney disease

CPAP: continuous positive airway pressure

CV: cardiovascular

DAG: diacylglycerol

DAN: diabetic autonomic neuropathy

DN: diabetic nephropathy

DPN: diabetic peripheral neuropathy

DR: diabetic retinopathy

E/I ratio: expiration/inspiration ratio

eGFR: estimated glomerular filtration rate

ESRD: end stage renal disease

ESS: epworth sleepiness scale

ET-1: endothelin-1

FDA: Food and Drug Administration

FFA: free fatty acids

FML: F-wave minimal latency

FRF: fundamental respiratory frequency

GAP: glyceraldehyde-3 phosphate

GAPDH: glyceraldehyde-3 phosphate dehydrogenase

GFAT: glutamine – fructose – 6 - phosphate amidotransferase

GIP: glucose dependant insulinotropic polypeptide

GIT: gastrointestinal tract

GLP-1: glucagon like peptide 1

GLUT-4: glucose transporters-4

HC: hip circumference

HDL: high density lipoprotein

HIF-1α: hypoxia inducible factor 1-α

HPA: hypothalamic-pituitary axis

HRV: heart rate variability

HSP: hexosamine pathway

ICAM-1: intercellular adhesion molecule-1

IENFD: intra-epidermal nerve fibre density

IGT: impaired glucose tolerance

IKK: inhibitor of nuclear factor κ-B kinase

IR: insulin resistance

IRS: insulin receptor substrates

LFa: low frequency area

LOX-1: oxidized LDL receptor-1

MAPK: mitogen activated protein kinase

MNCV: motor nerve conduction velocity

MNSI: Michigan Neuropathy Screening Instrument

MVC: microvascular complications

NCS: nerve conduction studies

NF-κB: nuclear factor-κB

NO: nitric oxide

Non-SA: non-South Asians

NOS: nitrous oxide synthase

ODI: oxygen desaturation index

OGT: O-GlcNAc transferase

OS: oxidative stress

OSA: obstructive sleep apnoea

PAI-1: plasminogen activator inhibitor-1

PARP: poly (ADP-ribose) polymers activation

PKC: protein kinase C

POMC: proopiomelanocortin

PPP: pentose phosphate pathway

PSQI: Pittsburgh Sleep Quality Index

QOL: quality of life

RAAS: renin–angiotensin–aldosterone system

RAGE: receptor of AGE

RBX: ruboxistaurin

RFa: respiratory frequency area

RNS: reactive nitrogen species

ROS: reactive oxygen species

RTA: road traffic accidents

SA: south Asians

SDH: sorbitol dehydrogenase

SGLT2: sodium glucose co- transporter 2

SOD: superoxide dismutase

STDR: sight threatening DR

STZ: streptozotocin

T1DM: type 1 diabetes

T2DM: type 2 diabetes

TCA: tricarboxylic acid cycle

TGFα: transforming growth factor-α

TGFβ_1: transforming growth factor-β_1

UCP: uncoupling proteins

UDP-GalNAc: UDP-N-acetylgalactosamine

VCAM-1: vascular cell adhesion molecule-1

VEGF: vascular endothelial growth factor

VPF: vascular permeability factor

WC: waist circumference

WHR: waist−hip ratio

Chapter One: Introduction

1.1 Type 2 Diabetes Mellitus

1.1.1 Overview and epidemiology

Type 2 diabetes (T2DM) is a complex metabolic disorder in which there is imbalance between insulin production from pancreatic β-cells and insulin action, mainly due to the impact of obesity on insulin resistance and β-cell function. Initially, insulin resistance (IR) is overcome by compensatory increase in insulin secretion leading to initial normoglycaemia in the presence of hyperinsulinemia. Subsequently hyperglycaemia ensues when the β-cells fail to secrete enough insulin to overcome IR resulting in increased endogenous hepatic glucose production (**Figure 1.1**) [1]. In addition to this, increased lipolysis, incretin deficiency, hyperglucagonaemia and reduced renal glucose excretion all play integral roles in the development of T2DM [1,2].

T2DM has reached epidemic proportions with an estimated 415 million people affected in the world with a UK prevalence of 9.1%, expected to rise to 10.7% by 2040 [3]. Patients are getting younger with 320 million patients with diabetes aged between 20 and 64 years, increasing to 440 million in 2040 [3]. This means patients would be living longer with the diabetes and its related complications, resulting in significant mortality and morbidity. Obesity is a major risk factor for the development of T2DM and many of its comorbidities [4] Overweight and obese patients are at 3 and 7 fold increase in risk of developing T2DM respectively [4]. Prevalence of obesity has risen from 20.2% in females and 20.9% in males in the year 2000 to 27% in both females and males in 2015 [5]. If continued at the same rate, it is estimated that by the year 2050, 50% of females and 60% of males will be affected by obesity [6]. Currently diabetes is one of the leading causes of mortality in the world [3]. The health spending on diabetes accounts for 11.6% of total health expenditure world wide

and 9% of total NHS budget in the UK [3]. In 2015, the global health spending to treat diabetes and prevent complications, is estimated to be USD 673 billion, increasing to USD 802 billion in 2040, indicating 1.2 fold increase in the diabetes cost despite 1.5 fold increase in diabetes prevalence [3]. This places a huge financial burden and substantial financial impact on countries and health systems, due to the cost of treatment, loss of productivity and multidisciplinary support needed in the case of microvascular complications [3].

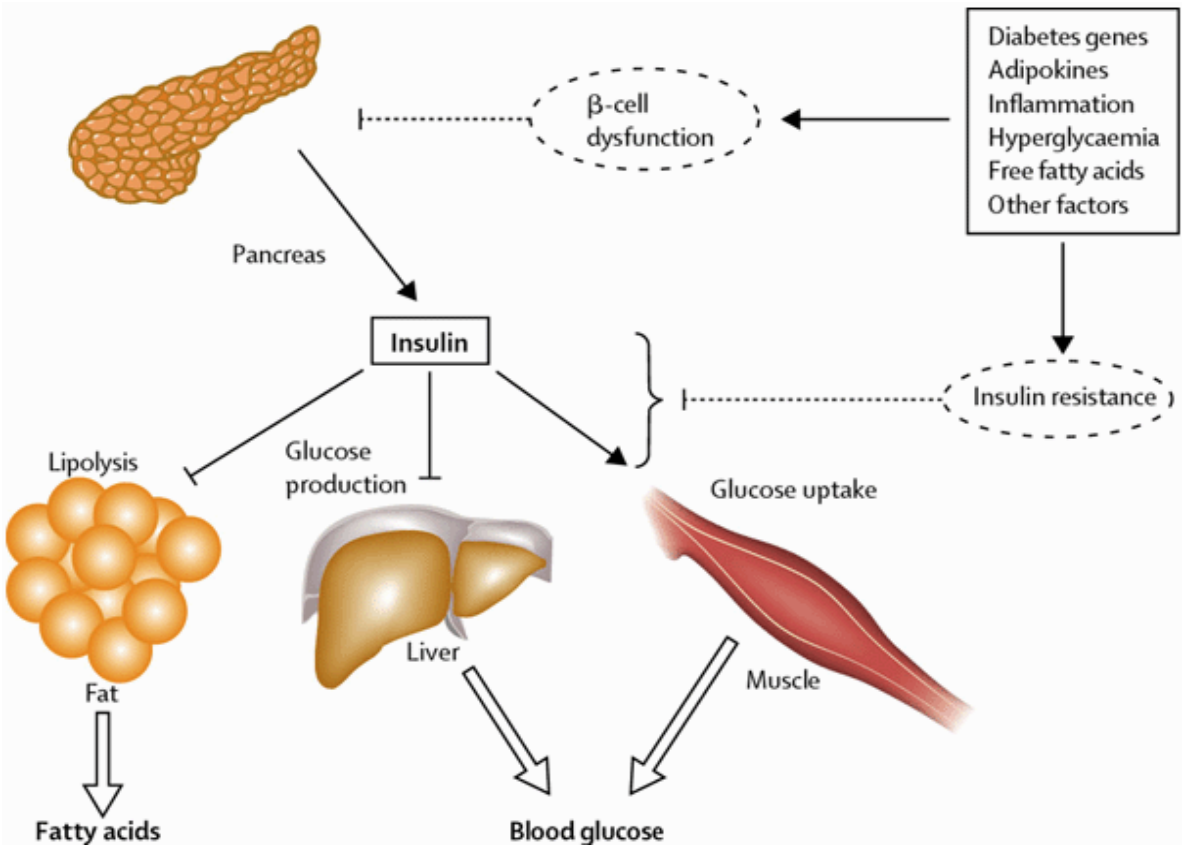

Figure 1.1 The complex pathophysiology of type 2 diabetes. Adapted with permission (1)

1.1.2 Pathogenesis of Type 2 Diabetes Mellitus

The maintenance of body glucose homeostasis depends on insulin secretion by pancreatic β- cells and glucose uptake by insulin sensitive tissues. Under basal condition, 75% of the total body glucose is utilised by brain, liver and gastrointestinal tract (GI) and is insulin independent. The remaining 25% is utilised by muscles and is insulin dependent. Majority of the endogenous glucose production is by the liver (glycogenolysis and gluconeogenesis), followed by the kidney (gluconeogenesis) [7,8].

After glucose ingestion, there is a rise of insulin secretion by pancreatic β-cells. Resultant hyperinsulinameia and hyperglycaemia stimulate glucose uptake by liver, GI tract and muscles and suppress glucagon production by pancreatic α-cells. This suppresses hepatic and renal glucose production by glycogenolysis and gluconeogenesis [8,9]. Hyperinsulinaemia also inhibits lipolysis and reduces plasma free fatty acid levels which stimulates muscle glucose uptake and inhibit endogenous glucose production [10].

1.1.2.1 Insulin Resistance

Insulin resistance (IR) is defined as subnormal biological response to a given concentration of insulin [11]. The insulin receptor is a trans-membrane receptor and a member of the tyrosine kinase family and has α and β subunits [12]. Activation of the receptor by insulin binding to the α-subunit results in the phosphorylation of the insulin receptor substrates (IRS) which leads eventually, after several steps, to increased protein kinase B (AKT) resulting in the translocation of the glucose transporters-4 (GLUT-4) to the cell surface allowing the entrance of glucose into the target cell and the storage of glucose as glycogen

by activating the enzyme glycogen synthase [1,13]. IR is usually the result of deficits at several levels of the insulin signalling pathway including the insulin receptor, IRS phosphorylation or post receptors signalling resulting in the inadequate action of insulin [2,14].

Several genetic (CAPN10, FTO, HHEXIIDE, KCNQ1, KCNJ11, MC4R, PPARG) and environmental factors (lifestyle, pregnancy, physical inactivity, ageing, drugs) contribute to the development of IR [2,15,16]. However, adiposity is the major contributor [1,2,17]. Obesity contributes to the development of IR via several mechanisms (**Figure 1.2**) [13,17,18]. Ectopic fat/lipid accumulation in the liver (non-alcoholic fatty liver disease) and skeletal muscle plays an important role in IR [13]. Intramyocellular triglyceride content is a stronger predictor of IR than circulating lipids [13,19]. Similarly, intrahepatic triglyceride content was better associated with IR than visceral fat and the surgical removal of visceral fat did not have an added metabolic benefit over that achieved by reduction in intrahepatic fat [20,21]. Free fatty acids (FFA), released from the adipose tissue, are esterified upon cellular entry to form acylglycerols (mono, di or tri) or ceramides (when NEFA esterified with sphingosine and are a precursor in the formation of sphingomyelin) [13] . Diacylglycerol activates PKC which reduces the insulin mediated phosphorylation of IRS-1/2 resulting in IR [22]. Ceramides, on the other hand, appear to reduce AKT activation/phosphorylation [23] . Obesity is also associated with increased inflammation and cytokine production which can lead to the activation of mitogen activated protein kinase (MAPK) pathways (such as JNK-1) and inhibition of nuclear factor κ-B kinase (IKK) leading to increased ceramides and impairment of the insulin-mediated IRS phosphorylation [13,24,25].

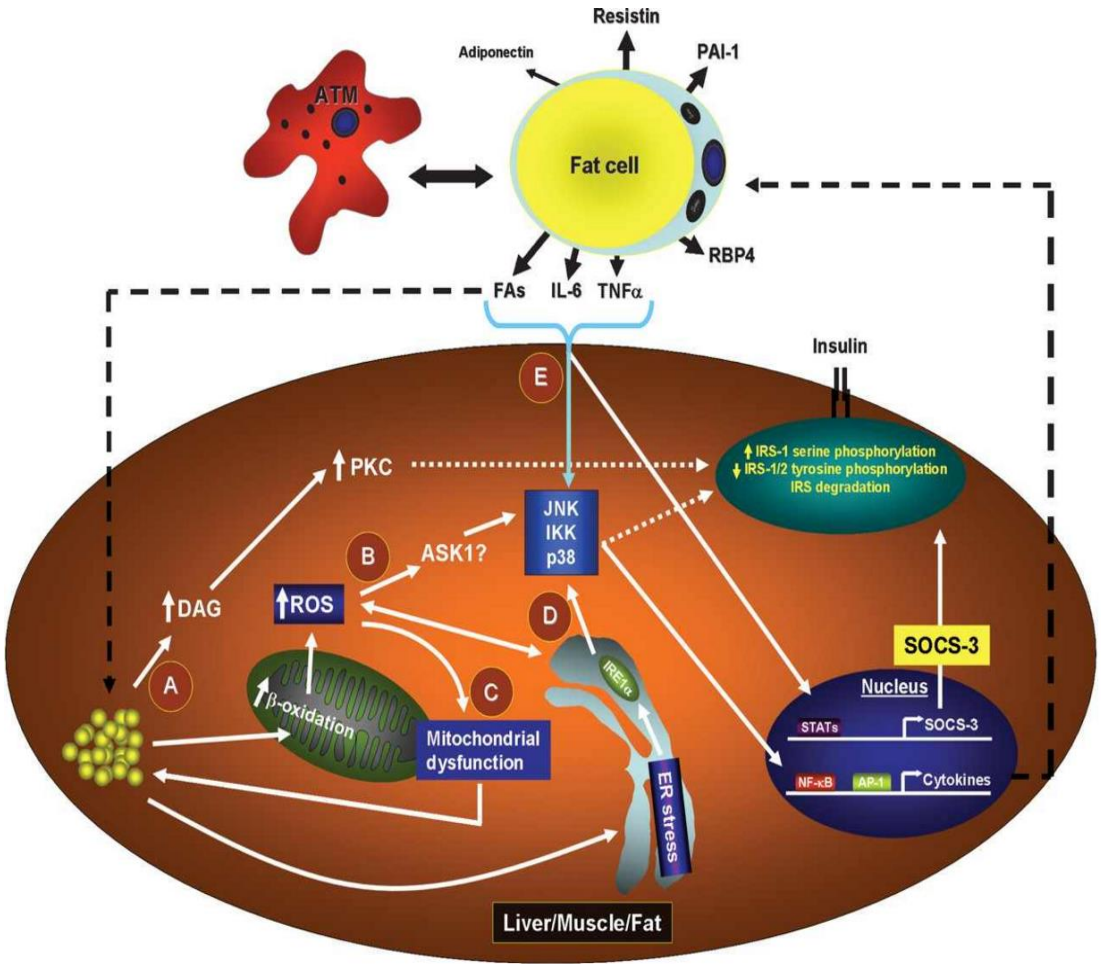

Figure 1.2 Obesity associated mediators of IR. Adopted with permission (18)

More recently, the role of circadian misalignment and other sleep related conditions, notably obstructive sleep apnoea (OSA) has been discussed in the literature. Shorter sleep duration is said to worsen IR and T2DM both in healthy volunteers and in patients with existing diagnosis of T2DM, partially because of its effect on adiposity [26].

1.1.2.2 Beta Cell Dysfunction

Under normal physiological conditions, effective β cell function is responsible to maintain euglycaemia, by secreting appropriate amount of insulin. This function is closely regulated

by glucose load itself by promoting insulin secretion from β-cells using ATP sensitive K^+ channel dependent pathway (which leads to an increase in the cytosolic Ca^{2+} concentration causing exocytosis of insulin granules) and augmenting the response using ATP sensitive K^+ channel independent pathway (which augments the secretory response of cytosolic Ca^{2+}) [27]. At the same time, transcriptional activity of insulin gene goes up remarkably, leading to increased translation of proinsulin molecules [28]. Amino acids and fatty acids, aided by glucokinase, also play a role in regulating insulin secretion from β cells at physiological glucose levels [27]. Therefore, optimum glucose concentration is the most important in regulating and preserving β-cell function.

Chronic hyperglycaemic exposure causes changes in gene and protein expression leading to β cell mass hypertrophy (**Figure 1.3**) [29,30]. Resultant changes lead to down regulation of transcriptional and translational genes and up regulation of pro-apoptotic and antioxidant genes [29,31]. Furthermore, presence of IR and obesity induce increased production of inflammatory cytokines resulting in mitochondrial, oxidative and nitrosative stress which lead to initially β cell loss and ultimately demise [31]. This is further compounded by increased levels of FFA due to lipolysis and IR. Short term exposure may lead to initial upregulation of insulin secretion following a meal but chronic exposure down regulates insulin secretion and glucose metabolism, resulting in further β cell loss [32]. This manifests itself as β cell demise. To counter this, certain transcription factors stimulate β cell proliferation and compensation. Chronic glucotoxicity and lipotoxicity exhaust the compensatory processes. T2DM results when β cell function loss exceeds more than 50% [31].

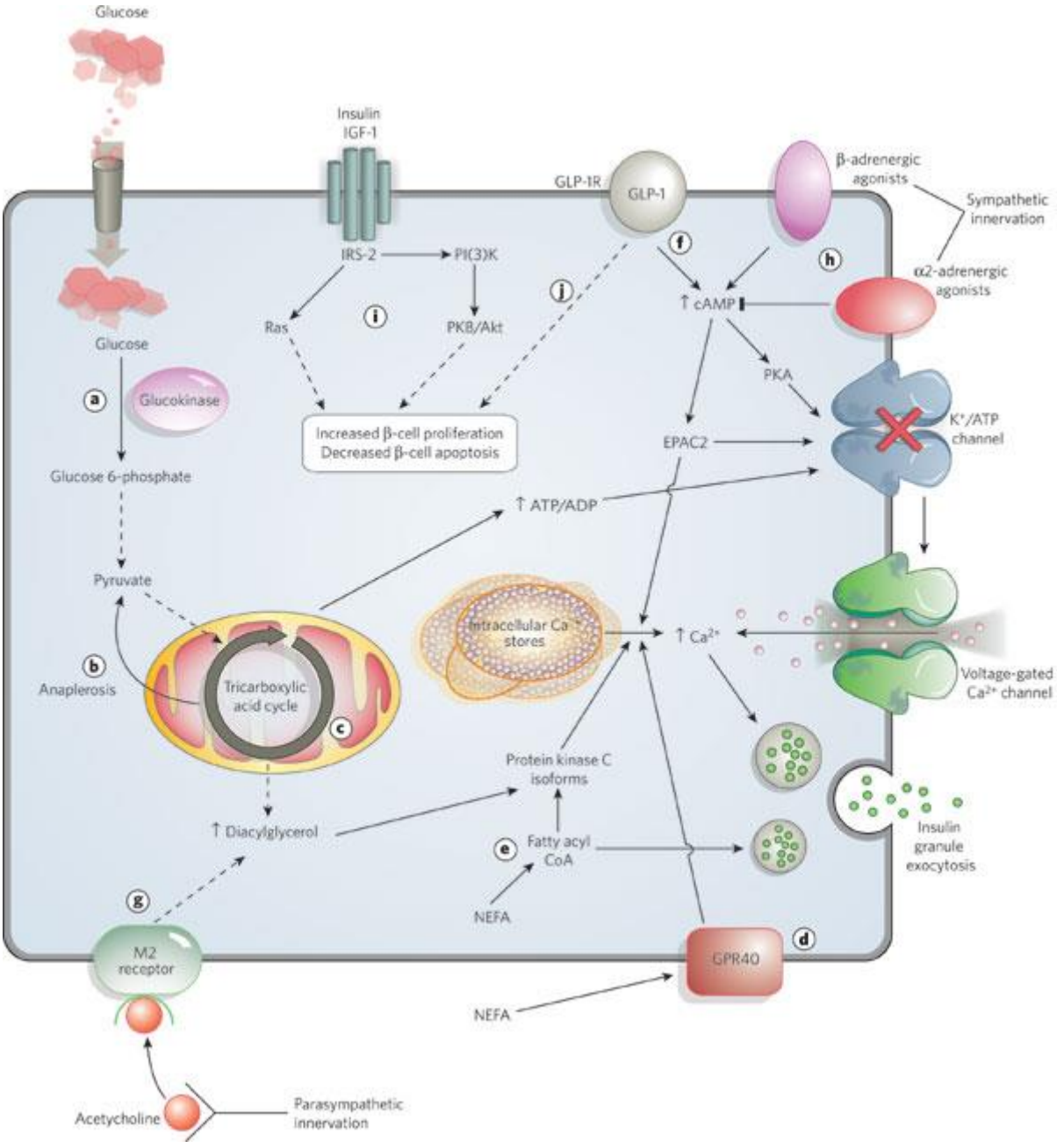

Figure 1.3 Mechanisms describing β cell adaptation in IR. Adopted with permission (30)

1.1.2.3 Other Important Considerations

1.1.2.3.1 Alpha Cell Dysfunction

Glucagon is the hormone released by α cells of pancreas. As with insulin, release of glucagon is also regulated by various hormonal and nutrient stimuli. The main stimulus is low glucose levels but amino acids, autonomic nervous system and gastric peptides also play a role. It promotes hepatic gluconeogenesis, glycogenolysis and fatty acid oxidation [33], thereby maintaining tight glucose homeostasis.

In patients with T2DM, the inhibition of glucagon to hyperglycaemia is diminished, resulting in fasting hyperglucagonaemia and paradoxical rise after meal leading to post prandial hyperglycaemia [33].

1.1.2.3.2 Loss of Incretin Effect

Incretins are the hormones, released by the gut in response to oral glucose load. In individuals without diabetes, following oral meal intake, incretin hormones, glucagon like peptide 1 (GLP-1) and glucose dependant insulinotropic polypeptide (GIP) promote three to four fold increase in insulin secretion by pancreatic β cells compared with insulin secretion observed after IV glucose infusion, the phenomenon also known as incretin effect [34,35]. GLP- 1 also suppresses glucagon secretion from pancreatic α cells and inhibit gastric emptying, promoting satiety [36].

In T2DM, GIP levels and effects have been found to be variable depending on the studies. It is possible that GIP has no significant role in the pathogenesis of T2DM [34,37,38]. Impaired GLP-1 secretion in response to a mixed meal, especially carbohydrates, have been found in

IR, obesity and T2DM [39,40]. GLP-1 when released following glucose load, significantly increases pancreatic β cell sensitivity, thus normalizing insulin secretion, even in the case of mild hyperglycaemia [41]. However, in patients with T2DM, low post prandial GLP-1 levels impair β cell function, losing incretin effect [41].

1.1.2.3.3 Role of Brain

Hypothalamus has been recognised to be playing an important role in glucose homeostasis [42]. Hypothalamic neurons exhibit both leptin receptors which promote satiety, and insulin receptors which improve insulin resistance, thereby maintaining glucose homeostasis [42]. Serotonin receptors on proopiomelanocortin (POMC) neurons regulate hepatic insulin sensitivity, independent of their effect on appetite [43]. Dopaminergic receptors have an inverse effect on glucose metabolism. Low dopamine levels are associated with IR and obesity [43]. In a double blind, placebo controlled study on 22 obese patients with T2DM, 16 week use of bromocriptine, dopamine receptor agonist, improved overall glycaemic control ($p=0.009$) and fasting hyperglycaemia ($p=0.02$) [44]. In 2009, Food and Drug Administration (FDA) approved the use of bromocriptine for the treatment of T2DM, both as monotherapy and in combination with other oral anti-hyperglycaemic agents [45]. In animal models, chronic hyperinsulinaemia, as seen in IR and T2DM, increased food intake and increased body fat [46,47]. It may be that similar mechanisms are at play in humans, contributing to development of IR and T2DM [42].

Brain derived neurotrophic factor (BDNF) is essential for survival and maintenance of neurons. In animal studies, lower levels of BDNF are associated with IR and obesity [48,49].

In humans, lower circulating levels of BDNF are associated with higher plasma glucose levels. No relationship was found between BDNF and insulin levels [50].

1.1.2.3.4 Role of Kidneys

Kidneys are responsible for 20% of total body glucose production. It also plays an important role in glucose homeostasis by glucose filtration and reabsorption. In hyperglycaemic state, renal gluconeogenesis and renal glucose reabsorption inappropriately increase, further increasing glucose load [51]. Chronic hyperglycaemia upregulates Sodium Glucose Co-Transporter 2 (SGLT2) activity, thereby exacerbation hyperglycaemia, rather than excreting increased glucose load and restoring normoglycaemia [52].

1.1.3 Diabetes-related microvascular complications

Although cardiovascular disease is the main cause of mortality in patients with T2DM; however, microvascular complications contribute significantly to the individual and economic burden of the disease [53-55]. Hence reducing the burden of vascular disease is a major aim in the management of patients with T2DM.

The pathogenesis of microvascular complications is complex and multi factorial and is driven by chronic hyperglycaemia and hypertension (please see below for details). However, despite our improved understanding of the pathogenesis of these complications and improved metabolic control, microvascular complications remain very common in patients with T2DM. A recent epidemiological study from the US showed that improved treatment of patients with T2DM has resulted in significant reduction in macrovascular disease in the US

between 1990 – 2010, but there was little impact on microvascular complications [54]. Hence, better understanding of the pathogenesis of these complications is needed on order to identify the treatment targets.

1.1.3.1 Diabetic Nephropathy and Chronic Kidney Disease

Diabetic nephropathy (DN) is the most common cause of chronic kidney disease (CKD) and end stage renal Disease (ESRD) in the western world [56] and is the cause of renal failure in 25% of the patients in the UK [57]. Patients with DN require 29% of the total health budget in the US, a 16 fold increase since 1993 [58].

DN and CKD share the similar pathophysiological mechanisms with other microvascular complications (please see below). However, specific pathological haemodynamic changes e.g. activation of renin–angiotensin–aldosterone system (RAAS) and endothelin system which lead to increased systemic and intra-glomerular pressures, contribute towards the development of DN [59]. Increased production of cytokines, growth factors, metalloproteinase and advanced glycation end-products (AGE) along with the activation of polyol, hexosamine and PKC pathways also play a crucial role in the development of DN [59]. Reduced resistance at both afferent and efferent arterioles of the glomeruli lead to hyper filtration and hyper perfusion of the glomeruli leading to subsequent glomerular hypertrophy and mesangial expansion [59].

The course of DN is slow but progressive, starting from microalbuminura; followed by proteinuria in 20 – 40% of the patients [56]. Out of these patients, 20% will go on to develop ESRD in 20 years' time. However, the rate of progression is highly variable and characteristically depends on glycaemic control and blood pressure [59]. According a meta-

analysis, microalbuminuria is a strong predictor of cardiovascular morbidity and mortality in patients with T2DM (OR=2.0; 95%CI 1.4, 2.7) [60]. Treatment involves optimum glycaemic control and metabolic control including hypertension [61]. RAAS blockade improves the micro albuminuria, thereby retarding the progression of DN [62].

1.1.3.2 Diabetic Retinopathy

Diabetic retinopathy (DR) is one of the leading causes of preventable visual impairment and loss [63]. Studies suggest that one third of diabetic population have DR; one tenth have sight threatening DR including proliferative retinopathy and maculopathy [63]. According to an estimate, the prevalence of DR in the US was 40.3% (95%CI 38.8%, 41.7%) and of sight threatening DR (STDR) was 8.2% (95%CI 7.4%, 9.1%)[64]. Patients with DR are more at risk of developing coronary heart disease (CHD), congestive heart failure and ischemic stroke [65]. The underlying mechanisms include increased oxidative stress and activation of multiple pathways (e.g. polyol pathway, hexosamine pathway and PKC pathway), resulting in increased vascular permeability (macular oedema) and ischaemia leading to increased vascular endothelial growth factor (VEGF), mRNA and retinal VEGF proteins which are thought to be responsible for neovascularization [65,66]. Laser therapy and vitrectomy have been the main stay of treatment so far [67]. Though Ruboxistaurin (RBX), a PKC inhibitor and Ranibizumab (an intra-ocular anti-VEGF) have shown promise in macular oedema and proliferative DR respectively [68-70]. In multicentre, parallel, placebo-controlled, double-masked Protein kinase C β Inhibitor-Diabetic Retinopathy Study 2 (PKC-DRS2), 36 month treatment of RBX resulted in less decline in visual acuity for any given duration of diabetic maculopathy (p=0.01) [69]. In another multicentre, randomised clinical trial, intravitreal

Ranibizumab treatment with prompt and deferred laser therapy for diabetic maculopathy involving central macula, resulted in significant improvement in visual acuity from baseline (p=<0.001) [70].

1.1.3.3 Diabetic Peripheral Neuropathy

Diabetic peripheral neuropathy (DPN) is common in patients with diabetes. Its prevalence has been estimated as 45% in patients with long standing diabetes [71]. Prevalence is variable depending on the study methods used, with one study estimating it to be 73% in patients with T2DM [72]. The pathogenesis of DPN is multifactorial, with activation of polyol and PKC pathway, increased oxidative and nitrosative stress and disruption in immune mechanisms at various levels, lead to the activation of poly ADP ribosylation and depletion of ATP, causing neuronal damage [73]. DPN when associated with pain, leads to considerable health burden leading to poor quality of life [74]. The symptomology varies greatly from the abnormal perception of temperature, paresthesia and dysesthesias to reduced light touch and proprioception sensations, depending on the type of nerve fibers involved [75]. DPN is the most difficult complication to treat. Treatment of hyperglycaemia and other modifiable factors remain central to the management. Several agents are still under development which hopefully will be more effective in the treatment [76].

1.1.3.4 Cardiac Autonomic Neuropathy

Cardiac Autonomic Neuropathy (CAN) is the lesser known of diabetes complications but has been shown to be associated with significant mortality and morbidity [77]. Autonomic neuropathy can occur as part of DPN or as a separate entity[72]. The prevalence has been

estimated between 20%-73% in patients with T2DM, depending on the populations and study methods used [78]. CAN shares its pathogenesis with DPN [78]. Parasympathetic denervation occurs first, leading to sympathetic overdrive, which in part, has been implicated in the pathogenesis of other microvascular complications as DN [78,79]. Sympathetic denervation occurs as the last step [78]. Though lifestyle modifications and hyperglycaemia treatment remain central, several specific treatments are also being considered in the management of CAN [78] such as α-lipoic acid and C-peptide treatment. [78]. Reno protective agents like ACE inhibitors or ARBs have shown to improve ventricular dysfunction and sympatho-vagal balance [78].

1.1.4 Pathogenesis of Microvascular Complications

Chronic hyperglycaemia is the defining feature of T2DM resulting in a variety of metabolic and molecular consequences such as oxidative stress, inflammation, and the activation of protein kinase C (PKC), the polyol and hexosamine pathways and AGE, which are associated with the development of microvascular complications and premature cardiovascular disease (**Figure 1.4**) [1,80,81].

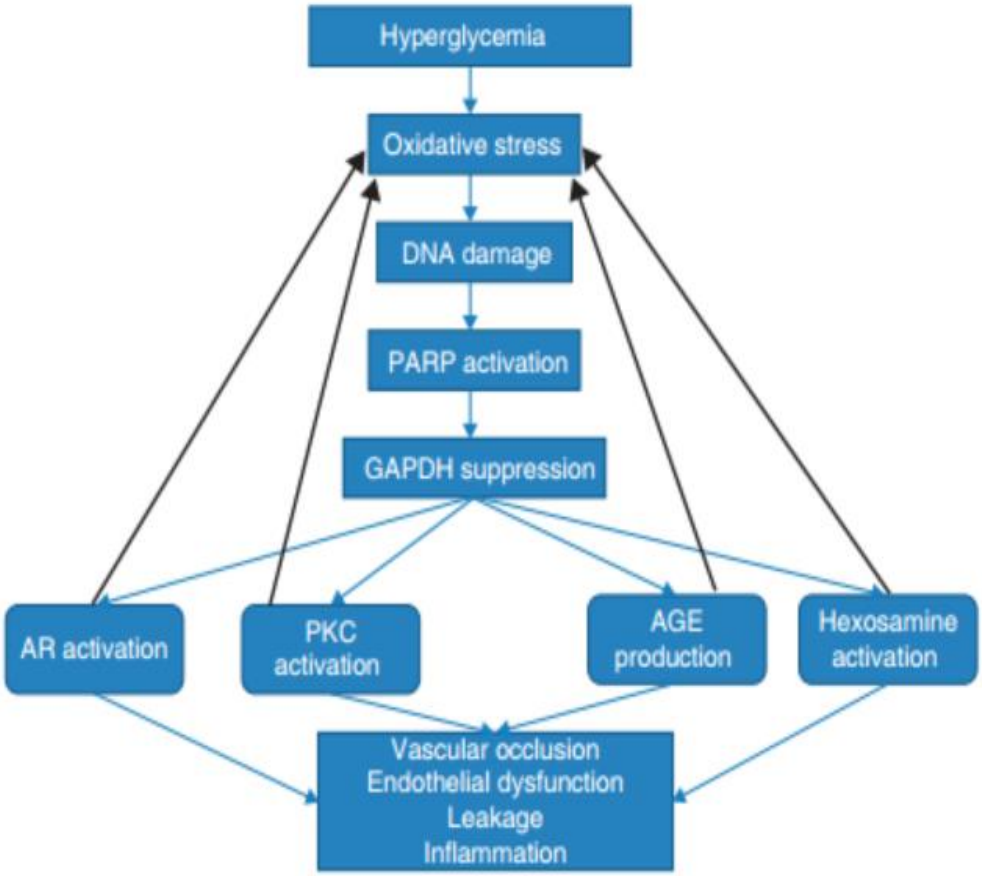

Figure 1.4 Summary of the mechanisms that relate hyperglycaemia to microvascular complications in patients with diabetes. Adopted with permission (75)

1.1.4.1 Advanced Glycation End Products

Advanced glycation end products (AGE) are modified proteins or lipids that have become glycated and oxidized non-enzymatically following exposure to aldose sugars. This results in the production of Schiff bases and Amadori products, the process also referred to as the Maillard reaction (**Figure 1.5**) [82].

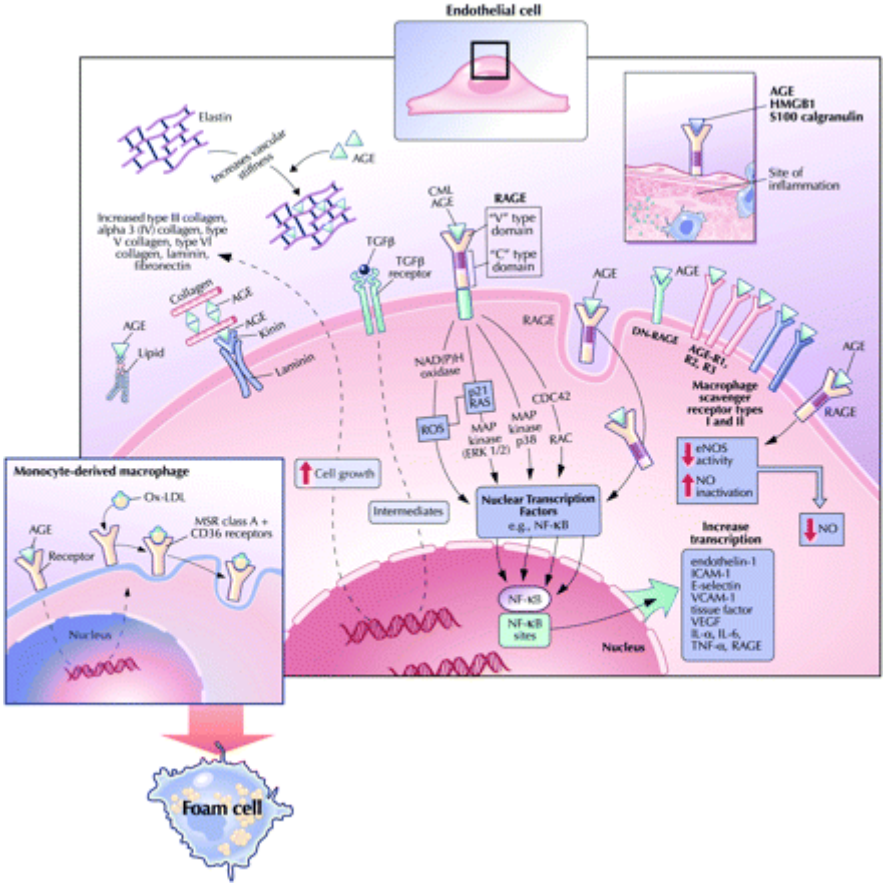

Figure 1.5 The cellular effects of AGEs. Adopted with permission (78)

The presence of increased rate of turnover of proteins, intra- and extracellular hyperglycaemia, and the oxidative stress in the environment are essential in the formation of AGE, which is invariably an irreversible and cumulative process, leading to the development of diabetic microvascular complications [82,83]. AGE exert their action by binding with the receptor of AGE (RAGE) which is a part of immunoglobulin receptor family [82]. RAGE, when bound with AGE, initiates an intracellular cascade that leads to cell function disruption. However, not all AGE receptors are disruptive. Certainly, other receptors like AGE-R1, R2, R3, and the class A macrophage scavenger receptor types I and II, when bound with AGE ligands, cause clearance and detoxification of AGE [82].

AGE formation on protein in extracellular matrix permanently alters cellular structure and function, while AGE formation on lipids leads to the formation of glycated LDL which reduces nitric oxide (NO) production and uptake and clearance of LDL [84]. Similarly, intracellular production of AGEs on proteins like basic fibroblast growth factor (bFGF) significantly reduces the mitogenic activity of endothelial cell cytosol [85]. Circulating AGEs transduce multiple signal pathways such as mitogen-activated protein kinases (MAPKs) which upregulate and translocate transcription factors like NF-κB, which after going through multiple pathways transcribe genes like vascular cell adhesion molecule-1 (VCAM-1), endothelin 1, intercellular adhesion molecule-1 (ICAM-1), vascular endothelial growth factor (VEGF), and proinflammatory cytokines [86,87]. All of which lead to endothelial dysfunction and increased vascular permeability which are the hallmark of diabetic microvascular complications.

In vitro studies and animal models have suggested that inhibition of AGEs with compounds like Aminoguanidine, ALT-946 (*N*-(2-Acetamidoethyl) hydrazine-carboximidamide hydrochloride), ALT-711 (3-phenyacyl-4, 5-dimethylthiazolium chloride) , improve arterial elasticity, reduce severity of atherosclerotic plaques, favour nitrous oxide activity and improve arterial stiffness [82,88-91].

Similar results have also been demonstrated in human studies. In a randomized, double-masked, placebo-controlled clinical trial on 690 patients with type 1 diabetes and diabetic nephropathy (DN), treatment with Pimagedine resulted in slow progression of DN, reduced proteinuria and statistically non-significant 13% risk-reduction of the combined end points of ESRD or death. It was also associated with reduction in the progression of diabetic retinopathy (DR), increase in high density lipoprotein (HDL), decrease in triglycerides and

decrease in sitting diastolic BP. Again, these results failed to achieve statistical significance [92].

1.1.4.2 Protein Kinase C Pathway

Protein Kinase C (PKC) family comprise 12 serine-threonine kinases which are divided into 3 sub-groups, depending on the activators. Classical PKC (α, β and γ) are activated by Ca^{2+} and diacylglycerol (DAG), novel PKC (δ, ε, η, and θ) are activated by DAG, and atypical PKC are activated independent of Ca^{2+} and DAG [93]. Activation of PKC family is essential for distinct cellular responses, for example cell proliferation, differentiation and apoptosis [93,94]. PKC β is specifically implicated in the hyperglycaemia induced vascular dysfunction [95]. Hyperglycaemia increases DAG, which activates PKC [95]. This activation stimulates the cellular signalling process for VEGF, which along with other growth factors like IGF -1, IGF – 2 and FGF, plays an important role in endothelial dysfunction and development of diabetic microvascular complications, specifically diabetic retinopathy (**Figure 1.6**) [87,95].

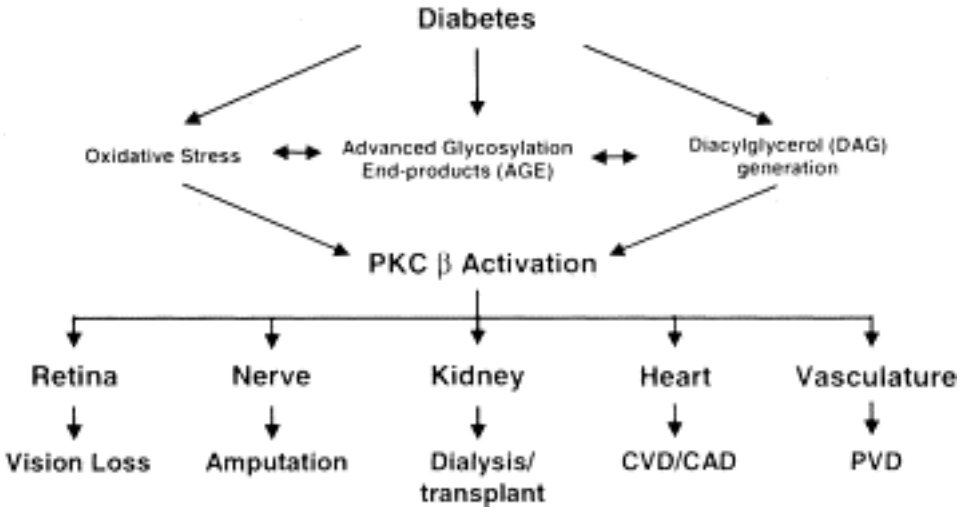

Figure 1.6 Diabetes induced activation of PKC. Adopted with permission (87)

PKC activation augments the oxidative stress (OS) by increased production of NF-KB and NADPH oxidase [80]. It also increases the expression of endothelin-1 (ET-1) and results in the increased concentrations of prostanoids (such as PG-E2 and PG-I2), which are implicated in the development and progression of diabetic retinopathy and diabetic nephropathy respectively [96].

Animal studies have demonstrated that inhibition of PKC could be potential therapeutic option for halting the progression of microvascular complications. After 16 weeks of treatment with ruboxistaurin (LY333531), a PKC β inhibitor, male db/db mice exhibited improved proteinuria and statistically non-significant improvement in systolic and diastolic blood pressure. These results were independent of any change in blood glucose levels [97].

Similarly in a randomised controlled trial of 123 adult patients with type 2 diabetes, a 12 month treatment with ruboxistaurin, improved proteinuria (p=0.02) and reduced the loss of eGFR (p=0.185) [98]. In another multicentre, double-masked, placebo-controlled study of 252 patients, ruboxistaurin was associated with reduced risk of moderate visual loss (p=0.25) in patients with baseline diabetic macular oedema. Ruboxistaurin significantly reduced the risk of moderate visual loss (HR=0.37; 95%CI 0.17, 0.80; p=0.012)] but did not prevent the progression of DR [99].

1.1.4.3 Aldose Reductase activation (polyol pathway)

Aldose Reductase (AR) is a part of monomeric, cytosolic oxidoreductase family that undertake the NADPH-dependent reduction of compounds such as glucose, ketones and steroids, to the corresponding alcohol product [100]. AR has a propensity for glycol

aldehydes and polyol aldehydes, and catalyses the first step of polyol pathway, reducing glucose to sorbitol initially, by an NADPH-dependent reaction [100,101]. Sorbitol is further oxidised to fructose by sorbitol dehydrogenase, increasing NADH concentration [101]. In the presence of excess glucose in the face of hyperglycaemia, the polyol pathway is activated many folds [101]. The resultant reduction in NADPH and increase in NADH lead to increased formation of AGE, increased oxidative stress and activation of pentose phosphate pathway (PPP) (**Figure 1.7**) [96,101,102].

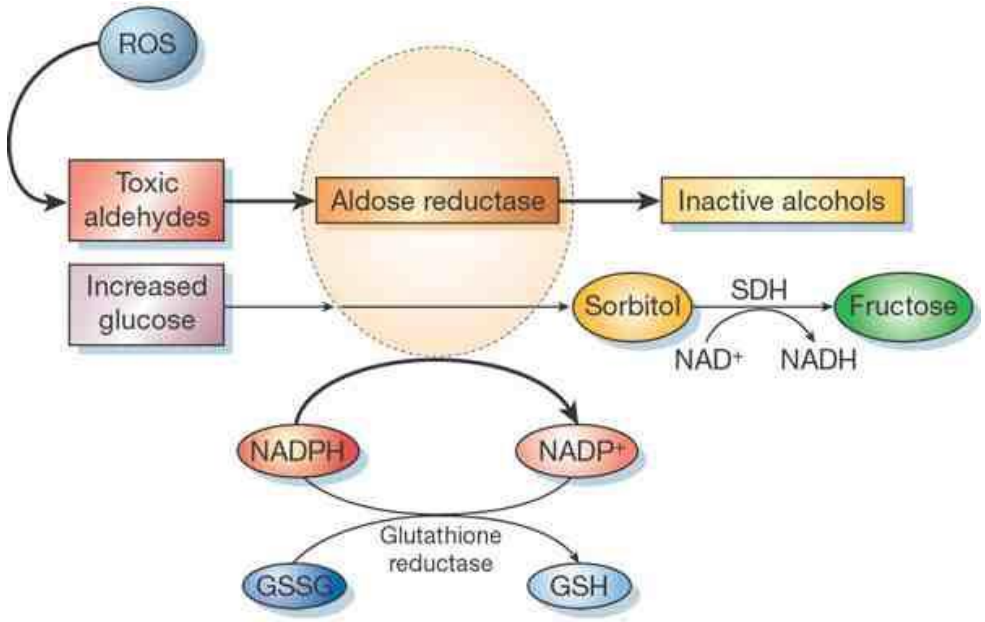

Figure 1.7 Aldose reductase and polyol pathway. Adapted with permission (96)

Increased flux of glucose through polyol pathway is thought to be responsible for the development of microvascular complications like diabetic nephropathy and diabetic neuropathy [101,103].

Inhibition of AR has been studied extensively, both in vitro and in vivo studies, in the context of slowing down the progression of microvascular complications. In an animal study, 15 month long administration of fidarestat (aldose reductase inhibitor) in streptozotocin (STZ)-induced diabetic rats, led to the improvement in the number of retinal pericytes and thickness of retinal basement membrane [104]. Zenarestat, another ARI studied in the similar context, improved motor nerve conduction velocity (MNCV) and F-wave minimal latency (FML), and reduced nerve sorbitol concentration, after 8 week course in Zucker diabetic fatty rats [105]. In a large phase 3, placebo controlled trial of patients with diabetes, 12 month administration of zenarestat resulted in the lack of progression of all the parameters of nerve conduction studies, when compared with placebo. This effect was independent of HbA1cHbA1c levels. The trial was terminated early due to rising levels of creatinine in some patients [106].

1.1.4.4 Hexosamine pathway

Hexosamine pathway (HSP) is a part of glycolysis, which utilises 3% of total body glucose [107]. Upon entry into the HSP, glucose is catalysed via glutamine–fructose–6-phosphate amidotransferase (GFAT) and after going through several steps, produce UDP-N-acetylgalactosamine (UDP-GalNAc) (**Figure 1.8**) [102,107]. UDP-GalNAc are not only the building blocks of glycoprotein and glycolipids, but also provide negative feedback to GFAT, thus regulating the entry of glucose into HSP [107]. UDP-GlcNAc provide substrate for O-GlcNAc transferase (OGT) which modify insulin receptor substrate–1 (IRS–1) and insulin receptor–2 (IRS–2), causing IR [107]. The flux of glucose into HSP increases the gene transcription of transforming growth factor-α (TGFα), transforming growth factor-β_1 (TGFβ_1)

and plasminogen activator inhibitor-1 (PAI-1) through unknown mechanisms. This gene transcription is implicated in the development of inflammation, IR and microvascular and macro vascular complications [80,102,108].

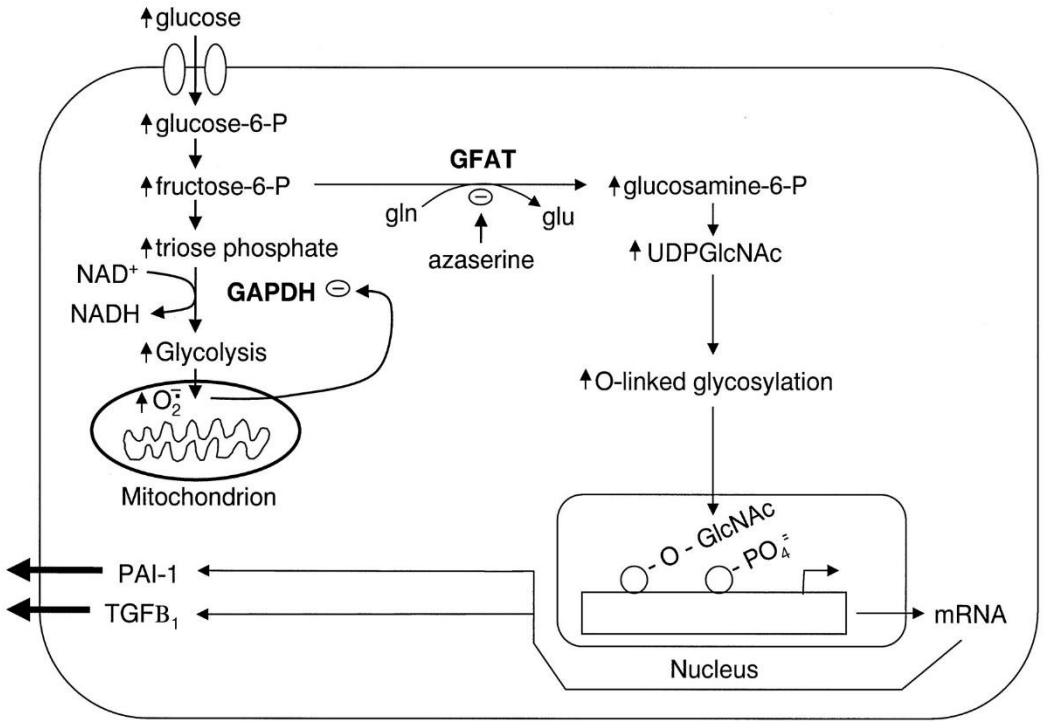

Figure 1.8 Schematic representation of Hexosamine pathway. Adopted with permission (102)

1.1.4.5 Oxidative Stress

Oxidative stress is defined as imbalance between the production of free reactive oxygen radicals and body's antioxidant mechanisms [109]. Mitochondrial electron transport chain consist of four protein complexes called complex I, II, III, and IV [80]. Under physiological conditions, glucose is metabolised through tricarboxylic acid (TCA) cycle, which generates electron donors. The electron donors comprise of NADH and $FADH_2$ which donate electrons to complex I and II respectively. From then, these electrons are transferred to coenzyme Q, complex III, cytochrome-C, complex IV and then to molecular oxygen, where they reduce to

water [80]. As the electrons are being passed through the complexes, some of the energy is utilised in pumping protons across mitochondrial membrane, effectively creating a voltage gradient [80]. This voltage gradient, along with uncoupling proteins (UCP) keep the generation of ATP constant [80]. In hyperglycaemic conditions, there is more glucose through TCA cycle, producing greater number of electrons and bigger voltage gradient across mitochondrial membrane [80] until a critical threshold is reached after which electrons start being transferred to coenzyme Q and molecular oxygen, giving rise to superoxide (**Figure 1.9**) [80,96].

Experimental studies also showed that production of superoxides or reactive oxygen species are the pre-requisite for the activation of polyol, PKC and hexosamine pathways and AGE production [80]. This suggests a crucial role of the oxidative stress in the development of microvascular complications.

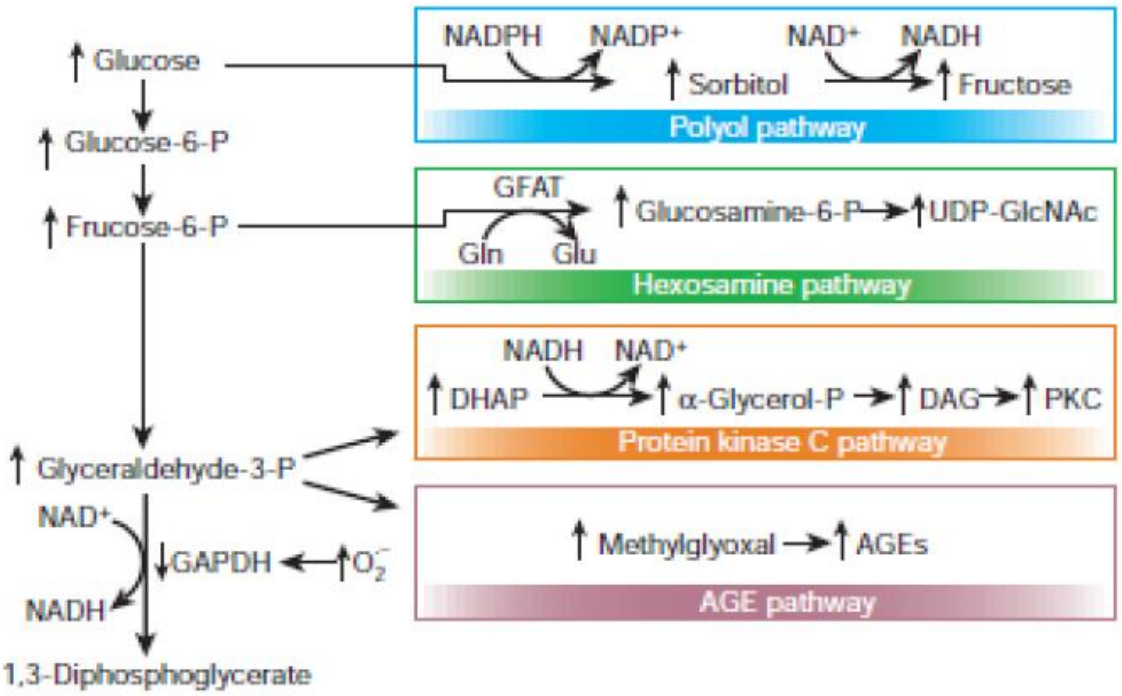

Figure 1.9 Mitochondrial overproduction of reactive oxygen species. Adopted with permission (96)

1.1.4.6 Glyceraldehyde-3 Phosphate Dehydrogenase Inhibition

Glyceraldehyde-3 Phosphate Dehydrogenase (GAPDH) catalyses the sixth step of glycolysis chain [110]. Hyperglycaemia decreases the activity of GADPH leading to an increase in the levels of glycolytic metabolites. Glycolytic metabolite Glyceraldehyde-3 Phosphate activates AGE production (due to increased production of methylglyoxal) and PKC (due to increased production of diacylglycerol) [80]. Glycolytic metabolite Fructose-6 Phosphate activates hexosamine pathway due to increased flux [80]. Furthermore, inhibition of GADPH leads to increased intracellular levels of glucose, increasing flux through polyol pathway [80].

1.1.4.7 Polymers of ADP-ribose polymerase Activation

Poly (ADP-ribosyl)ation is a process of protein modification which form polymers of poly(ADP-ribose) (PAR). These PARPs are attached with each other via glutamic acid, aspartic acid and lysine target proteins [111]. Out of eighteen PARP family members, only 2 are activated in response to DNA damage [111].

Hyperglycaemia is the main stimulus for PARP activation [80]. Increased oxidative stress secondary to production of reactive oxygen molecules induce DNA damage, activating PARP [80]. PARP splits NAD^+ molecules into nicotinic acid and ADP-ribose which form polymers. These polymers inhibit GADPH, which activates the polyol, PKC and hexosamine pathways and increase production of AGE (**Figure 1.9**) [80,96]. In an experimental study on STZ-diabetic rats, 10 week treatment with oral PARP inhibitor (GPI-15,427) improved motor and

sensory nerve conduction velocities and nitrosative stress. It also prevented TNF-α accumulation and axonal atrophy of large myelinated nerve fibres [112].

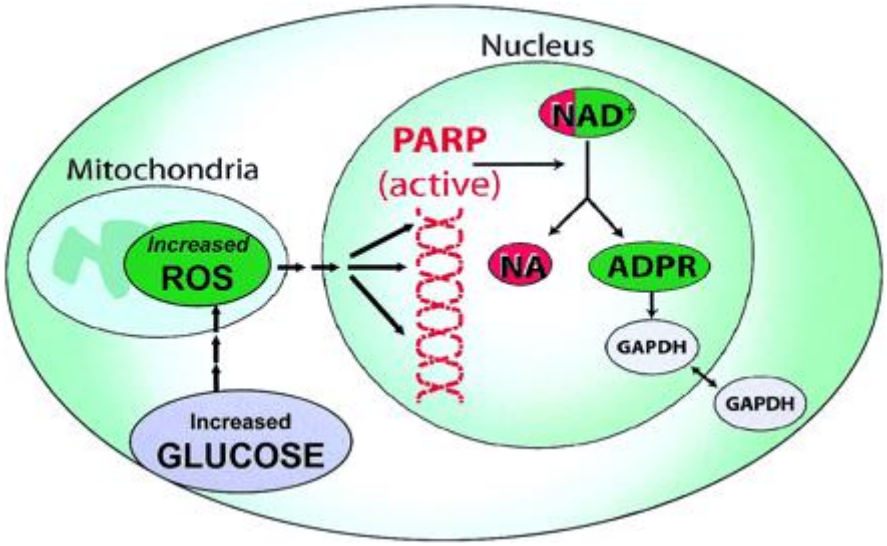

Figure 1.10 PARP activation secondary to DNA damage. Adopted with permission (96)

1.1.4.8 Role of Hyperglycaemia

Hyperglycaemia is crucial in initiating the polyol, hexosamine and PKC pathways and increase the production of AGE. For details, please see above. Several prospective studies have demonstrated that hyperglycaemia can lead to the development and progression of macrovascular and microvascular complications and increased all-cause mortality as is associated with T2DM [113-118].

In the landmark UKPDS trial, improved HbA1c lowered the risk for all-cause mortality by 6% (95%CI 10, 20; p=0.44) [119] and for any diabetes related death by 10% (95%CI 11, 27; p=0.34). There was a risk reduction of 25% (95%CI 7, 40; p=0.009) in microvascular end points, mainly the need for retinal photocoagulation [119]. In DCCT trial, intense treatment of diabetes reduced the mean adjusted risk of incidence of microalbuminuria by 34%

(p=0.04) and reduced the albumin excretion rate by 15% (p=<0.01) [120]. In longitudinal analysis of DCCT cohort, patients with previous optimum glycaemic control had a 25% prevalence of diabetic neuropathy when compared with 35% in patients who had standard glycaemic control (p=<0.001) [121]. In the follow up of randomised, open-label, blinded STENO-2 trial, intensive treatment resulted in the reduction of cardiovascular mortality (HR= 0.43; 95%CI 0.19, 0.94; p=0.04), cardiovascular morbidity (HR=0.41; 95%CI 0.25, 0.67; p=<0.001) and all-cause mortality (HR=0.54; 95%CI 0.32, 0.89, p=0.02). There was less progression of DN (RR=0.44; 95%CI 0.25, 0.77; p=0.04), DR (RR=0.57; 95%CI 0.37, 0.88, p=0.01) and autonomic neuropathy (RR=0.53; 95%CI 0.34, 0.81; p=0.004) in intense treatment group [122].

1.1.4.9 Role of Hypertension

T2DM and hypertension are closely linked together, carrying the increased risk of renal cardiovascular morbidity and mortality [123]. In patients with better blood pressure control, there was a 24% risk reduction in all diabetes-related end points (95%CI 8, 38; p=0.004), 32% risk reduction in diabetes related deaths (95%CI 6, 51; p=0.019) and 37% risk reduction in microvascular end points (95%CI 11, 56; p=0.009), mainly owing to reduced risk of retinal photocoagulation [123]. In a prospective study on patients with diabetic nephropathy, aggressive blood pressure treatment reduced urinary albumin excretion from 977 µg/minute to 433 µg/minute after a 39 month period of treatment with multiple anti-hypertensive agents [124]. The rate of GFR decline reduced from 0.91 ml/min/month to 0.39 ml/min/month [124]. In a prospective, controlled, randomized trial of 5 year duration on normotensive patients with T2DM, aggressive blood pressure treatment significantly

lowered the progression of normoalbuminuria to microalbuminuria (p=0.012) and microalbuminuria to overt albuminuria (p=0.028) [125]. There was less progression of diabetic retinopathy at two years (13% vs 21%, p=0.046) and five years (34% vs 46%, p=0.019) [125]. There was no difference in diabetic neuropathy [125].

1.1.4.10 Role of Lipids

Dyslipidaemia, especially hypertriglyceridaemia, has been brought into light recently as a significant risk factor, along with hyperglycaemia, for diabetic complications [126]. Chemical modification of the lipid produce dicarbonyl intermediates, glyoxal and methylglyoxal [126] which contribute to the production of advanced lipoxidation end-product (ALE) [126]. This process is catalysed by hyperglycaemia and oxidative stress [126]. Low density lipoproteins (LDL) oxidize in the presence of ROS to form oxLDL which are not only cytotoxic but are also involved in endothelial dysfunction [127]. Increased levels of oxLDL upregulate oxidized LDL receptor-1 (LOX-1) which is the primary cell surface receptor [127]. LOX-1 is found in abundance in neurons [127]. Following activation of LOX-1, neurons activate NADPH oxidase, increasing superoxide generation, leading to neuronal injury [127].

In EURODIAB study, higher levels of total cholesterol, LDL and triglycerides were associated with increased incidence of diabetic neuropathy, despite adjustments for duration of diabetes and glycaemic control [128]. In animal models, treatment with HMG-CoA reductase inhibitors has been shown to be associated with restoration of vasa nervosum, possibly by upregulating nNOS/NOS in Schwann cells via the P13K/Akt signalling pathway [129].

1.1.4.11 Role of Obesity

Obesity share multiple complex mechanisms linking it to the pathogenesis of T2DM. These include increased inflammation, IR, low adiponectin, endothelial dysfunction and activation of autonomic nervous system amongst others [130]. Obese non-diabetic patients are found to have reduced amplitude of sensory/mixed nerve responses on nerve conduction studies, probably due to thickness of sub cutaneous tissue [131]. These changes are associated with hyperinsulinaemia and IR [131]. In SOS study which was a prospective randomized, controlled interventional trial in obese patients with T2DM, bariatric surgery reduced incidence of fatal and nonfatal cardiovascular events (HR=0.53; 95%CI 0.35, 0.79; p=0.002) [132]. In a retrospective cohort analysis of obese patients with T2DM who underwent bariatric surgery showed that bariatric surgery improved existing DN significantly especially in patients with CKD 3a (p=0.004). The change in eGFR was greater in patients with eGFR ≤60 mL/min/1.73m^2 (95%CI -6.8, 10, p=0.009) [130]. In an observational cohort study on patients with T2DM, obesity (BMI >30 kg/m^2) was a significant risk factor for developing DR (OR=3.52; p=<0.0001). This remained independently associated despite adjustment for multiple confounders [133]. There was no association of obesity with diabetic maculopathy [133].

In multicentre, randomised Look AHEAD trial, exploring the effect of weight loss through intensive life style intervention (LSI) in obese patients with T2DM, LSI resulted in significant weight loss of 8.6% (95%CI -8.9%, -8.4%) compared with 0.7% (95%CI -0.9%, -0.4%) in diabetes support and education (DSE) group at year 1 (p=<0.001). 11.5% of the participants in LSI group experienced partial or complete remission of T2DM (95%CI 10.1%, 12.8%;

p=<0.001) [134]. LSI was associated with reduced incidence of high risk CKD (HR=0.69; 95%CI 0.55, 0.87, p=0.002) after 13 years of follow up [135].

However, not all measures of adiposity are associated with worse metabolic outcomes. Visceral adiposity, rather than abdominal subcutaneous fat, are associated with IR, hyperglycaemia and hyperinsulinaemia [136].

1.2 Obstructive Sleep Apnoea

1.2.1 Overview

Obstructive sleep apnoea (OSA) is characterised by repeated episodes of apnoea and hypopnoea during sleep [137]. This results in deranged gas exchange and recurrent arousals from sleep [138]. Prevalence of OSA of varying degrees has been estimated from 2% - 28% depending on the population studied and the study designs [139]. According to an estimate, the prevalence of OSA is 79% in patients with impaired glucose tolerance (IGT) and diabetes [140]. Men seem to be more at risk of developing OSA than women; post-menopausal women are more at risk than pre-menopausal women [139]. OSA has been associated with hypertension, cardiovascular disease, insulin resistance and impaired glucose tolerance [137,138,141].

OSA has been linked with non-dipping of nocturnal blood pressure, leading to increased risk of developing hypertension and cardiovascular diseases as well as target tissue damage [142]. Non dipping of blood pressure is thought to be secondary to sympathetic overdrive resulting from apnoeic episodes throughout the night [142]. The Wisconsin Sleep Cohort Study estimated the adjusted odds ratio (95%CI) of systolic non-dipping for baseline AHI 5-

14.9 and ≥ 15, vs. AHI < 5 to be 3.1 (1.3, 7.7) and 4.4 (1.2, 16.3), respectively [142]. In a prospective, population-based sub-study of the Wisconsin Sleep Cohort, dose–response association was found between OSA at base line and the development of hypertension and subsequent increased cardiovascular mortality after 4 years of follow up [143].

In another study of males with and without OSA but without other co-morbidities, 36.7% of the patients with OSA were found to have at least one cardiovascular (CV) event compared with 6.6% patients without OSA (p=<0.001) [144]. In OSA group, 56.8% sub optimally treated patients (either with continuous positive airway pressure (CPAP), surgery (uvulopalatopharyngoplasty, or oral appliance) developed CV event compared with 6.7% efficiently treated patients (p=<0.001) [144]. Significant risk reduction for CV events was observed post treatment (OR 0.1; 95%CI 0.0, 0.7) after adjusting for multiple confounders in patients with OSA [144].

However, treatment of OSA with CPAP therapy has not shown significant impact on cardiovascular morbidity and mortality [145-147]. In multicentre, randomised, parallel-group, open label SAVE study on patients with pre-existing diagnosis of cardiovascular or cerebrovascular disease and moderate to severe OSA, there was no difference in cardiovascular mortality and morbidity and cerebrovascular morbidity in patients who were treated with CPAP compared to patients who had usual care of OSA. However, in propensity score-matched analysis, patients on CPAP were less likely to have a cerebral event compared with patients who only received usual care with no impact on cardiac events (HR=0.52; 95%CI 0.3, 0.9; p=0.02) [145].

Undiagnosed OSA is associated with increased risk of road traffic accidents (RTA). In a sub-study on Wisconsin Study Cohort, it was found that patients with mild OSA are at risk of

having at least one RTA in 5 years (adjusted OR=4.2 for AHI 5 – 15; adjusted OR=3.4 for AHI >15) compared with patients with no OSA [148]. There is limited data about OSA and its association with poor cognitive functions and quality of life (QOL). In Wisconsin Study Cohort, it was found that an increase in AHI of 15 was equivalent to the 5 years of aging on psychomotor function; with no effect on the memory [139]. Similarly, in the same cohort, a linear association of OSA severity was described with decrements on the eight SF-36 scales, indicating impaired QOL [139].

1.2.2 Role of hypoxia

The mechanisms linking OSA and T2DM are complex and multifactorial. Although, intermittent hypoxia is believed to contribute significantly to its pathological consequences. Hypoxia can reduce insulin sensitivity, suggesting an impact on β-cell function [149]. The intermittent hypoxia and the re-oxygenation simulate ischaemic–reperfusion injury and result in the production of oxidative and nitrosative stress causing cellular and DNA damage (**Figure 1.11)** [149] .

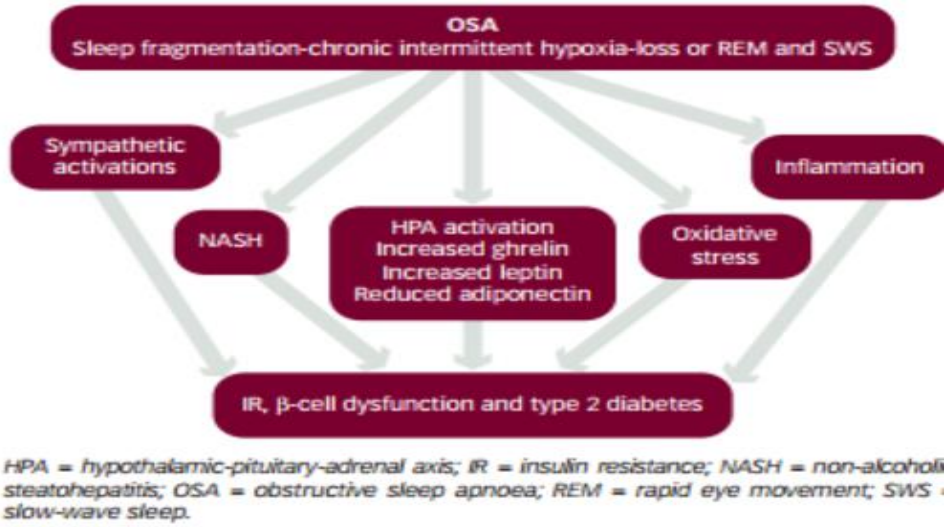

Figure 1.11 Possible mechanisms linking OSA and T2DM. Adopted with permission (148)

Hypoxia (as part of OSA) results in the activation of hypoxia-inducible factor-1 (HIF-1) which is responsible for the activation of cellular responses e.g. production of VEGF, cytokines and other growth factors [150]. HIF-1$\propto$ binds with HIF-1β, forming active HIF complex. This results in the formation of hypoxia response element (HRE) which is responsible for multiple biologic responses e.g. transcriptional activity of iNOS expression which is essential for the production of oxidative stress [151,152]. Oxidative stress remain one of the most important common metabolic imbalance resulting from both hyperglycaemia and hypoxia [153]. Hypoxia impairs the utilization of electrons by mitochondrial NADH [153]. The resultant accumulation of electrons stop the transfer of the electrons from the cytosol to mitochondria, resulting in the increased cytosolic NADH [153]. In addition, both hypoxia and hyperglycaemia increase the production of triose phosphates and glycolysis via different additive mechanisms [153]. Furthermore, reduced oxidative phosphorylation and increased anaerobic metabolism, which are the adaptive responses to the hypoxia, are attenuated in the presence of hyperglycaemia, resulting in a further increase in oxygen consumption and worsening of intra-cellular and mitochondrial hypoxia [154]. This results in mitochondrial dysfunction and uncoupling of oxygen consumption from ATP production [154].

1.2.3 OSA and T2DM

So far, we have ascertained that OSA is closely related to increased cardiovascular morbidity and mortality. There is a growing body of evidence that OSA is associated with IR and T2DM. In a single-blinded study on healthy non-diabetic volunteers, 5 hours of intermittent hypoxia was associated with reduced insulin sensitivity (p=0.017) [155]. Hypoxia was also associated with shift in sympathovagal balance towards increased sympathetic activity [155]. In

longitudinal analysis of Wisconsin Sleep Cohort, patients with higher apnoea-hypopnoea index (AHI) of ≥15 were at greater risk of developing T2DM in the long term (OR=2.3; 95%CI 1.28, 4.11; p=0.005). The results remained significant even after adjustments for age, sex and BMI [156].

In a case-control study of patients with OSA only, it was found that OSA was independently associated with hypertension, hyperinsulinameia, hypertriglyceridemia and higher HOMA levels, suggesting the presence of metabolic syndrome, even after adjusting for multiple confounders [137]. Similarly, in a sub-study of Sleep Heart Health Study (SHHS) cohort, it was found that patients with moderate to severe OSA, had higher HOMA values, suggesting an insulin resistance state [157]. OSA has also been associated with impaired β – cell function and β – cell death [158,159].

In patients with pre-existing diagnosis of T2DM, prevalence of OSA has been described from 48% to 86%, depending on the population size and characteristics studied [160-162]. In a study on patients with T2DM, presence of OSA was associated with poorer glycaemic control, higher BMI (unadjusted p=0.00042), greater waist circumference (unadjusted *P* = 0.00038) and more diabetic microvascular complications compared with patients with no OSA [163]. In a meta-analysis of 6 prospective studies including 6000 patients, patients with moderate to severe OSA was at higher risk of developing T2DM (RR=1.63; 95%CI 1.09, 2.45; p=0.018) [164].

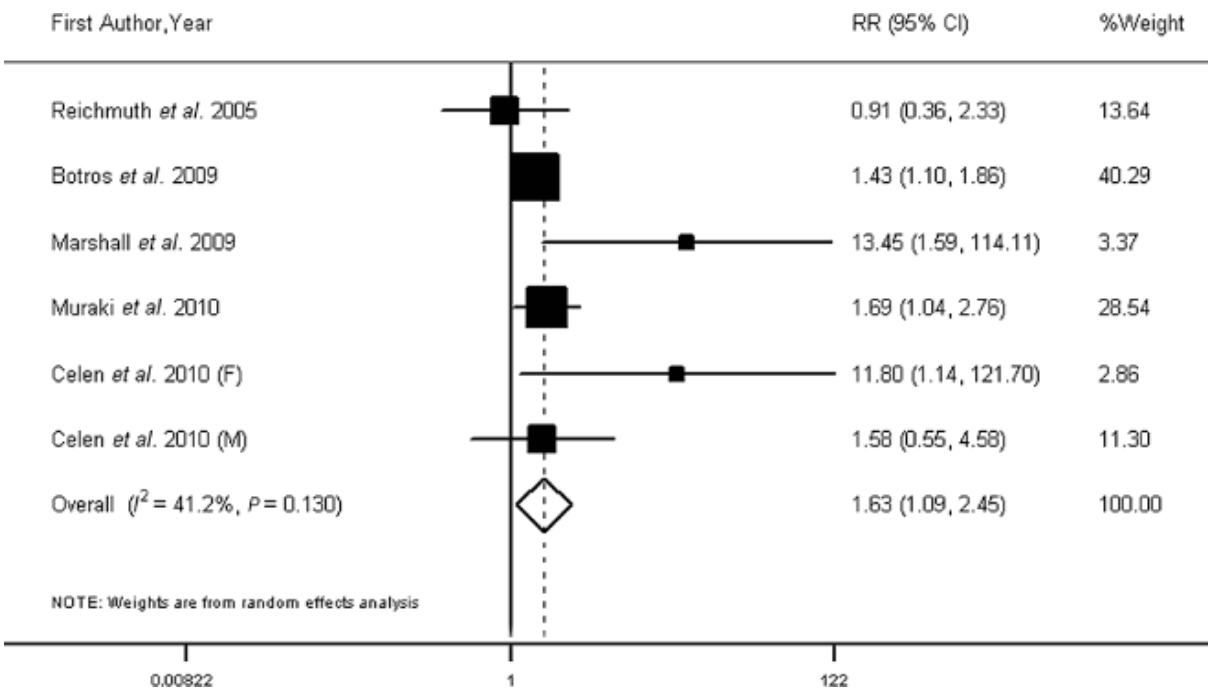

Figure 1.12 Association between moderate to severe OSA and incident T2DM. Adopted with permission (162)

1.2.4 OSA and Diabetic Microvascular Complications

We have described above the possible links between OSA and diabetic microvascular complications. Epidemiologically several studies have confirmed the association between OSA and diabetic microvascular complications. These studies, however, are mostly cross-sectional, showing association rather than causation. There is also a lack of clarity whether OSA contribute to the development or the progression of these complications (or both). A major confounding factor for the association between OSA and microvascular complications is obesity. Statistical adjustments and matching were used in some studies to take into account this confounding effect. Longitudinal studies and interventional trials assessing the impact of OSA on the diabetes-related microvascular outcomes are currently ongoing.

1.2.4.1 OSA and Diabetic Nephropathy

Studies regarding the relationship between OSA and DN are limited. Our group was the first to report a comprehensive cross-sectional data to show that OSA is associated with albuminuria (micro and macro), lower eGFR and more diabetic nephropathy in South Asians and White Europeans with T2DM. It also proved that OSA was independently associated with DN despite adjustments for multiple confounders [165].

In another cross-sectional study of Japanese patients with T2DM, ODI ≥5 was independently associated with micro albuminuria and DN in women but not in men [166]. Similar studies were found in another cross-sectional study in which snoring (as surrogate marker of OSA) was found to be independently associated with micro albuminuria in patients with diabetes [167]. However, another cross-sectional study found that OSA was not associated with micro albuminuria in patients with T2DM [168]. These findings could be due to the small sample size of this study (n=52) or to methodological issues as polysomnography was performed in high risk patients only.

1.2.4.2 OSA and Diabetic Retinopathy

OSA has been shown to be associated with DR. In Japanese patients undergoing vitreous surgery, the ODI was higher in patients with compared to those without proliferative DR and higher oxygen saturations were protective against proliferative DR after adjustment for age, HbA1c and hypertension [169]. OSA was also associated with angle neovascularisation in Japanese patients with proliferative DR [170]. Our work has shown that in a cross sectional

study, OSA was found to be independent predictor of maculopathy (OR=3.320; 95% CI 1.59, 6.93; p=0.001), advanced retinopathy (OR=6.065; 95% CI 1.91, 19.23, p=0.002) and sight threatening DR (OR=3.68; 95% CI 1.77, 7.69; p=0.001) after adjustment for possible confounders [171].

1.2.4.3 OSA and Diabetic Peripheral Neuropathy

Our group assessed this association in a cross sectional study and found OSA to be independently associated with DPN (adjusted OR=2.82; 95%CI 1.44, 5.52) and foot insensitivity (adjusted OR=3.97; 95%CI 1.80, 8.74) after adjustment for confounders [172]. AHI and nocturnal hypoxemia were also associated with DPN [172]. OSA was associated with lower intra-epidermal nerve fibre density (IENFD) (p=<0.001) [173]. AHI was associated with percentage of PARP stained nuclei indicating PARP activation (B=13.67; p=0.025) [173]. OSA was associated with increased prevalence of diabetic foot ulceration (DFU) (OR=3.34; 95%CI 1.19, 9.38; p=0.022) [173].

1.2.4.4 OSA and Cardiac Autonomic Neuropathy

OSA is also associated with CAN and increased sympathetic activity [174]. It is likely that both, the recurrent hypoxia [175] and recurrent arousals [176] are contributing to the activation of the sympathetic system in patients with OSA. However, the relationship with OSA and CAN is likely to be bidirectional as obese patients with CAN develop more frequent and more prolonged hypopnoea/apnoea in comparison to those without CAN whether or not they had T2DM [177]. The impact of OSA on CAN might be modulated by diabetes

duration. Whilst early in the course of the disease (i.e. pre diabetes/short diabetes duration) OSA is associated with sympathetic over activity, in patients with long diabetes duration we found that OSA was associated with sympathetic and parasympathetic withdraw [171]. Both OSA and CAN share the similar mechanisms that link OSA to other diabetic microvascular complications e.g. oxidative and nitrosative stress [171]. Interestingly, our group and others showed that CAN contribute to the development and progression of microvascular complications (particularly nephropathy); whether the impact of OSA on microvascular complications is mediated by or independent of CAN is currently being examined.

1.2.5 Effect of CPAP on T2DM

While OSA is very common in patients and is associated with vascular disease, the impact of OSA treatment in patients with T2DM is unclear with limited evidence available.

Treatment of OSA with CPAP in patients without diabetes, results in the better systolic, diastolic and mean blood pressure control [178]. In a meta-analysis of studies looking at effect of CPAP on IR, it was found that the difference in means of HOMA IR between patients with CPAP and sham CPAP, was −0.44 (95%CI −0.82, −0.06; p=0.02), indicating a modest effect [179]. Similarly, another meta-analysis showed that treatment with CPAP improved IR in patients without diabetes and with moderate to severe OSA [180]. CPAP treatment improves IR but its effect on glycaemia is still unclear. Several studies have shown benefit of CPAP on glycaemia [181]. In an open label, parallel and randomized clinical trial, 6 month treatment with CPAP not only improved HbA1c but also mean nocturnal oxygen saturation and baseline IL-1 levels independent of HbA1c change (R^2 =0.51; p=0.002) [182].

However, another RCT in patients with T2DM and OSA failed to show such difference when patients on CPAP were compared with patients on sham CPAP [183]. In a meta-analysis of 6 prospective studies including patients with T2DM, treatment of OSA with CPAP did not improve HbA1c (p=0.42) and BMI (p=0.3) [184]. However, insulin sensitivity was improved (p=0.05) [184].

1.3 Sleep Duration, Sleep Quality and Adiposity Measures

1.3.1 Sleep Duration

Sleep patterns have changed drastically over the centuries due to behavioural, cultural, social and environmental factors. About, one third of adult population sleep an average of 6 hours per night [185]. In a trend analysis as part of National Health Interview Survey (NHIS) on 33,000 healthy adults over a period of 28 years (1985 – 2012), prevalence of short sleep duration was higher in 2012 compared with 1985 with prevalence ratio of 1.32 (95%CI 1.27, 1.37) [186]. This has resulted in poor general health and well-being [187]. Due to this, Centres for Disease Control and Prevention (CDC) has declared sleep restriction as a public health epidemic in 2015 [188]. Furthermore, this has cause deleterious effects on metabolism and endocrine function. Individuals with poor sleep have been found to have increased insulin resistance, activation of sympathetic nervous system and dysregulation of negative glucocorticoid feedback [189-191]. Increased secretion of pro-inflammatory cytokines (IL-6, TNF-α), increased ghrelin and reduced leptin levels have all been implicated as possible mechanisms as clear reasons remain unknown [191]. This could potentially promote obesity, glucose intolerance and hypertension as part of metabolic syndrome and changes in energy balance equation.

In an experimental study on 12 healthy normal weight adults, acute sleep restriction of 4 hours/night for 2 nights resulted in 18% reduction in leptin (p=0.04), 28% rise in ghrelin (p=<0.04), 24% increase in global hunger (p=<0.01) and 33% increased consumption of carbohydrate dense foods (p=0.02) [192]. Epidemiological data in children and adolescents have shown that shorter sleep duration is associated significantly with higher adiposity measures, especially waist circumference (p=<0.05) [193]. In cross sectional analysis of Wisconsin Sleep Cohort, a U-shaped association was found between sleep duration and adiposity measures. Short sleep (<5 hours/night) was associated with 15.5% lower leptin levels (p=0.01) and 14.9% higher ghrelin levels (p=0.008) [194], possibly explaining the link between sleep deficit and increased adiposity through increased food intake and reduced satiety.

In a meta-analysis of 17 studies in adults, a significant association was found between short sleep duration and obesity (OR=1.55; 95%CI 1.43, 1.68) [195].

Similar to adiposity measures, a meta-analysis of 10 prospective studies looking at the relationship between sleep duration and risk of T2DM, observed a U-shaped relationship. Each 1-hour reduction in sleep duration compared with a reference point of 7 hours/night, resulted in a relative risk of 1.09 (95%CI 1.04, 1.15) for developing T2DM [196]. In a cross sectional study on patients with pre-existing diagnosis of diabetes, individuals who slept longer were found to have worse glycaemic control (OR=1.11; 95%CI 1.05, 1.18) [191].

1.3.2 Sleep Quality

Similar to sleep duration, sleep quality also plays an important role in maintaining normal physiology [197]. Poor sleep quality is a hall mark of OSA with or without changes in sleep duration [197]. Metabolic, physiologic and clinical consequences of OSA are described above in this chapter. Prevalence of poor sleep quality varies between 10%-48% depending on the population studied [198]. In a population based study of 700 adults, the prevalence of poor sleep quality (defined as Pittsburgh Sleep Quality Index (PSQI) score >5) was found to be 34.7% [199]. Poor sleep quality was associated with obesity and high fat mass (OR=1.07; 95%CI 1.01, 1.13), despite adjustments [199].

In an experimental study on 11 healthy volunteers, sleep fragmentation (using auditory and mechanical stimuli) resulted in reduced insulin sensitivity by 25.5% (p=<0.001) [197]. There was sympathovagal dysregulation with 12.8% reduction in vagal tone and 16.8% increase in sympathetic tone (p=<0.001) [197]. Dysregulation of cortisol was also observed with 12.5% higher early morning values (p=<0.015) [197]. These findings suggest a negative role of poor sleep quality in glucose metabolism [197].

In the Cardiovascular Health Epidemiology Study on 1500 healthy individuals, poor sleep quality, as assessed by PSQI, was associated with obesity, defined by BMI, among women (OR=1.08; 95%CI 1.03, 1.12) but not with men (OR=0.98; 95%CI 0.89, 1.09) [200]. This gender difference has previously been described in literature with women reporting more sleep-related complaints compared with men [201]. In a cross-sectional study on 16000

In longitudinal Nurse Health Study I on 200,000 healthy women, doing rotating night shift work, 20 years of shift work resulted in increased risk of developing T2DM (HR=1.58; 95%CI 1.43, 1.74; p=<0.001) and weight gain (HR=1.24; 95%CI 1.13, 1.37; p=<0.001) [205].

Whether manipulating the master clock will result in improvement of IR, T2DM and adiposity, remain to be seen and will require interventional studies [26]

1.4 Hypotheses

While the association between OSA and microvascular complications in patients with T2DM has been described by our group and others; the longitudinal impact of OSA on microvascular complications has not been explored before. In addition, while the relationship between sleep duration and obesity is established in general population, there are no data about the relationship between sleep duration and obesity and diabetes in T2DM; which is important considering the impact of obesity on diabetes related outcomes.

Based on the above review, I hypothesised the following:

1. OSA is associated with greater decline in eGFR and the development of CKD in T2DM.
2. OSA is associated with development and progression of sight threatening DR in T2DM.
3. OSA is associated with longitudinal worsening of DPN, foot insensitivity and CAN in T2DM.

healthy individuals, self-reported poor sleep quality was associated with higher prevalence of T2DM despite adjustments for relevant confounders (OR=1.76; 95%CI 1.14, 2.71) [202].

The relationship between poor sleep (duration and quality) and measures of adiposity and metabolic parameters in patients with pre-existing T2DM has not been explored widely.

1.3.3 Circadian Misalignment

Circadian rhythm is defined as 24 hour rhythms of sleep/wake, behaviour, hormones secretion and metabolism, regulated and controlled by the master clock located in the hypothalamus [26]. The master clock uses light signals to synchronise neuro-hormonal mechanisms of peripheral organs e.g. liver, muscles, adipose tissues and pancreas [26]. Advent of modern-day life including longer working hours, shift patterns and increasing use of information technology in our lives, coupled with unhealthy eating and sedentary life style lead to inappropriately synchronised endogenous rhythms, leading to circadian misalignment [26]. The role of the Clock genes in neuro-behaviours and glucose metabolism has been complex and can result in IR [26].

In a study on 10 healthy volunteers, experimental circadian misalignment, resulted in 17% lower levels of leptin (p=<0.001), 6% increase in glucose levels, secondary to exaggerated post-prandial rise (p=<0.001), 22% rise in insulin levels (p=0.006), reduction in insulin sensitivity (p=0.04) and dysregulation of cortisol (p=<0.001) [203]. The effect on leptin levels were independent of sleep deficit, usually seen in the case of circadian misalignment [203].

In a large Finnish epidemiological study, individuals with circadian misalignment (evening chronotype) had 2.6 fold increase in the prevalence of T2DM (p=<0.001), were more likely to be obese (p=<0.05) and had increased waist circumference (p=<0.05) [204]

4. Poor sleep quality and long and short sleep duration are associated with increased adiposity measures and worsened metabolic parameters in young patients with T2DM.

1.5 Aims

1.5.1 Primary Aim

To assess the longitudinal impact of OSA on the progression and development of microvascular complications in patients with T2DM (including nephropathy, neuropathy and retinopathy).

To assess the relationship between sleep duration, sleep quality and adiposity measures.

1.5.2 Secondary Aims

To explore whether CPAP treatment has an impact on microvascular complications in patients with T2DM.

To assess the relationship between sleep duration, sleep quality and metabolic parameters.

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Chapter Two: Methods

2.1 Introduction

We conducted two studies in order to explain the previously described aims. The first was the longitudinal follow-up of patients who were recruited from 2008 to 2011 as part of Dr. Tahrani's PhD project as explained below. The second study was a cross-sectional analysis utilising the baseline assessments of the Young Type 2 Diabetes study (CI Dr. Sri Bellary, Aston University) in order to assess the relationship between sleep duration and quality and adiposity measures, the details of which follow below.

2.2 Study population

2.2.1 The OSA study

The baseline data for this study was collected between 2008 and 2011 as part of the PhD of my supervisor (Dr Tahrani) which was funded as an NIHR doctoral fellowship. The focus of Dr. Tahrani's project was to explore the impact of OSA and ethnicity on diabetes-related microvascular complications in patients with T2DM. I have utilized this study population and followed them up prospectively to assess the impact of OSA on the development and progression of microvascular complications in T2DM. Patients for the baseline study were recruited consecutively from the outpatient diabetes departments at Birmingham Heartlands Hospital. Patients were approached prior to their clinic appointment in the waiting area of the outpatients department by either Dr. Tahrani or a research assistant. Patients who agreed to participate to the study were booked for the baseline assessments which were obtained during 1-to-1 interview with Dr. Tahrani or a member of the study team. I have recollected the data between 2012 and 2014, utilizing one-to-one interviews

with the patients and the patients' electronic records. All participating patients consented at baseline and re-consented during the follow-up visit. For non-English speaking study participants, most of the communications were carried out by the researcher who was fluent in the mother tongue. The baseline project and the longitudinal follow up were approved by the Warwickshire Research Ethics Committee (REC number 08/H1211/145).

2.2.1.1 Inclusion Criteria

1. Adult patients with T2DM
2. White Europeans and South Asian ethnic origins
3. Able to give consent

2.2.1.2. Exclusion Criteria

1. Past medical history of OSA or other sleep or respiratory disorder
2. The use of sleeping tablets
3. Pregnancy
4. Patients receiving dialysis

2.2.2 The Sleep Duration and Sleep Quality Study

We have utilised the baseline data for an ongoing observational study of patients with young onset T2DM (CI Dr Sri Bellary, Aston University). For this study, patients were recruited from out-patients area from Birmingham Heartlands Diabetes Centre by either myself (Dr Altaf), research assistant (Mrs. Safia Begum) or research nurse (Mrs Helen

Jenner). Patients who were willing to participate had a 1-to-1 appointment. Consent was obtained from all study participants. For non-English speaking study participants, most of the communications were carried out by the researcher who was fluent in the mother tongue. The project was approved by NRES Committee West Midlands - Staffordshire (REC – 12/WM/0166) and funded by Heart of England NHS foundation Trust.

Patients who agreed to take part were extensively characterised in terms of demographics and metabolic profile. Data collected included sleep, mental health and physical activity assessments. I utilised the demographics, metabolic profile and sleep data (using PSQI questionnaire) collected from this cohort for this cross-sectional study.

2.2.2.1 Inclusion Criteria

1. Patients with the diagnosis of T2DM
2. Patients who were diagnosed to have T2DM before the age of 40 years
3. Patients who were diagnosed with T2DM after December 2000.
4. Patients who are able to consent

2.2.2.2 Exclusion Criteria

1. Patients with diagnoses other than T2DM
2. Pregnancy

2.3 Assessments

For the OSA study, this data were collected at baseline by Dr. Tahrani and his study team and by me at the follow-up visit. For the sleep duration and sleep quality study, baseline data were collected by me.

2.3.1 The OSA Study

The following assessments were performed at baseline and study-end.

2.3.1.1 General and Clinical Assessment

Age, gender, ethnicity, diabetes duration, medications.

Ethnicity was defined as per Office for National Statistics 2011 census data. Patients were categorised as South Asians (SA) and non-South Asians (non-SA) based on their ancestry.

2.3.1.2 Anthropometry

Height, weight, Body mass index (BMI), waist circumference (WC), hip circumference (HC), waist−hip ratio (WHR).

Height and weight were measured with patient standing without shoes in a relaxed position. BMI was calculated as weight divided by height squared. Waist circumference was

measured at the midpoint between the inferior border of the ribcage and the superior aspect of the iliac crest [1]. Hip circumference was measured at the widest circumference over the greater trochanters [2]. Both the measurements were taken with an inelastic measuring tape, without outer garments, with patient in an upright position. WHR was calculated as WC divided by hip circumference. Measurements were taken twice and the average was used in the analysis.

2.3.1.3 Metabolic Assessment

Blood pressure, HbA1c, lipid profile, urea and electrolytes.

Blood pressure was measured using an automated device. Total of 2 readings were taken at least 10 minutes apart, with patient sitting down in a chair with arm resting on the table. First reading was usually taken around 30 minutes after patient's arrival to the department. An average of 2 readings were entered into database as study end BP. HbA1c was measured using high performance liquid chromatography method (HPLC-Tosoh inc.) at the clinical laboratory at Birmingham Heartlands Hospital. Urea, electrolytes and lipid profile were measured by colorimetric method (Roche diagnostics). The clinical laboratory measurements were performed as part of patients' routine clinical care whilst attending Diabetes clinics at Birmingham Heartlands Hospital.

2.3.1.4 Nephropathy and chronic kidney disease Assessment

Plasma urea and creatinine, estimated glomerular filtration rate (eGFR), urinary albumin/creatinine ratio (ACR).

These clinical laboratory investigations were performed as patients' routine clinical care at Birmingham Heartlands Hospital. Estimated GFR was calculated based on MDRD equation ($186 \times (Creat / 88.4)^{-1.154} \times (Age)^{-0.203} \times (0.742$ if female) x (1.210 if black) [3]. Urinary albumin was measured by an immunoturbidimetric assay. Urinary creatinine was measured by a kinetic Jaffe assay. ACR was calculated as ratio between urinary albumin and urinary creatinine excretion. Diabetic nephropathy was assessed using eGFR and urinary ACR. Microalbuminuria was present if urinary ACR was >2.5 mg/mmol in men, and >3.5 mg/mmol in women [4]. DN was diagnosed when either eGFR was <60 ml/min/1.73m^2 or ≥60 ml/min/1.73m^2 in the presence of albuminuria [5]. In the study, we used a single random measurement of urine ACR, taken during patients' visits to the follow-up appointments of the diabetes clinic, to assess and make the diagnosis of microalbuminuria, instead of repeated samples [6,7]. However, this approach has been used by other researchers. In a large multicentre RIACE trial, single measurement of ACR demonstrated good performance in predicting microalbuminuria (AUC=0.93; 95%CI 0.92, 0.94) and macro albuminuria (AUC=0.95; 95%CI 0.93, 0.97) as compared with 24-hour urine collection for albumin excretion [8]. This suggests a role for single ACR measurement for classification of DN in clinical and epidemiological studies [8-10].

2.3.1.5 Retinopathy Assessment

Images from the National English retinal screening program were obtained. DR was assessed by reviewing the images from retinal screening database, which were taken as part of patients' routine clinical care at Birmingham Heartlands Hospital. DR was assessed using two 45 degree digital photographic images per eye as per National Retinal Screening

Programme. As part of the screening programme, images are initially graded by a primary retinal grader as per National Retinal Screening guidelines (**Table 2.1)** [11]. All abnormal grades and 10% normal grades are then passed onto the second grader for second opinion and for quality assurance respectively. In case of a discrepancy, the images are then graded by either a senior grader or an ophthalmologist for a final decision. For ungradable images, slit lamp examination is performed by the ophthalmologist. All graders were blinded to patients' OSA status. We defined sight threatening diabetic retinopathy (STDR) as the presence of pre proliferative or proliferative DR, maculopathy or photocoagulation [11].

No retinopathy	No lesion
Background retinopathy	Microaneurysm(s); retinal haemorrhage(s) ± any exudate
Pre-proliferative retinopathy	Venous beading; venous loop or reduplication; intraretinal microvascular abnormality (IRMA); multiple deep, round or blot haemorrhages; cotton wool spots (CWS)
Proliferative retinopathy	New vessels on disc (NVD); new vessels elsewhere (NVE); pre-retinal or vitreous haemorrhage; pre-retinal fibrosis ± tractional retinal detachment
Maculopathy (M)	Exudate within 1 disc diameter (DD) of the centre of the fovea; circinate or group of exudates within the macula; retinal thickening within 1 DD of the centre of the fovea; any micro-aneurysm or haemorrhage within 1 DD of the centre of the fovea only if associated with a best VA of ≤ 6/12
Photocoagulation (P)	Focal/grid to macula

Table 2.1 National Retinal Screening Grading Guidelines. Adopted with permission [11]

2.3.1.6 Peripheral Neuropathy Assessment

Michigan Neuropathy Screening Instrument (MNSI) - questionnaire and examination, 10g monofilament test.

For our study, we used Michigan Neuropathy Screening Instrument (MNSI) to assess DPN and 10 gram monofilament to assess foot insensitivity.

MNSI is a 2 component tool comprising of a questionnaire and feet examination. The questionnaire component consists of 15 questions that aim to assess the individual for sensory impairment, peripheral vascular disease and general asthenia (**Figure 2.1**)[12]. A "yes" response to question 1–3, 5–6, 8–9, 11–12 and 14–15 will score 1 point each. A "no" response to question 7 and 13 will score 1 point each. Question 4 is a measure of peripheral vascular disease; and question 10 is a measure of general asthenia. Therefore, these 2 questions do not count towards the total score which is a maximum of 13.

The examination component requires examiner to inspect the feet to look for evidence of any deformity, dry skin, callus, infection, fissure and ulceration. Presence of any abnormality score 1 point on each foot.

The ankle reflexes are measured using 128-Hz tuning fork. The tuning fork is placed over the boney prominence of distal interphalangeal joint (DIP) of unsupported great toe. The patient is asked to indicate when he can stop feeling the vibration sense from the vibrating tuning fork. Ideally, the examiner should be able to feel vibration from the tuning fork for 5 seconds longer on his distal interphalangeal joint of the first finger than a patient can at the great toe. If the examiner feels vibration for 10 or more seconds on his finger, then vibration

is considered reduced. Vibration is scored as present (scored as 0) if the examiner can sense the vibration on his finger for < 10 seconds, reduced (scored as 0.5) if sensed for ≥ 10 or absent (scored as 1) if no vibration is sensed. Both feet are examined and scores added.

The ankle reflexes are examined using a reflex hammer. The ankle reflexes are elicited with patient relaxed in a sitting position with dependent foot. For the reflex, the foot is passively positioned and dorsi-flexed slightly to obtain optimal stretch of the muscle. The Achilles tendon is percussed directly. If the reflex is obtained, it is graded as present (scored as 0). If the reflex is absent, the patient is asked to perform the Jendrassic manoeuvre (i.e., hooking the fingers together and pulling). Reflexes elicited with the Jendrassic manoeuvre alone are designated "present with reinforcement" and scored as 0.5. If the reflex is absent, even with Jendrassic manoeuvre, the reflex is considered absent and scored as 1. Both the feet are examined in turn and scores added.

Monofilament testing is used to assess foot insensitivity. Patient's foot is supported on a flat, warm surface. The 10 gram monofilament is then applied at 10 points in each foot. They include plantar aspect of the first, third and fifth toes, the first, third and fifth metatarsal heads, the medial and lateral mid-foot, the calcaneus and the dorsal mid-foot. The filament is applied perpendicularly and briefly, with an even pressure. When the filament bends, the force of 10 grams has been applied. The patient is asked to say yes if he feels the filament. Eight correct responses out of 10 applications is considered normal. Zero to seven correct responses indicate absent/reduced sensation.

In the study, we diagnosed DPN if MNSI questionnaire score is ≥7 or MNSI examination score is ≥2.5 [12]. Foot sensitivity is said to be reduced if 10 gram monofilament testing was abnormal (<8 positions) [13].

MNSI has been validated against nerve conduction studies (NCS) in different studies, in different populations [14,15]. In a study of 80 diabetic patients, MNSI score of ≥2.5 was proved to be reliable for ambulatory screening of suspected DPN with specificity of 75% and sensitivity of 78.6% [16]. It was also reported that MNSI score of ≥2.5 showed high inter- and intra-observer reproducibility (88.8% and 95% respectively) [16]. In DCCT trial, MNSI score of ≥2.5 showed 61% sensitivity, 79% specificity and had a positive predictive value of 55% and negative predictive value of 83% in establishing the diagnosis of DPN [17]. In ACCORD study, MNSI scoring was used to establish the diagnosis of DPN and to assess the impact of intense glycaemic control in longitudinal analysis (5% relative risk reduction after a follow up of 3.7 years) [18].

In a case-control study on 200 patients with T2DM, use of monofilament as a marker of foot insensitivity had a sensitivity of 85.7% and a false positive rate of 16% in predicting the development of foot ulcer [19]. According to a meta-analysis of 16 cohort studies, inability to feel a monofilament was associated with higher risk of foot ulceration (OR=3.19; 95%CI 2.65, 3.82) [20]. In epidemiological study from Pittsburgh in patients with DPN, monofilament test showed the highest sensitivity (98% for DPN), and positive predictive value (89% for DPN) [13]. In The Seattle Diabetic Foot Study, monofilament insensitivity predicted the development of foot ulceration (HR=2.03; 95%CI 1.5, 2.76) [21].

Patient Version

MICHIGAN NEUROPATHY SCREENING INSTRUMENT

A. History (To be completed by the person with diabetes)

Please take a few minutes to answer the following questions about the feeling in your legs and feet. Check yes or no based on how you usually feel. Thank you.

1.	Are you legs and/or feet numb?	☐ Yes	☐ No
2.	Do you ever have any burning pain in your legs and/or feet?	☐ Yes	☐ No
3.	Are your feet too sensitive to touch?	☐ Yes	☐ No
4.	Do you get muscle cramps in your legs and/or feet?	☐ Yes	☐ No
5.	Do you ever have any prickling feelings in your legs or feet?	☐ Yes	☐ No
6.	Does it hurt when the bed covers touch your skin?	☐ Yes	☐ No
7.	When you get into the tub or shower, are you able to tell the hot water from the cold water?	☐ Yes	☐ No
8.	Have you ever had an open sore on your foot?	☐ Yes	☐ No
9.	Has your doctor ever told you that you have diabetic neuropathy?	☐ Yes	☐ No
10.	Do you feel weak all over most of the time?	☐ Yes	☐ No
11.	Are your symptoms worse at night?	☐ Yes	☐ No
12.	Do your legs hurt when you walk?	☐ Yes	☐ No
13.	Are you able to sense your feet when you walk?	☐ Yes	☐ No
14.	Is the skin on your feet so dry that it cracks open?	☐ Yes	☐ No
15.	Have you ever had an amputation?	☐ Yes	☐ No

Total: _____

MICHIGAN NEUROPATHY SCREENING INSTRUMENT

B. Physical Assessment (To be completed by health professional)

1. Appearance of Feet

	Right	Left
a. Normal	☐ 0 Yes ☐ 1 No	Normal ☐ 0 Yes ☐ 1 No
b. If no, check all that apply:		If no, check all that apply:
Deformities	☐	Deformities ☐
Dry skin, callus	☐	Dry skin, callus ☐
Infection	☐	Infection ☐
Fissure	☐	Fissure ☐
Other	☐	Other ☐
specify: _____		specify: _____

	Right		Left	
	Absent	Present	Absent	Present
2. Ulceration	☐ 0	☐ 1	☐ 0	☐ 1

	Right			Left		
	Present	Present/ Reinforcement	Absent	Present	Present/ Reinforcement	Absent
3. Ankle Reflexes	☐ 0	☐ 0.5	☐ 1	☐ 0	☐ 0.5	☐ 1

	Right			Left		
	Present	Decreased	Absent	Present	Decreased	Absent
4. Vibration perception at great toe	☐ 0	☐ 0.5	☐ 1	☐ 0	☐ 0.5	☐ 1

Figure 2.1 Michigan Neuropathy Screening Instrument. Adopted with permission [12]

2.3.1.7 Cardiac Autonomic Assessment

Cardiac Autonomic Neuropathy (CAN) was assessed by Dr. Tahrani, using the ANX-3.0 software, (ANSAR Inc., Philadelphia, PA), as part of his PhD project [22]. I used the same software and methodology to collect follow-up data. This software analyzes heart rate variability (HRV) using the continuous wavelet transform methods to generate numerical and graphical data. The R-R intervals are recorded by electrocardiography. HRV is plotted in the frequency domain. Spectral analysis is then performed by separating the respiratory

frequency components (Rfa, 0.15 to 0.4 Hz) from the low-frequency (Lfa, 0.04 to 0.15 Hz) components. Patient is asked to sit quietly and HRV and BP are recorded whilst patient is resting, deep breathing, performing Valsalva maneuver and standing [23]. Data recorded included the E/I ratio, 30:15 ratio, Valsalva ratio, frequency domain analysis after respiratory adjustment (Lfa, Rfa and Lfa/Rfa) and blood pressure measurements.

CAN was diagnosed when 2 or more of the following tests were abnormal: E/I ratio, 30:15 ratio, Valsalva ratio, and postural drop in BP (drop of 20mmHg in systolic or 10mmHg in diastolic BP) [22,24]. Cut off values for E/I ratio, 30:15 ratio and Valsalva ratio were defined as previously described in the literature [22,25].

The ANX performs frequency analysis of HRV by incorporating the respiratory signal (ANSAR's patented MIT-based technology) which reflects parasympathetic activity. During deep breathing, parasympathetic activity moves into the low frequency area (0.04 - 0.10 Hz) and the heart rate slows down. The ANX also corrects for ectopic beats throughout the procedure by using a proprietary algorithm based on spline interpolation. The technology behind ANX software is summarized in **figure 2.2** [22].

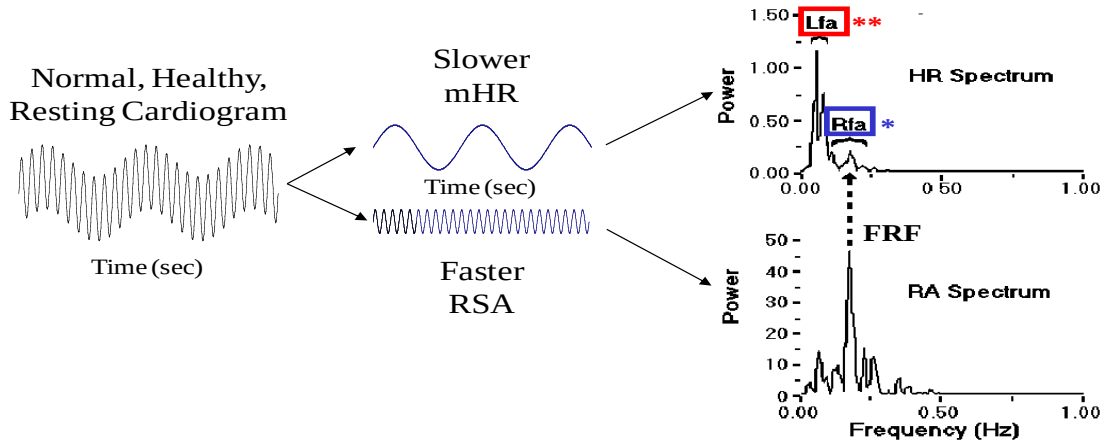

Figure 0.1 The principles of the ANX software.

mHR: mean heart rate, RSA: respiratory sinus arrhythmia, Rfa: parasympathetic, Lfa: sympathetic.

2.3.1.7.1 Parameters Used

Fundamental Respiratory Frequency (FRF):

Fundamental Respiratory Frequency is defined as the frequency corresponding to the highest peak on a respiratory activity spectrum. In a healthy individual, FRF at rest is equal to one divided by the respiratory rate (seconds per breaths). This indicates the frequency range over which the parasympathetic nervous system is controlling the heart rate. The normal range for FRF during deep breathing is 0.09 to 0.15 Hz for 6 breaths per minute.

Low Frequency Area (LFa)

The LFa defines the tone of the sympathetic nervous system as mediated by the parasympathetic modulation with the frequency range from 0.04 Hz to 0.10 Hz. At Baseline and during Standing the LFa is mostly sympathetic driven. During Deep Breathing and Valsalva, the LFa is a mixture of sympathetic and parasympathetic activity. During baseline assessment, the LFa should be between 0.5 and 10.0. A baseline LFa less than 0.1 is a sign of cardiac sympathetic denervation. During Valsalva assessment, the LFa is expected to be >28.0 but is age related.

Respiratory Frequency Area (RFa)

The RFa determines parasympathetic power. This relationship is independent of patient's respiratory activity. During baseline assessment, the RFa is expected to be > 0.5. A baseline RFa less than 0.1 is a sign of cardiac parasympathetic denervation. During Deep Breathing assessment, the RFa is expected to be > 28.0 but is age related.

Lfa/Rfa Ratio

It is a measure of the balance between sympathetic nervous system and parasympathetic nervous system. Normal range of Lfa/Rfa ratio is 0.4 – 3.0. In a young, healthy patient, Lfa/Rfa ratio is expected to be around 2.0 while awake and at rest; and around 0.5 while asleep.

A copy of the report generated by the ANX is shown in **figure 2.3**

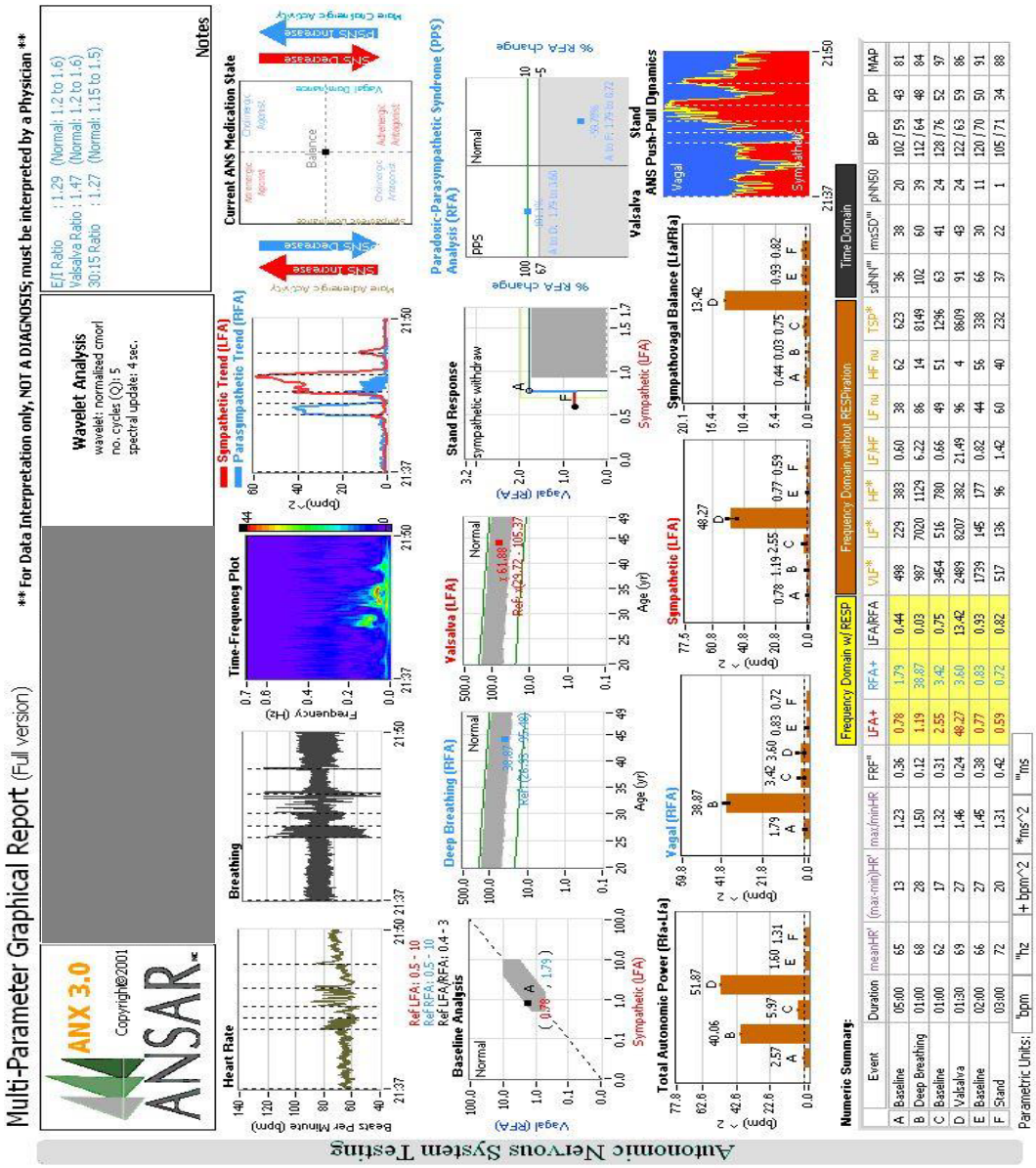

Figure 0.2 A copy of the report of the CAN test from a normal patient. Adopted with permission [22].

Top row

- Patient demographics, medications, medical history, analysis technique, signal processing
- Standard ANS ratios

  E/I Ratio (measure of sympathovagal response to Deep Breathing assessment)

  Valsalva Ratio

  30:15 Ratio (measure of sympathovagal response to Stand assessment),
- Notes from the technician indicating any difficulty during the study
- Number of possible premature beats as detected from the cardiac monitoring during the study.

Second row

- Heart rate variability
- Respiratory activity. The depth of respiration should increase during deep breathing phase and Valsalva assessment.
- A colour plot of the heart rate variability
- The "Trends Graph". A plot of the LFa and RFA changes.

Third row

- Baseline Analysis plot. This plot describes the balance between parasympathetic and sympathetic systems. The broken line indicates a ratio of 1.0. The grey area indicates a ratio between 0.5 and 2.0 and is normal.
- Deep Breathing Response (RFa) plot. This assesses parasympathetic tone in a challenged situation. RFa response is plotted against age. Therefore, RFa response

adjusted for age is indicated by a solid black line. The gray area indicates one standard deviation of the normal data and is considered normal.

- Valsalva Response (LFa) plot - This assesses sympathetic tone in a challenged situation. LFa response is plotted against age. Therefore, LFa response adjusted for age is indicated by a solid black line. The gray area indicates one standard deviation of the normal data and is considered normal.
- Stand Response plot – This assesses autonomic balance when challenged and is plotted as LFa/RFa ratio. 'A' describes Initial Baseline LFa response and 'F' describes the Stand LFa response. The gray area is normal. Outside the gray area can indicate parasympathetic excess, Orthostatic Hypotension or some form of Syncope.
- ANS Push-Pull Dynamics Plot - It measures continuous changes in the ratio throughout the study.

Fourth row

- Graph of Total Autonomic Power (LFa + RFa) as assessed during all 6 stages of the test. Total power increases significantly during Deep Breathing as there is an increase in RFa only, increases significantly during Valsalva as there is an increase in LFa increase, and stay the same or increases slightly during Standing as there is an increase in LFa and decrease in RFa.
- Bar graph of the parasympathetic power (RFa) as assessed during all 6 stages of the test. RFa increases significantly during Deep Breathing, stay the same or decreases slightly during Valsalva and Standing.

- Bar graph of the Sympathetic power (LFa) as assessed during all 6 stages of the test. LFa stays the same or decreases slightly during Deep Breathing, and increases significantly during Valsalva and Standing.
- Bar graph of the Sympathovagal Power (LFa/RFa) as assessed during all 6 stages of the test. Ratio should be low during Deep Breathing and high during Valsalva and Standing.

Fifth row

Table showing the results for each of the six stages of the study including: the study stage, duration of the study, mean heart rate during each stage, heart rate variability in beats per minute (Normal Ranges: Resting = 10-50 bpm; Deep Breathing, Valsalva, Standing = 15-50 bpm), FRF, Lfa, Rfa, Lf/Rfa, blood pressure and mean arterial pressure.

2.3.1.7.2 Protocol

Patients were instructed to attend for the test without having consumed caffeinated drinks. CAN testing was performed while the patient was in sitting position and lasted for about 16 minutes. Patient was connected to the cardiac monitor as shown in **figure 2.4**. BP cuff was placed on either arm. We guided the patient through different stages of the test (Baseline 5 minutes, deep breathing 1 minute, baseline 1 minute, Valsalva manoeuvre 2 minutes, baseline 2 minutes and Standing 5 minutes). For Valsalva manoeuvre, we asked the patient to blow into a syringe connected to a gauge. Patient was asked to achieve a pressure of 20mmHg and to maintain it for about 15 seconds. If there were artefacts in cardiac and respiratory monitoring, then we ensured the absence of electrical interference from other equipment (including mobile phones) and firm attachments of the leads before considering

a re-run of the test.

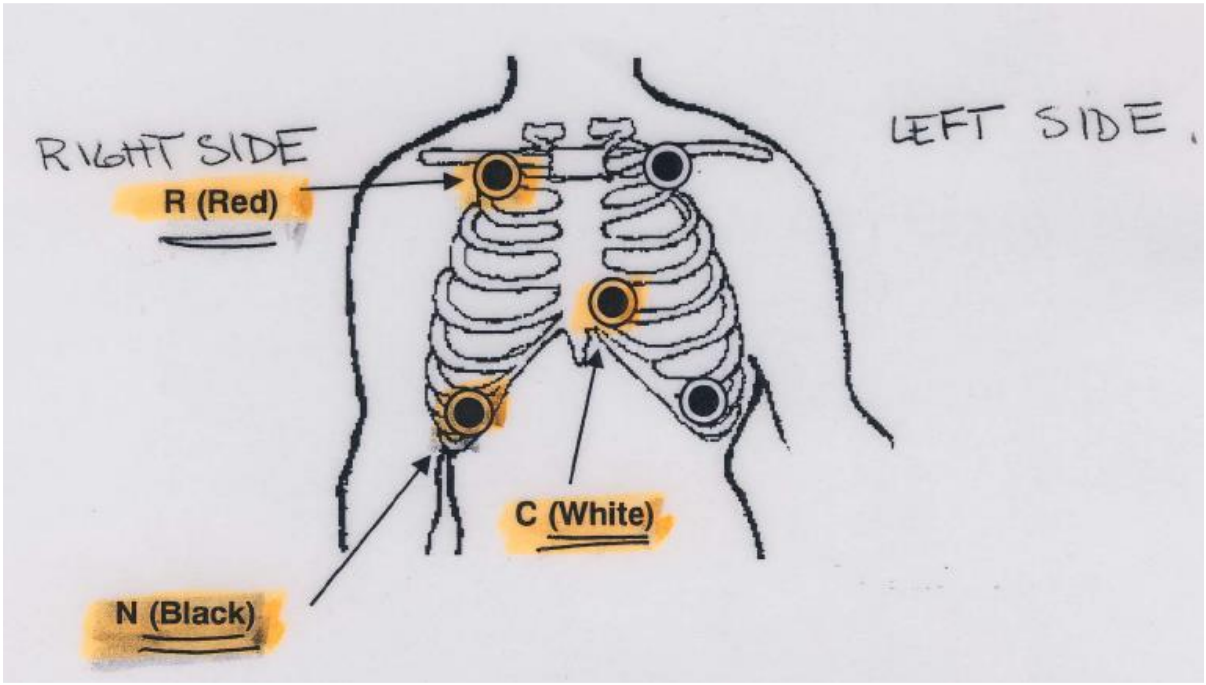

Figure 0.3 Lead positions for the CAN test. Adopted with permission [22]

2.3.1.8 Sleep and Obstructive sleep apnoea

Information regarding patients' sleepiness and OSA status were obtained from Dr. Tahrani's NIHR funded cross sectional database. Sleepiness was assessed using Epworth sleepiness scale (ESS) questionnaire. Patients were screened for OSA using Berlin questionnaire. OSA was diagnosed by performing home-based sleep study using a portable multi-channel respiratory device.

ESS is a self-administered questionnaire that aims to assess patient's chances of dozing off in 8 commonly occurring day to day activities (**figure 2.5**) [26]. ESS score ranges between 0 – 24, with higher score representing increased sleepiness [26]. Patients with OSA tend to have

a higher ESS score than healthy controls, which improves after treatment with CPAP [27]. ESS score >10 was consistent with excessive daytime sleepiness [22].

Berlin questionnaire aims to look at some of the known risk factors for OSA. Questions address presence of sleepiness in the context of hypertension and obesity (**figure 2.6**) [22,28]. Based on the scores, patients can be classifies as high risk or low risk for OSA [22]. Berlin questionnaire has been validated as a tool to screen for OSA in different populations [22]. In a study on 180 SA middle aged adults, the use of modified Berlin questionnaire in which obesity was defined as BMI of 25 kg/m^2 (instead of 30, due to low cut off for diagnosing obesity in SA patients), the sensitivity and specificity remained significant at 86% and 95% respectively [22,28]. We used modified Berlin questionnaires in SA patients [22].

We have used home based sleep studies at baseline to assess OSA. These methods are widely used in research and clinical practice due to ease of use, minimal cost and better patient satisfaction [22,28]. The disadvantage is a higher number of technically ungradable studies due to lack of technician supervision which require either a repeat study or the performance of gold standard polysomnography [22,28].

Dr Tahrani used the Alice PDX (Philips Resporinics, USA) device to assess the presence of OSA (**figure 2.7**) [22]. This device records flow and oral pressure, snoring signal, abdominal and thoracic movements, oxygen saturations, pulse rate, body position, ECG, EEG and electro-oculogram [22], but the EEG and electro-oculogram were not recorded in this study. Data are downloaded and scored using specific sleepware software [22]. The Alice PDX conforms with American Academy of Sleep Medicine (AASM) guidelines for Level III diagnostic device and is comparable with gold standard in-patient polysomnography [22].

Data was analysed in accordance with AASM guidelines [29]. Inadequate sleep recordings were repeated and were excluded if remained poor [22]. Patients with AHI ≥ 5 events/hour were diagnosed with OSA [22]. OSA severity was described based on AHI, ODI, time spent with oxygen saturation of <90% and the nadir oxygen levels during sleep (**figure 2.8**) [22]. Sleep studies were scored by Dr Ali (Consultant in respiratory medicine at the University Hospital of Coventry and Warwickshire) and Mr Nicholls (senior Sleep technician at Birmingham Heartlands Hospital) at baseline [22]. Apnoea was defined as cessation or ≥ 90% reduction in airflow for at least 10 seconds [22]. Hypopnea was defined as ≥ 30% reduction in airflow for ≥ 10 seconds associated with ≥ 4% drop in oxygen saturations [22]. The newer version of the AASM guidelines included the presence of micro arousals in the definition of hypopnea, but this study was conducted prior to the release of these latest AASM guidelines.

Epworth Sleepiness Scale

Name: _____ Today's date: _____

Your age (Yrs): _____ Your sex (Male = M, Female = F): _____

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired?

This refers to your usual way of life in recent times.

Even if you haven't done some of these things recently try to work out how they would have affected you.

Use the following scale to choose the **most appropriate number** for each situation:

0 = would **never** doze
1 = **slight chance** of dozing
2 = **moderate chance** of dozing
3 = **high chance** of dozing

It is important that you answer each question as best you can.

Situation	**Chance of Dozing (0-3)**
Sitting and reading	___
Watching TV	___
Sitting, inactive in a public place (e.g. a theatre or a meeting)	___
As a passenger in a car for an hour without a break	___
Lying down to rest in the afternoon when circumstances permit	___
Sitting and talking to someone	___
Sitting quietly after a lunch without alcohol	___
In a car, while stopped for a few minutes in the traffic	

THANK YOU FOR YOUR COOPERATION

Figure 0.5 The Epworth Sleepiness Score. Adopted with permission [22]

BERLIN QUESTIONNAIRE

Height (m) _____ Weight (kg)_____ Age_____ Male / Female

Please choose the correct response to each question.

CATEGORY 1

1. **Do you snore?**
   - ☐ a. Yes
   - ☐ b. No
   - ☐ c. Don't know

If you snore:

2. **Your snoring is:**
   - ☐ a. Slightly louder than breathing
   - ☐ b. As loud as talking
   - ☐ c. Louder than talking
   - ☐ d. Very loud – can be heard in adjacent rooms

3. **How often do you snore**
   - ☐ a. Nearly every day
   - ☐ b. 3-4 times a week
   - ☐ c. 1-2 times a week
   - ☐ d. 1-2 times a month
   - ☐ e. Never or nearly never

4. **Has your snoring ever bothered other people?**
   - ☐ a. Yes
   - ☐ b. No
   - ☐ c. Don't Know

5. **Has anyone noticed that you quit breathing during your sleep?**
   - ☐ a. Nearly every day
   - ☐ b. 3-4 times a week
   - ☐ c. 1-2 times a week
   - ☐ d. 1-2 times a month
   - ☐ e. Never or nearly never

CATEGORY 2

6. **How often do you feel tired or fatigued after your sleep?**
   - ☐ a. Nearly every day
   - ☐ b. 3-4 times a week
   - ☐ c. 1-2 times a week
   - ☐ d. 1-2 times a month
   - ☐ e. Never or nearly never

7. **During your waking time, do you feel tired, fatigued or not up to par?**
   - ☐ a. Nearly every day
   - ☐ b. 3-4 times a week
   - ☐ c. 1-2 times a week
   - ☐ d. 1-2 times a month
   - ☐ e. Never or nearly never

8. **Have you ever nodded off or fallen asleep while driving a vehicle?**
   - ☐ a. Yes
   - ☐ b. No

If yes:

9. **How often does this occur?**
   - ☐ a. Nearly every day
   - ☐ b. 3-4 times a week
   - ☐ c. 1-2 times a week
   - ☐ d. 1-2 times a month
   - ☐ e. Never or nearly never

CATEGORY 3

10. **Do you have high blood pressure?**
    - ☐ Yes
    - ☐ No
    - ☐ Don't know

Figure 0.6 The Berlin questionnaire. Adopted with permission [22]

Figure 0.4 The Alice PDX. Adopted with permission [22]

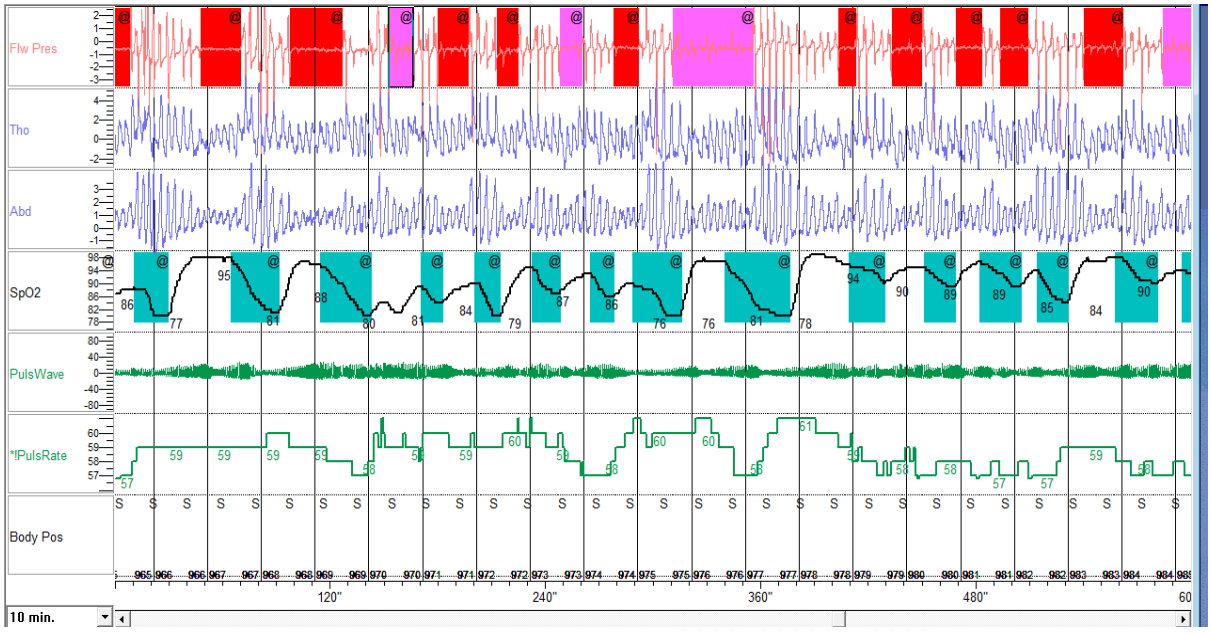

Figure 0.8 Apnoea, hypopnea and oxygen desaturations on data downloaded from Alice PDX. Adopted with permission [22]

2.3.2 The Sleep Duration and Sleep Quality Study

2.3.2.1 General and clinical assessment

Age, gender, ethnicity, diabetes duration, medications.

2.3.2.2 Anthropometry

Height, weight, Body mass index (BMI), waist circumference (WC), total body fat%.

Height, weight, waist circumference and BMI were measured and calculated as described in the OSA study. Total body fat% was measured using TANITA body fat composition analyser.

2.3.2.3 Metabolic assessment

Blood pressure, HbA1c, lipid profile, urea and electrolytes.

Blood pressure was measured using the ANX-3.0 software which was used to examine for cardiac autonomic neuropathy. HbA1c, urea, electrolytes and lipid profile were measured at clinical laboratory at Birmingham Heartlands Hospital as described in the OSA study.

2.3.2.4 Sleep assessment

Sleep quality and duration were assessed by using Pittsburgh Sleep Quality Index (PSQI). PSQI is a self-reported questionnaire that examines sleep quality over a period of 1 month [30]. The questionnaire consists of 19 self-rated items, which look at 7 components and give a global score to rate sleep quality. These components include subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping

medication and daytime dysfunction [30]. PSQI questionnaire has been validated in different populations and has shown good test-retest reliability (r=0.87; p=<0.001) [31-35]. Sleep duration has been measured as reported as part of PSQI questionnaire. Poor sleep quality was defined as global score of >5 [30].

2.4 Statistical Analysis

Data analysis was performed using SPSS 22 software (SPSS Inc, Chicago, USA). Depending on distribution, data are presented as mean (SD) or median (IQR). Histograms and the Shapiro-Wilk test were used for normality testing. Independent t-test and the Mann-Whitney test were used to compare independent continuous variables. Chi-square test was used to compare categorical variables. In case of multiple comparisons, Bonferroni correction was used as post-hoc test.

Analysis of variance (ANOVA) with post-hoc analysis or the Kruskal–Wallis test were used to assess the differences between independent groups. Pearson or Spearman tests were performed to assess the correlations between continuous variables. To assess the impact of confounders on correlation between two continuous variables, partial correlation was used. All assumption of statistical tests used were adhered to. In regression models, multicollinearity was assessed using simple correlations between variables, the tolerance and VIF values, and the condition indices. Residuals were also examined in multiple linear regression models. In all the models included, residuals showed a normal distribution with uniform variance. Logistic regression (forced entry method) was used to assess the independent association of either a categorical or a scale variable if the outcome was a categorical dichotomous variable. Linear regression (forced entry method) was used to

assess the independent association of either a categorical or a scale variable if the outcome was a continuous variable. Variables included in the regression models were either known to be outcome-related risk factors or variables that were found to be different between patients. A p value < 0.05 was considered significant. Detail discussion of statistical techniques used can be found in the relevant chapters.

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Chapter Three: The impact of obstructive sleep apnoea on chronic kidney disease in patients with Type 2 Diabetes

3.1 Introduction

3.1.1 Epidemiology

Diabetic Nephropathy (DN) and chronic kidney disease (CKD) are few of the leading causes of end-stage renal disease (ESRD) worldwide [1] . CKD is diagnosed in the presence of micro or macro albuminuria and/or impaired eGFR (< 60 mL/min/1.73m^2) [2,3]. Normoalbuminuria is defined as urine albumin excretion <30 mg/g Cr [2]. Microalbuminuria is defined as urine albumin 30-299 mg/g Cr [2]. Macro albuminuria is defined as urine albumin ≥300 mg/g Cr [2]. DN progresses slowly, in which albuminuria progresses to overt proteinuria in 20-40% of patients with a further 20% progressing to ESRD within 20 years [4]. This progression depends on metabolic factors including blood pressure, BMI, glycaemic control, male sex and ethnicity [5]. DN is more prevalent in South Asians (SA) and Afro-Caribbeans than Caucasians [1].

Incidence and prevalence of DN vary greatly depending on the population studied. In UKPDS, prevalence of DN (defined as microalbuminuria or worse), at the time of diagnosis of T2DM was 7.3% rising to 28% after 15 years [5]. There was the progression of 2% (95%CI 1.9%, 2.2%) per year from no nephropathy to microalbuminuria, 2.8% (95%CI 2.5%, 3.2%) per year to macro albuminuria and 2.3% (95%CI 1.5%, 3.0%) per year to elevated plasma creatinine or renal replacement therapy [5]. The annual death rate from any cause at no nephropathy stage was 1.4% (1.3% to 1.5%), increasing to 3.0% (2.6% to 3.4%) at microalbuminuria stage, 4.6% (3.6% to 5.7%) at macro albuminuria stage, and 19.2% (14% to 24.4%) at renal replacement therapy stage [5]. In a prospective observational study in Denmark, incidence of DN (defined as microalbuminuria or worse) after a follow up period of 5 years was estimated at 23% [6]. DN is associated with significantly increased

cardiovascular and cerebrovascular morbidity and mortality [5,7]. In a prospective observational case control study on patients with T1DM, 43.4% patients with DN suffered a non-fatal or fatal cardiovascular event during a median follow up period of 10 years (adjusted HR=2.05; 95%CI 1.31, 3.20; p=0.002) [8]. In longitudinal analysis of National Health and Nutrition Examination Survey (NHANES III) cohort, in patients with T2DM and DN, standardized all-cause mortality was significantly increased at 31.1% (95%CI 24.7%, 37.5%) with absolute risk difference of 23.4% (95%CI 17.0%, 29.9%) after multiple adjustments [9]. Similar patterns were observed for both cardiovascular and non-cardiovascular mortality with 10 year standardized cumulative incidence of 19.6% (95%CI 14.7%, 24.4%) and 23.3% (95%CI 16.5%, 29.9%) respectively [9]. Albuminuria is an independent risk factor for increased cardiovascular mortality. Microalbuminuria is associated with 2.4 fold (95%CI 1.8%, 3.1%) increased risk of cardiovascular mortality in patients with T2DM [10] and 4 fold increased risk in patients without diabetes [11].

This presents significant economic burden to the health systems worldwide. In US, 20% of the Medicare health expenditure is for patients with CKD [12]. The total cost of CKD management in UK is about £1.45 billion which is 1.3% of all NHS spending [13].

3.1.2 Pathogenesis of DN and CKD

Hyperglycaemia is the most important triggering factor for the development of microvascular complications. Hyperglycaemia causes metabolic and molecular damage through AGE formation, PKC, polyol and hexosamine pathways and oxidative stress. For details, refer to chapter 1.

3.1.2.1 Role of HbA1c

Glycaemic control plays an important role in the development and progression of DN through various metabolic pathways as discussed above. In DCCT, intensive treatment reduced the incidence of microalbuminuria by 34% (95%CI 2, 56; p=0.04) and decreased albumin excretion rate (AER) by 15% after first year (95%CI 6.5, 7.7; p=<0.001) [14]. UKPDS found a relative risk reduction of 34% for albuminuria, 67% for two-fold increase in plasma creatinine and 74% for doubling of plasma urea in patients with improved glycaemic control [15]. In ADVANCE trial, intensive glycaemic control showed a relative risk reduction of 21% (95%CI 7, 34; p=0.01) for new or worsening diabetic nephropathy [16].

3.1.2.2 Role of Blood Pressure and Renin Angiotensin System

In patients with diabetes, hypertension has been an independent risk factor which leads to development and progression of micro and macro vascular complications of diabetes. Hypertension usually precedes T2DM. The prevalence of hypertension in T2DM is estimated from 58%-70% depending on the population studied [17]. Multiple factors link T2DM and hypertension e.g. oxidative stress and endothelial dysfunction [17]. In UKPDS, for every 10 mmHg reduction in systolic blood pressure, there was a 13% reduction in microvascular end points including albuminuria (p=<0.001) [18].

Activation of renin-angiotensin-aldosterone system (RAAS) also plays an important role [17]. Angiotensin II after binding to angiotensin type 1 receptors cause vasoconstriction, increased sodium reabsorption and increased aldosterone production, leading to hypertension. Although renin activity is suppressed, RAAS activity is inappropriately high

given the high intra cellular volume in the presence of hypertension [17]. Therefore, using angiotensin-converting enzyme inhibitor (ACEi) or angiotensin II receptor blockade (ARB) are the preferred way of treating hypertension in DN [19]. In post hoc analysis of double blind, randomised, placebo controlled Reduction of End pints in NIDDM with Angiotensin II Antagonist Losartan (RENAAL) study, reduction of blood pressure and albuminuria using ARB were associated with positive renal outcome for ESRD [20]. In Microalbuminuria Reduction with Valsartan (MARVAL), randomised controlled trial, intensive blood pressure control (systolic BP <120 mmHg) was associated with reduction in albuminuria (p=0.007) [21]. In Irbesartan Diabetic Nephropathy Trial (IDNT), Irbesartan has shown to reduce the risk of ESRD by 23% (p=0.07) after a follow up period of 2.6 years [22].

3.1.3 CKD and OSA

CKD and OSA share several similar pathological and molecular mechanisms e.g. oxidative and nitrosative stress, AGE production and PKC activation (**Figure 3.1**) [23]. Please see chapter 1 for details. Moreover, OSA induces endothelial dysfunction, sympathetic activation and lipid metabolism dysfunction which increase the risk of hypertension [24]. Sympathetic activation and inflammatory cytokines production in the presence of OSA rapidly progress the loss of renal function [25]. Also, severity of OSA is directly correlated to the loss of renal function [25]. In the cross sectional analysis of the current study population, Dr Tahrani showed in his thesis that OSA was independently associated with CKD in patients with T2DM [23]. Patients with T2DM and OSA had more albuminuria and lower eGFR compared to patients without OSA [23].

Data from patients without diabetes suggest that OSA is associated with CKD [26]. In a cross sectional study on obese non-diabetic adults, patients with OSA had higher serum creatinine than patients without (p=0.013) [26]. Rise in serum creatinine was associated with severity of OSA (p=0.044) [26]. In a cross-sectional study on patients with CKD, a 10 ml/min/1.73m^2 drop in eGFR was associated with 42% increased risk of OSA after adjustments [27]. In Sleep-SCORE study, patients with advanced CKD (eGFR ≤40 ml/min/1.73m^2) and those on haemodialysis were at increased risk of OSA (OR=2.19; 95%CI 1.22, 3.92; OR=4.14; 95%CI 2.26, 7.60 respectively) [27]. There are many cross sectional studies examining the relationship between OSA on albuminuria and eGFR [28-30], but there is no data that examined the longitudinal impact of OSA on CKD in patients with T2DM.

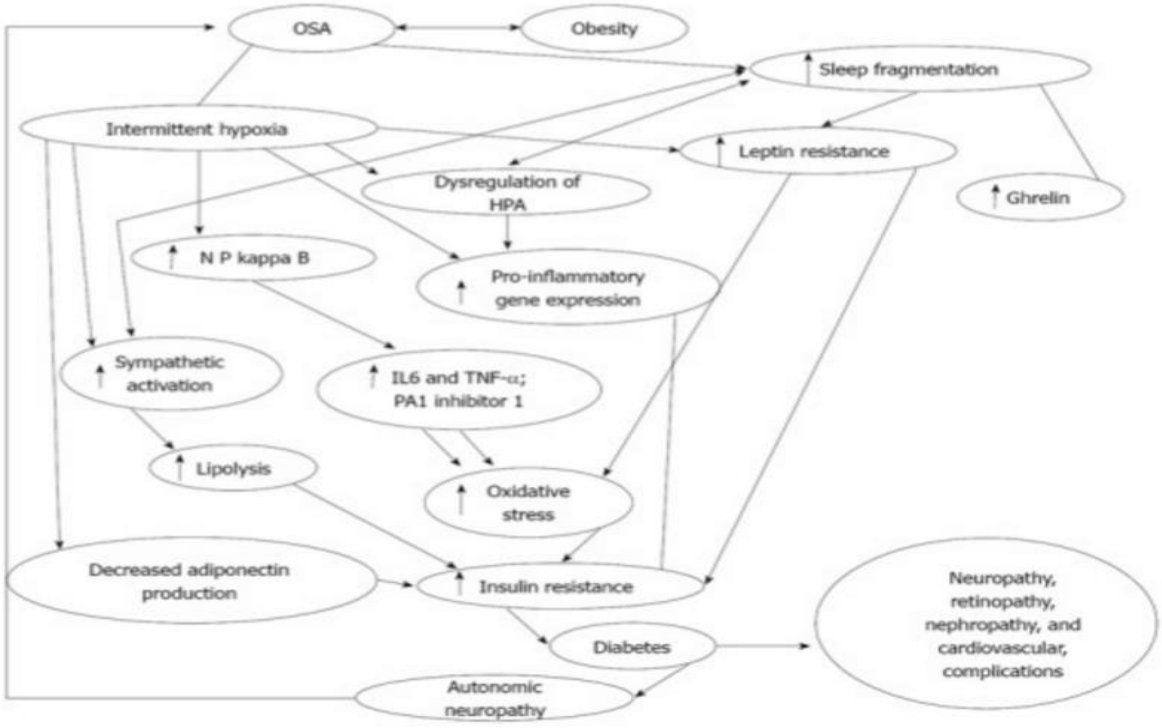


Figure 3.1. Flow diagram demonstrating the relationship of OSA and T2DM. Adopted with permission (44)

3.1.4 CAN, OSA and CKD

Our group have previously shown that presence of OSA is associated with CAN [44]. This association is likely to be bi-directional as discussed in chapter 5. This association is found not only in patients with T2DM but only lean patients with T1DM [84]. In a case control study on non-obese patients with T1DM, 67% patients with CAN had OSA as diagnosed by polysomnography (p=0.01) [84]. We also shown that CAN is an independent predictor of the eGFR decline (B=-3.5; p=0.03) [70]. However, the decline of eGFR in patients with CAN is mediated by OSA is unclear.

3.2 Hypothesis

Based on the above, I hypothesised that OSA is associated with greater decline in eGFR and the development of CKD in T2DM. I also hypothesised that the combination of OSA and CAN has greater effect on eGFR than either alone in patients with T2DM.

3.2.1 Primary Aim

To assess the longitudinal impact of OSA on the eGFR decline in patients with T2DM.

3.2.2 Secondary Aims

1. To assess the impact of OSA on albuminuria.
2. To assess the impact of CPAP treatment on DN progression.
3. To assess the impact of the interaction between OSA and cardiac autonomic neuropathy on eGFR changes longitudinally.

3.3 Methods

3.3.1 Study population

Patients for baseline data collection were recruited between 2008 and 2011, to explore the links between OSA and CKD in T2DM. I have utilized this study population and followed them up prospectively to assess the impact of OSA on the development of CKD. Patients for the study were recruited consecutively from outpatient diabetes departments at Birmingham Heartlands Hospital (BHH) and Royal Stoke University Hospital. I have collected the follow up data from BHH between 2012 and 2014, utilizing patients' electronic records. This analysis is only based on the data from patients recruited at BHH.

3.3.2 Inclusion Criteria

1. Adult patients with T2DM
2. White Europeans and South Asian ethnic origins
3. Able to give consent

3.3.3 Exclusion Criteria

1. Past medical history of OSA or other sleep or respiratory disorder
2. The use of sleeping tablets
3. Pregnancy
4. Patients with ESRD or receiving dialysis

3.3.4 CKD assessment

Renal function was assessed using eGFR, calculated using the 4-variable MDRD equation [GFR (mL/min/1.73m^2)=175 x (S_{cr})$^{-1.154}$ x (Age)$^{-0.203}$ x (0.742 if females) x (1.212 if African American)] [31]. Albuminuria was assessed using a single early morning urine measurement for urinary albumin creatinine ratio (ACR). Microalbuminuria was defined as ACR > 3.4 mg/mmol [32] and macro albuminuria was defined as ≥ 30 mg/mmol [32]. CKD was defined as the presence of albuminuria (micro or macro) or an eGFR < 60 ml/min/1.73 m^2. To assess eGFR progression, only patients with both baseline and study-end eGFR were included in the analysis. Rapid eGFR decline was defined as 4% decline of eGFR/year [33]. To assess the progression of albuminuria, only patients with normal baseline ACR were included.

3.3.5 OSA assessment

Patients were assessed at the baseline for the presence of OSA. This was examined using a portable multichannel respiratory device (Alice PDX, Philips Resporinics, USA); an AHI ≥ 5 events/hour was used to diagnose OSA [34]. OSA severity was assessed based on the AHI, ODI, time spent with oxygen saturations < 90%, time spent with oxygen saturations < 80% and the nadir nocturnal oxygen saturation during sleep [35]. Data was analysed in accordance with AASM guidelines [36]. OSA was further classified into mild (AHI 5- <15), moderate (15- <30) and severe (≥30). Inadequate sleep recordings (sleep duration <4 hours) were repeated and were excluded if remained poor [35]. Sleep studies were scored by Dr Ali (Consultant in respiratory medicine at the University Hospital of Coventry and Warwickshire) or Mr Nicholls (senior Sleep technician at Birmingham Heartlands Hospital) [35]. Apnoea was defined as cessation or ≥ 90% reduction in airflow for at least 10 seconds.

Hypopnea was defined as ≥ 30% reduction in airflow for ≥ 10 seconds associated with ≥ 4% drop in oxygen saturations [35]. For details, please see chapter 2.

Patients who were diagnosed to have OSA were referred to sleep clinic at Birmingham Heartlands Hospital. Patients with moderate to severe OSA were offered CPAP as part of their routine clinical care. These patients were then divided into compliant group who were using CPAP ≥4 hours/night for 70% of the time [37]; and non-compliant group who either declined CPAP, could not tolerate it or used it for <4 hours/night. Data regarding CPAP usage was downloaded from CPAP machine.

3.3.6 Cardiac Autonomic Neuropathy

Cardiac autonomic neuropathy (CAN) was diagnosed based on heart rate variability, as assessed by ANX-3.0 software, (ANSAR Inc., Philadelphia, PA). Data recorded included the E/I ratio, 30:15 ratio, Valsalva ratio, frequency domain analysis after respiratory adjustment (Lfa, Rfa and Lfa/Rfa) and blood pressure measurements.

CAN was diagnosed when 2 or more of the following tests were abnormal: E/I ratio, 30:15 ratio, Valsalva ratio, and postural drop in BP (drop of 20mmHg in systolic or 10mmHg in diastolic BP). For further details, please refer to chapter 2 (pages 87-94).

3.3.7 Statistical Analysis

Data analysis was performed using SPSS 22.0 software (SPSS Inc, Chicago, USA). Data are presented as mean (SD) or median (IQR). Independent continuous variables were compared using the Student's t-test or the Mann-Whitney test. Categorical variables were compared using the Chi-squared test. Correlations between continuous variables were performed

using the Pearson or Spearman tests. Differences between independent groups were assessed by ANOVA. To assess the independently association of a categorical or scale variable with a particular outcome, logistic regression (forced entry method) was used if the outcome was a dichotomous variable and linear regression (forced entry method) was used if the outcome was a continuous variable.

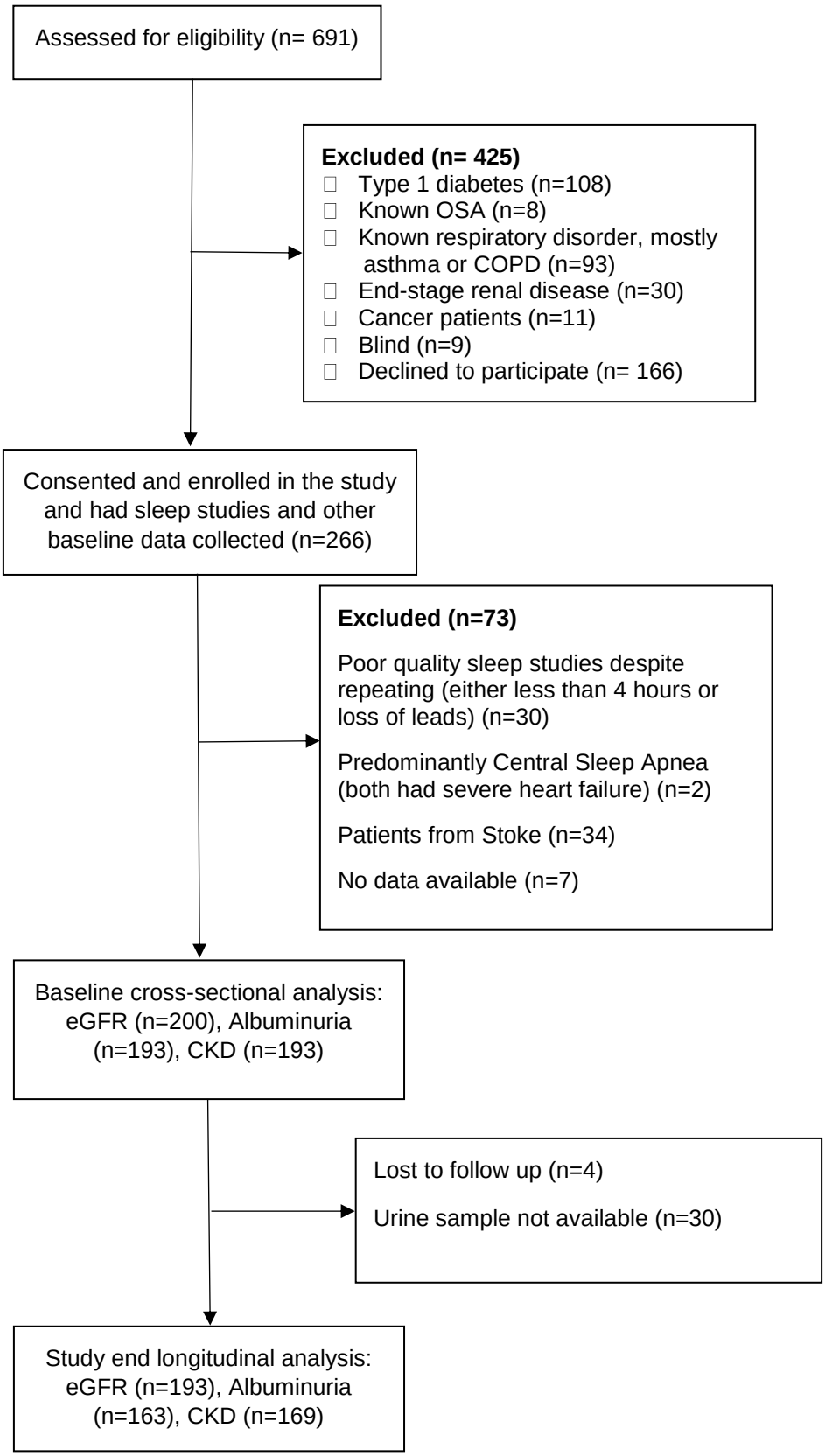
To assess the impact of OSA and AHI on CKD, logistic regression (forced entry method) was used. To assess independent association of continuous variables, multiple linear regression (forced entry method) was used. To assess the impact of OSA on eGFR progression, only those patients were included in the analysis who had baseline and study end eGFR measurements. Linear regression (forced entry method) was used with eGFR at study end and eGFR change as outcome measures. To assess the impact of interaction of OSA and CAN on progression of CKD, patients were divided into 4 groups (no OSA and no CAN; CAN with no OSA; OSA with no CAN; OSA and CAN). Linear regression was used to explore the relationship between these groups (using dummy variables) with study end eGFR, eGFR change and eGFR change% as outcome measures. To assess the impact of OSA on progression of albuminuria, only patients with normal ACR at baseline were included in the logistic regression analysis.

Collinearity and residuals were assessed and considered in the model fit. Removing variables responsible for collinearity did not have significant impact on model estimates. Therefore, final models were adjusted for variables which were either clinically significant or differed between patients with and without OSA. A p value of <0.05 was considered statistically significant.

3.4 Results

A total of 200 patients agreed to take part at BHH. Cross-sectional and longitudinal analyses were carried out on patients who had complete set of data. The average follow-up period was 2.5 (0.7) years (**Figure 3.2**). These results have been published in Diabetes Care in 2013 [5].

Figure 3.2 Study flow diagram



3.4.1 Baseline Analysis

The prevalence of OSA was 63% [n=126]) (mild 38.5% [n=77], moderate to severe 24% [n=48]). The prevalence of CKD was 41.5% (n=83), albuminuria was 34% (n=68) and macro albuminuria was 11% (n=22). The eGFR was ≥90 in 46% (n=92), 60-89 in 37% (n=74), 30-59 in 15.5% (n=31), 15-29 in 1.5% (n=3) and <15 mL/min/1.73m^2 in 0%. Baseline characteristics are described in **Table 3.1**. Patients with OSA were more likely to be male and white European. They were older, obese and had longer diabetes duration. They were more likely to have worse blood pressure control. They were more likely to be on more insulin, GLP-1 analogues and anti-hypertensive agents. Patients with OSA had a higher prevalence of DN and albuminuria **(Table 3.2)**.

Table 3.1 – Participant Characteristics in relation to OSA status
Data presented as median (IQR), mean (SD) or % (n). Analysis performed using the Chi-square test for categorical variables, the independent t test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables.

	OSA- (n=74)	OSA+ (n=126)	p value
Male	43.2% (32)	65.9% (83)	0.002
White European	31.1% (23)	57.1% (72)	0.0001
Age (years)	54.6 (11.9)	59 (11.7)	0.01
Diabetes duration (years)	9 (6, 15)	12 (7.5, 18.5)	0.02
Smoking (current or ex-smoker)	37.8% (28)	38.1% (48)	0.97
Alcohol (consumes alcohol)	9.5% (7)	28.6% (36)	0.001
Body Mass Index (kg/m^2)	30 (26.7-34.5)	33.6 (29.9-39.1)	<0.001
Waist circumference (cm)	105.4 (13.7)	115.6 (15.7)	<0.001
Waist-to-hip ratio	0.9 (0.1)	1 (0.1)	0.23
Neck circumference (cm)	38.3 (3.1)	41.9 (4.3)	<0.001
Systolic blood pressure (mmHg)	125.3 (15.9)	132.7 (17.7)	0.003
Diastolic blood pressure (mmHg)	77.3 (10.1)	78.1 (9.8)	0.5
HbA1c (%)	7.6 (6.9-8.4)	8.1 (7.2-9.3)	0.03
Total cholesterol (mmol/L)	3.7 (3.4-4.4)	3.7 (3.3-4.2)	0.6
Triglycerides (mmol/L)	1.5 (1.1-2.3)	1.8 (1.3-2.5)	0.2
Epworth sleepiness score	5 (1, 11.3)	8 (4, 13)	0.002
Oral hypoglycaemic agents	98.6% (73)	90.5% (114)	0.02
Insulin	40.5% (30)	57.9% (73)	0.02
GLP-1 analogue	5.4% (4)	10.3% (13)	0.23
Anti-hypertensive agents	74.3% (55)	85.7% (108)	0.05
Anti-platelet agents	62.2% (46)	73% (92)	0.1
Lipid lowering therapy	87.8% (65)	84.9% (107)	0.6
CKD	26.4% (19)	52.9% (64)	<0.001
Microalbuminuria	22.2% (16)	43% (52)	0.004
Macroalbuminuria	5.6% (4)	14.9% (18)	0.05

Table 3.2 Relationship between OSA and DN in patients with T2DM in total study population and in ethnicity sub groups

Data presented as median (IQR), mean (SD) or % (n). Analysis performed using the Chi-square test for categorical variables, the independent t test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables.

	OSA-	OSA+	p value
Total Cohort			
CKD (n=72 vs. 121)	26.4% (19)	52.9% (64)	<0.001
Albuminuria (n=72 vs. 120)	22.2% (16)	43% (52)	0.004
Macroalbuminuria (n=72 vs. 120)	5.6% (4)	14.9% (18)	0.05
Serum creatinine (µmol/L) (n= 74 vs. 126)	75.3 (23.2)	91.4 (38.3)	0.001
eGFR (mL/min/1.73m^2) (n=74 vs. 126)	91.7 (23.4)	81.9 (27.6)	0.01
eGFR <60 mL/min/1.73m^2 (n=74 vs. 126)	6.8% (5)	23% (29)	0.003
White Europeans			
CKD (n=22 vs. 67)	22.7% (5)	52.2% (35)	0.02
Albuminuria (n=22 vs. 67)	18.2% (4)	38.8% (26)	0.08
Macroalbuminuria (n=22 vs. 67)	0% (0)	13.4% (9)	0.07
Serum creatinine (µmol/L) (n=23 vs. 72)	75.3 (27.8)	94.9 (39.2)	0.03
eGFR (mL/min/1.73m^2) (n=23 vs. 72)	83.7 (24.3)	77.9 (27.6)	0.39
eGFR <60 mL/min/1.73m^2 (n=23 vs. 72)	22.7% (5)	25.4 (17)	0.8
South Asians			
CKD (n=50 vs. 54)	28% (14)	53.7% (29)	0.01
Albuminuria (n=50 vs. 53)	24% (12)	48.1% (26)	0.01
Macroalbuminuria (n=50 vs. 53)	8% (4)	16.7% (9)	0.2
Serum creatinine (µmol/L) (n=51 vs. 54)	75.5 (21.3)	86.9 (28)	0.1
eGFR (mL/min/1.73m^2) (n=51 vs. 54)	94.6 (22.8)	85.9 (28)	0.1
eGFR <60 mL/min/1.73m^2 (n=51 vs. 54)	4% (2)	14.8% (8)	0.1

3.4.2 Longitudinal Analysis

As indicated in **figure 3.2**, follow up data was available for 196 out of 200 patients for eGFR progression, 163 out of 193 patients for albuminuria progression and 169 out of 200 patients for CKD progression.

Change in eGFR was noted in both OSA+ and OSA- groups but was significant in only OSA+ group [OSA+: 81.7 (27.8) vs.75.2 (28.8) mL/ min/1.73 m^2 ; p=0.001; OSA-: (90.9 (23.2) vs. 89.2 (21.9) mL/ min/1.73 m^2, p=0.1]. The change in eGFR was greater in patients with OSA [-5.0 (-14, 1.8) vs -1.5 (-8.5, 4.0) mL/ min/1.73 m^2; p=0.006). When eGFR change was calculated as a percentage of baseline eGFR, greater decline was noted in OSA+ group than OSA- group [-6.8% (-16.1, 2.2%) vs. -1.6% (-7.7, 5.3), p=0.002) in a step-wise manner [-1.4% (-7.7, 5.2) vs. -5.3% (-16.5, 2.7) vs. -8.7% (-16.1, 2.0); p=0.003] for no OSA vs. mild vs. moderate to severe OSA). Rapid eGFR decline, was noted in OSA+ group (39.5% vs. 20.8%, p=0.007).

In linear regression model (R^2 =0.84), baseline eGFR was a predictor of study-end eGFR (B=0.94, p=<0.001). After addition of age at diagnosis, sex, ethnicity, diabetes duration, BMI, mean arterial pressure (MAP), HbA1c, total cholesterol, triglycerides, use of insulin, lipid lowering treatment, anti-hypertensive treatment use, anti-platelets, oral anti-hyperglycaemic agents and smoking, as potential confounders, OSA remained independent predictor of study-end eGFR (r^2 =0.86, B=-3.8; p=0.04) (**table 3.3**). Even after replacing BMI with WC, OSA remained independent predictor (R^2 =0.86, B=-4.2, p=0.03). Similarly, after replacing OSA with AHI, baseline AHI was an independent predictor of study end eGFR (B= -4.6; p=0.02). There was no relationship between hypoxemia measures and study end eGFR. Similar analysis by replacing study end eGFR with eGFR change confirmed that OSA was

independent predictor of eGFR decline (r^2 =0.08; B=-5.5, p=0.001). This remained significant despite adjustments (r^2 =0.17; B=-3.8, p=0.04). Of the patients with no CKD at baseline, 23.1% in OSA+ group progressed to CKD as compared with 12.2% in OSA- group without OSA, but this difference was not statistically significant (p=0.18). Patients with OSA at baseline were more likely to develop albuminuria during follow up but this was not statistically significant (22.6% vs. 13.3%, p=0.23).

Table 3.3. Assessing the relationship between OSA and CKD using linear regression model
Model adjusted for age, sex, ethnicity, diabetes duration, BMI, MAP, HbA1c, total cholesterol, triglycerides, insulin use, lipid lowering treatment, anti-hypertensive use, anti-platelets, oral anti diabetic agents, and smoking

Outcome measures	R^2	Adjusted R^2	B	p value
Study end GFR	0.86	0.85	-3.8	<0.001
eGFR change	0.17	0.09	-3.8	0.04

3.4.3 Interaction between OSA and CAN

In order to assess the impact of OSA and CAN on CKD progression, data were available for 193 patients who were divided in 4 groups. Baseline characteristics of the patients are as indicated in **table 3.4**. Patients were divided in 4 groups: group 1 included patients with no OSA and no CAN (n=45); group 2 included patients with CAN but no OSA (n=27); group 3 included patients with OSA but no CAN (n=67); group 4 included patients with both OSA and CAN (n=54).

Patients who had both OSA and CAN were more likely to have CKD, microalbuminuria and macroalbuminuria (**Table 3.4**). Longitudinally, eGFR decline was greater in patients with both OSA and CAN (-9.4 (13.2) mL/ min/1.73 m^2 ; p=0.002) compared to the other groups

[(No OSA and no CAN: -1.9 (6.5) mL/ min/1.73 m^2 vs. CAN with no OSA: -1.4 (11.7) mL/ min/1.73 m^2) vs. OSA with no CAN -3.2 (10.6) mL/ min/1.73 m^2]. Rapid eGFR decline was more common in patients with OSA and CAN as compared with other groups [51% (n=26) vs 21.4% (n=9) for patients with no OSA and no CAN; 24% (6) for patients with CAN only; 25% (n=15) for patients with OSA only; p=0.005].

The relationship between the OSA and CAN interaction and study-end eGFR and eGFR decline is summarised in **Table 3.5.** In order to explore the relationship between the interaction of OSA and CAN and study-end eGFR, linear regression was used. The outcome measure was study-end eGFR. Group 1 (patients with no OSA and no CAN) was used as reference category. After adjusting for age at diagnosis, sex, ethnicity, diabetes duration, BMI, MAP, HbA1c, total cholesterol, triglycerides, use of insulin, lipid lowering treatment, anti-hypertensive treatment use, anti-platelets, oral anti-hyperglycaemic agents and smoking, presence of both OSA and CAN was associated with lower study-end eGFR at study end (R^2 =0.9; B=-7.2, p=0.006) compared to the other groups. Similar results were found when study end eGFR was replaced by eGFR change as the outcome measure (R^2 =0.13; B=-6.7, p=0.01) or eGFR change percent (R^2 =0.2; B=-0.1, p=0.005). In order to assess the relationship between the interaction of OSA and CAN and rapid eGFR decline, logistic regression was used. After adjusting for the same variables as described above, presence of both OSA and CAN was associated with rapid eGFR decline (OR=3.3; 95% CI 1.08, 9.9; p=0.04).

Table 3.4 Characteristics of patients in relation to interaction between OSA and CAN
Data presented as median (IQR), mean (SD) or % (n). Analysis performed using the Chi-square test for categorical variables, the independent t test for normally distributed variables and the Kruskal-Wallis test for non-normally distributed variables.

	OSA-, CAN- n=45	CAN+, OSA- n=27	OSA+, CAN- n=67	OSA+, CAN+ n=54	p value
Male	48.9% (22)	37% (10)	62.7% (42)	72.2% (39)	0.009
White European	28.9% (13)	40.7% (11)	62.7% (42)	55.6% (30)	0.003
Age (years)	52.2 (11.6)	57.6 (12.9)	56.3 (11.9)	59.8 (10.4)	0.01
Diabetes duration (years)	8 (4, 12)	11.5 (7.8, 21)	10 (6, 14.8)	15 (10, 20)	0.001
Smoking (current/ex)	26.7% (12)	59.3% (16)	43.3% (29)	25.9% (14)	0.008
Alcohol (consumes alcohol)	8.9% (4)	14.8% (4)	32.8% (22)	29.6% (16)	0.01
Body Mass Index (kg/m^2)	30 (27.3-34.1)	31 (24.9-34.9)	34.5 (30.2-40.4)	33.2 (29.4-37.9)	0.001
Waist circumference (cm)	106.5 (16.8)	106 (15.4)	116 (13.7)	116.2 (15)	0.002
Neck circumference (cm)	38.5 (2.9)	38.2 (3.4)	41.6 (4.6)	42.3 (3.9)	0.03
Systolic blood pressure (mmHg)	127.6 (16.1)	122.5 (15.4)	134 (16.3)	133.9 (20)	0.01
Diastolic blood pressure (mmHg)	78.1 (10.6)	74.2 (9.2)	81.3 (9)	77.3 (10.8)	0.01
HbA1c (%)	7.5 (7-8.4)	7.5 (6.6-9.2)	8.2 (7.2-9.1)	8.3 (7.3-9.7)	0.3
Total cholesterol (mmol/L)	3.7 (3.4, 4.2)	3.6 (3.2, 4.7)	3.8 (3.3, 4.5)	3.7 (3.3, 4.2)	0.8
Triglycerides (mmol/L)	1.4 (1.1, 2.1)	1.6 (1, 2.5)	1.8 (1.3, 2.6)	1.9 (1.3, 2.8)	0.09
Oral hypoglycaemic agents	97.8% (44)	96.3% (26)	92.5% (62)	90.7% (49)	0.5
Insulin	31.1% (14)	59.3% (16)	43.3% (29)	25.9 (14)	0.01
GLP-1 analogue	4.4% (2)	3.7& (1)	14.9% (10)	11.1% (6)	0.2
Anti-hypertensive agents	68.9% (31)	77.8% (21)	80.6% (54)	88.9% (48)	0.1
Anti-platelet agents	60.5% (26)	65.4% (17)	73.3% (44)	73.6% (39)	0.4
Lipid lowering therapy	84.4% (38)	85.2% (23)	83.6% (56)	85.2% (46)	0.9
CKD	20% (9)	25.9% (7)	35.8% (24)	63% (34)	<0.001
Microalbuminuria	20% (9)	18.5% (5)	26.9% (18)	51.9% (28)	0.001
Macroalbuminuria	6.7% (3)	3.7% (1)	4.5% (3)	22.2% (12)	0.004

Table 3.5 Assessing the relationship between OSA and CAN interaction and eGFR change using linear regression model

Model adjusted for age, sex, ethnicity, diabetes duration, BMI, MAP, HbA1c, total cholesterol, triglycerides, insulin use, lipid lowering treatment, anti-hypertensive use, anti-platelets, oral anti diabetic agents, and smoking. The reference category for all models was patients who had neither OSA nor CAN

Outcome measures	OSA and CAN	R for the model	R square for the model	B	p value
Study end eGFR	CAN, no OSA	0.93	0.87	-0.7	0.8
	OSA, no CAN			-0.43	0.8
	OSA and CAN			-7.2	0.006
eGFR change	CAN, no OSA	0.36	0.13	1.6	0.6
	OSA, no CAN			-1.3	0.6
	OSA and CAN			-6.7	0.01
eGFR change%	CAN, no OSA	0.41	0.17	0.05	0.23
	OSA, no CAN			-0.001	0.98
	OSA and CAN			-0.1	0.005

3.4.4 Possible Impact of CPAP

Forty seven patients were diagnosed to have moderate to severe OSA and were offered CPAP treatment; only 16 were compliant. eGFR declined by -1.4% (-7.7%, 5.2%) vs. -5.3% (-16.5%, 2.7%) vs. -7.7% (-15.9%, -1.8%) vs. -10.0% (-17.2%, 2.3%) for no OSA vs. mild OSA vs. moderate to severe OSA CPAP-compliant vs. moderate to severe OSA non-compliant with CPAP respectively (p=0.01 for the trend). Similar trend was observed when albuminuria progression was analysed. Of the patients in moderate to severe OSA, CPAP non-compliant

group, 33% developed albuminuria during follow up, as compared with 11.1% in moderate to severe OSA, CPAP compliant group, but this was not statistically significant (p=0.4).

3.5 Discussion

We found baseline OSA status and AHI to be independent predictors of future eGFR and eGFR change over the follow up period of 2.5 years. Presence of both OSA and CAN was associated with greater eGFR decline.

Our study population characteristics were similar to other cohorts in secondary care in the U.K. as reported by a study from a different region [38]. Whether we could extrapolate the findings to patients in the primary care with shorter duration of diabetes remains to be seen. High prevalence of OSA noted in our cohort was similar to other studies [39,40]. Prevalence of DN and albuminuria in our study was also consistent with other reports on patients with T2DM [41].

There were demographic and metabolic differences between patients with and without OSA. Despite these, OSA remained independently associated with DN even after multiple adjustments as discussed above. In longitudinal analysis, after adjustments, OSA remained an independent predictor of study-end eGFR and eGFR change. Similar results were found in the multivariable analysis.

As far as we are aware, this is the first study to assess the impact of OSA on CKD in patients with T2DM. In few studies, sleep disorders including OSA have been found to be a modifiable risk factor for the development and progression of CKD, but there is little evidence to support such findings [42]. Little evidence that is out there is mainly on patients

without diabetes and is cross-sectional [28-30,43-46]. In a study of 500 elderly men, there was no association between eGFR and OSA after adjustments of relevant confounders [28,43]. In a study of 91 obese adults, AHI was independently associated with serum creatinine after adjustment but no association was noted between albuminuria and OSA [29]. In one study, AHI was independently associated with albuminuria after adjustments [44]. However, other studies failed to identify such association [30]. Another cross-sectional study found that albuminuria was 57% greater in patients with OSA and albuminuria correlated with AHI and OSA severity [45].

Our data showed that even mild OSA is associated with DN and worsening eGFR long term. We postulate that this could be due to intermittent hypoxemia, which is exaggerated in the presence of chronic hyperglycaemia. This finding is important in the context of planning for future interventional studies; as current guidelines do not recommend treating patients with mild OSA. Though we found that OSA was an independent predictor of worsening eGFR, OSA was not a predictor of the development of either CKD or albuminuria. We hypothesize that OSA may not necessarily result in the development of CKD, but once CKD is present, OSA results in rapid decline in renal function.

To our knowledge, this is the first study that has looked at the interaction between OSA and CAN and progression of CKD. OSA and CAN often co-exist [47]. Pathogenesis of CAN is multifactorial, with hyperglycaemia being the initiating event [48]. As with other microvascular complications; oxidative stress, endothelial dysfunction, AGE production and PARP activation have all been implicated in the pathogenesis [48]. In addition, over activation of sympathetic drive is usually observed during apnoeic episodes of OSA and is also a hallmark of CAN [49]. In our study, the combination of OSA and CAN have shown to

be the major contributing factor in the progression of CKD compared with OSA and CAN alone. We have previously established that CAN on its own is associated with eGFR decline [50]. It is plausible that presence of 2 risk factors with similar underlying pathogenic mechanisms has an additive effect in the progression of CKD but, as with OSA alone, does not lead to the development of CKD in the context of T2DM. Whether OSA has a bigger role in the progression of CKD than CAN or vice versa, need to be explored further.

Our group have previously identified that OSA is associated with increased nitrosative and oxidative stress and impaired endothelial function in patients with T2DM [51]. Studies in non-diabetic patients with OSA have demonstrated that intermittent hypoxia and OSA are associated with altered PKC signalling [52], increased AGE [53], increased endothelin-1 levels [54] , decreased endothelial nitric oxide synthase [54], increased inflammation [55], hypercoagulability (increased plasminogen activator inhibitor-1) [56] and generation of ROS [55]. We have already established that OSA, nocturnal hypoxemia and AHI are independently associated with elevated serum nitrotyrosine levels after adjustments in patients with T2DM [51]. It is plausible that nitrosative stress can mediate the relationship between OSA and eGFR, [57].

The impact of CPAP treatment was not one of the aim of this study. However, in the sub-group, the decline in eGFR was bigger in the CPAP non-compliant group than those who were compliant with CPAP. A smaller proportion of patients in the compliant CPAP group developed albuminuria during follow-up than those who were non-compliant with CPAP. However, it is difficult to assess CPAP efficacy from small observational data owing to the small numbers of patients who were compliant with CPAP treatment. These data provide

further scope for assessing the impact that CPAP can have on the progression of DN but also highlight the challenges of CPAP compliance in patient with T2DM.

Our study has strengths and limitations. We used home-based portable multichannel respiratory devices rather than inpatient overnight polysomnography which according to some, may be considered a limitation, but this approach is validated in the literature [58]. We used single measurement of ACR, instead of repeated sample. However, this approach has been used by other researchers and has been validated for clinical and epidemiological studies [59-61]. In an observational prospective cohort study on patients with T2DM, single urinary albumin measurement was accurate in identifying microalbuminuria (AUC=0.93; 95%CI 0.92, 0.94; p=<0.001) and macro albuminuria (AUC=0.95; 95%CI 0.93, 0.97; p=<0.001) [62]. This longitudinal analysis of the impact of OSA on worsening DN can implicate a cause-effect relationship, although this needs to be tested in interventional studies. The missing follow-up albuminuria data, is a potential source for bias but no differences in characteristics of participants versus patients lost to follow-up were observed. A strength of our study was the well-characterized population, which helped us to adjust for a wide range of potential confounders and allowed the assessment of the impact of OSA on eGFR decline.

In the end, we conclude that patients with T2DM and OSA are more likely to have CKD compared with those with T2DM but without OSA. CKD progressed rapidly (assessed by eGFR) in patients with T2DM and OSA compared with those with T2DM alone. This finding was clinically relevant, as more patients in the OSA group had a rapid decline in eGFR (≥10% over 2.5 years). This study could potentially form the basis for interventional studies to examine the impact of OSA treatment on the development and progression of CKD in patients with T2DM.

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Chapter Four: The impact of obstructive sleep apnoea on diabetic retinopathy in patients with Type 2 Diabetes

4.1 Introduction

Diabetic retinopathy (DR), affects 40-50% of patients with diabetes and is one of the leading cause of blindness in the Western world and results in significant morbidity and economic burden [1,2]. Important DR risk factors include age, hyperglycaemia, hypertension, diabetes duration, dyslipidaemia and genetic factors [1,3]. Although the precise aetiology of DR remains debated, increased inflammation, oxidative stress, and activation of multiple pathways, are thought to result in functional and/or structural defects involving the microvasculature. This increases vascular permeability (macular oedema) or causes ischaemia leading to increased vascular endothelial growth factor (VEGF) and neovascularisation [1,4]. Despite improvements in the control of metabolic and vascular risk factors, DR remains very common [5]. A significant proportion of DR progresses to sight-threatening diabetic retinopathy (STDR) [1]. Hence, improved understanding of the pathogenesis of DR is important in order to identify new treatment targets/strategies.

4.1.1 Pathogenesis of DR

Chronic hyperglycaemia has been confirmed as one of leading underlying mechanisms for the development and progression of DR. The mechanisms that underlie the development of microvascular changes which remain the hallmark of DR, remain unclear [6].

4.1.1.1 Role of Angiotensin II

Recent studies have indicated a role of Angiotensin II (AT-II) in the development of endothelial dysfunction which is independent of effect on blood pressure [7]. AT-II stimulates non-phagocytic NADPH oxidase protein, producing reactive oxygen species which

add to the cellular oxidative burden [7]. In addition, it also produces peroxy-nitrites and other reactive nitrogen species [7]. All of this activate PARP, PKC and other pathways resulting in endothelial dysfunction [7].

In an animal study on hypertensive, non-diabetic rats, treatment with enalapril was associated with PARP inhibition and improvement in endothelial dysfunction (p=<0.05) [8].

In MICRO-HOPE study, treatment with ACE inhibitor Ramipril (10 mg) was associated with 22% relative risk reduction (95%CI -9, 28, p=0.24) in requiring laser treatment for DR. However, results were statistically not significant [9]. In a 2-year randomised double-blind placebo-controlled trial on patients with T1DM, treatment with Lisinopril was associated with significant reduction in retinopathy incidence (OR=0.69; 95%CI 0.30, 1.59; p=0.4) and progression (OR=0.50; 95%CI 0.28, 0.89; p=0.02) [10].

4.1.1.2 Role of HbA1c

As discussed above, hyperglycaemia remains the most important triggering factor for the activation of bio chemical and cellular pathways leading to the development and progression of microvascular complications related to diabetes. In DCCT, 10% lowering of HbA1c reduced the risk of retinopathy progression by 41% (95%CI -54, -24; p=<0.001) [11]. In UKPDS, DR was associated with hyperglycaemia and poor HbA1c (p=<0.001 and p=<0.02 respectively) [12]. In multivariate analysis, increasing severity of DR was associated with worsening hyperglycaemia (OR=1.39; 95%CI 1.13, 1.71; p=<0.001 for CBG ≥14.4 mmol/L) [12]. In a retrospective analysis of patients with T2DM who did not have DR at baseline, poor glycaemic control was associated with the development of DR (p=<0.001) [13]. Chronic

poor glycaemic control (HbA1c >8.3%) was associated with increased risk of developing DR (RR=7.2; 95%CI 1.61, 32.4) [13].

4.1.1.3 Role of Hypertension

The association between T2DM and hypertension has been described in the past. Patients with T2DM have higher prevalence of hypertension (32%), mainly as part of metabolic syndrome [14]. The underlying mechanism could be the lack of functioning sympathetic nerve fibre. This means that the control of retinal blood flow is entirely dependent on autoregulation. Under normoglycaemic conditions, high BP results in vasoconstriction, thereby maintaining constant retinal blood flow[15]. In the case of poor glycaemic control and DR, retinal blood flow is increased and autoregulation is lost [16]. This results in stress damage to retinal blood vessel walls and worsen retinopathy [16].

A 10 mmHg reduction in systolic BP and 5 mmHg reduction in diastolic BP results in 38% reduction in microvascular disease [14]. In UKPDS, a long term tight BP control (<150/85 mmHg) resulted in fewer micro aneurysms (RR=0.66; p=<0.001), fewer hard exudates and cotton wool spots (RR=0.53; p=<0.001 for both) and less progression of DR (RR=0.75; p=0.02) [17]. In another prospective randomised controlled trial on normotensive patients with T2DM (<140/90 mmHg), intensive treatment of BP (<128/75 mmHg) was associated with less progression of DR (p=0.019) [18].

4.1.1.4 Role of Lipids

Chronic hyperglycaemia has been described as the major contributor towards the development of microvascular complications, both on bio-chemical and physiological levels. In addition to hypertension (as discussed above), an association between lipids (triglycerides and HDL-cholesterol) and DR has also been described [19]. In a multi-centre case-control study on patients with T2DM, in group matched analysis, 0.5 mmol/L increase in triglycerides and 0.2 mmol/L reduction in HDL cholesterol was associated with increased risk of DR (OR=1.09; 95%CI 1.02, 1.16 for triglycerides; OR=0.93; 95%CI 0.86, 1.0 for HDL cholesterol). DR was associated with higher levels of triglycerides (OR=1.04; 95%CI 0.98, 1.11; p=0.30) and lower levels of HDL cholesterol (OR=0.97; 95%CI 0.90, 1.05; p=0.08) [20]. In ACCORD Lipid Study, which was a multicentre, randomised trial on patients with T2DM, 4 year treatment with fenofibrate was associated with a reduced rate of progression of retinopathy (6.5% vs 10.2% in placebo; OR=0.60; 95%CI 0.42, 0.87; p=0.006) [19] and reduction in moderate vision loss (HR=0.95; 95%CI 0.79, 1.14; p=0.57) [19].

4.1.2 DR and OSA

Obstructive sleep apnoea (OSA) is very common in patients with T2DM [21-25]. We have previously reported that OSA is associated with peripheral neuropathy, nephropathy and estimated glomerular filtration (eGFR) decline in patients with T2DM independently of obesity [25,26]. We have also shown that OSA is independently associated with increased nitrosative and oxidative stress and impaired microvascular regulation in patients with

T2DM [25]. T2DM is also a risk factor for severe nocturnal hypoxaemia [27]. Hence, it seems reasonable to speculate that OSA could play an important role in the pathogenesis STDR particularly since OSA is also associated with many of the pathophysiological deficits that are found in DR (including inflammation, oxidative stress and increased VEGF) [28-33]. Recurrent hypoxia and re-oxygenation, the hallmark of OSA, activates NF-κB pathway [34]. This activation leads to the increased expression of iNOS protein, adding to nitrosative stress and inflammation [34]. Cyclical hypoxia with re-oxygenation also act as ischaemic re-perfusion damage which add to oxidative stress [32]. The presence of oxidative stress increase the production of AGE and expression of RAGE, which are associated with endothelial dysfunction [33,35]. The levels of AGEs correlate with severity of OSA [33]. Recurrent hypoxia increase DAG levels and activate PKC isoforms [36], increase endothelin-1 [36], VEGF [37] and PAI-1 levels [38]. The levels of Inflammatory cytokines e.g. TNF-α and IL-6 and cellular adhesion markers e.g. ICAM-1 and VCAM-1 are also found to be high during hypoxic, apnoeic stage of OSA [32].

4.2 Hypothesis

Based on the above discussion, I hypothesised that OSA is associated with progression of DR in T2DM.

4.2.1 Primary Aim

1. To assess whether OSA is associated with DR progression.

4.2.2 Secondary Aim

1. To assess the impact of CPAP on DR progression

4.3 Methods

Patients for cross-sectional data analysis were recruited between 2008 and 2011 as part of Dr. Tahrani's NIHR funded, cross-sectional study to explore the links between OSA and MVC in T2DM. I have utilized this study population and followed them up prospectively to assess the impact of OSA on the development of DR. Patients were recruited consecutively from outpatient diabetes departments at Birmingham Heartlands Hospital (BHH) and Royal Stoke University Hospital. I have collected the follow up data from BHH between 2012 and 2014, utilizing patients' electronic records. This analysis is only based on the data from patients recruited at BHH.

4.3.1 Inclusion Criteria

1. Adult patients with T2DM
2. White Europeans and South Asian ethnic origins
3. Able to give consent

4.3.2 Exclusion Criteria

1. Past medical history of OSA or other sleep or respiratory disorder
2. The use of sleeping tablets
3. Pregnancy

4. Patients with ESRD or receiving dialysis

4.3.3 Diabetic Retinopathy Assessment

DR/STDR was assessed using two 45 degrees digital retinal images per eye as per the English National Screening programme guidelines (see **Table 2.1** in chapter 2) [39]. All retinal images were graded at least twice with further grading performed in cases of discrepancy by a consultant ophthalmologist. Patients with ungradable images were examined by a consultant ophthalmologist. STDR was defined as the presence of pre-proliferative or proliferative DR, maculopathy or photocoagulation (**Table 2.1**) [39]. Advanced DR was defined as having pre-proliferative (R2) or proliferative (R3) DR. All patients who had at least one retinal screening following the baseline visit were included in the longitudinal analysis. All images between baseline and end of follow-up were reviewed and the worst retinal grades prior to receiving DR treatment were included in the analysis. Progression to maculopathy was assessed by examining the progression from no maculopathy (M0) to maculopathy (M1) after excluding patients with M1 at baseline. Progression to advanced DR was examined by assessing the progression from no (R0) or background (R1) DR to pre-proliferative (R2) or proliferative (R3) DR after excluding patients who had R2 or R3 at baseline. In analysing retinal imaging grading, the worst eye grade was used.

4.3.4 Obstructive Sleep Apnoea Assessment

OSA was assessed by a single overnight home-based cardio-respiratory study using a portable multi-channel device (Alice PDX, Philips Respironics). Sleep studies were scored in accordance with the American Academy of Sleep Medicine (AASM) guidelines using the

hypopnea definition of ≥ 4% oxygen desaturation and ≥ 30% reduction in nasal air flow signal [40]. Sleep studies of <4 hours of adequate recordings were repeated and if the quality remained poor, they were excluded from analysis. All sleep studies were double scored manually and further scoring was performed in cases of discrepancy (by a consultant in sleep medicine, Dr Asad Ali). An apnoea hypopnea index (AHI) ≥ 5 events/hour was consistent with OSA diagnosis [41]. OSA severity was assessed based on the AHI and the oxygen desaturation index (ODI) based on 4% oxygen desaturation. OSA was classified into mild, moderate and severe based on AHI ≥ 5 - < 15, 15 - < 30 and ≥ 30 events/h respectively. Data regarding continuous positive airway pressure (CPAP) treatment was collected as part of routine care. CPAP was offered to all patients with moderate to severe OSA. CPAP usage > 4 hours/night on 70% of days was considered to indicate compliance [42].

4.3.5 Statistical Analysis

Data analysis was performed using SPSS 22.0 software (SPSS Inc., Chicago, USA). Data are presented as mean (SD) or median (IQR) depending on data distribution. Independent continuous variables were compared using the Student's t-test or the Mann-Whitney test. Categorical variables were compared using the Chi-squared test. Correlations between continuous variables were performed using the Pearson or Spearman tests. All statistical tests conditions/assumptions were adhered to throughout the analysis.

To assess whether OSA and/or hypoxaemia measures are independently associated with STDR, Advanced DR and maculopathy, multiple logistic regression (forced entry method) was used, in which STDR, advanced DR and maculopathy status were the outcome measures respectively and OSA and other possible confounders were the covariates.

To assess the predictors of DR progression, multiple logistic regression was used with progression to maculopathy, progression to STDR and progression to advanced DR as the outcomes and OSA and other confounders as the covariates.

Collinearity was considered in assessing fit of models to data. Based on the tolerance and the VIF tests there was no evidence of collinearity. However, based on the condition index there was evidence of collinearity with condition index value > 30 but there was no variance proportions >0.5. Sequentially removing variables involved in multicollinearity had limited impact on models estimates for the main exposure. Hence, final models presented included variables based on the known outcome-related risk factors and/or possible confounders and/or variables that differed between patients with and without OSA, regardless of the presence of collinearity. A p value < 0.05 was considered significant.

4.4 Results

One hundred and ninety nine patients were included in the baseline analysis (**Figure 4.1**). Of these patients, 57.3% (n=114) were men and 47.7% (n=95) were White Europeans. There were no differences between those who were included and those excluded in regards to the prevalence of DR, maculopathy or STDR. OSA prevalence was 62.8% (n=125). The prevalence of no OSA, mild, moderate and severe OSA were 37.7% (n=75), 38.2% (n=76) and 24.1% (n=48) respectively. STDR prevalence was 37.2% (n=74), DR prevalence was 65.3% (n=130) (R0 32.7% (n=65), R1 52.8% (n=105), R2 6.0% (n= 12), R3 8.5% (n= 17). Prevalence of advanced DR was 14.6% (n=29) and maculopathy prevalence was 32.7% (n=65). The prevalence of OSA was higher in White Europeans. Patients with OSA (OSA+) were older, more obese and had higher systolic BP compared to those without OSA (OSA-) (**Table 4.1**).

The use of antihypertensive agents and insulin was higher in OSA+ patients, whilst there were no differences in the use of lipid-lowering therapy (**Table 4.1**).

Figure 4.1 Study flow diagram

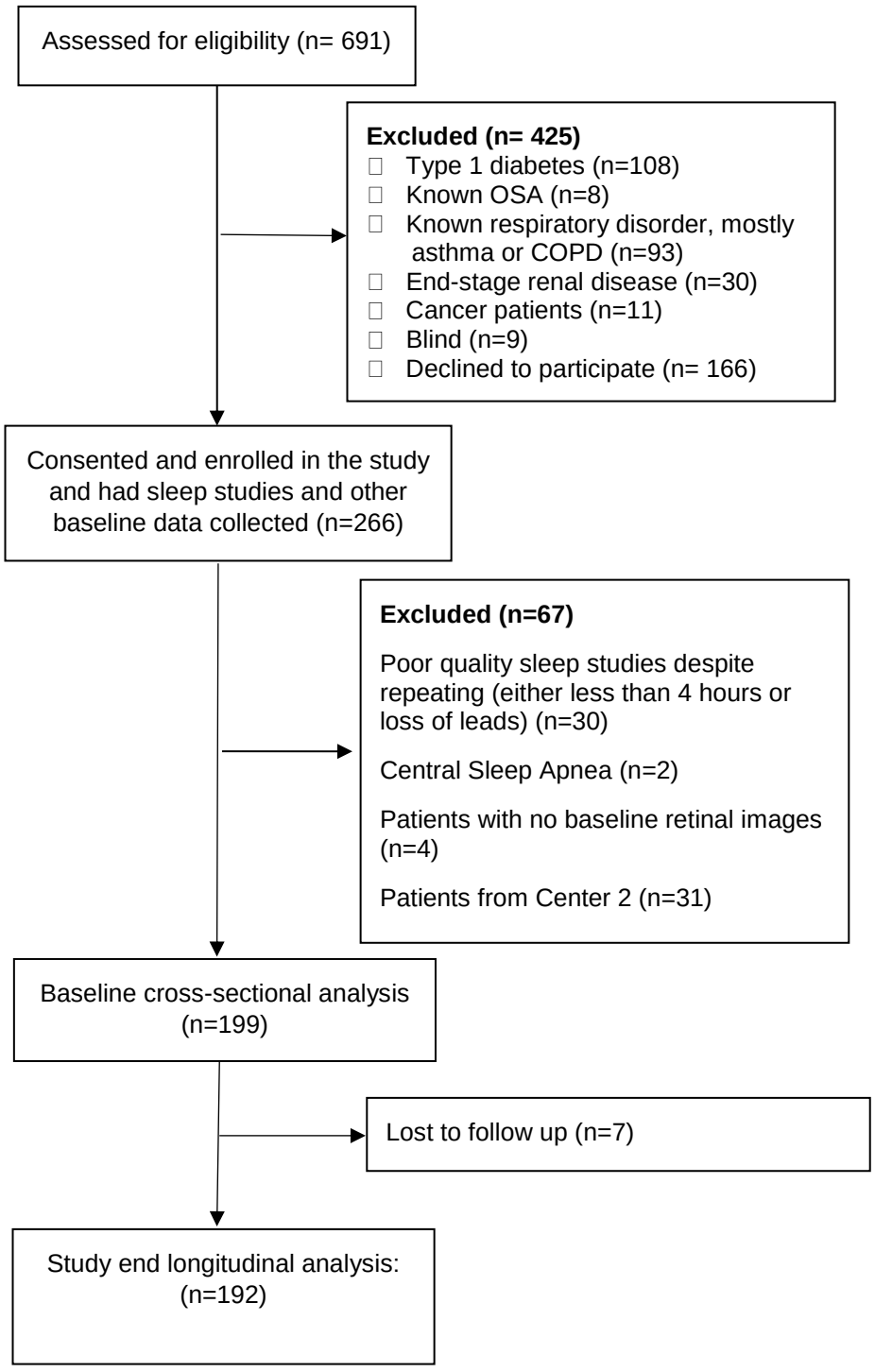

Table 4.1 Participant characteristics in relation to OSA status.

Data presented mean (SD), median (IQR) or % (n). GFR: Glomerular Filtration Rate. Analysis performed using the Chi-square test for categorical variables, the independent t test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables.

	OSA- (n=74)	OSA+ (n=125)	p value
Male	43.2% (32)	65.6% (82)	0.002
White European	31.1% (23)	57.6% (72)	<0.001
Age (years)	54.6 (11.9)	59.1 (11.7)	0.01
Diabetes duration (years)	9 (6-15)	12 (7.5-19)	0.02
Body Mass Index (kg/m^2)	31.5 (7.2)	35.5 (9.1)	0.001
Waist circumference (cm)	105.4 (13.7)	115.6 (15.7)	<0.001
Systolic blood pressure (mmHg)	125.3 (15.9)	132.8 (17.8)	0.003
Diastolic blood pressure (mmHg)	77.3 (10.1)	78.2 (9.8)	0.5
HbA1c (%)	7.6 (6.9-8.4)	8.1 (7.2-9.3)	0.02
Total cholesterol (mmol/L)	3.7 (3.4-4.4)	3.7 (3.3-4.2)	0.2
Triglycerides (mmol/L)	1.5 (1.1-2.3)	1.8 (1.3-2.5)	0.2
Estimated GFR (ml/min/1.73 m^2)	91.4 (23.9)	81.4 (26.7)	0.008
Epworth sleepiness score	6.4 (5.8)	8.9 (5.9)	0.003
Smoking (current or ex-smoker)	37.8% (28)	38.4% (48)	0.9
Alcohol (consumes alcohol)	9.5% (7)	28.8% (36)	0.001
Oral hypoglycaemic agents	98.6% (73)	90.4% (113)	0.02
Insulin	40.5% (30)	58.4% (73)	0.02
GLP-1 analogue	5.4% (4)	10.4% (13)	0.2
Anti-hypertensive agents	74.3% (55)	85.6% (107)	0.05
Lipid lowering therapy	87.8% (65)	85.6% (107)	0.6
Fibrates	5.4% (4)	4.8% 96)	0.9
Diabetic nephropathy	30.9% (21)	57.1% (64)	0.001

4.4.1 Cross-sectional analysis

The prevalence of STDR, advanced DR (R2 or R3) and maculopathy were significantly higher in OSA+ patients compared to those without OSA (**Table 4.2**).

Table 4.2 Relationship between OSA status and sight threatening diabetic retinopathy, retinopathy and maculopathy (unadjusted analysis).
Data presented as % (n) of the respective OSA category.

Total cohort		**OSA- (n=74)**	**OSA+ (n=125)**	**p value**
Sight threatening diabetic retinopathy*		25.7% (19)	44% (55)	0.01
Advanced retinopathy		8.1% (6)	18.4% (23)	0.05
Retinopathy	R0	40.5% (30)	28% (35)	0.12
	R1	51.4% (38)	53.6% (67)	
	R2	2.7% (2)	8% (10)	
	R3	5.4% (4)	10.4% (13)	
Maculopathy		20.3% (15)	40% (50)	0.004
South Asians		**OSA- (n=51)**	**OSA+ (n=53)**	
Sight threatening diabetic retinopathy*		27.5% (14)	35.8% (19)	0.4
Advanced retinopathy		9.8% (5)	15.1% (8)	0.4
Retinopathy	R0	39.2% (20)	26.4% (14)	0.5
	R1	51% (26)	58.5% (31)	
	R2	3.9% (2)	7.5% (4)	
	R3	5.9% (3)	7.5% (4)	
Maculopathy		21.6% (11)	34% (18)	0.2
White Europeans		**OSA- (n=23)**	**OSA+ (n=72)**	
Sight threatening diabetic retinopathy*		21.7% (5)	50% (36)	0.02
Advanced retinopathy		4.3% (1)	20.8% (15)	0.06
Retinopathy	R0	43.5% (10)	29.2% (21)	0.2
	R1	52.2% (12)	50% (36)	
	R2	0% (0)	8.3% (6)	
	R3	4.3% (1)	12.5% (9)	
Maculopathy		17.4% (4)	44.4% (32)	0.03

***** pre-proliferative or proliferative retinopathy, maculopathy or laser treatment

4.4.2 Longitudinal Analysis

We hypothesise that retinopathy progression from background to more advanced stages is accelerated by the presence of OSA. Therefore in order to explore this construct, a longitudinal analysis was conducted in 192 patients. The average follow up was 4.4±1 years. There was no significant difference in the follow up duration between patients with (4.4±0.9 year) and without (4.3±1 year) OSA (p=0.5).

Examining the progression from R0 or R1 to advanced DR (R2 or R3), 164 cases were available for analysis after excluding 35 patients who had advanced DR at baseline. The proportion of patients progressing to advanced DR was higher in OSA+ patients (18.4% (n=18) vs. 6.1% (n=4), p=0.02 for OSA+ and OSA- respectively) with similar trends in South Asians (14.0% (n=6) vs. 8.9% (n=4), p=0.45) and White Europeans (21.8% (n=12) vs. 0.0% (n=0), p=0.02) **(Table 4.3)**.

Data from 129 (out of 199) subjects were available to examine the progression to maculopathy (M0 to M1) after excluding 70 patients with M1 at baseline. There was no significant difference in maculopathy progression between OSA+ and OSA- patients (20.8% (n=15) vs. 19.3% (n=11), p=0.83 for OSA+ and OSA- respectively) **(Table 4.3)**.

Similarly, data from 121 patients (out of 199) was available to examine the progression to sight threatening DR. The proportion of patients progressing to sight threatening DR was not statistically different between OSA+ and OSA- patients (20.6% (n=14) vs. 13.2% (n=7), p=0.29 for OSA+ and OSA- respectively), with trend failing to achieve significance in South Asians (21.2% (n=7) vs. 19.4% (n=7), p=0.86), but significant in White Europeans (20.0% (n=7) vs. 0.0% (n=0), p=0.05) **(Table 4.3)**.

After adjustment for ethnicity, gender, diabetes duration, age at diabetes diagnosis, systolic blood pressure, HbA1c, eGFR, BMI and insulin and number of anti-hypertensive medications, OSA remained an independent predictor of progression to advanced DR (OR 5.15, 95%CI 1.15-22.9, p=0.03) (**Table 4.4**).

Table 4.3 Relationship between OSA status and progression of sight threatening diabetic retinopathy, retinopathy and maculopathy (unadjusted analysis).
Data presented as % (n) of the respective OSA category.

Progression of outcome of interest	**OSA-**	**OSA+**	**p value**
Advanced DR (n=164)	6.1% (4)	18.4% (18)	0.02
Maculopathy (n=129)	19.3% (11)	20.8% (15)	0.83
STDR (n=121)	13.2% (7)	20.6% (14)	0.29

Table 4.4: Assessing the association between OSA and STDR, maculopathy and advanced diabetic retinopathy (DR) (R2 or R3) based on the longitudinal analysis using logistic regression models (forced entry method).
The odds ratios (OR) reported are the odds for having the outcome of interest (progression of STDR, Maculopathy or advanced DR) in OSA+ compared to OSA- patients. Model is adjusted for OSA, ethnicity, age at diabetes diagnosis, diabetes duration, gender, HbA1c, BMI, systolic blood pressure, insulin use, number of anti-hypertensive agents, oral anti-hyperglycaemic agents and eGFR. Replacing BMI with waist circumference or waist/hip ratio does not change the results significantly. Inserting BMI and waist circumference together into the model did not have an impact on the OR.

Progression of outcome of interest	Nagelkerke R Square	Odds ratio	95% confidence interval	P value
Advanced DR (R2 or R3)	0.42	5.15	1.15, 22.9	0.03
STDR	0.22	1.98	0.57, 6.82	0.28
Maculopathy	0.24	1.05	0.35, 3.17	0.94

4.4.3 CPAP and DR progression

Out of 178 patients that were included in the progression to advanced DR analysis, 43 had moderate to severe OSA and only 15 were CPAP-compliant. Summary of patient characteristics according to CPAP compliance can be found in **Table 4.5**. There were largely no differences between patients who were and were not compliant with CPAP; apart from that the AHI was higher in the CPAP compliant group.

Progression to advanced DR occurred in 2.9% (n=2) vs. 17.9% (n=12) vs.17.9% (n=5) vs. 0% (n=0) in patients with no OSA, mild OSA, moderate to severe OSA non-compliant with CPAP and moderate to severe OSA compliant with CPAP respectively (p=0.01).

Out of 150 patients included in the progression to maculopathy analysis, 33 patients had moderate to severe OSA, of which 13 were CPAP-compliant. Progression to maculopathy occurred in 12.9% (n=8), 14.5% (n=8), 25.0% (n=5) and 15.4% (n=2) in patients with no OSA, mild OSA, moderate to severe OSA non-compliant with CPAP and moderate to severe OSA compliant with CPAP respectively (p=0.6).

Table 4.5: Comparison of the characteristics of patients who were and were not compliant with CPAP treatment.
Data presented as mean (SD) or n (%) of the respective CPAP group.

	CPAP non-compliant (n=28)	CPAP compliant (n=15)	p value
Male	22 (78.6%)	9 (60.0%)	0.3
White Europeans	17 (60.7%)	11 (73.3%)	0.4
Age (years)	62.0 (10.4)	61.9 (7.0)	1.0
Diabetes Duration (years)	14.7 (8.1)	13.1 (7.9)	0.6
Body Mass Index (kg/m^2)	35.7 (8.4)	39.4 (10.2)	0.2
Waist circumference (cm)	117.9 (14.3)	119.4 (19.3)	0.8
Systolic blood pressure (mmHg)	131.7 (20.9)	135.6 (14.5)	0.5
Diastolic blood pressure (mmHg)	76.9 (10.2)	78.5 (6.5)	0.6
HbA1c (%)	8.1 (1.4)	7.7 (1.0)	0.3
Total cholesterol (mmol/L)	3.7 (0.7)	4.0 (1.2)	0.3
Triglycerides (mmol/L)	2.1 (1.0)	1.5 (0.8)	0.03
HDL (mmol/L)	1.1 (0.2)	1.3 (0.3)	0.01
AHI (events/hour)	29.5 (17.5)	43.7 (25.1)	0.04
Epworth sleepiness score	10.9 (6.6)	10.6 (4.7)	0.9
Smoking (current or ex-smoker)	15 (53.6%)	5 (33.3%)	0.2
Oral anti-diabetes treatment	26 (92.9%)	12 (80.0%)	0.3
Insulin	16 (57.1%)	6 (40.0%)	0.3
Anti-hypertensive agents	25 (89.3%)	14 (93.3%)	1.0
Lipid lowering treatment	24 (85.7%)	15 (100.0%)	0.3

4.5 Discussion

T2DM and OSA frequently co-exist and can result in a range of metabolic and physiological perturbations implicated in the pathogenesis of DR. Our study demonstrates that OSA (even when mild) and its severity are independently associated with STDR, maculopathy and advanced DR in patients with T2DM. We have also shown that OSA is an independent predictor of progression to R2 or R3 over an average 4.5 year period and that CPAP treatment might have a beneficial impact on DR progression.

The population in our report comprises subjects attending large inner city, hospital-based diabetes clinics in which the known duration of diabetes was approximately 10 years. Many of the subjects already exhibited established diabetes complications (such as DN as indicated in **Table 4.1**). The participant characteristics are similar to those reported previously from a different region in the UK [43], suggesting that the current study sample was representative of the wider T2DM population in secondary care. However, whether our findings are applicable to patients typically managed in primary care and those with a shorter duration of diabetes remains to be examined. The high prevalence of OSA in our sample is consistent with other studies in subjects with T2DM [44-47]. The prevalence of STDR in our cohort (36.1%) is higher than that reported in the literature (5%-15%) [48,49], reflecting differences in the cohorts and DR risk factors in the various studies.

OSA+ patients differed from those without OSA in regards to multiple demographic and metabolic factors. Nevertheless, the association between OSA and advanced DR remained independent despite adjustment for these confounders. STDR, and maculopathy were also associated with mild OSA.

OSA+ patients differed from those without OSA in regards to multiple demographic and metabolic factors. Nevertheless, the association between OSA and advanced DR remained independent despite adjustment for these confounders, although these differences contributed to the observed relationship. STDR, maculopathy and advanced DR were also associated with both mild and moderate to severe OSA as well as AHI. This is important as it suggests that the adverse impact of OSA in patients with T2DM occurs even in patients with mild degrees of OSA and it is possible that the impact of mild OSA is magnified in tissue that is already predisposed to damage because of chronic hyperglycaemia. This is further exacerbated by the increased retinal oxygen requirements during night adaptation, hence even mild hypoxia can result in major adverse consequences to the retina [50]. In addition, there was a dose-response relationship in the association between OSA and progression to advanced DR as this association was statistically significant mainly in patients with moderate to severe OSA, suggesting potentially that OSA exacerbated retinal ischaemia which is an integral part of the development of pre-proliferative and proliferative DR.

We have previously hypothesized that OSA might be associated with microvascular complications in patients with T2DM since the molecular perturbations of OSA are similar to those of hyperglycaemia including activation of PARP, PKC, the polyol pathway, VEGF and AGE production and inflammation [25]. We have also shown that OSA is associated with increased oxidative and nitrosative stress and impaired microvascular regulation in patients with T2DM [25], all of which can contribute to the observed relationship between OSA and advanced DR.

Two previous studies have shown an association between OSA and DR, but there are important differences between these studies and ours. These studies were cross-sectional

and did not perform a longitudinal analysis. Additionally, the previous studies included highly selected populations such as Japanese patients who underwent vitreous surgery [51], or only White European men [52], while our study was comprehensive and included patients regardless of their ethnicity, gender or the severity of retinopathy. Moreover, in prior reports the diagnosis of DR was based on case records rather than using retinal images as utilized in our study [51]. OSA assessment also differed between the studies; one study used pulse oximetry to diagnose OSA [51]. whilst another used a complex multi-step approach based on questionnaires followed by pulse oximetry on a selected subgroup [52]. Our study, however, performed a more in depth assessment using a multi-channel device in all the study participants. Critically, the previous studies did not adjust for important possible confounders such as blood pressure, BMI, medication use, or used suboptimal measures, such as self-reported BMI [51,52]. In contrast, our extensive data ascertainment allowed us to adjust for a wide range of possible confounders. This is also the first report to examine the relationship between OSA and DR longitudinally.

Despite the association between OSA and advanced DR, changes similar to advanced DR have not been described in OSA patients without diabetes. OSA, however, is associated with several other ocular pathologies in patients without diabetes [53-57].

Although DR was more common in OSA+ patients, there was a lower prevalence of background (R1) DR and a higher prevalence of advanced DR (R2 and R3) in OSA+ compared to OSA- patients. Hence, it is tempting to speculate that the development of background DR critically requires hyperglycaemia, and the impact of OSA is to accelerate the progression of DR towards more advanced forms. This construct is supported by our longitudinal analysis which demonstrated that OSA was an independent predictor of the progression to advanced

DR. This is biologically plausible, particularly since the intermittent hypoxia associated with OSA can result in retinal ischemia and increased VEGF production resulting in the development of advanced DR [58]. The lack of OSA effect on maculopathy development longitudinally could be due to differences in the pathogenesis between maculopathy and pre-/proliferative DR or due to the methods used to diagnose maculopathy in this study (i.e. images) rather than the actual measurement of macular thickness using ocular coherence tomography.

Assessing the impact of CPAP treatment on DR and DR progression was not the focus of this study. The available observational data however suggest that CPAP-compliance might have a favourable impact on DR progression although these patients had worse AHI. The progression to maculopathy in the CPAP-compliant group was lower than that in non-compliant group and similar to patients with mild OSA. However, it is difficult to assess CPAP efficacy from observational data due to the small numbers of patients who were compliant. Nonetheless, these data provide further justification for future research to assess the impact of CPAP on the development and progression of DR, and highlight the challenges of CPAP compliance in patient with T2DM.

Our study has several limitations. We have used home-based portable multi-channel respiratory devices rather than in-patient overnight polysomnography. However, this approach is well established [59,60]. Our sample population is also drawn from hospital-based diabetes centres; hence we cannot necessarily extend our conclusions to other patient populations. We have used 2-field images to assess DR, rather than 7-field images, which might result in missing peripheral retinal lesions. This is unlikely to affect the results unless patients with OSA are more likely develop more peripheral lesions compared to

patients without OSA (or vice versa), but there is no evidence to support this. The limited number of events over the follow-up period did not allow us to perform more in depth analysis about the relationship between OSA and DR progression. OSA status was ascertained during and not prior to the follow-up period, but as OSA is closely related to obesity which remained constant over the study period, it is reasonable to assume that OSA was present in the majority of subjects for the 2 years prior to OSA diagnosis [61].

In conclusion, we have identified a relationship between OSA, its severity and advanced DR in patients with T2DM. OSA was an independent predictor for the development of advanced DR. CPAP-compliance was associated with reduction in the development of advanced DR. Interventional studies are needed to assess the impact of OSA treatment on STDR.

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Chapter Five: The impact of obstructive sleep apnoea on peripheral and cardiac autonomic neuropathies in patients with Type 2 Diabetes

5.1 Introduction

5.1.1 Epidemiology

Diabetes-related neuropathy is one of the most common but under recognised and poorly treated microvascular complication of T2DM [1]. Broadly speaking, it includes diabetic peripheral neuropathy (DPN) affecting the peripheral nerves and diabetic autonomic neuropathy (DAN) affecting autonomic nervous system. Cardiac autonomic neuropathy (CAN) is one of the most studied and well understood form of DAN [1]. Both DPN and CAN are associated with increased morbidity and mortality [1,2]. DPN is associated with foot ulceration and painful neuropathy [3]. CAN, on the other hand, is associated with potential life threatening complications e.g. arrhythmias, myocardial ischaemia and sudden death [4]

Prevalence of DPN varies between 45% - 73% depending on the study methods used [5,6]. Prevalence of CAN varies between 7.7% to 90% depending on the population studied and criteria used to define CAN [7-9]. CAN is significantly associated with age and longer diabetes duration [10,11]. In DCCT/EDIC, 25% of patients with CAN diagnosed based on HRV, experienced cardiovascular events as compared with 10% of patients without CAN (HR=2.79; 95%CI 1.91, 4.09) [12]. Patients with CAN were more likely to have LV hypertrophy and increased cardiac output (p=<0.001) [13]. In ACCORD trial on patients with T2DM, presence of CAN was associated with increased all-cause mortality (HR=2.22; 95%CI 1.45, 3.39; p=0.0002) and cardiovascular mortality (HR=2.55; 95%CI 1.41, 4.6; p=0.002) [14].

The clinical manifestations of DPN vary from pain, paraesthesia and hyperaesthesia (typically worse at night, usually in feet and lower limbs, occasionally affecting hands) to painless foot ulcer (usually associated with sensory loss of vibration, pressure and temperature perception and absent ankle reflexes) [15].

CAN is characterised by resting tachycardia (heart rate of 90-130 beats per minute), impaired response of blood pressure, HR and cardiac stroke volume to exercise in the absence of structural or coronary cardiac disease and postural hypotension (reduction in systolic blood pressure by > 20 mmHg or in diastolic blood pressure by > 10 mmHg on standing) [16]. Patients with CAN are more at risk of silent ischaemia (due to prolonged subjective angina threshold), diabetes-related cardiomyopathy (due to changes in the biochemical signalling resulting from sympathovagal dysbalance), systolic and most importantly, diastolic dysfunction [16].

With increasing diabetes prevalence, the prevalence of DPN and CAN are likely to increase too. Currently, NHS spend £639 million to £662 million/year on diabetic foot care [17]. Percentage of total NHS health expenditure on cardiovascular disease ranges between 6.3% in London to 7.9% in the South East and equates to £7.2 billion/year [18].

5.1.2 Pathogenesis of DPN and CAN

DPN and CAN share the same underlying pathogenic mechanisms, with hyperglycaemia as the initiating trigger. Activation of polyol and PKC pathways, increased oxidative and nitrosative stress, increased production of inflammatory cytokines and PARP activation have been described in the development of DPN and CAN [1]. In addition, CAN is also associated with denervation of parasympathetic nervous system and activation of sympathetic nervous system [1,16]. For details of the above mechanisms, please refer to chapter 1. In this chapter, I will focus on the mechanisms relating to DPN and CAN.

5.1.2.1 Role of HbA1c

Hyperglycaemia remains one of the most important factor in the pathogenesis of microvascular complications associated with T2DM. In the context of DPN, elevated HbA1c can be associated with asymptomatic disease. In a clinical study, HbA1c was the most important factor in predicting the risk of sub clinical asymptomatic neuropathy (OR=10.7; 95%CI 2.49, 46; p=<0.005) as diagnosed by nerve conduction studies [19]. In DCCT/EDIC study, intensive diabetes therapy in patients with T1DM reduced the risk of developing DPN and CAN by 64% and 34% (p=<0.01) respectively after a follow up period of 14 years [20]. In randomised, multi-centre STENO 2 trial, after a follow up of 13 years, patients on conventional therapy for diabetes were more likely to experience progression of autonomic neuropathy (RR=0.53; 95%CI 0.34, 0.81; p=0.004) and DPN (RR=0.97; 95%CI 0.62, 1.51; p=0.89) as compared with patients on intensive therapy [21]. In a longitudinal study on 1000 patients with T2DM, after a follow up period of 7.5 years, patients with poor glycaemic control were more likely to develop CAN in the future [(HbA1c 9%-11%: OR=2.6; 95%CI 1.5, 4.3; p=<0.001); (HbA1c >11%: OR=2.8; 95%CI 1.1, 7.0; p=0.04)] [11].

5.1.2.2 Role of OSA

Our group has already shown that OSA is associated with DPN, possibly via increasing the nitrosative stress and oxidative stress, PARP activation secondary to oxidative stress-induced DNA damage, impaired vascular regulation and endothelial dysfunction [22-24]. Moreover, OSA shares several other possible mechanisms with T2DM, namely increased AGE production and PKC activation [22], which lead to increased IR and metabolic

syndrome. In addition, recurrent hypoxia and recurrent arousals, as seen in OSA, lead to sympathetic overdrive [24]. Sympathetic over activity is also seen in CAN, associated with T2DM. In patients with IGT and/or shorter duration of diabetes tend to have sympathovagal dysbalance resulting in over activity of sympathetic nervous system. However, during the later stages of T2DM, there is withdrawal of both sympathetic and parasympathetic nervous response [24]. The relationship between OSA and CAN seem to be bi-directional as recurrent hypoxaemia result in increased oxidative and nitrosative stress leading to CAN; however, patients with CAN tend to have changes in upper airway tone and respiratory drive, which could lead to OSA [25]. Moreover, patients with CAN have severe OSA with prolonged apnoeas/hypopnoes than patients without CAN [26].

5.2 Hypothesis

Based on the above, I hypothesised that OSA is associated with progression in DPN and CAN in T2DM.

5.2.1 Primary Aim

To assess the longitudinal impact of OSA on the progression of DPN and CAN in patients with T2DM.

5.2.1 Secondary Aim

To assess the impact of CPAP treatment on DPN and CAN progression.

5.3 Methods

5.3.1 Study population

Patients for baseline data collection were recruited between 2008 and 2011 by Dr. Tahrani as part of NIHR funded, cross-sectional study looking at the links between OSA and MVC in T2DM. I have utilized this study population and followed them up prospectively to assess the impact of OSA on the progression of DPN and CAN. Patients for the study were recruited from outpatient diabetes departments at Birmingham Heartlands Hospital (BHH) and Royal Stoke University Hospital. I have collected the follow up data from BHH between 2012 and 2014 during 1-to-1 interview with the patients. This analysis is only based on the data from patients recruited and followed up at BHH.

5.3.2. Inclusion Criteria

1. Adult patients with T2DM
2. White Europeans and South Asian ethnic origins
3. Able to give consent

5.3.3. Exclusion Criteria

1. Past medical history of OSA or other sleep or respiratory disorder
2. The use of sleeping tablets
3. Pregnancy
4. Patients with ESRD or receiving dialysis

5.3.4. DPN and CAN assessment

DPN was assessed using Michigan Neuropathy Screening Instrument (MNSI) and foot sensitivity was assessed using 10 gram monofilament. DPN was diagnosed if MNSI questionnaire score was ≥7 or MNSI examination score was ≥2.5 [27]. Foot sensitivity was said to be reduced if 10 gram monofilament testing was abnormal (< 8 positions) [28].

CAN was assessed using the ANX-3.0 software, (ANSAR Inc., Philadelphia, PA), which uses heart rate variability (HRV) to generate data. Data recorded included the E/I ratio, 30:15 ratio, Valsalva ratio, frequency domain analysis after respiratory adjustment (Lfa, Rfa and Lfa/Rfa) and blood pressure measurements.

CAN was diagnosed when 2 or more of the following tests were abnormal: E/I ratio, 30:15 ratio, Valsalva ratio, and postural drop in BP (drop of 20mmHg in systolic or 10mmHg in diastolic BP) [24,29].

To assess DPN, CAN and foot insensitivity progression, only patients with both baseline and study-end data were included in the analysis.

5.3.5 OSA Assessment

Patients were assessed at the baseline for the presence of OSA using a portable multichannel respiratory device (Alice PDX, Philips Resporinics, USA). An AHI ≥ 5 events/hour was used to diagnose OSA [30]. OSA severity was assessed based on the AHI, ODI, time spent with oxygen saturations < 90%, time spent with oxygen saturations < 80%

and the nadir nocturnal oxygen saturation during sleep [24]. Data was analysed in accordance with AASM guidelines [31]. OSA was further classified into mild (AHI 5- <15), moderate (15- <30) and severe (≥30) [24]. Inadequate sleep recordings (sleep duration <4 hours) were excluded if they remained poor despite repeating [24]. Sleep studies were scored by Dr Ali (Consultant in respiratory medicine at the University Hospital of Coventry and Warwickshire) [24]. Apnoea was defined as cessation or ≥ 90% reduction in airflow for at least 10 seconds. Hypopnea was defined as ≥ 30% reduction in airflow for ≥ 10 seconds associated with ≥ 4% drop in oxygen saturations [24]. For details, please see chapter 2.

Patients who were diagnosed to have OSA were referred to sleep clinic at Birmingham Heartlands Hospital. Patients with moderate to severe OCA were offered CPAP as part of their routine clinical care. Patients were then divided into compliant group (using CPAP ≥4 hours/night for 70% of the time) [32]; and non-compliant group (either declined CPAP, could not tolerate it or used it for <4 hours/night). Data regarding CPAP usage was downloaded from CPAP machine.

5.3.6 Statistical Analysis

Data analysis was performed using SPSS 22.0 software (SPSS Inc, Chicago, USA). Data are presented as n (%), mean (SD) or median (IQR). Independent continuous variables were compared using the Student's t-test, the Mann-Whitney test or the Kruskal-Wallis test. Categorical variables were compared using the Chi-squared test.

To assess the impact of OSA on DPN, CAN and foot insensitivity, logistic regression (the enter method) was used. To assess independent association of continuous variables, multiple linear regression (forced entry method) was used. To assess the impact of CPAP

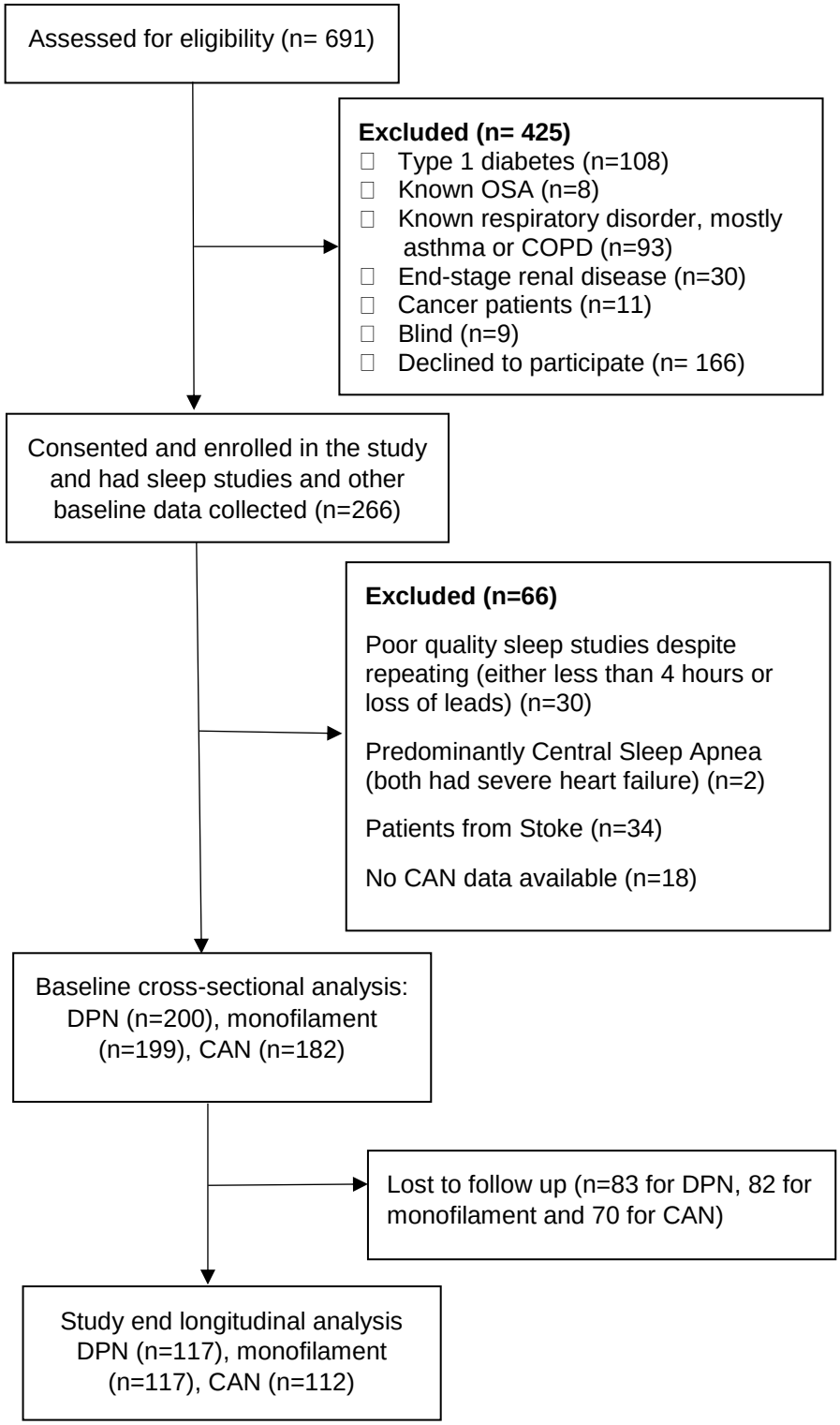
treatment on CAN parameters, linear regression models were used. Non-normally distributed variables were log transformed to satisfy the linear regression assumptions. Only those patients who had both baseline and study-end data were included in the analysis. Group with patients with no OSA were used as reference group.

Collinearity and residuals were assessed and considered in the model fit. Removing variables responsible for collinearity did not have significant impact on model estimates. Therefore, final models were adjusted for variables which were either clinically significant or differed between patients with and without OSA. A p value of <0.05 was considered statistically significant.

5.4 Results

Two hundred patients agreed to take part at BHH. Analysis were carried out on patients who had baseline and study end data. The average follow-up period was 4.6 (0.5) years (**Figure 5.1**).

Figure 5.1 Study flow diagram



5.4.1 DPN Analysis

5.4.1.1 Baseline Analysis

DPN analysis data were available for 117 patients. Prevalence of OSA was 65% (n=76) [mild 35% (n=41), moderate to severe 29.1% (n=34)]. Prevalence of DPN was 47.9% (n=56), abnormal monofilament was 36.8% (n=43). Baseline characteristics of the patients included in DPN analysis are summarized in **Table 5.1**. Patients with OSA were more likely to be older, obese and have longer duration of diabetes. Patients with OSA were also more likely to have worse glycaemic control and worse renal functions. They were more likely to be on oral anti-hyperglycaemic agents, insulin and blood pressure medications.

Patients with OSA were more likely to have DPN and foot insensitivity compared to those without OSA (**Table 5.2**). All the MNSIe components were more likely to be abnormal in patients with OSA compared to those without OSA (**Table 5.2**).

Table 5.1 – Characteristics of the participants included in DPN analysis in relation to OSA status
Data presented as median (IQR), mean (SD) or % (n). Analysis performed using the Chi-square test for categorical variables, the independent t test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables.

	OSA- (n=41)	OSA+ (n=76)	p value
Male	43.9% (18)	67.1% (51)	0.02
White European	24.4% (10)	61.8% (47)	<0.001
Age (years)	53.6 (11.1)	57.8 (10.6)	0.05
Diabetes duration (years)	9 (5-13.5)	11 (6-16.8)	0.04
Smoking (current or ex-smoker)	37.5% (15)	33.8% (26)	0.7
Alcohol (consumes alcohol)	7.3% (3)	31.6% (24)	0.003
Body Mass Index (kg/m^2)	30.3 (28-34.9)	33.5 (29.2-38.7)	0.02
Waist circumference (cm)	106.3 (14.1)	114.6 (15.3)	0.004
Systolic blood pressure (mmHg)	121 (112.3-138.5)	132 (122.6-140.8)	0.01
Diastolic blood pressure (mmHg)	79 (73.8-87)	78.8 (72.8-84.5)	0.9
HbA1c (%)	7.6% (6.7-8.5)	8.1 (7.2-9.4)	0.06
Total cholesterol (mmol/L)	3.8 (3.4-4.4)	3.7 (3.3-4.2)	0.8
Triglycerides (mmol/L)	1.5 (1.0-2.1)	1.8 (1.3-2.5)	0.2
Oral hypoglycaemic agents	97.6% (40)	90.8% (69)	0.2
Insulin	39% (16)	60.5% (46)	0.03
GLP-1 analogue	4.9% (2)	11.8% (9)	0.2
Anti-hypertensive agents	73.2% (30)	80.3% (61)	0.4
Lipid lowering therapy	90.2% (37)	86.8% (66)	0.6
Baseline eGFR (ml/min/1.73m^2)	90.9 (24.2)	84.1 (26.6)	0.2

Table 5.2. Characteristics of the patients in relation to OSA and DPN

*DPN defined as MNSIq score ≥7 or MNSIe score ≥2.5

	OSA- (n=41)	OSA+ (n=76)	p value
DPN*	29.3% (12)	57.9% (44)	0.003
Inspection (abnormal)	36.6% (15)	60.5% (46)	0.01
Ulcer (present)	0% (0)	5.3% (4)	0.1
Ankle reflex (abnormal)	34.1% (14)	64.5% (49)	0.002
Vibration (abnormal)	26.8% (11)	63.2% (48)	<0.001
Foot insensitivity	19.5% (8)	46.1% (35)	0.004

5.4.1.2 Longitudinal Analysis

At study-end, DPN and monofilament data were available for 117 patients (**Figure 5.1**). Longitudinal analysis was carried out on 61 patients for DPN progression and 74 patients for foot insensitivity progression after excluding patients who had DPN or foot insensitivity respectively at baseline.

Progression to DPN was similar in patients without and with OSA (62.1% [n=18] vs 46.9% [n=15]; p=0.2). There was no significant difference in progression to foot insensitivity in patients with vs. without OSA (9.8% [n=4] vs 6.1% [n=2]; p=0.6). Consistent with the non-significantly higher progression to foot insensitivity in patients with OSA vs. no OSA, the development of new foot ulcers during the follow up was also non-significantly higher in patients with OSA vs. no OSA (7.0% (n=5) vs. 2.4% (n=1), p=0.3)

To assess the impact of CPAP use on the outcome measures, we compared progression to DPN and foot insensitivity across the following groups: no OSA, mild OSA, moderate to

severe OSA not on CPAP and moderate to severe OSA compliant with CPAP. There was no difference in progression to DPN between these categories (63.3% [n=19] vs 50% [n=8] vs 40% [n=4] vs 40% [n=2]; p=0.5 for no OSA, mild OSA, moderate to severe OSA not on CPAP and moderate to severe OSA compliant with CPAP respectively). There was a non-significant trend of less progression to foot insensitivity in patients complaint with CPAP (5.9% [n=2] vs 10% [n=2] vs 16.7% [n=2] vs 0% [n=0]; p=0.5 for mild OSA, moderate to severe OSA not on CPAP and moderate to severe OSA compliant with CPAP respectively).

Consistent with the statistically non-significant favourable impact on progression to foot insensitivity, CPAP seems to have a favourable impact on the development of foot ulceration during the follow up (no OSA 2.4% [n=1]; mild OSA 7.7% [n=3]; moderate to severe OSA no CPAP 11.8% [n=2]; moderate to severe OSA on CPAP 0% [n=0]; p=0.3)

Due to the small number of events, we combined the development of new foot ulcer and new foot insensitivity as one outcome measure and compared the development of this combined outcome between patients with and without OSA. There was a non-significant association between OSA and the progression to foot insensitivity or development of new ulcer (OSA 20% [n=9] vs no OSA 6.1% [n=2]; p=0.08). Similar to the progression of foot insensitivity, CPAP treatment had a statistically non-significant favourable impact on the combined outcome of progression to foot insensitivity or development of new ulcers (no OSA 5.9% [n=2]; mild OSA 21.7% [n=5]; moderate to severe OSA no CPAP 30.8% [n=4]; moderate to severe OSA on CPAP 0% [n=0); p=0.06).

As both the results did not show a relationship and failed to achieve statistical significance, multivariate regression analysis was not performed.

5.4.2 CAN Analysis

5.4.2.1 Baseline Analysis

CAN analysis data were available for 112 patients. Prevalence of OSA was 65.2% (n=73) [mild 35.7% (n=40), moderate to severe 29.5% (n=33)]. Prevalence of CAN was 43.8% (n=49). Baseline characteristics of the patients included in CAN analysis are summarized in **table 5.3**. Patients with OSA were more likely to be male white Europeans with longer duration of diabetes. They were more likely to be obese and have worse glycaemic control. Baseline HRV parameters are summarized in table 5.4. Patients with OSA had lower ratios and lower sympathetic and parasympathetic tone suggesting sympathetic and parasympathetic withdraw but the results were not statistically significant. Similarly, LFA/RFA ratios were not different suggesting that the sympathovagal balance was not affected by OSA.

Table 5.3 – Characteristics of the participants included in CAN analysis in relation to OSA status

Data presented as median (IQR), mean (SD) or % (n). Analysis performed using the Chi-square test for categorical variables, the independent t test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables.

	OSA- (n=39)	OSA+ (n=73)	p value
Male	46.2% (18)	67.1% (49)	0.03
White European	20.5% (8)	63% (46)	<0.001
Age (years)	53.2 (11.3)	57.5 (10.6)	0.05
Diabetes duration (years)	9 (5-12)	11 (6-16.5)	0.04
Smoking (current or ex-smoker)	35.9% (14)	32.9% (24)	0.7
Alcohol (consumes alcohol)	5.1% (2)	30.1% (22)	0.002
Body Mass Index (kg/m^2)	30.2 (28-33.8)	33.8 (29.4-39.5)	0.006
Waist circumference (cm)	105.1 (13.3)	114.9 (15.7)	0.001
Systolic blood pressure (mmHg)	120.5 (112-139.5)	132 (123.3-140.5)	0.008
Diastolic blood pressure (mmHg)	79 (73-86)	79.5 (73.8-85)	0.5
Baseline eGFR	92.1 (24.1)	84.7 (26.7)	0.1
HbA1c (%)	7.6 (6.7-8.5)	8.1 (7.2-9.3)	0.07
Total cholesterol (mmol/L)	3.7 (3.3-4.4)	3.7 (3.3-4.2)	0.8
Triglycerides (mmol/L)	1.4 (1-2.1)	1.8 (1.3-2.5)	0.1
Oral hypoglycaemic agents	97.4% (38)	91.8% (67)	0.2
Insulin	41% (16)	60.3% (44)	0.05
GLP-1 analogue	5.1% (2)	12.3% (9)	0.2
Anti-hypertensive agents	71.8% (28)	79.5% (58)	0.4
Lipid lowering therapy	89.7% (35)	87.7% (64)	0.7

Table 5.4: Relationship between OSA and CAN parameters at baseline

Data presented as median (IQR). Analysis performed using Mann-Whitney U test for non-normally distributed variables.

	OSA- (n=39)	OSA+ (n=73)	p value
CAN	41% (16)	45.2% (33)	0.7
E/I ratio (abnormal)	69.2% (27)	65.8% (48)	0.7
Valsalva ratio (abnormal)	38.5% (15)	43.8% (32)	0.6
30:15 ratio (abnormal)	15.4% (6)	20.5% (15)	0.5
Postural drop (abnormal)	2.6% (1)	13.7% (10)	0.06
E/I ratio	1.1 (1.1-1.2)	1.1 (1-1.1)	0.3
Valsalva ratio	1.3 (1.1-1.5)	1.2 (1.1-1.3)	0.2
30:15 ratio	1.2 (1.1-1.7)	1.2 (1.1-1.4)	0.2
Baseline LFa	0.8 (0.5-2.3)	0.6 (0.3-1.5)	0.1
Baseline RFa	0.4 (0.2-1.2)	0.3 (0.1-0.7)	0.2
Baseline LFa/RFa	3.5 (1.8-5.6)	2.9 (1.5-6.8)	0.9
Deep Breathing LFa	0.7 (0.3-1.7)	0.5 (0.2-1.3)	0.3
Deep Breathing RFa	3.9 (0.5-9.8)	2.8 (0.6-6)	0.4
Deep Breathing LFa/RFa	0.2 (0.1-0.7)	0.2 (0.1-0.7)	0.7
Valsalva LFa	19.9 (3.7-46.5)	11.9 (2.9-24.4)	0.07
Valsalva RFa	1.6 (0.5-7.3)	1.3 (0.4-3.4)	0.2
Valsalva LFa/RFa	22.8 (7.9-48)	21.7 (8.4-44.5)	0.9
Standing LFa	1.2 (0.8-3.2)	0.7 (0.2-1.5)	0.003
Standing RFa	0.5 (0.1-1.5)	0.3 (0.1-0.6)	0.07
Standing LFa/RFa	4.7 (2.4-8.9)	3.5 (1.7-9.3)	0.6

5.4.2.2 Longitudinal Analysis

At study-end, CAN data was available for 112 patients. Data were available for 63 patients to assess CAN progression, 37 patients for E/I ratio progression, 65 patients for Valsalva progression, 91 for 30:15 progression and 100 patients for postural drop progression.

Progression to CAN was similar in patients without OSA compared with patients with OSA (39.1% [n=9] vs 37.5% [n=15]; p=0.9). There was no difference in the progression to abnormal standardized HRV ratios between patients with vs. without OSA (OSA vs. no OSA: progression to abnormal E/I ratio: 28% [n=7] vs 33.3% [n=4], p=0.7; progression to BP postural drop: 7.9% [n=5] vs. 13.5% [n=5]; p=0.4; progression to abnormal Valsalva ratio: 39% [n=16] vs. 37.5% [n=9]; p=0.9; progression to abnormal 30:15 ratio: 34.5% [n=20] vs. 24.2% [n=8]; p=0.3).

In order to assess the impact of OSA and the potential role of CPAP on CAN parameters, patients were divided in the following groups: no OSA, mild OSA, moderate to severe OSA not on CPAP and moderate to severe OSA compliant with CPAP (**Table 5.5**).

Table 5.5 Impact of CPAP on CAN parameters
Data presented in median (IQR). Analysis performed using Kruskal-Wallis test for non-normally distributed variables. CAN parameters presented as change from baseline.

	No OSA (n=39)	Mild OSA (n=40)	Moderate-severe OSA no CPAP (n=19)	Moderate-severe OSA on CPAP (n=14)	p value
E/I ratio (abnormal)	69.2% (27)	65% (26)	68.4% (13)	64.3% (9)	0.9
Valsalva (abnormal)	38.5% (15)	42.5% (17)	42.1% (17)	50% (7)	0.9
30:15 (abnormal)	15.4% (6)	30% (12)	15.8% (3)	0% (0)	0.07
Postural drop (abnormal)	2.6% (1)	15% (6)	5.3% (1)	21.4% (3)	0.1
E/I ratio	-0.01 (-0.05, 0.02)	0.004 (-0.02, 0.03)	-0.01 (-0.03, 0.02)	0.01 (-0.02, 0.06)	0.1
Valsalva ratio	-0.03 (-0.2, 0.1)	-0.02 (-0.1, 0.1)	-0.01 (-0.1, 0.02)	-0.005 (-0.1, 0.1)	0.9
30:15 ratio	-0.1 (-0.3, -0.03)	-0.1 (-0.3, 0.04)	-0.1 (-0.2, -0.01)	-0.1 (-0.2, -0.02)	0.7
Baseline LFa	-0.3 (-0.7, 0.3)	-0.3 (-0.7, 0.5)	-0.4 (-0.7, 0.2)	0.3 (-0.4, 4.9)	0.2
Baseline RFa	-0.3 (-0.7, 0.3)	-0.04 (-0.5, 1.3)	-0.1 (-0.5, 0.2)	0.2 (-0.5, 4.2)	0.2
Baseline LFa/RFa	-0.4 (-0.8, 0.1)	-0.5 (-0.7, -0.1)	-0.6 (-0.8, -0.4)	-0.4 (-0.7, -0.1)	0.4
Deep Breathing LFa	-0.2 (-0.6, 1.3)	0.01 (-0.5, 1.4)	0.2 (-0.5, 2)	1.1 (-0.5, 15.5)	0.3
Deep Breathing RFa	0.04 (-0.5, 0.8)	0.4 (-0.6, 2.9)	-0.4 (-0.6, 0.2)	0.5 (-0.4, 8.3)	0.2
Deep Breathing LFa/RFa	0.0 (-0.8, 2.4)	0.02 (-0.9, 0.9)	0.8 (-0.4, 5.7)	-0.3 (-0.5, 2.7)	0.2
Valsalva LFa	-0.3 (-0.8, 1.6)	-0.4 (-0.7, 0.8)	-0.7 (-0.9, 1.2)	-0.01 (-0.7, 2.6)	0.4
Valsalva RFa	0.1 (-0.8, 1.4)	-0.3 (-0.6, 0.5)	-0.6 (-0.9, 1.0)	0.1 (-0.5, 1.7)	0.5
Valsalva LFa/RFa	-0.6 (-0.9, 0.6)	-0.8 (-0.9, -0.5)	-0.8 (-0.9, 0.2)	-0.6 (-0.8, 0.4)	0.6
Standing LFa	-0.7 (-0.8, -0.2)	-0.3 (-0.8, 0.1)	-0.3 (-0.7, 0.2)	-0.4 (-0.6, 2.2)	0.04
Standing RFa	-0.5 (-0.7, -0.1)	-0.2 (-0.7, 0.7)	0.1 (-0.6, 1.4)	-0.5 (-0.6, 0.2)	0.2
Standing LFa/RFa	-0.6 (-0.9, -0.03)	-0.6 (-0.8, -0.2)	-0.5 (-0.8, -0.3)	-0.3 (-0.8, 0.5)	0.2

Patients with moderate to severe OSA, not on CPAP treatment were more likely to have abnormal E/I ratio and 30:15 ratio as compared with patients who were on CPAP treatment. Patients on CPAP treatment were more likely to have better E/I ratio and Valsalva ratio when described as change from baseline. These results did not achieve statistical significance.

In order to explore the impact of OSA status and CPAP treatment on the progression frequency domain parameters, linear regression models were used where the outcome is the frequency domain measure of interest. Models were adjusted for the baseline value of the outcome measure, ethnicity, gender, age at diagnosis, BMI, baseline eGFR, diabetes duration, HbA1c, systolic blood pressure, triglyceride levels, use of insulin and anti-hyperglycaemic agents. CPAP compliance was associated with higher study-end log E/I ratio (B=0.06; p=0.002), study-end log baseline LFA (B=0.7; p=0.001), study-end log baseline RFA (B=0.6; p=0.006), study-end log deep breathing LFA (B=0.5; p=0.03), study-end log deep breathing RFA (B=0.4; p=0.03) and study-end log standing LFA (B=0.6; p=0.01). These results suggest that patients with OSA who were compliant with CPAP had better autonomic function than patients with moderate to severe OSA who were not compliant with CPAP or those with mild OSA who were not CPAP treated. There was no impact of CPAP on progression of other CAN parameters.

5.5 Discussion

5.5.1. DPN

We have shown previously and in this analysis that OSA was associated with DPN and foot insensitivity in patients with T2DM [24]. However, longitudinal analysis failed to show an impact of OSA on the development of DPN or foot insensitivity in patients with T2DM.

There are multiple potential reasons for the observed lack of impact. It is possible that OSA has no impact on the development of DPN and foot insensitivity. A potential explanation is that the recurrent ischaemic and hypoxic injuries to the peripheral nerve cells, as seen in the case of T2DM and OSA, can lead to the development of adaptation and resistance to ischaemic conduction failure (RICF) in which there is persistence of abnormal action potentials despite the anaerobic metabolism and endoneural hypoxia [33]. Another possible explanation could be the increased protein and gene expressions of hypoxia inducible factor 1-α (HIF-1α) which is typically seen in ischaemic and hypoxic injury of axonal and endoneural cells . HIF-1α promotes the production of VEGF which may promote nerve regeneration and tissue survival. However, I think that lack of impact of OSA on DPN is unlikely to be due to true lack of effect in my study as my results suggest a trend of association with development of foot insensitivity and diabetic foot ulceration and hence this could be due to the small sample size of patients in which I was able to assess progression.

The lack of impact of OSA on progression to DPN could also be due to the confounding effect of CPAP treatment. If OSA was to cause DPN/foot insensitivity then CPAP would be expected to have a favorable impact on the progression to DPN and foot insensitivity. My analysis showed that the progression to foot insensitivity or foot ulceration was lower in patients with moderate to severe OSA who were compliant with CPAP compared to patients

with mild OSA or those non-compliant with CPAP. This difference was not statistically significant due to the small sample size but it suggests that there might be a link between OSA and foot insensitivity and foot ulceration that might be modulated by CPAP treatment and need to be explored in further studies. Another important factor to consider when interpreting the impact of OSA on DPN progression is the relatively large loss of follow up which left a small sample size to analyze progression to DPN after excluding patients with DPN at baseline.

Another potential factor to consider is that OSA may not cause DPN but might accelerate the progression after DPN developed i.e. the development of DPA is the function of hyperglycaemia but OSA might affect the progression. The data presented in the DR chapter support such notion but we could not assess this in our study as the MNSI is not a sensitive measure for the assessment for changes in neuropathy severity. To assess this we need better measures such as nerve conductions velocities or small fiber function testing or quantitative sensory testing in order to test this hypothesis.

5.5.2 CAN

The results shown suggest that OSA is associated with worse CAN parameters at baseline and the OSA might affect the progression of HRV standardized ratios and frequency domain parameters in patients with T2DM. In this study, I found that patients who were complaint with CPAP treatment had better study-end E/1 ratio, and baseline and deep breathing LFA and RFA after adjustment. However, I found no impact of OSA on the development of CAN over the study period. Again, similar to DPN this could be because of the sample size available but also could be a true lack of effect. Another explanation is that the effect of OSA

on CAN in patients with T2DM might be related to progression rather than the development of CAN, which might explain our findings showing no impact of OSA on CAN progression but improvements in CAN parameters in patients compliant with CPAP.

In conclusion, this study did not show an impact of OSA on the development of DPN or CAN in patients with T2DM. However, CPAP compliance was associated with better HRV parameters at study-end, and there was a trend of favourable effect of CPAP on the progression to foot insensitivity or the development of diabetic foot ulceration. Adequately powered cohort studies and interventional RCTs are needed to assess the impact of OSA and its treatment on DPN and CAN in patients with T2DM.

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Chapter Six: The Relationship between Adiposity and Sleep quality and duration in patients with Young-onset Type 2 Diabetes: A cross-sectional exploratory study

6.1 Introduction

Sleep patterns are influenced by behavioural, cultural, social and environmental factors [1].While obesity and T2DM prevalence was increasing over the last few decades, sleep duration for the population was getting shorter. The sleep duration was lessened by 1.5 hour over the past century [2,3]. Presently, one third of adult population sleep less than 7 hours per night [2,4]. This is mainly due to longer working hours, shift patterns and increased use of information technology and social media [5]. Modern life style with 24 hour access to light, coupled with shift working, often leads to a mismatch between endogenous circadian rhythm (controlled by the master clock located in the suprachiasmatic nuclei in the hypothalamus) and metabolic response (regular sleep/wake cycle, feeding behaviour, hormones secretion and metabolism) [6]. Sleep restriction, both acute and chronic, has deleterious effect on metabolism and endocrine function, leading to increased insulin resistance and activation of sympathetic nervous system, similar to age related chronic disorders [10]. Partial and total acute sleep deprivation delay the recovery of hypothalamic-pituitary axis (HPA) from early morning circadian cortisol secretion by at least an hour, leading to dysregulation of negative glucocorticoid feedback. Resultant cortisol excess could accelerate the metabolic consequences including impaired glucose tolerance [11]. Similar results can be found in studies which looked at chronic or extended sleep restriction. In an experimental study on healthy individuals, extended partial sleep restriction was associated with impaired glucose tolerance ($p=<0.02$), elevated cortisol levels ($p=0.0001$) activation of sympathetic nervous system ($p=<0.02$) [10]. Chronic sleep deprivation cause over production of inflammatory cytokines e.g. IL-1, IL-6, IL7, CRP and TNF-α, which could explain the increase in cardiovascular and chronic inflammatory diseases [12,13]. Moreover, day

time secretion of IL-6 and TNF-α (as opposed to night-time secretion) is thought to be responsible for increased fatigue and performance decrements [14]. In addition, sleep restriction results in lower adiponectin levels and increased ghrelin [15]. All of this suggest that sleep restriction would be expected to be associated obesity and T2DM.

In a pooled analysis of 10 prospective studies looking at the association between sleep quality and quantity and incidence of T2DM, sleeping ≤5-6 hours/night and >8- 9 hours/night were associated with increased incidence of T2DM ([RR=1.28; 95%CI 1.03, 1.60, p=0.024], and [RR=1.38; 95%CI 1.15, 1.65; p=0.0006] respectively) [16]. Patients who struggled to maintain sleep (self-reported sleep quality) also had increased risk of T2DM (RR=1.67; 95%CI 1.30, 2.14; p=<0.0001) [16]. The relative risk increment of developing T2DM per year of follow up was 2% for people with short sleep duration (RR=1.02; [95%CI 0.93, 1.12), 7% for people with long sleep duration (RR=1.07; 95%CI 0.99, 1.16) and 12% for people struggling to maintain sleep (RR=1.12; 95%CI 1.00, 1.24, p=0.04) [16]. In Massachusetts Male Aging Study, a significant U-shaped relationship was also found between short (less than 5 hours per night) or long (more than 8 hour per night) sleep and incidence of T2DM. In regression models examining the incidence of T2DM in relation of sleep duration, RR were as follow: ≤5 hours per night: 2.59 (95%CI 1.28, 5.23); 6 hours per night: 1.91 (95%CI 1.05, 3.48); 8 hours per night: 1.40 (95%CI 0.78, 2.51); and ≥8 hours per night: 3.69 (95%CI 1.83, 7.46) when 7 hours/night was used as reference point. These results remained significant despite adjustments for baseline age decade, self-rated health status, smoking, hypertension, and waist circumference multiple confounders [17]. In a Swedish study on middle aged population, men who reported new onset of diabetes at the end of 12 year follow up typically slept less than 5 hours per night (16% vs. 5.9%, p=<0.01) and had difficulties in initiating (16% vs. 3.1, p=<0.001) and maintaining sleep (28% vs. 6.3%,

p=<0.001). The relative risk (RR; 95%CI) for developing diabetes was higher in patients with short sleep duration (2.8; 1.1, 7.3) and patients with poor sleep quality (4.8; 1.9, 12.5) after adjusting for relevant confounders [18]. In a Japanese prospective occupational-based study, people with poor sleep quality were more at risk of developing T2DM at a 4 year follow up (OR=3.71; 95%CI 1.37, 10.07) [19]. Similar results have been found by other studies in different populations [20-23].

Adults with short sleep duration (5-6 hour sleep) were found to have 15.5% lower leptin levels (p=0.01) and 14.5% higher ghrelin levels (p=0.008) when compared with adults who sleep an average of 8 hours [24,25].

I have discussed in Chapter 1 (pages 55-59) the metabolic consequences of sleep duration, and quality and circadian misalignment and the epidemiological and experimental evidence linking sleep duration and quality to obesity and T2DM. Briefly, sleep restriction results in increased insulin resistance and impaired glucose tolerance in laboratory studies as well as increased appetite and increased preference to calorie dense food; which predisposes the patients to obesity and T2DM which has been shown in several epidemiological studies and meta-analyses. In addition, poor sleep quality has been shown to be associated with T2DM and obesity as discussed in chapter 1.

The relationship between sleep duration and obesity is stronger for younger patients than older patients. Moreover, despite the above described epidemiological links between sleep duration and quality and obesity and T2DM, there is no data in the literature regarding these associations in patients who already have T2DM. Identifying these relationships (if any) is important as it might offer new therapeutic targets to improve glycaemia and reduce weight in patients with T2DM.

6.2. Hypothesis

Poor sleep quality and long and short sleep duration are associated with increased adiposity measures and worsened metabolic parameters in patients with T2DM.

6.2.1 Primary Aim

To assess the relationship between sleep duration and quality and adiposity measures in patients with T2DM.

6.2.2. Secondary Aim

To assess the relationship between sleep duration and quality and metabolic parameters (HbA1c, total cholesterol, triglycerides, systolic blood pressure and diastolic blood pressure) in patients with T2DM.

To assess the interaction between sleep quality and sleep duration on adiposity measures in patients with T2DM

To assess the interaction between sleep quality and sleep duration on metabolic parameters (HbA1c, total cholesterol, triglycerides, systolic blood pressure and diastolic blood pressure) in patients with T2DM.

6.3 Methods

We have utilised the baseline data for an ongoing observational cohort study of patients with young onset T2DM (CI Dr Sri Bellary, Aston University). For details, please refer to

chapter 2. The project was approved by NRES Committee West Midlands - Staffordshire (REC – 12/WM/0166) and funded by Heart of England NHS foundation Trust as part of a cohort study which aimed to extensively characterise young patients with T2DM.

6.3.1 Inclusion Criteria

1. Patients with the diagnosis of T2DM
2. Patients who were diagnosed to have T2DM before the age of 40 years
3. Patients who were diagnosed with T2DM after December 2000
4. Patients who are able to consent

6.3.2 Exclusion Criteria

1. Patients with diagnoses other than T2DM
2. Pregnancy

6.3.3 Data collected

For details on the methods used to collect data, please refer to chapter 2.

General and clinical assessment

Age, gender, ethnicity, diabetes duration, medications.

Anthropometry

Height, weight, Body mass index (BMI), waist circumference (WC), total body fat %.

Metabolic assessment

Blood pressure, HbA1c, lipid profile, urea and electrolytes.

Sleep assessment

Sleep quality and duration were assessed by using Pittsburgh Sleep Quality Index (PSQI). For details please refer to Chapter 2. PSQI >5 was consistent with poor sleep quality. Sleep duration was based on the PSQI in which the patients self-report their average sleep duration/night over the last month

6.3.4 Statistical Analysis

Data analysis was performed using SPSS 22 software (SPSS Inc., Chicago, USA). Depending on distribution, data are presented as mean (SD) or median (IQR). Histograms and the Shapiro-Wilk test were used for normality testing. Independent t-test and the Mann-Whitney test were used to compare independent continuous variables. Chi-square test was used to compare categorical variables. In case of multiple comparisons, Bonferroni correction was used as post-hoc test.

Analysis of variance (ANOVA) with post-hoc analysis was used to assess the differences between independent groups. Pearson or Spearman tests were performed to assess the correlations between continuous variables. Logistic regression (forced entry method) was used to assess the independent association with dichotomous outcome variables. Linear regression (forced entry method) was used to assess the independent association of continuous outcome variables variable. Variables included in the regression models were either known to be outcome-related risk factors or variables that were found to be different

between patients with or without poor sleep quality and patients with different sleep duration. If the outcome measure of the linear regression was not normally distributed, then it was log transformed to improve data distribution. To assess the relationship between sleep duration and different outcomes, sleep duration quartiles were created using dummy variables. Each quartile represented the specific sleep duration vs rest of the sleep duration. In regression models, quartile 3 was used as reference point. To assess the interaction between sleep quality and duration, data was divided into 3 groups, with each group looking at sleep quality and sleep duration of varying length. In regression models, group 1 (normal sleep quality and any sleep duration) was used as reference point. For statistical purposes, when normality was assumed, non-normally distributed data was normalized by log transformation. A p value < 0.05 was considered significant.

All assumption of statistical tests used were adhered to. In regression models, multicollinearity was assessed using simple correlations between variables, the tolerance and VIF values, and the condition indices. No tolerance values were < 0.1 and no variables had strong correlations ($r > 0.8$). Condition indices > 30 were used to represent collinearity. Variances proportions > 0.5 were used to indicate the variables involved. There was no collinearity observed in variables used in the models. Residuals were also examined in multiple linear regression models.

6.4 Results

One hundred patients were recruited to the Young onset T2DM study, of which 96 patients had PSQI data. The 4 patients excluded for not completing the PSQI form appropriately. Out

of 96 patients 38.8% (n=40) were men and 53.4% (n=55) were South Asians. The clinical and biochemical characteristics of the study population are summarised in **Table 6.1**.

Table 6.1 Baseline characteristics of the study population

Data presented as % (n), median (IQR) or mean (SD)

Baseline characteristics	**n=96**
Age	40.2 (7.2)
Gender (male %)	38.8% (40)
Ethnicity (SA %)	53.4% (55)
Diabetes duration (years)	8 (5-11)
Alcohol (ex/current)	22% (22)
Smoking (ex/current)	40% (40)
Insulin (%)	61.2% (63)
GLP-1 agonist (%)	9% (9)
Lipid lowering treatment (%)	64.1% (66)
Anti-hypertensives (%)	54.4% (56)
CKD (%)	20.4% (21)
Blood pressure (systolic mmHg)	121.4 (16)
Blood pressure (diastolic mmHg)	70.7 (10.6)
Total cholesterol (mmol/L)	4.4 (1.1)
Triglycerides (mmol/L)	1.6 (1.2-2.7)
HbA1c (mmol/mol)	72 (56-92)
BMI (kg/m^2)	32.9 (28.1-38.5)
Waist Circumference	111 (19.3)
Total body fat%	37.6 (11.5)

6.4.1 Sleep Quality

Poor sleep quality (PSQI >5) was very common with a prevalence of 64.6% (n=62). The clinical characteristics of the study population according to sleep quality status are as described in **Table 6.2**. There were more women in the poor sleep quality group (vs. normal sleep quality). There was no difference in insulin, anti-hypertensive and lipid lowering

treatment use in patients with and without poor quality sleep. Patients with poor quality sleep were more likely to be male, and non-SA.

There were significant differences in adiposity measures between patients with poor vs. normal sleep quality (**Table 6.2**). Patients with poor sleep quality were more obese and had higher BMI, waist circumference and total body fat% than patients with normal sleep quality. This suggests that patients with poor sleep quality had increased total and visceral adiposity compared to patients with normal sleep quality. This might explain the non-significant trend of higher HbA1c in the poor sleep quality group.

In order to assess whether the observed relationship between poor sleep quality and obesity are independent of potential confounders we used multiple linear regression where the outcome variables were the adiposity measure (BMI, waist circumference, total body fat%) and the independent variables were age, alcohol consumption, smoking, diabetes duration, ethnicity, gender, use of GLP-1 analogue, insulin use, and poor sleep quality. Having a PSQI >5 (i.e. poor sleep quality) was significantly associated with higher total body fat% (B=5.54; p=0.02) but not with BMI. There was a trend towards an association between poor sleep quality and increased WC (B=8.16; p=0.06). Summary of the regression findings can be found in **table 6.3**. The only other significant association with adiposity measures was the use of GLP-1 receptor analogues. The results suggest that the use of GLP-1 RA was associated with higher BMI (p=0.01), WC (p=0.007) and total body fat% (p=0.01), which might reflect prescribing practices using GLP-1 RA in heavier patients.

There was no significant differences in HbA1c, lipids profile, and BP between patients with and without poor sleep quality. After adjustment for potential confounders, there remained no relationship between poor sleep quality and BP and triglycerides but there was a non-

significant trend of association between poor sleep quality and higher HbA1c and total cholesterol level. Summary of these models can be found in **Table 6.4.**

Table 6.2 Characteristics of patients in relation to sleep quality
Data presented as % (n), median (IQR) or mean (SD). Analysis performed using the Chi-square test for categorical variables, the independent t test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables.

	Normal sleep quality (n=34)	**Poor sleep quality (n=62)**	**p value**
Age	40.9 (7.6)	39.6 (7.1)	0.4
Gender (male %)	52.9% (18)	32.3% (20)	0.05
Ethnicity (SA %)	70.6% (24)	48.4% (30)	0.09
Diabetes duration (years)	9 (6.0-12)	7 (4.8-10)	0.13
Alcohol (ex/current)	15.6% (5)	25.8% (16)	0.26
Smoking (ex/current)	26.5% (9)	45.9% (28)	0.06
Insulin (%)	61.8% (21)	61.3% (38)	0.96
GLP-1 agonist (%)	5.9% (2)	11.3% (7)	0.39
Lipid lowering treatment (%)	61.8% (21)	67.7% (42)	0.55
Anti-hypertensives (%)	52.9% (18)	58.1% (36)	0.63
CKD (%)	23.5% (8)	19.4% (12)	0.63
Blood pressure (systolic mmHg)	121.5 (18.5)	120.8 (14.7)	0.86
Blood pressure (diastolic mmHg)	69 (12)	71 (14.25)	0.98
Total cholesterol (mmol/L)	4.5 (1.11)	4.3 (1.03)	0.59
Triglycerides (mmol/L)	1.59 (1.2-2.7)	1.65 (1.2-2.7)	0.73
HbA1c (mmol/mol)	70 (54.5-88)	75 (57.5-93)	0.12
BMI (kg/m^2)	30.3 (25.1-35.4)	33.33 (29.3-39.7)	0.02
Waist Circumference	104.5 (18.2)	113.9 (19.42)	0.03
Total body fat%	31.9 (11.1)	40.7 (10.73)	<0.0001

Table 6.3 Assessing the impact of poor sleep quality as categorical variable on adiposity measures including BMI, total body fat% and WC.

Model adjusted for age, alcohol consumption, smoking, diabetes duration, ethnicity, gender, use of GLP-1 and insulin use

BMI – Body Mass Index; WC – Waist Circumference

There was no impact when BMI was replace by WC in the models

Outcome measure	R for the model	R square for the model	Beta	p value
Total body fat%	0.60	0.36	5.54	0.02
WC	0.47	0.22	8.16	0.06
Log BMI	0.48	0.23	0.04	0.14

Table 6.4 Assessing the impact of poor sleep quality as categorical variable on metabolic parameters including HbA1c, total cholesterol, triglycerides, systolic and diastolic BP

HbA1c - adjusted for age, diabetes duration, ethnicity, gender, BMI, use of GLP-1 and insulin use

Total Cholesterol - adjusted for age, diabetes duration, ethnicity, gender, alcohol, lipid lowering treatment, BMI

Triglycerides – adjusted for age, diabetes duration, ethnicity, gender, alcohol, lipid lowering treatment, BMI

Systolic Blood Pressure – adjusted for age, diabetes duration, ethnicity, gender, smoking, anti-hypertensive treatment

Diastolic Blood Pressure – adjusted for age, diabetes duration, ethnicity, gender, smoking, anti-hypertensive treatment

There was no impact when BMI was replace by WC in the models

Outcome measure	R for the model	R square for the model	Beta	p value
HbA1c	0.38	0.14	7.93	0.09
Total Cholesterol	0.43	0.18	-0.20	0.07
Triglycerides	0.45	0.20	0.05	0.62
Systolic Blood Pressure	0.38	0.14	-0.08	0.45
Diastolic Blood Pressure	0.42	0.18	-0.003	0.98

6.4.2 Sleep Duration

Sleep duration was divided in quartiles (≤330 minutes [n=27], 331-390 minutes [n=24], 391-450 minutes [n=23], and ≥451 minutes [n=23]). The clinical characteristics of the study population according to sleep duration are as described in **table 6.5.** Patients with shorter sleep duration (in the first quartile, ≤330 minutes) were more likely to be on lipid lowering treatment and have higher total body fat%.

Considering quartile3 of the sleep duration as the reference point, and following adjustments for age, alcohol consumption, smoking, diabetes duration, ethnicity, gender, use of GLP-1 and insulin use (R^2 =0.37), shorter sleep duration (quartile 1, ≤330 minutes) was significantly associated with higher total body fat% (B=5.92; p=0.04) but not with WC (B=2.01; p=0.71) or BMI (B=0.03; p=0.37) as shown in **table 6.6**.

As with sleep quality, and after adjustment for potential confounders, there were no relationships between sleep duration quartiles and HbA1c (mmol/mol), total cholesterol (mmol), triglycerides (mmol), systolic and diastolic blood pressure (mmHg) (**Table 6.6)**.

Table 6.5 Characteristics of patients in relation to sleep duration
Data presented as % (n), median (IQR) or mean (SD). Analysis performed using the Chi-square test for categorical variables, ANOVA (analysis of variance) for continuous normally distributed variables and Kruskal-Wallis for continuous non-normally distributed variables

	Sleep duration - ≤330 minutes	Sleep duration – 331-390 minutes	Sleep duration – 391-450 minutes	Sleep duration – ≥451 minutes	p value
Age (years)	39.8 (7.3)	42.5 (7.8)	39.1 (7.6)	39.0 (6.0)	0.30
Diabetes duration (yrs)	6 (4-11)	8 (5-10)	7 (3.5-11.5)	10 (5-12)	0.7
Ethnicity (SA)%	48.1% (13)	45.8% (11)	52.2% (12)	78.3% (18)	0.09
Gender (male)	25.9% (7)	50.0% (12)	34.8% (8)	47.8% (11)	0.25
Alcohol (ex/current)%	19.2% (5)	34.8% (8)	21.7% (5)	13% (3)	0.34
Smoking (ex/current)%	38.5% (10)	54.2% (13)	34.8% (8)	30.4% (7)	0.36
Oral anti-hyperglycaemic agents (%)	96.3% (26)	95.8% (23)	91.3% (21)	100% (23)	0.53
GLP-1 (%)	14.8% (4)	8.3% (2)	8.7% (2)	4.3% (1)	0.64
Insulin (%)	63% (17)	54.2% (13)	73.9% (17)	56.5% (13)	0.51
Lipid lowering treatment (%)	55.6% (15)	91.7% (22)	60.9% (14)	56.5% (13)	0.02
Anti-hypertensive agents (%)	55.6% (15)	75% (18)	56.5% (13)	39.1% (9)	0.10
CKD (%)	18.5% (5)	20.8% (5)	30.4% (7)	17.4% (4)	0.69
HbA1c (mmol/mol)	75 (60-92)	74 (57.5-91.5)	75.5 (61-93)	67 (51-90)	0.4
Cholesterol (mmol)	4.4 (0.95)	4.3 (1.2)	4.5 (1.0)	4.3 (1.2)	0.92
Triglycerides (mmol)	1.8 (1.2-2.7)	1.7 (1.1-2.5)	1.6 (1.3-2.9)	1.6 (1.1-2.9)	0.9
Blood pressure (systolic mmHg)	121.3 (12.7)	122.6 (17.1)	123.5 (18.9)	117.5 (15.8)	0.63
Blood pressure (diastolic mmHg)	70.2 (10.8)	70.5 (11.8)	72.3 (11.1)	69.5 (9.5)	0.85
BMI (kg/m^2)	34.6 (29.6-43.7)	31.7 (27.9-37.4)	32.6 (27-38.6)	32.7 (25.4-38)	0.4
Total body fat%	44.1 (11.7)	34.2 (10.7)	36.3 (11.2)	34.9 (10.6)	0.01
Waist circumference	113.6 (19.8)	107.5 (14.9)	109.9 (22.2)	111.5 (21.4)	0.75

Table 6.6 Assessing the impact of sleep duration on adiposity measures (BMI, Total body fat% and WC) using sleep quartiles

Model adjusted for age, alcohol consumption, smoking, diabetes duration, ethnicity, gender, use of GLP-1 and insulin use

BMI – Body Mass Index; WC – Waist Circumference

There was no impact when BMI was replace by WC in the models

Sleep duration 391-449 minutes was used as reference point

Outcome measure	Sleep duration (minutes)	R for the model	R square for the model	Beta	p value
Total body fat%	≤330	0.61	0.37	5.92	0.04
	331-390			-0.79	0.79
	≥450			1.42	0.64
Log BMI	≤330	0.47	0.22	0.03	0.37
	331-390			0.01	0.83
	≥450			0.02	0.53
WC	≤330	0.45	0.21	2.01	0.71
	331-390			-2.96	0.61
	≥450			4.05	0.49

Table 6.7 Assessing the impact of sleep duration as quartiles on metabolic parameters.
HbA1c - adjusted for age, diabetes duration, ethnicity, gender, use of GLP-1 and insulin use

Total Cholesterol - adjusted for age, diabetes duration, ethnicity, gender, alcohol, lipid lowering treatment, BMI

Triglycerides – adjusted for age, diabetes duration, ethnicity, gender, alcohol, lipid lowering treatment, BMI

Systolic Blood Pressure – adjusted for age, diabetes duration, ethnicity, gender, smoking, anti-hypertensive treatment

Diastolic Blood Pressure – adjusted for age, diabetes duration, ethnicity, gender, smoking, anti-hypertensive treatment

There was no impact when BMI was replace by WC in the models

Sleep duration 391-449 minutes was used as reference point

Outcome measure	Sleep duration (minutes)	R for the model	R square for the model	Beta	p value
HbA1c	≤330	0.35	0.12	1.88	0.75
	331-390			1.78	0.77
	>450			-8.06	0.21
Total cholesterol	≤330	0.39	0.15	-0.04	0.90
	331-390			-0.02	0.95
	>450			-0.01	0.97
Triglycerides	≤330	0.45	0.21	-0.18	0.52
	331-390			-0.26	0.39
	>450			-0.18	0.54
Systolic Blood Pressure	≤330	0.38	0.14	-2.22	0.63
	331-390			-3.58	0.47
	>450			-3.08	0.54
Diastolic Blood Pressure	≤330	0.44	0.20	-1.96	0.51
	331-390			-3.91	0.21
	>450			-1.74	0.59

6.4.3 Interaction between sleep quality and sleep duration

To assess the interaction between sleep quality and duration on adiposity measures and metabolic parameters, data was divided into 3 groups. Group 1 (n=34) included patients with normal sleep quality irrespective of sleep duration. Group 2 (n=37) included patients with poor sleep quality and normal or long sleep duration (these were combined due to small numbers). Group 3 (n=25) included patients with poor sleep quality and short sleep duration (<= 330 minutes per night). The demographics and clinical characteristics of the patients according to the groups are described in **Table 6.8**. Patients in group 3 (poor sleep quality and short sleep) were more likely to be women and have higher total body fat%.

In order to explore this relationship further and using group 1 as reference point in linear regression, patients in group 3 were more likely to have higher total body fat% (B=-8.15, p=0.004), despite adjustments for age, alcohol consumption, smoking, diabetes duration, ethnicity, gender, use of GLP-1 and insulin use (Table 6.9). There was no associations with WC (B=-7.58; p=0.15) and BMI (B=-0.05; p=0.08).

As with sleep quality and duration and after adjustments as described above, no relationship was found between group 2 and 3 and metabolic parameters including HbA1c, total cholesterol, triglycerides, systolic blood pressure and diastolic blood pressure.

Table 6.8 Characteristics of patients in relation to sleep quality and duration

Data presented as % (n), median (IQR) or mean (SD). Analysis performed using the Chi-square test for categorical variables, ANOVA (analysis of variance) for continuous normally distributed variables and Kruskal-Wallis for non-normally distributed variables

Group 1 – Normal sleep quality and any sleep duration

Group 2 – Poor sleep quality and normal sleep duration (quartile 2, 3 and 4)

Group 3 – Poor sleep quality and shorter sleep duration (quartile 1)

	Group 1 (n=34)	**Group 2 (n=37)**	**Group 3 (n=25)**	**p value**
Age (years)	40.9 (7.6)	39.1 (7.0)	40.3 (7.2)	0.58
Diabetes Duration (years)	9 (6-12)	8 (5-10)	6 (3.5-11.5)	0.31
Ethnicity (SA)%	70.6% (24)	45.9% (17)	52% (13)	0.09
Gender (male)	52.9% (18)	37.8% (14)	24% (6)	0.07
Alcohol (ex/current)%	15.6% (5)	29.7% (11)	20.0% (5)	0.35
Smoking (ex/current)%	26.5% (9)	48.6% (18)	41.7% (10)	0.15
Oral anti hyperglycaemic agents (%)	100% (34)	91.9% (34)	96% (24)	0.23
GLP-1 (%)	5.9% (2)	8.1% (3)	16% (4)	0.39
Insulin (%)	61.8% (21)	62.2% (23)	60% (15)	0.98
Lipid lowering treatment (%)	61.8% (21)	75.7% (28)	56% (14)	0.23
Anti-hypertensive agents (%)	52.9% (18)	59.5% (22)	56% (14)	0.86
CKD (%)	23.5% (8)	21.6% (8)	16% (4)	0.77
Blood pressure (systolic mmHg)	121.5 (18.5)	120.8 (15.9)	121 (13.2)	0.98
Blood pressure (diastolic mmHg)	70.9 (9.6)	70.4 (11.7)	70.6 (10.7)	0.98
HbA1c (mmol/mol)	70 (54.5-88)	78 (57-93)	72 (57-92)	0.27
Total cholesterol (mmol)	4.5 (1.1)	4.4 (1.2)	4.3 (0.8)	0.84
Triglycerides (mmol)	1.6 (1.2-2.7)	1.6 (1.2-2.9)	1.8 (1.3-2.5)	0.81
BMI (kg/m^2)	30.3 (25.1-35.4)	33 (29.3-38.9)	34.6 (29.2-44)	0.04
Total body fat%	31.9 (11.2)	38.5 (9.2)	44.4 (12.2)	<0.001
Waist circumference	104.5 (18.2)	113.8 (19.1)	114.2 (20.4)	0.09

Table 6.9 Assessing the impact of sleep quality and duration on adiposity measures (BMI, Total fat% and WC)

Model adjusted for age, alcohol consumption, smoking, diabetes duration, ethnicity, gender, use of GLP-1 and insulin

BMI – Body Mass Index; WC – Waist Circumference

Group 1 – Normal sleep quality and any sleep duration (quartile 1, 2, 3 and 4). Used as reference point.

Group 2 – Poor sleep quality and normal sleep duration (quartile 2, 3 and 4)

Group 3 – Poor sleep quality and shorter sleep duration (quartile 1)

There was no impact when BMI was replace by WC in the models

Outcome measure		R for the model	R square for the model	Beta	p value
Total body fat%	Group 2	0.62	0.39	4.27	0.09
	Group 3			8.15	0.004
Log BMI	Group 2	0.48	0.23	0.03	0.2
	Group 3			0.05	0.2
WC	Group 2	0.47	0.22	8.55	0.07
	Group 3			7.58	0.15

Table 6.10 Assessing the impact of sleep quality and duration on metabolic parameters.

HbA1c - adjusted for age, diabetes duration, ethnicity, gender, use of GLP-1 and insulin use

Total Cholesterol - adjusted for age, diabetes duration, ethnicity, gender, alcohol, lipid lowering treatment, BMI

Triglycerides – adjusted for age, diabetes duration, ethnicity, gender, alcohol, lipid lowering treatment, BMI

Systolic Blood Pressure – adjusted for age, diabetes duration, ethnicity, gender, smoking, anti-hypertensive treatment

Diastolic Blood Pressure – adjusted for age, diabetes duration, ethnicity, gender, smoking, anti-hypertensive treatment

Group 1 – Normal sleep quality and any sleep duration (quartile 1, 2, 3 and 4). Used as reference point.

Group 2 – Poor sleep quality and normal sleep duration (quartile 2, 3 and 4)

Group 3 – Poor sleep quality and shorter sleep duration (quartile 1)

Outcome measure		R for the model	R square for the model	Beta	p value
HbA1c	Group 2	0.38	0.14	-9.72	0.06
	Group 3			-8.84	0.13
Total cholesterol	Group 2	0.43	0.18	0.42	0.12
	Group 3			0.45	0.12
Triglycerides	Group 2	0.43	0.20	-0.15	0.54
	Group 3			-0.06	0.84
Systolic Blood Pressure	Group 2	0.38	0.14	3.31	0.43
	Group 3			2.12	0.64
Diastolic Blood Pressure	Group 2	0.43	0.18	0.42	0.88
	Group 3			-0.50	0.87

6.5 Discussion

This is the first report of examination of the relationships between sleep quality and duration on one hand and obesity and metabolic parameters on the other hand in patients with young onset T2DM. My study showed that poor sleep quality and shorter sleep duration (≤330 minutes) were associated with increased adiposity in patients with young onset T2DM. In addition, poor sleep quality and shorter sleep duration were more likely to be associated with adiposity compared with patients with poor sleep quality with any sleep duration. This suggests that the impact of poor sleep quality on adiposity markers is far greater than any sleep duration.

Our findings are consistent with other studies that examined the relationship between sleep-related disorders and obesity in the general population that showed that short sleep duration is associated with obesity and incipient diabetes. It is plausible that the short sleep duration and poor sleep quality in our study might have been responsible for the younger onset of diabetes in a sub-set our study population; however, this cannot be proved due to the cross-sectional design of the study.

The observed relationship between obesity and sleep duration and quality in this cohort need to be examined in longitudinal studies to assess whether poor sleep quality and/or short sleep duration can actually contribute to the weight gain in some patients with T2DM. If this is proven, then interventional studies are needed to assess whether improving sleep

quality and manipulating sleep duration might potentially be used as treatment strategies to reduce weight in patients with T2DM.

The relation between sleep duration, sleep quality, adiposity and T2DM is likely to be multi-factorial. Short sleep duration results in increased appetite and reduction in energy expenditure which will cause an increase in weight and obesity. Previous studies suggested that total sleep deprivation results in increased food intake and increased ghrelin/leptin ratio secondary to increased ghrelin and reduction in leptin levels [15,26,27]. Furthermore, individuals who sleep for short duration might have more time available for calorie consumption. This could be potentiated further by orexins which were found to be elevated following sleep restriction in animal studies [28]. In addition, short sleep duration is also associated with decreased 24 h mean thyrotropin concentration [10], changed 24-h plasma cortisol profile including raised concentrations in the afternoon and early evening [10,29], inhibition of the HPA axis and increased GH levels (particularly in the first 4 hours of night time sleep) and increased catecholamine levels [29-31]. On the other hand, poor sleep quality appears to be associated with activation of the HPA axis and suppression of the GH axis and lower IGF-1 levels [30,32-34] . A meta-analysis has suggested a relationship between sleep duration (at least short sleep) and obesity in adults. Pooled OR for short sleep and obesity in adults was 1.55 (95% CI 1.43, 1.68; p=< 0.0001) [37]. However, the direction of this relation cannot firmly be determined, although the limited prospective studies suggest that short sleep duration might cause increased obesity. It should be noted, that no observational studies have reported that change in sleep duration from a baseline was associated with weight loss [38].

Patients with self-reported poor sleep quality are more prone to report increased hunger, and emotional eating [35] which could lead to increased adiposity and increased incidence of T2DM. In Helsinki Health Study, after 5-7 years of follow up, patients with poor sleep quality were more likely to gain 5 or more kg than patients without sleep problems [36].

Sleep deprivation or shorter sleep duration and poor sleep quality is associated with increased insulin resistance in patients without T2DM [27, 39]. In an experimental study on healthy volunteers, poor sleep quality resulted in marked decrease in insulin sensitivity without adequate compensatory increase in insulin release [40]. The magnitude of reduction in insulin sensitivity was comparable to a gain of 8-13 kg in weight [40]. In another study even a short period of sleep deprivation (two nights) resulted in significant changes in glucose metabolism [41,42]. After 2 days of short bedtimes (4 hours), glucose levels were higher and insulin levels were lower than after 2 days of long bedtimes (10 hours) [41,42].

In patients with existing T2DM, this could potentially, further lead to worsening of metabolic control and promote adiposity.

Shorter sleep duration and poor sleep quality have also been postulated to increase sympathetic activity in laboratory and population studies which lead to increased sympatho-vagal balance resulting in cardiac autonomic neuropathy which has been discussed in detail in previous chapter of this thesis [10,43].

After adjustment for confounders, there was no significant relationship between short sleep duration and poor sleep quality and BMI and WC. This might be due to our small sample size as the p value suggested a trend of association between poor sleep quality and waist circumference. In addition, while total body fat% reflects total adiposity, BMI might be affected by muscle mass, and patients with shorter sleep duration have lower muscle mass

[47] which will “artificially” lower the BMI which might explain why we found a relationship between sleep duration and total body fat% but not with BMI.

Our study has several limitations. The small sample size and the cross sectional nature of the study does not allow us to confirm causation. However, the current cohort is being followed up with the same assessments being undertaken after a period of 2 years. The main strength of our study was that it is the first study that we are aware of that looked at sleep quality and sleep duration in adults with T2DM. Patients were well characterised and represented the usual cohort of patients seen in UK hospitals therefore data can be extrapolated to the general population.

In conclusion, poor sleep quality and shorter sleep duration is associated with increased adiposity measures in patients with T2DM. Whether it impacts on development of micro and macrovascular complications remains to be seen. Further interventional studies to look at the impact of improving sleep health in patients with T2DM in the long term are needed.

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Chapter Seven: Future Directions

7.1 Summary of findings

T2DM, adiposity and sleep related disorders are closely linked together. In this study, I have shown that OSA was associated with greater eGFR decline and was an independent predictor of study-end eGFR. OSA was associated with progression to advanced DR. There was a trend of association between OSA and the development of foot insensitivity and diabetic foot ulceration. The presence of both OSA and CAN resulted in greater eGFR decline as compared to patients with OSA or CAN only. Compliance with CPAP treatment was associated with less eGFR decline, less progression to advanced DR, less development of foot ulceration and foot insensitivity and was associated with better HRV parameters. I have also shown that poor sleep quality and shorter sleep duration were associated with increased total body fat%.

This project has several limitations. Obesity remained one of the main confounding factor. However, the association between OSA and the progression of microvascular complications remained significant or unchanged after adjustments for clinically significant variables. We saw a significant loss of follow up data especially in the neuropathy cohort which could explain the non-significant trends observed in both DPN and CAN cohorts.

This work highlights several important questions and the need for future research projects.

7.1.2 Future studies

Future studies need to address whether the impact of OSA on microvascular complications in patients with T2DM is related to the development of the disease or the progression of the disease. This will require larger cohort of patients that are well characterised at baseline to be followed up longitudinally.

OSA is significantly associated with obesity and has been one of the important confounding factor in the research examining the association of OSA and T2DM. However, there is limited observational data about the association of OSA in lean patients with T1DM, suggesting that obesity is not the only association between OSA and T1DM and T2DM [1]. CAN may play an important role in the pathophysiology of OSA in lean patients with T1DM [2]. This association needs to be examined further in larger epidemiological and longitudinal studies. This research will help in improving our understanding about the underlying mechanisms tying OSA and diabetes together. These studies need to take into account the impact of ethnicity, gender and duration of diabetes.

Studies exploring the relationship between OSA and CKD need to not only include eGFR change but also albuminuria to assess the progression of CKD based on both eGFR and albuminuria. We used MDRD equation to calculate eGFR. However, there is evidence that cystatin-C estimate is a better assessor of glomerular filtration than creatinine [3]. Therefore, studies undertaking robust assessment of kidney functions are needed.

We used 2 retinal images (as per National Retinal Screening guidelines) to score the images and diagnose DR. Robust assessment of DR including 7 field images (to include peripheral retina) and ocular coherence tomography to measure the thickness of macula are needed to characterise DR and assess the progression.

We used MNSI to diagnose DPN and monofilament to diagnose foot insensitivity. MNSI is a validated tool to assess large nerve fibre function. Studies exploring the relationship and association between OSA and DPN using in-depth analysis of DPN e.g. nerve conduction studies (gold standard), quantitative sensory testing (large and small nerve fibre functions) and SUDO scan (peripheral autonomic function) are needed. In addition, CAN needs to be assessed using night time HRV to examine the impact of nocturnal hypoxia and CPAP treatment on CAN parameters.

Assessing more important outcomes such as end-stage renal disease, blindness, amputations, macrovascular complications, glycaemic control and glycaemic variability will require longer follow up that in the studies presented in this thesis.

In addition, future studies need to assess the impact of OSA treatment (i.e. CPAP) on the development and progression of microvascular complications in patients with T2DM. This is likely to be challenging due to the poor compliance with CPAP treatment.

The association between sleep quality, sleep duration and adiposity in patients with T2DM that I described in this study, need to be examined in larger epidemiological studies to determine the direction of this relationship and to assess whether sleep duration manipulation can have favourable impact on obesity and metabolic profile in patients with T2DM.

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