THE IMPACT OF OBSTRUCTIVE SLEEP APNOEA IN EXTREME OBESITY: THE IMPACT ON ETHNICITY, GLYCAEMIA AND DIABETES RELATED MICROVASCULAR COMPLICATIONS

By

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A thesis submitted to

The University of Birmingham

for the degree of

DOCTOR OF MEDICINE

College of Medical and Dental Sciences

School of Health and Population Sciences

University of Birmingham

2014

ABSTRACT

Obesity is known to be associated with obstructive sleep apnoea (OSA) and Type 2 diabetes mellitus (T2DM). The effect of OSA in very severely obese individuals is not well documented. In this thesis, I compared the effect of OSA in South Asians and white Europeans, examined the effect of OSA on glycaemic control among T2DM, and explored the relationship between OSA and diabetic retinal and kidney diseases in a severely obese population. I also systematically reviewed the effect of OSA on diabetic kidney and retinal diseases. Findings from this thesis were 1) severely obese South Asians had greater severity of OSA compared to white Europeans and the mechanisms mediating this require further investigation, 2) a high OSA prevalence in T2DM individuals with a positive relationship between nocturnal hypoxia and glycaemic control, 3) severity of hypoxaemia during sleep may be an important factor in the development of diabetic retinal complications, 4) duration of hypoxaemia during sleep were inversely associated with renal function in T2DM and 5) from the systematic review, there is a need for future large cohort studies with long term follow-up data to examine the long-term effects of OSA and other sleep parameters on diabetic retinal and kidney diseases.

ACKNOWLEDGEMENTS

The majority of the work in this thesis is part of theme 8 (Diabetes) National Institute for Health Research (NIHR) funded Collaborations for Leadership in Applied Health care (CLAHRC) for Birmingham and Black Country.

I would like to express my upmost gratitude to Dr Shahrad Taheri for accepting me as his clinical fellow at the Heart of England NHS Foundation and provided me the great opportunity to be part of NIHR CLAHRC theme 8. Furthermore, he has provided tremendous support and guidance to me from the beginning as a clinical fellow and continue to do so. I am also thankful for his unconditional believe in me and my capabilities and without him, the thesis would not have happened.

I would also like to sincerely thank my supervisors, Professor Peymané Adab and Dr Neil Thomas who provided constant supervision, guidance, patience and encouragement. Special thanks go to Dr Antje Lindenmeyer, my mentor for her valuable insights and unfailing support during hard times.

I am also grateful to Dr Yen-Fun Chen for his expert guidance towards my systematic review chapter and Mr Ferozkhan Jadhakhan for his time and help as a second reviewer. I am indebted to all fellow students residing in Room 122 of Public Health Building as well as everyone from NIHR CLAHRC theme 8 for their generous friendship, kindness, compassion, understanding and support of the thesis writing process.

Finally, I would like to acknowledge and express my thanks to my husband Alvyn, my daughter Ellie, my parents and siblings as well as my parents-in-law for their presence and the generous love towards me.

STATEMENT OF CONTRIBUTIONS

The research works from the thesis were published in peer-reviewed journals. Below are the summary of my contributions:

Chapter 1

The introduction chapter is my own work with valuable comments from my supervisors.

Chapter 2

Leong W, Arora T, Jenkinson D, Thomas A, Punamiya V, Banerjee D, Taheri S. The prevalence and severity of obstructive sleep apnoea in severe obesity: the impact of ethnicity. Journal of Clinical Sleep Medicine 2013;9(9):853-858

The hypothesis was generated in conjunction with my clinical supervisor (Shahrad Taheri/ST). Melissa Nolen/MS, Ajit Thomas/AT, Vikas Punamiya/VP and I performed data collection. I carried out data cleaning. I performed data analysis with valuable advice from ST, Teresa Arora/TA and David Jenkinson/DJ. I wrote the manuscript and received specialist input from Dev Banerjee/DB and ST.

Chapter 3

Leong WB, Banerjee D, Nolen M, Adab P, Thomas GN, Taheri S. Hypoxia and glycaemic control in Type 2 diabetes mellitus with extreme obesity. Journal of Clinical Endocrinology and Metabolism 2014; Jun 17:jc20141260. [Epub ahead of print]

The hypothesis was generated in conjunction with my clinical supervisor (Shahrad Taheri/ST). Melissa Nolen/MS and I performed data collection. I carried out data cleaning and data analysis with valuable advice from ST. I wrote the manuscript and received specialist input from Dev Banerjee/DB and ST. I also received valuable comments from my supervisors G Neil Thomas/GNT and Peymané Adab/PA.

Chapter 4

Banerjee D**, Leong WB**, Arora T, Nolen M, Punamiya V, Grunstein R, Taheri S. The potential association between obstructive sleep apnoea and diabetic retinopathy in severe obesity – The role of hypoxaemia. PLoS One 2013;8(11):e79521.

The hypothesis was generated by ST and DB. Melissa Nolen/MS, Vikas Punamiya/VP and I performed data collection. I carried out data cleaning. Data analysis was performed by myself and TA with valuable advice from ST. I wrote part of the manuscript and went on maternity leave unexpectedly. DB completed the manuscript. Ron Grunstein/RG and ST also contributed expert input into the manuscript.

Chapter 5

Leong WB, Banerjee D, Nolen M, Thomas GN, Adab P, Taheri S. The impact of hypoxaemia on nephropathy in extremely obese patients with type 2 diabetes mellitus. Journal of Clinical Sleep Medicine. 2014 Jul 15;10(7):773-8

The hypothesis was generated in conjunction with my clinical supervisor (Shahrad Taheri/ST). Melissa Nolen/MS and I performed data collection. I carried out data cleaning

and data analysis with valuable advice from ST. I wrote the manuscript and received specialist input from Dev Banerjee/DB and ST. I also received valuable comments from my supervisors G Neil Thomas/GNT and Peymané Adab/PA.

Chapter 6

Leong WB, Jadhakhan F, Taheri S, Chen YF, Adab P, Thomas GN. The effect of obstructive sleep apnoea on diabetic retinopathy and maculopathy: a systematic review and meta-analysis. (submitted to Diabetic medicine)

Leong WB, Jadhakhan F, Taheri S, Thomas GN, Adab P. The association between obstructive sleep apnoea and diabetic kidney disease: a systematic review and meta-analysis. (plan submission to Sleep Journal)

The research questions for the systematic review were generated by myself. I wrote the study protocol, designed data extraction form, devised a quality assessment form, and carried out database searches. Study selection was performed by myself and Ferozkhan Jadhakhan/FJ. Data extraction was carried out by myself and checked by other authors. Both FJ and I did the quality assessment. All meta-analyses were performed by myself with YFC provided valuable advice. ST provided expert sleep input. Manuscript for the systematic review was drafted by me with valuable input from other authors.

Chapter 7

The conclusion chapter is my own work with valuable comments from my supervisors.

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ABBREVIATIONS

A	AASM	American Academy of Sleep Medicine
	ACCORD	Action to Control Cardiovascular Risk in Diabetes
	ACE	Angiotensin-converting enzyme
	ACR	Albumin creatinine ratio
	ADA	American Diabetes Association
	ADVANCE	Action in Diabetes and Vascular disease: preterAx and diamicoN modified release Controlled Evaluation
	AHI	Apnoea-hypopnoea index
	ARB	Angiotensin receptor blocker
	ARIC	Atherosclerosis Risk in Communities
B	BMI	Body mass index
	BP	Blood pressure
C	CAD	Coronary artery disease
	CHD	Coronary heart disease
	CHS	Cardiovascular Health Study
	CI	Confidence intervals
	CKD	Chronic kidney disease
	CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
	CPAP	Continuous positive airway pressure
	CVD	Cardiovascular disease
D	DCCT	Diabetes Control and Complications Trial
	DIRECT	DIabetic REtinopathy Candesartan Trials
	DM	Type 2 Diabetes Mellitus
	DMac	Diabetic maculopathy
	DKD	Diabetic kidney disease
	DR	Diabetic retinopathy
E	ETDRS	Early Treatment Diabetic Retinopathy Study
	eGFR	Estimated glomerular filtration rate
	ESRD	End stage renal disease
	ESS	Epworth's Sleepiness Score
	EUCLID	Eurodiab Controlled Trial of Lisinopril in Insulin-Dependent Diabetes
F	FIELD	Fenofibrate Intervention and Event Lowering in Diabetes
	FOSQ	Functional Outcomes of Sleep Questionnaire
G	GLP-1	Glucagon-like Peptide-1
	GIP	Glucose-dependent Insulinotropic polypeptide
H	HbA_{1c}	Glycosylated haemoglobin A_{1c}
	HIF-1α	Hypoxia-inducible factor-1
	HTN	Hypertension
I	IDF	International Diabetes Federation
	IGT	Impaired glucose tolerance
	IL-6	Interleukin-6
	IQR	Interquartile range
M	MDRD	Modification of Diet in Renal Disease

	MSLT	Multiple Sleep Latency Test
N	NHANES	National Health and Nutrition Examination Survey
	NHS	National Health Service
	NICE	National Institute for Health and Care Excellence
	NO	Nitrite oxide
O	ODESS	Oxfordshire Diabetic Eye Screening Service
	ODI	Oxygen desaturation index
	OR	Odds ratio
	OSA	Obstructive Sleep Apnoea
	O_2	Oxygen saturation
P	PDR	Proliferative diabetic retinopathy
	PPAR-α	Peroxisome proliferator-activated receptor alpha
	PSG	Polysomnography
R	RAAS	Renin-angiotensin-aldosterone system
	RCT	Randomised controlled trial
	REM	Rapid eye movement sleep
	RIACE	Renal Insufficiency And Cardiovascular Events study
	RR	Relative risk
	RRT	Renal replacement therapy
	ROS	Reactive oxygen species
	RYGB	Roux-en-Y gastric bypass surgery
S	SD	Standard deviation
	SHHS	Sleep Heart Health Study
T	T1DM	Type 1 diabetes mellitus
	T2DM	Type 2 diabetes mellitus
	TNFα	Tumour necrosis factor alpha
U	UKPDS	United Kingdom Prospective Diabetes Study
V	VEGF	Vascular endothelial growth factor
W	WC	Waist circumference
	WESDR	Wisconsin Epidemiologic Study of Diabetic Retinopathy
	WHO	World Health Organisation
Others	%TST<90	Percentage time spent under 90% oxygen saturation
	8-OhdG	8-hydroxy-2-deoxyguanosine
	95% CI	95 percent confidence intervals

CHAPTER 1 – INTRODUCTION

1.1 Obesity

1.1.1 Obesity classification: body mass index (BMI)

Obesity is crudely due to chronic surplus of energy intake with inadequate energy expenditure. The term 'morbid obesity' was introduced by Scott and Law in 1970 to describe individuals with excess weight as well as adverse health issues.[1] This term is now used in parallel with 'extreme obesity', 'clinically severe obesity' or class III obesity' in the literature.[2] The lack of a reliable method to determine body fat in a practical, cost-effective and reliable way has led to the utilisation of the simple anthropometric measures as alternatives to determine the levels of obesity.

The commonest anthropometric definition of obesity is based on the body mass index (BMI). Due to the close association between BMI and body fat,[3] as well as the ability to provide a simple and objective measure without bias for comparison between populations, the BMI system is endorsed by the World Health Organisation (WHO)[4] and several other international organisations.[5,6] The BMI system is also cheap and non-labour intensive to measure and therefore has been widely used in large epidemiological studies.[7,8] A BMI of greater or equal to ($\geq$) 30Kg/m^2 is considered to be obese. Table 1-1 shows the WHO classification of obesity for Caucasian adults.[4] The obesity classification for adult Asians is different as the risk for the development of co-morbidities is at a lower weight.[9] This is summarised in Table 1-2.

Table 1-1: The WHO classification of obesity for adult white Europeans [4]

BMI (Kg/m^2)	Classification	Risk of co-morbidities
25 – 29.9	Pre-obese	Increased
30 – 34.9	Obese I	Moderate
35 – 39.9	Obese II	Severe
≥ 40	Obese III	Very severe

WHO = World Health Organisation, BMI = body mass index

Table 1-2: The WHO classification of obesity for adult Asians [9]

BMI (Kg/m^2)	Classification	Risk of co-morbidities
23.0 – 27.5	Overweight	Increased
27.5 – 32.5	Obese I	Moderate
32.5 – 37.5	Obese II	Severe
≥37.5	Obese III	Very severe

WHO = World Health Organisation, BMI = body mass index

BMI has been found to have a 'U' shape relationship with morbidity and mortality.[10,11] Several studies have shown that there is an increased risk of premature cardiovascular events,[12,13] stroke[14] and mortality[11,15-17] in overweight and obese individuals based on BMI calculations, compared to those who have a healthy weight. Although BMI is a widely used measurement for obesity, it has limitations, as it is a measurement of both fat and fat free mass including muscle, body fluids and organs. For muscular individuals such as body builders or rugby players, the BMI system may misclassify them to be either overweight or obese because it takes into account both lean mass as well as fat mass therefore it is a poor measure of obesity on an individual level. One study found that BMI lacked accuracy (sensitivity of 44% in males and 52% in females) in identifying obese individuals when compared to body fat percentage.[18] BMI however, is highly accurate in excluding obesity.[18] The National Institute for Health and Care Excellence (NICE) has therefore recommended that BMI should be interpreted with caution as it is a surrogate measurement of adiposity.[19]

1.1.2 Other anthropometric measurements

Adipose tissue is usually deposited either in the subcutaneous or visceral tissues. A high level of visceral adipose tissue (VAT) has been found to be associated with increased metabolic risk.[20] Because of this, waist circumference (WC) and waist-to-hip ratio (WHR) are now increasing being used as an indirect measurement of visceral adiposity. Similar to BMI, population studies have shown that both WC and WHR are predictors for hypertension, Type 2 diabetes mellitus (T2DM), cardiovascular diseases (CVD) and all-cause mortality.[10,21-24] Currently, two major guidelines are using WC as part of the diagnosis for metabolic syndrome.[25,26] However, both guidelines implement different cut off points for WC.[25,26]

The U.S. National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) for the treatment of dyslipidaemia defines central obesity as ≥102cm for males and ≥88cm for females.[25] The International Diabetes Federation (IDF) uses ethnic-specific levels for WC as shown in Table 1-3.[26] For white Caucasians, the NCEP and IDF cut points differs because the NCEP uses a level corresponds to a BMI ≥30Kg/m^2 whilst the IDF uses a cut point corresponds to BMI ≥25Kg/m^2.[26] If an individual has a BMI of ≥30Kg/m^2, then central obesity can be assumed based on the IDF guideline.[26] Diagnosis of metabolic syndrome based on the NCEP and IDF guidelines are shown in Table 1-4.

Table 1-3: The IDF waist circumference cut off points according to ethnicity[26]

Ethnicity	Male	Female
- White Caucasians	≥ 94 cm	≥ 80 cm
- Sub-Saharan African	≥ 94 cm	≥ 80 cm
- Eastern Mediterranean	≥ 94 cm	≥ 80 cm
- Middle East (Arab) populations	≥ 94 cm	≥ 80 cm
- South Asians	≥ 90 cm	≥ 80 cm
- South and Central American	≥ 90 cm	≥ 80 cm
- Chinese	≥ 90 cm	≥ 80 cm
- Japanese	≥ 90 cm	≥ 80 cm

IDF = International Diabetes Federation

Table 1-4: Diagnosis of metabolic syndrome based on the IDF[26] and NCEP[25] guidelines

	Obesity	Lipid	Blood pressure	Glucose
IDF – Obesity plus 2 factors	Waist circumference (ethnic specific) or BMI ≥30Kg/m^2	Raised Tg ≥150mg/dl or HDL-chol <50 (M) or <40mg/dl (F) or on therapy	≥130/85 mmHg or on therapy	Fasting glucose > 100mg/dl or known T2DM
NCEP -any 3 criteria	Waist circumference >102cm (M) or >88cm (F)	Raised Tg ≥150mg/dl or HDL-chol <40mg/dl (M) or <50mg/dl (F) or on therapy	≥130/85 mmHg or on therapy	Fasting glucose >110mg/dl or known T2DM

IDF=International Diabetes Federation, NCEP= National Cholesterol Education Programme, BMI=body mass index, M=Male; F=Female, Tg=triglycerides, HDL-chol=high-densitiy- lipoprotein cholesterol, T2DM=type 2 diabetes mellitus.

1.1.3 Classification based on obesity related complications

Although both BMI and WC are good surrogate measures in population-based studies, they lack accuracy to stratify patient's risk individually for obesity related co-morbidities.[2] This has led to the proposed adoption of a clinical staging system to identify high risk obese individuals for weight management treatment. The aim of the clinical staging is similar to that of utilising the tumour, node and metastasis (TNM) staging system for individual cancer care[27] or the New York Heart Association (NYHA) functional classification for heart failure patients.[28] This approach adopts a standardised framework in order to describe individuals' risk and to ease communication between healthcare practitioners. Currently, there are four staging systems available in the literature and they are: the Edmonton Obesity Staging System (EOSS),[29] the Cardiometabolic Disease Staging (CMDS) system,[30] the King's Obesity Staging Criteria[31] and the Dudley Bariatric Surgery Comorbidity (DUBASCO) score.[32]

The EOSS assessed 3 components with each component rated using a 5-point ordinal scale (please refer to Table 1-5).[2] The components are categorised into medical, mental and functional. Stage 0 applies to asymptomatic patients with no obesity-related health risk factors, a full functional capacity and absence of mental health issues. Stage 1 consists of individuals with either a sub-clinical risk factor (e.g. borderline hypertension, impaired glucose tolerance), the presence of mild physical symptoms such as breathlessness on moderate exertion, mild psychopathology or the presence of mild functional limitation. Individuals with established obesity related co-morbidity such as hypertension or presence of moderate psychopathology/functional limitation causing an impact on well-being are classify in to Stage 2.

Stage 3 includes patients with disease specific end-organ damage such as renal impairment or significant psychopathology or function impairment. The final stage, Stage 4 involves individuals with debilitating illness either from chronic disease, mental health or severe functional impairments. Using data from three large population survey data: the National Health and Human Nutrition Examination Surveys (NHANES) III (1988 to 1994), NHANES 1999-2004[29] and the Aerobics Centre Longitudinal Study,[33] the EOSS classification system had been shown to be a better predictor of mortality compared with the BMI system.

Using the EOSS stage 0 and 1 as the reference, the results from the NHANES data demonstrated a step-wise increase in mortality (please refer to Figure 1-1): EOSS stage 2 HR 1.62 (95% CI: 1.19 to 2.21) and EOSS stage 3 HR 2.78 (95% CI: 2.07 to 3.74). This is compared to using the BMI system whereby overweight individuals were used as reference, there are no difference in the risk for mortality according level of obesity: class I obesity HR

1.26 (95% CI: 1.03 to 1.55), class II obesity HR 1.81 (95% CI: 1.27 to 2.57) and class III obesity HR 1.61 (95% CI: 1.02 to 2.56).[29] Due to this reason, the Royal College of Physicians have recently recommended the use of EOSS to stratify individual patients' risk in their report Action on obesity: comprehensive care for all.[34]

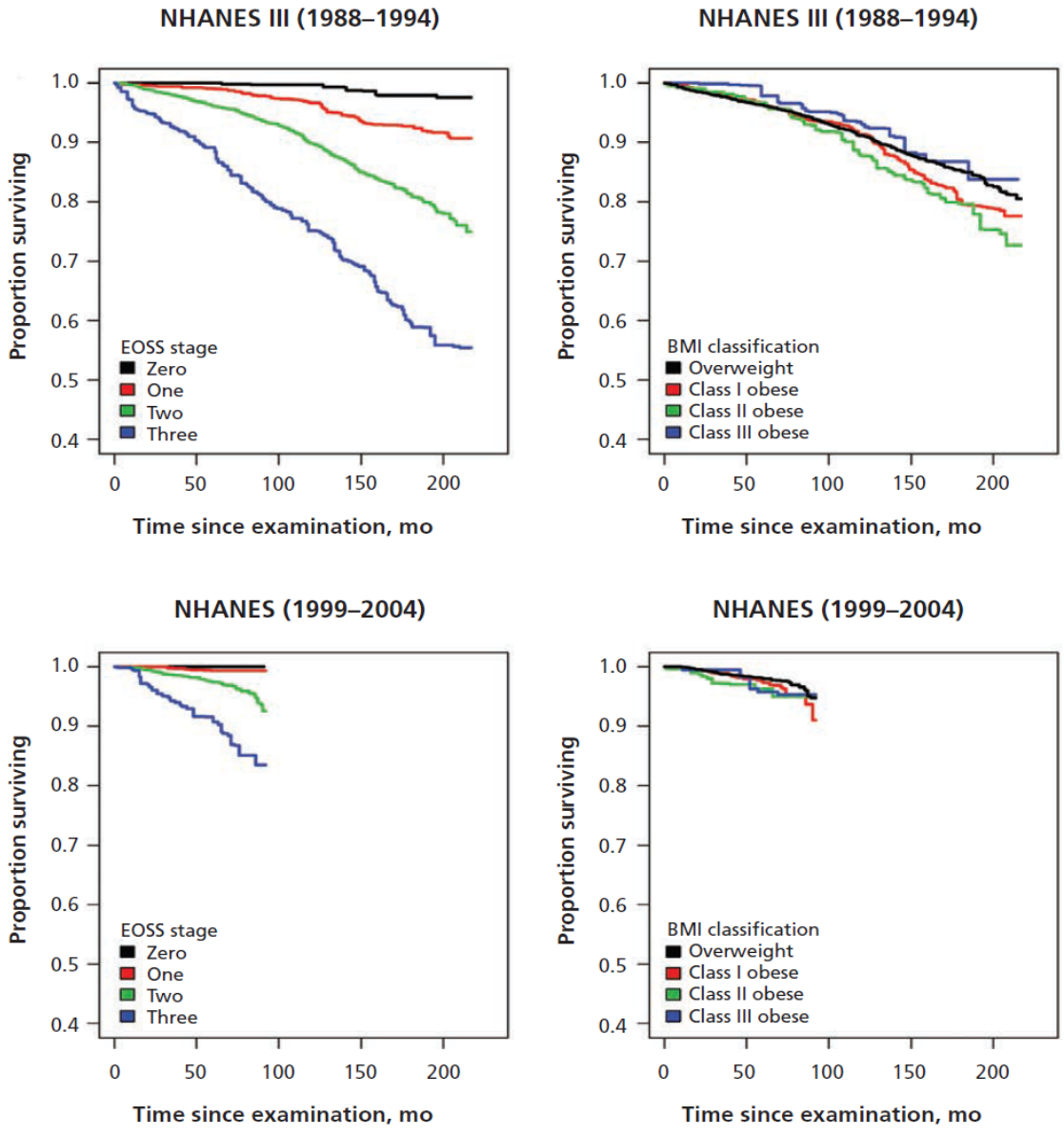

Figure 1-1: The prediction of all-cause mortality for overweight and obese individuals based on the Edmonton obesity staging system and the body mass index system.
"Using the Edmonton obesity staging system to predict mortality in a population-representative cohort of people with overweight and obesity" – Reprinted from, Canadian Medical Association Journal 2011; (14), (183), E1059-66 by permission of the publisher. ©2014 Canadian Medical Association

Table 1-5: The Edmonton Obesity Staging System. Reprinted by permission from Macmillan Publishers Ltd: [International Journal of Obesity],[2] copyright 2009

Stage	Description	Management
0	No apparent obesity-related risk factors (e.g., blood pressures, serum lipids, fasting glucose, etc. within normal range), no physical symptoms, no psychopathology, no functional limitations and/or impairment of well being	Identification of factors contributing to increased body weight. Counselling to prevent further weight gain through lifestyle measures including healthy eating and increased physical activity
1	Presence of obesity-related subclinical risk factors (e.g., borderline hypertension, impaired fasting glucose, elevated liver enzymes, etc.), mild physical symptoms (e.g., dyspnoea on moderate exertion, occasional aches and pains, fatigues, etc.), mild psychopathology, mild functional limitations and/or mild impairment of well being	Investigation for other (non-weight related) contributors to risk factors. More intense lifestyle interventions, including diet and exercise to prevent further weight gain. Monitoring of risk factors and health status.
2	Presence of established obesity-related chronic disease (e.g., hypertension, type 2 diabetes, sleep apnoea, osteoarthritis, reflux disease, polycystic ovary syndrome, anxiety disorder, etc.), moderate limitations in activities of daily living and/or well being	Initiation of obesity treatments including considerations of all behavioural, pharmacological and surgical treatment options. Close monitoring and management of comorbidities as indicated.
3	Established end-organ damage such as myocardial infarction, heart failure, diabetic complications, incapacitating osteoarthritis, significant psychopathology, significant functional limitations and/or impairment of well being	More intensive obesity treatment including considerations of all behavioural, pharmacological and surgical treatment options. Aggressive management of comorbidities as indicated.
4	Severe (potentially end-stage) disabilities from obesity-related chronic diseases, severe disabling psychopathology, severe functional limitations and/or severe impairment of well being	Aggressive obesity management as deemed feasible. Palliative measures including pain management, occupational therapy and psychosocial support.

Similar to the EOSS, the CMDS system has been shown to predict all-cause mortality using the NHANES III and the Collaborative Assessment of Research Data Infrastructure and Objectives (CARDIO) data.[30] However, the CMDS system is a very recent classification and there is no study which compares its predictability for mortality with the BMI or other anthropometric systems. The CMDS system is devised from the criteria used for metabolic syndrome and the diagnosis of type 2 diabetes mellitus (T2DM). Individuals in Stage 0 have no metabolic risk factors. Criteria for Stages 1, 2, 3 and 4 are shown in Table 1-6.

Table 1-6: The Cardiometabolic Disease Staging (CMDS) system. Adapted from Guo et al.[30]

Stage	Description	Components
0	Metabolically healthy	No metabolic risk factors
1	One or two risk factors	(a) Waist circumference ≥112cm in men or ≥88cm in women (b) Blood pressure (systolic ≥130mmHg and/or diastolic ≥85mmhg) or on antihypertensive medication (c) Low serum HDL-cholesterol (<1.0 mmol/l in men or <1.3mmol/l in women) or on lipid lowering medication (d) Raised fasting serum triglycerides (≥1.7mmol/l) or on lipid lowering medication
2	Metabolic syndrome or prediabetes state	Any one of the following conditions: (a) Metabolic syndrome: based on the ≥3 of the above risk factors (b) Impaired fasting glycaemia (fasting serum glucose ≥5.6mmol/l) (c) Impaired glucose tolerance (post-prandial glucose ≥7.8mmol/l)
3	Metabolic syndrome with prediabetes state	Have two of the Stage 2 conditions
4	Type 2 diabetes mellitus and/or cardiovascular disease (CVD)	Have either one of the following: (a) Type 2 diabetes mellitus or on anti-diabetic treatment (b) Active CVD (angina pectoris or post CVD event)

HDL= high-density-lipoprotein, CVD = cardiovascular disease

Both the Kings Obesity Staging system[31] and the DUBASCO score[32] are devised to aid decision making for bariatric surgery taking into account adiposity levels as well as co-morbidities. Similar to the CMDS system, there is no research comparing the predictive performance (on mortality and morbidity risks) for both the scoring systems with other anthropometry measurements. The Kings Obesity Staging system[31] contains 9 components with 4 ordinal score for each component. The nine components in alphabetical order are airways, BMI, cardiovascular disease, diabetes, economic complications, functional limitations, gonadal axis, health status, and image (body) as shown in Table 1-7.

Table 1-7: The King's Obesity Staging Criteria. Adapted from Aaesheim et al.[31]

	Stage 0 'Healthy'	Stage 1 'At risk'	Stage 2 'Established disease'	Stage 3 'Advanced disease'
Airway	Normal	Snorer	On CPAP	Cor pulmonale
BMI (Kg/m^2)	<35	35 – 40	40 – 60	>60
Cardiovascular	<10% risk	10-20% risk	Heart disease	Heart failure
Diabetes	No diabetes	IGT/IFG	T2DM	Uncontrolled T2DM
Economic	Normal	Expensive travel/clothes	Workplace discrimination	Unemployed due to obesity
Functional	Manage 3 flights of stairs	1/2 flights of stairs	Walking aids/wheel chair	House bound
Gonadal	No problem	PCOS	Infertility	Sexual dysfunction
Health	Normal	Low QoL or mood	Poor QoL of depression	Severe depression
Image (body)	Normal	Dislikes own body	Body image dysphoria	Eating disorder

CPAP=continuous positive airway pressure, BMI=body mass index, IGT=impaired glucose tolerance, IFG=impaired fasting glycaemia, T2DM=type 2 diabetes mellitus, PCOS=polycystic ovarian syndrome, QoL=quality of life

The DUBASCO score[32] contained 5 components, each with a four level ordinal scale as shown in Table 1-8. The researchers for DUBASCO score have recommended the cut point of ≥10 as a cut-off for considering bariatric surgery. The benefit of using a scoring system is that the level of cut point can be altered depending on the available resources. The scoring system also aids healthcare professionals in the prioritisation of patients who have the greatest needs for bariatric surgery in the face of the current NHS with limited healthcare access and scarce resources.

Table 1-8: The Dudley Bariatric Surgery Comorbidity (DUBASCO) score. Adapted from Labib et al.[32] (with permission from Dr M. Labib)

Component	**1**	**2**	**3**	**4**
Age (years)	>60	51-60	41-50	≤40
BMI (Kg/m^2)	≥35.0 to 40.0	>40.0 to 50.0	>50.0 to 60.0	60.0
Comorbidities:				
T2DM	IFG/IGT	HbA_{1c} ≤7.0%	HbA_{1c} 7.1-9.0%	HbA_{1c} >9.0% or on insulin
Hypertension	Well-controlled on one drug	Well-controlled on 2 drugs	Poorly controlled on 2 drugs	Poorly controlled on >2 drugs
Dyslipidaemia	No treatment	Well-controlled on 1 drug	Well-controlled on 2 drugs	Poorly controlled on 2 drugs
OSA	Neck circumference >43 cm or snorer	ODI 5-14 events/hour	ODI 15-30 events/hour	ODI >30 events/hour or on CPAP

BMI = body mass index, T2DM= type 2 diabetes mellitus, IFG=impaired fasting glycaemia, IGT=impaired glucose tolerance, HbA_{1c}=glycosylated haemoglobin A_{1c}, OSA=obstructive sleep apnoea, ODI=oxygen desaturation index assessed by 2 overnight oximetry readings, CPAP=continuous positive airway pressure

1.1.4 Epidemiology of obesity

The prevalence of obesity is increasing in both developing and developed countries. A report using data from 199 countries and territories with 9.1 million participants found that the mean BMI has risen by $0.4Kg/m^2$ per decade (95% uncertainty interval: 0.2 to 0.6) for males and

0.5Kg/m^2 per decade increase (95% uncertainly interval: 0.3 to 0.7) for females from 1980 to 2008.[35] Moreover, the report estimated that the age-standardised prevalence of obesity is 9.8% (95% CI: 9.2 to 10.4) for men and 13.5% (95% CI: 13.1 to 14.7) for women in 2008. Recently, the WHO estimated that globally over 1.4 billion adults are now considered obese.[36] In the UK, The 2012 Health Survey for England reported that 24% of adult men and 25% of adult women over the age of 16 were obese.[37] Furthermore in 2030, it is projected that an extra 11 million adults will be obese[38] and the Foresight report estimated that by 2050, more than half of the UK population will be obese.[39] The Foresight report also evaluated that in 2050, both the obese and overweight adults, will cost the country £50 billion per year[39] if weight preventative measures are not successful.

1.1.5 Obesity related diseases

It is well-recognised that obese individuals suffer from premature mortality,[17,40] have increased risk for cardiovascular disease (CVD)[41-43] and several different types of malignancy.[44] The National Institute of Health-American Association of Retired Persons (NIH-AARP) Diet and Health study followed-up 527,265 US individuals aged between 50 and 71 years old for a maximum of 10 years reported a 'J' shaped relationship between BMI and death.[40] The Framingham Heart Study followed-up 3457 individuals from 1948 to 1990 demonstrated that a 40-year-old obese non-smoker female loses 7.1 years while a 40-year-old obese non-smoker male loses 5.8 years of their respective life expectancy compared to their normal weight (BMI between 18.5 and 24.9Kg/m^2) counterparts.[17] The premature mortality is likely to be related to the increase risk in both malignancy[44] and CVD.[42]

A systematic review of 141 studies which included 282,137 cancer cases demonstrated an association between BMI and several carcinomas.[44] For example, a 5Kg/m^2 increase in BMI was associated with a higher risk of colon (RR1.24, 95% CI: 1.15 to 1.34), thyroid (RR 1.33, 95% CI: 1.04 to 1.70) and oesophageal (RR 1.52, 95% CI: 1.33 to 1.74) malignancies in men. In women, a 5Kg/m^2 increase in BMI was associated with a higher risk of oesophageal (RR 1.51, 95% CI: 1.31 to 1.74), gallbladder (RR 1.59, 9% CI: 1.02 to 2.47) and endometrial (RR 1.59, 95% CI: 1.50 to 1.68) carcinomas. A Swedish longitudinal study (n=22,025, 26 year follow-up) reported that obese men had a 76% higher risk (RR 1.76, 95% CI: 1.49 to 2.08) of CVD compared to those with normal BMI.[42]

One of the mechanisms linking obesity with CVD as well as premature death is the association of obesity with several cardiovascular risk factors. The NHANES (1999 to 2004) study consisted of 13,745 US individuals, and found that when compared to individuals with normal BMI, those with BMI ≥40Kg/m^2 had a higher risk of hypertension (OR 4.8, 95% CI: 3.8 to 5.9), dyslipidaemia (OR2.2, 95% CI: 1.7 to 2.4), metabolic syndrome (OR 2.0, 95% CI: 1.4 to 2.8) and diabetes mellitus (DM) (OR 5.1, 95% CI: 3.7 to 7.0).[45] Moreover, treatment for obesity especially bariatric surgery has been shown to result in improvements in these cardiovascular risks, especially T2DM. The Swedish Obese Subjects study followed-up 1703 participants who had had bariatric surgery and shown that after 10 years, improvements in hypertriglyceridaemia (OR 2.56, 95% CI: 1.85 to 3.57), hypertension (OR 1.68, 95% CI: 1.09 to 2.58) and T2DM (OR 3.45, 95% CI: 1.64 to 7.28)[46] occurred in bariatric patients compared with those medically managed patients. Another chronic condition associated with obesity is obstructive sleep apnoea (OSA). The association between obesity and OSA will be discussed in section 1.3.

1.2 Type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is a condition characterised by a chronic hyperglycaemic state secondary to insulin resistance and beta cell dysfunction. The International Diabetes Federation (IDF) estimates that there are at least 366 million individuals with diabetes mellitus (DM) worldwide and this is projected to rise to 552 million in 2030.[47] In the UK, there are over 3 million individuals known to have DM and an approximately 850,000 people with undiagnosed DM[48] making the overall prevalence of diabetes in the UK to be 4.5%.[48] DM is an important chronic condition partly because it is associated with a considerable economic burden to the healthcare system. A recent report from the London School of Economics (LSE) estimated that DM costs the UK economy at least £13.8 billion annually and the cost of each DM patient was estimated to be £3,717[49] with a large proportion of the cost attributed to the treatment and management of T2DM complications.

Apart from economic cost, T2DM also is associated with increased morbidity and premature mortality secondary to the macro- and micro-vascular complications.[50,51] Macro-vascular complications of DM include ischaemic heart disease, cerebrovascular disease and peripheral vascular disease. A recent meta-analysis on 26 studies compared mortality risk of DM and non-DM individuals. There was a total of 220,689 participants with a mean follow-up of 10.7 years and DM was found to be an independent predictor for all-cause mortality (RR 1.85, 95% CI: 1.79 to 1.92).[52] The UK Prospective Diabetes Study (UKPDS) recruited 4585 newly diagnosed T2DM participants found that each 1% rise of glycosylated haemoglobin A_{1c} (HbA_{1c}) was associated with 21% (95% CI: 15% to 27%) increase in DM related deaths, 14%

increase in myocardial infarction (95% CI: 8% to 21%) and 37% increase in microvascular complications (95% CI: 33% to 41%).[51]

1.2.1 Natural history of Type 2 diabetes mellitus (T2DM)

The underlying pathophysiology of T2DM is chronic hyperglycaemia and this disturbance in glucose homeostasis is likely to have started several years before the onset of DM.[53] A study which recruited T2DM from the US and Australia to investigate the prevalence of retinopathy found that by extrapolating the linear relationship between the diagnosis of diabetic retinopathy and the duration of T2DM, it was estimated that onset of T2DM most likely occurred 9 to 12 years before a clinical diagnosis was made.[53] The combination of hereditary influences and other environmental factors such as sedentary lifestyle leading to obesity have contributed to the development of insulin resistance state in which normal glucose tolerance is maintained through a compensatory greater production of insulin by the pancreatic beta-cell resulting in a hyperinsulinaemic state. Studies on high risk pre-diabetes individuals have revealed that insulin resistance is most likely the initial pathognomonic phase for the development of T2DM.[54] Over time, insulin resistance progressively worsened and the pancreas is not able to supply the required amount of insulin to match demand from the insulin resistant organs. This causes elevation of glucose level commonly known as the impaired fasting and glucose tolerance (IGT) state.

The chronic excess glucose from the IGT state induced excess formation of reactive oxygen species (ROS) causing oxidative stress. Pancreatic beta-cells are very sensitivity to the insult from oxidative stress. Therefore, this leads to damage and subsequently dysfunction of the pancreatic beta cells. The term glucotoxicity has been utilised to describe this process.

Furthermore, excess glucose also affects intracellular lipid synthesis with accumulation of metabolic products causing lipotoxicity. Together, gluco- and lipotoxicity impose deleterious effects to insulin signalling, expression and apoptosis of the pancreatic beta-cells.[54,55] Indeed, it has been demonstrated that at the onset of T2DM, there is at least 50-60% loss of beta-cell function.[54] The progressive decompensation of pancreatic beta-cell ultimately led to overt T2DM. Although beta-cells continue to secrete some insulin in T2DM state, over time, insulin production decreases. At this stage, without the adequate inhibitory action of insulin, hepatic gluconeogenesis ensues causing a greater level of hyperglycaemia. This process is summarised in Figure 1-1.

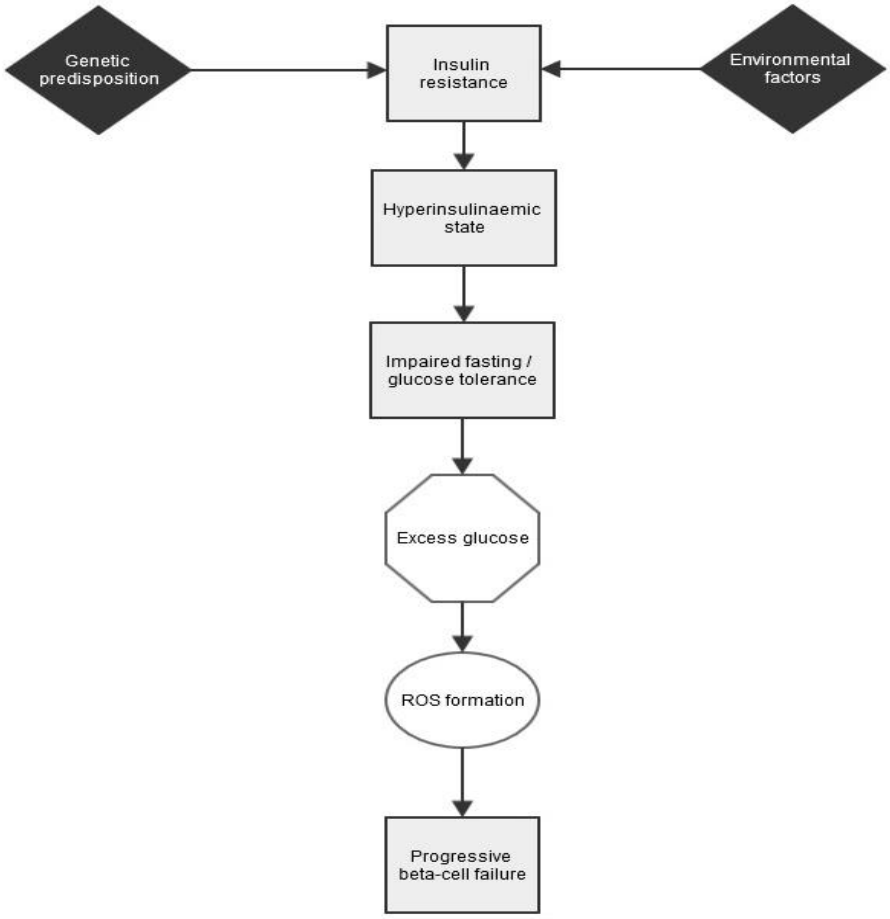

Figure 1-2: Pathogenesis of beta-cell failure

Apart from beta-cell dysfunction, a reduction in incretin effect also plays a role. The ability of gastrointestinal system to secrete incretin hormones after a meal, especially after a high carbohydrate meal, is termed the incretin effect. The incretin hormones are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). These hormones are released after a meal and exert different effects on several target organs. GIP inhibits beta cell apoptosis and increase beta-cell replication. It also increases glucagon secretion as well as lipogenesis. On the other hand, GLP-1 stimulates insulin release, suppresses glucagon secretion, increase beta-cell replication and inhibits beta-cell apoptosis. It also promotes satiety by acting on the central nervous system, delays gastric emptying thus promoting early satiety and helps in weight reduction. In addition, it also has been found to reduce post-prandial fatty acid levels. Collectively, these effects help in insulin sensitivity and secretion[56] as summarised in Table 1-9. In T2DM, it has been proposed that a progressive ineffective response to the incretin hormones occur leading to the decline in incretin effect.[57,58]

Table 1-9: Effect of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) actions in Type 2 diabetes mellitus

Action	**GIP**	**GLP-1**
Insulin secretion	No effect	Increased
Glucagon secretion	Increased	Reduced
Beta-cell	Reduced apoptosis Increased replication	Reduced apoptosis Increased replication
Obesity	Increased lipogenesis	Early satiety via appetite centre, reduced in body weight
Gastric emptying	No effect	Decreased rate of gastric emptying
Insulin resistance	No effect	Increased insulin sensitivity

GIP = glucose-dependent insulinotropic polypeptide; GLP-1 = glucaton-like peptide-1

1.2.2 Obesity and Type 2 diabetes mellitus

As mentioned in section 1.1.5, T2DM is one of the cardiometabolic risk factors associated with obesity leading to premature death. Additional information on the effect of obesity on T2DM will be covered in the introduction section of Chapter 3 to avoid repetition.

1.3 Obstructive sleep apnoea (OSA)

1.3.1 Obstructive sleep apnoea: definition and symptoms

Obstructive sleep apnoea (OSA) is characterised by the intermittent repetitive complete or partial collapses of the upper airway or pharynx. It is an increasingly common chronic respiratory disorder. It has been recognised as a significant health problem from the late 20th century. The term 'Picwickian' was first used to describe this syndrome in the 19th century after Charles Dickens' description of the character Fat Boy Joe in '*The Posthumous Papers of the Pickwick Club* (1837)'. Fat Boy Joe is a remarkably obese boy with an inclination to fall asleep at any time of the day in any situation. This has led to the detailed depiction of the Picwickian syndrome by Bickelmann and colleague in 1956,[59] referring to an obese individual who has hypersomnolence with hypercapnia which may result in cor pulmonale and erythrocytosis.[59] Currently, this syndrome is redefined as obesity hypoventilation syndrome, which represents between 9% and 20% of OSA patients.[60] OSA is characterised by the intermittent repetitive complete or partial collapses of the upper airway or pharynx. The collapses lead to increase in airflow resistance, raised intra-thoracic pressure, reduction in arterial oxygenation, hypercapnia and subsequently arousal from sleep to re-establish ventilation. These respiratory disturbances result in sleep fragmentations as well as activation of sympathetic activities.[61,62]

Evaluation of OSA as in many other diseases involves history taking, physical examination and laboratory investigations. There are several symptoms found to be associated with OSA, most commonly, snoring. In the Sleep Heart Health Study (SHHS) with 5615 participants, 61% of OSA individuals were habitual snorers, defined as snoring between 3 to 7 nights per week.[63] Snoring may gradually progress to be significantly worse over time and can be aggravated by alcohol intake or sedative medications. In some situations, snoring may even disrupt relationships as some bed partners are compelled to sleep in a separate bedroom. One issue with snoring is that it is also a very common presentation within the general population. The Health Improvement Network (THIN) database, a longitudinal UK primary care database, found that of the 1,073,116 individuals, 0.6% (6,527 individuals) had a diagnosis of OSA while 1.2% (12,499 individuals) was reported to be snorers.[64] A recent meta-analysis of 6 studies investigating the accuracy of clinical examination in the diagnosis of OSA concluded that snoring alone was not a predictive factor for OSA individuals with an AHI $\geq$10 events/hr (likelihood ratio 1.1, 95% CI: 1.0 to 1.1).[65]

Apart from snoring, episodes of breathing pauses during sleep witnessed by bed partners have also been associated with OSA. In a study of 394 hypertensive individuals, witnessed apnoeas were reported in 18% (95% CI: 11 to 25) of individuals with mild OSA and 11% (95% CI: 28 to 45) of those with moderate to severe OSA.[66] A clinical study of 250 patients who were seen in a sleep centre found that those with OSA (n=134) had double the risk for observed apnoeas (OR 2.0 95% CI: 1.1 to 3.8) compared with those who did not have OSA.[67]

In addition, the Wisconsin Sleep Cohort Study included 388 women found that up to 6% of females within the general population may report apnoea episodes.[68] Other non-specific

symptoms have also been shown to be associated with OSA and can be categorised into nocturnal or daytime symptoms. Nocturnal symptoms besides snoring and apnoeas include dyspnoea which may present as choking or gasping sensation and usually results in arousal, nocturia, gastro-oesophageal reflux, dry mouth and restless sleep. Daytime symptoms include excessive daytime somnolence, impairment in neurocognition such as poor concentration and memory impairment, mood change, headache upon waking as well as sexual dysfunction including decrease in libido and impotence. A summary of the OSA symptoms is shown in Table 1-9.

Table 1-10: Nocturnal and daytime symptoms of obstructive sleep apnoea

Nocturnal symptoms	Day time symptoms
Snoring	Excessive daytime somnolence
Observed apneoas	Poor concentration
Dyspnoea	Memory impairment
Nocturia	Mood change
Gastro-oesophageal reflux	Early morning headache
Dry mouth	Sexual dysfunction
Restless sleep	

Not only are symptoms for OSA non-specific, but physicians' impression based on symptoms and signs, has also been shown to have poor predictive ability for OSA. In an observational study of 594 patients referred to the sleep clinic in Canada with standard sleep-related questions, subjective physician impression had a 60% sensitivity and 63% specificity for the diagnosis of OSA compared to the gold standard.[69] In another study of 37 patients who had had polysomnography (PSG), which is the gold standard investigation for OSA, the researchers found that a brief clinical assessment had 65% sensitivity and 70% accuracy for

the diagnosis of OSA.[70] Therefore, screening tools may be useful in identifying high risk individuals.

1.3.2 Screening and diagnosis of obstructive sleep apnoea

As symptoms for OSA and clinicians' impression have lower accuracy for OSA, several screening questionnaires have been developed. Screening for OSA usually involves administrating validated questionnaires such as the Epworth's Sleepiness Score (ESS)[71] and the Berlin Questionnaire.[72] ESS was first designed to assess daytime somnolence[71] and has a maximum score of 24. A total of 184 adults which consisted of 150 patients with sleep disorders were compared with 30 normal individuals. All participants rated the probability of falling asleep in eight different situations. An ESS score of $\geq$16 was found to be present only in patients with excessive daytime somnolence (idiopathic hypersomnia, moderate-to-severe OSA and narcolepsy).[71] The correlation between ESS and apnoea-hypopnoea index (AHI), a measure for OSA (see below for further explanation), was reported to be ($r=0.55$, $p<0.001$).[71] However, another study of 54 OSA patients from a sleep clinic found that the correlation between ESS and AHI was ($r=0.28$, $p<0.05$).[73] The inconsistencies in the results limit the use of the ESS screening, rather than a diagnostic test for OSA.

The Berlin questionnaire is an alternative questionnaire for OSA. It contains 3 categories; namely snoring, wake time sleepiness or drowsy driving and hypertension or obesity.[72] The presence of persistent symptoms in at least 2 of the categories indicates a patient has a high risk of OSA. The Berlin questionnaire was designed based on the consensus of 120 U.S. and German primary care physicians on the Conference on Sleep in Primary Care held in Berlin, Germany in 1996.[72] The questionnaire was subsequently tested on 744 adults from 4 primary

care centres in Cleveland, Ohio.[72] Compared to a portable home monitoring device, the Berlin questionnaire was found to have a sensitivity and specificity of 0.17 and 0.97, respectively for AHI >30 events/hr. For AHI >15 events/hr, sensitivity was 0.54 and specificity was 0.97 and for AHI >5 events/hr, sensitivity was 0.86 with specificity of 0.77.

Another study of 2,467 elective surgical patients compared the Berlin questionnaires with in-hospital full polysomnography, and reported an area under the receiver operating characteristic curve (AUC) of 0.668 in those with AHI >30 events/hr, AUC of 0.672 in those with AHI >15 events/hr and AUC of 0.690 for AHI >5 events/hr.[74] The sensitivity for Berlin questionnaire was 87.2% (95% CI: 72.6 to 95.7), 78.6% (95% CI: 67.1 to 87.5) and 68.9% (95% CI: 59.8 to 76.9) for AHI >30 events/hr, >15 events/hr and >5 events/hr respectively. The specificity was 46.4% (95% CI: 37.9 to 55.1) for AHI >30 events/hr, 50.5% (95% CI: 40.6 to 62.3) for AHI >15 events/hr and 56.4% (95% CI: 42.3 to 69.7) for AHI >5 events/hr.

The questionnaires are ideal for stratifying OSA risk and for screening, but they are not designed to diagnose OSA. According to the American Academy of Sleep Medicine (AASM) guidelines for OSA diagnosis, a diagnosis of OSA is based on apnoea-hypopnoea index (AHI).[75] An apnoea occurs when there is a complete absence of airflow for at least 10 seconds while hypopnoea occurs when there is at least 30% reduction in airflow in conjunction with at least 4% arterial oxygen desaturation with evidence of abdominal or thoracic movement.[75] AHI is the calculated as the average number of apnoea or hypopnoea episodes occurring per hour of sleep. An AHI cut off point of greater or equal (≥) to 5 is diagnostic of OSA. AHI is further used to classify OSA into mild, moderate and severe disease: AHI of between 5 and 14events per hour (events/hr) is classified as mild; AHI between 15 and 29 events/hr is

moderate and AHI ≥30 events/hr is classified as severe OSA.[61,76] OSA syndrome is a diagnosis of OSA with sleep symptoms.[77]

The gold standard and the recommended test to diagnose OSA is overnight sleep polysomnography (PSG) which includes electroencephalogram (EEG), electroculogram (EOG), chin electromyogram, electrocardiography, oxygen saturation, respiratory airflow, thoracic-abdominal movements, monitoring for episodes of snoring and body position.[61,76] A basic sleep study should consist of at least assessment of respiration, snoring and abdominal-thoracic movement.[76] A full PSG is costly and needs special technical expertise to perform therefore some sleep centres might not offer it routinely to patients.[78] Portable home sleep monitors such as a Level III device with data available for oxygen saturation, respiratory airflow and thoracic-abdominal movement, provide an alternative option for sleep studies.

1.3.3 Complications of obstructive sleep apnoea

Apart from the association with obesity, OSA has been linked to and is likely an important risk factor for type 2 diabetes mellitus (DM),[79,80] hypertension,[81-83] CVD[84,85] and premature mortality either due to road traffic accidents (RTA)[86,87] or through CVD-related death.[84,85] The SHHS[79] has 2656 patients with fasting blood glucose (FBG) results, 1930 patients with prandial blood glucose (PBG) results and 1144 data on fasting insulin levels. The study found those with moderate to severe OSA have greater insulin resistance as measured by the HOMA index after adjustment for age, gender, ethnicity, BMI, waist circumference, smoking status and sleep duration. Individuals with abnormal FBG (includes impaired fasting glycaemia and DM) had a 46% (OR 1.46, 95% CI: 1.09 to 1.97) increase in the risk for moderate and severe OSA. Similarly, PBG (includes impaired glucose tolerance and DM) is associated with 44%

(OR 1.44, 95% CI: 1.11 to 1.87) increase in the risk for experiencing AHI ≥ 15, independent of confounding factors.[79] A recent meta-analysis of 6 cohort studies aims to assess the association between T2DM and OSA severity, found that of a total of 5953 participants, there was 332 incident cases of DM.[80] The follow-up period was between 2.7 to 16.0 years. The researchers concluded that patients with moderate and severe OSA have a 63% (RR 1.63, 95% CI: 1.09 to 2.45) increase in risk of DM compared to those without OSA.[80]

Several studies have examined the associations between OSA and hypertension, this includes large population studies,[81-83] sleep clinics[4,88,89] and case control studies.[90] Large cross-sectional population studies such as the SHHS,[81] the Wisconsin Sleep Cohort Study[82] and the Pennsylvania study[83] have all reported strong associations between hypertension and OSA. Longitudinal research has strengthened the association further. Wright et al. examined 82 patients with OSA with 5 years follow-up and found a linear relationship between AHI and 24 hour mean arterial BP (β=0.18, SE=0.09, p=0.04) and maximum systolic BP (β=0.56, SE=0.24, p=0.02).[91] Wisconsin Sleep Cohort group followed 706 patients up for 4 years and found a stepwise increase in risk of hypertension: OR 1.42 (95% CI: 1.13 to 1.78) for AHI between 0.1 and 4.9 events/hr, OR 2.03 (95% CI: 1.29 to 3.17) for AHI between 5.0 and 14.9 events/hr, OR 2.89 (95% CI: 1.46 to 5.64) for AHI >15 events/hr.[92]

OSA also results in an increased risk of stroke. An observational cohort study of 697 patients followed up over 3.4 years with mean AHI of 35 events/hr found that the hazard ratio for stroke is 1.97 (95% CI: 1.12 to 3.48).[93] There is also a trend (p=0.005) towards a dose response relationship between severity of OSA and risk of stroke: HR 1.75 (95% CI: 0.88 to

3.49) for AHI between 4 and 12 events/hr, HR 1.74 (95% CI: 0.87 to 3.51) for AHI between 13 and 36 events/hr and HR 3.30 (95% CI: 1.74 to 6.26).

Apart from stroke, OSA also increases risk of fatal as well as non-fatal cardiovascular events.[84,85] A study from Spain[85] followed up 264 healthy men, 377 simple snorers, 403 untreated mild-moderate OSA, 235 untreated severe OSA, 372 treated severe OSA with CPAP for 10 years. Patients were age matched. They found that those with untreated severe OSA had a markedly increased risk for non-fatal CVD after adjusting for potential confounders (OR 3.17, 95% CI: 1.2 to 7.51). This study also reported a higher risk for CVD related death for the untreated severe OSA group (OR 2.87, 95% CI: 1.17 to 7.51). Another study followed 168 OSA patients up for 7.5 years; CVD death was significantly higher in the untreated OSA group compared to those treated with CPAP (14.8% vs. 1.9%).[84] The total CVD events and death were also greater in the untreated group (31% vs. 18%).[84]

Other than death from premature CVD events, OSA is also associated with higher risk of road traffic accidents (RTA), likely due to sleepiness. A Canadian group compared the RTA data of 783 patients with suspected OSA with sleepiness and 783 controls matched for age and gender.[86] They reported an increased risk for RTA in mild (RR 2.6, 95% CI: 1.7 to 3.9), moderate (RR 1.9, 95% CI: 1.2 to 2.8) and severe (RR 2.0, 95% CI: 1.4 to 3.0) OSA compared to age and gender matched controls. Of those patients who had RTA, the likelihood of personal injury from the RTA was also examined.[86] Unsurprisingly, the risk of personal injury was also increased with RR of 4.8 (95% CI: 1.8 to 12.4), 3.0 (95% CI: 1.3 to 7.0) and 4.3 (95% CI: 1.8 to 8.9) for mild, moderate and severe OSA, respectively.[86] A case-control study of 102 drivers with OSA and 152 aged and sex-matched controls found that those with

AHI of 10 and above had a 6 fold increase in risk (OR 6.3, 95% CI: 2.4 to 16.2) for RTA after adjustment for several confounders.[87] As OSA is associated with these potentially life-threatening complications, it is important for the clinician to be aware and initiate appropriate treatment when necessary.

1.3.4 Obesity and obstructive sleep apnoea

Several risk factors are linked to the development of OSA. Documented risk factors in the literature for OSA include male gender,[94-99] increasing age,[97,100-102] and smoking.[103,104] Obesity is also one of the well-recognised risk factors for OSA.[63,94,105,106] The Wisconsin Sleep Cohort documented that a 1 standard deviation (SD) increase in BMI was associated with a three times increased risk of OSA.[94] Davies and colleagues studied 66 OSA patients and shown a moderate to high correlation between OSA and obesity (r=0.54, 95% CI: 0.39 to 0.69).[106]

Further evidence on the influence of adiposity on OSA has also been shown in longitudinal studies.[92,107] In the longitudinal Sleep Heart Health cohort study, 2968 middle and older age patients were followed up for 5 years. The study reported a weight gain of ≥10Kg led to a significant rise in the risk (OR for males 5.21, 95% CI: 2.35 to 11.53) of AHI ≥15 events/hr.[107] The Wisconsin Sleep Cohort Study followed up 690 patients for 4 years and found that a 10% weight gain resulted in 32% (95% CI: 20% to 45%) increase in AHI and a six-fold increase in the risk for moderate-to-severe OSA (95% CI: 2.2 to17.0).[92]

Likewise, weight reduction improves the severity of OSA.[108-114] The impact of weight loss on AHI is seen after lifestyle intervention,[110,111] drug therapy[112] as well as post bariatric

surgery.[109,113,114] The Sleep AHEAD study, a randomised control trial (RCT) of 264 DM obese patients with OSA received either intensive lifestyle intervention (ILI) or diabetes support and education (DSE). After 4 years of follow-up, ILI group lost 5.2Kg and the DSE group lost <1Kg in weight with a 5 fold greater remission of OSA in ILI compared to DSE group (20.7% vs. 3.6%).[110] A recent meta-analysis of dietary interventions for weight loss on OSA found that a pooled mean reduction of 4.8Kg/m^2 (95% CI: 3.8-5.9) in BMI resulted in a weighted mean difference of 14.3 events/hr (95% CI: -23.5 to-5.1) reduction in AHI.[111] A small RCT on 22 obese patients receiving phentermine/topiramate, medication for weight reduction (licensed in the US), and 23 obese patients on placebo found that after 28 weeks, there was a 10% weight reduction in those receiving phentermine/topiramate compared to 4.3% (p=0.0006) in the placebo group. This was associated with a 31.5 events/hr reduction in AHI for the drug treatment compared to a reduction of 16.6 events/hr (p=0.0084) in the placebo group.[112]

Bariatric surgery is currently the most effective means for weight reduction and has been shown to improve OSA.[109] Common procedures performed for bariatric surgery are laparoscopic Roux-en-Y gastric bypass (RYGB), laparoscopic adjustable gastric band (LAGB) and laparoscopic sleeve gastrectomy (LSG). A study comparing 59 patients with an ILI and 74 in RYGB resulted in 8% and 30% weight reduction respectively after 1 year found a reduction in AHI of 6 and 13 events/hr (p=0.017) respectively. There is also significant difference in the remission of OSA (40% vs. 66% in RYGB, p=0.028).[113] A RCT including 30 obese individuals who received a lifestyle intervention and 30 on LAGB found a 5.1Kg and 27.8Kg (p<0.001) reduction in weight after 2 years, respectively. AHI reductions were 14.0 and 25.5 events/hr (p=0.18) for lifestyle and LAGB, respectively.[114] A systematic review

of 69 studies on the effect of bariatric surgery on OSA found that RYGB results in 79% remission or improvement in OSA compared to 77% in LAGB and 86% in LSG.[109] Overall, these data support that obesity is a risk factor for OSA, and weight reduction is an effective treatment for OSA.

Studies on patients with OSA have provided insight into the mechanisms behind the link between obesity and OSA.[106,115] These may involve adverse anatomical changes and airway collapsibility. Imaging studies on the pharyngeal anatomy has revealed specific anatomical characteristics of OSA individuals, including narrow pharyngeal airway space either due to larger soft tissue area or a smaller skeletal enclosure,[116] which may be accentuated in obesity.[117] Excess fat deposition at the upper airway has been shown in OSA patients when compared to matched non-OSA individuals (matched for BMI, neck circumference and age).[118] Therefore a greater neck circumference, which may be a surrogate marker for fat deposition within the airway or on the lateral pharyngeal wall, is a feature of OSA.[106,115]

Apart from pharyngeal adiposity, truncal adiposity may also play a part. Obesity is associated with lower functional residual capacity, lung compliance[119] and lung volume.[120] Hoffstein and colleagues showed that in obese OSA patients, changes in the cross-sectional pharyngeal area is associated with changes in lung volume.[120] This may signifies that obese individuals with lower lung volumes are more prone to greater collapsibility of the airway impeding airflow. Likewise, an improvement in lung volume improves OSA. Heinzer and colleagues studied 12 OSA patients exposed to negative extra-thoracic pressure at 3 different levels (no negative pressure, CPAP-like negative pressure and 500ml above CPAP-like pressure) and found that greater lung volume reduces AHI events.[121] During inspiration, negative thoracic pressure

produced by the diaphragm and intercostal muscles causes the trachea to be pulled caudally and this increases the upper airway traction therefore making it less collapsible. However, the diaphragm of an obese individual with high truncal fat level is more likely to be pushed upwards in a supine position compared with a non-obese person and this reduces the intra-thoracic pressure resulting in less traction and increases the collapsibility of the upper airway.[121]

1.4 Aim and objectives

The effect of OSA in very severe obesity individuals (BMI $\geq$40Kg/m^2), however, is not well documented in the literature. South Asians have an increased cardio-metabolic risk with BMI of 30Kg/m^2 compared with their white European counterparts therefore lower BMI cut points were introduced. As OSA is also a risk factor for cardiovascular disease and there is no research on how South Asians ethnicity might impact on the prevalence and severity of OSA amongst extreme obese individuals, we aim to address that in this thesis. For individuals with diabetes mellitus with extreme obesity, there is also limited evidence on the effect of nocturnal hypoxaemia on glycaemia, and more importantly on the microvascular complications. The aim of this thesis is therefore to improve the understanding of the effect of OSA in very severe obese individuals. The following are the objectives of the thesis:

1. Chapter 2 of the thesis will aim to compare the prevalence and severity of OSA, co-morbidities and glycaemic control in two different ethnic groups with severe obesity. Part of the chapter will also demonstrate the difference in sleep parameters between genders;

2. Chapter 3 aim to examine the effect of OSA on glycaemia among DM with severe obesity;
3. Chapter 4 aim to investigate the prevalence, risk factors and relationship between OSA and diabetic retinal disease in a severely obese population;
4. Chapter 5 will aim to demonstrate the prevalence and relationship between OSA and diabetic kidney disease in severe obesity; and
5. Chapter 6 will aim to systematically review the effect of OSA on diabetic kidney and retinal diseases.

In summary, the rise in the prevalence of obesity globally has significant impact on healthcare services worldwide. One of the condition associated with obesity is OSA, this thesis aims to describe the effect of OSA on people with extreme obesity.

CHAPTER 2 - THE PREVALENCE AND SEVERITY OF OBSTRUCTIVE SLEEP APNOEA IN SOUTH ASIANS WITH SEVERE OBESITY

2.1 Introduction

2.1.1 Prevalence of obstructive sleep apnoea (OSA)

Several reports from the literature described the prevalence of OSA[94,96,97,100,102,122-135] in the Western,[94,100,123-125,131,133-135] Middle Eastern[122,129] and Asian populations.[96,97,102,126-128,130,132] As PSG is not easily performed and is costly for health services, a variety of approaches were utilised including using questionnaires;[100,122,124,128-130] screening questionnaires followed by PSG later;[94,123,126,127,131] portable home overnight sleep monitoring device.[134,135] In those studies which performed PSG, the criterion for the diagnosis of OSA based on AHI cut off points varied including AHI ≥5 events/hr[94,126,127,134] or 15 events/hr.[131] No data are available on the incidence of OSA. In this chapter, we described OSA prevalence in the general population, effect of age on OSA prevalence, OSA prevalence in obese populations and prevalence of OSA in South Asians.

2.1.2 Prevalence of OSA in the general populations

The prevalence of OSA in Western populations has been reported to be between 1 and 28%.[94,123-125,134] The large difference in the prevalence rate is likely to be due to heterogeneity on the diagnosis of OSA and populations studied. For example, some studies reported the prevalence rate of OSA while others reported prevalence of OSA syndrome. A diagnosis of OSA with sleep symptoms has been referred to as OSA syndrome in some studies.[77] The Wisconsin Sleep Cohort study reported a prevalence of 16.5% amongst middle-age adults

(24% in males and 9% in females).[94] The prevalence is lower at about 3% (about 4% in males and 1 to 2% in females) for OSA syndrome.[94,98] A review of 12 studies performed by Davies and colleagues concluded that the prevalence of OSA syndrome in Western population is likely to be between 1 and 5% in males but much lower rate amongst females (effect size not reported) in the general population.[125] Possible reasons for the gender variation include anatomical differences and hormonal influences. The effects of gender on OSA will be elaborated further in the discussion section.

Two studies from Australia reported very different prevalence rates: 3.6% by Olson et al.[131] and 26% by Bearpark and colleagues.[135] This wide difference can be explained by the cut off points of AHI as the diagnosis of OSA. Olson et al. used AHI ≥15 events/hr[131] while Bearpark et al. engaged AHI ≥5 events/hr.[135] A study from Spain showed a prevalence of 27% for AHI ≥5 events/hr.[134] Therefore, the true prevalence of OSA in the general population is likely to be between 16 and 27% while OSA syndrome is likely to be 1 and 5%.

2.1.3 Prevalence of OSA and the effect of aging

The prevalence of OSA increases with aging and peaks at middle-age as illustrated in Table 2-1. A Spanish study performed PSG on 555 individuals aged between 30 and 70 years.[134] OSA was defined as AHI ≥5 events/hr. The researchers reported a gradual increase in the prevalence of OSA as age rises. The SHHS study however, demonstrated a gradual increase in OSA prevalence until the age 60 years whereby the prevalence plateaued.[63] The San Diego study conducted PSG on 420 individuals aged ≥65 years.[136] Based on the diagnosis of AHI ≥10 events/hr, overall prevalence was 62% (70% in men and 56% in women). Also, the prevalence of OSA remains almost stagnant across age group 60-69, 70-79 and 80-89 years.

Table 2-1: Age-specific prevalence of OSA from four different studies

	Prevalence of OSA Study			
	Spain*‡ [134]	SHHS* [63]	San Diego⌈ [136]	Pennsylvania‡‖ [123]
Age group (years)				
20-29	-	-	-	0.4%
30-39	9%	-	-	1.5%
40-49	46%	29%	-	2.8%
50-59	28%	40%	-	5.5%
60-69	52%	51%	61%	4.1%
70-79	-	54%	62%	2.5%
80-99	-	56%	65%	-

OSA = obstructive sleep apnoea, SHHS = Sleep Heart Health Study.
‡Percentage represents the males only
*OSA diagnosis based on AHI ≥5 events/hr
⌈OSA diagnosis based on AHI ≥10 events/hr
‖ OSA diagnosis based on AHI ≥10 events/hr with sleep symptoms (OSA syndrome)

The Pennsylvania study was specifically designed to examine the effect of age on OSA syndrome.[123] Only men were recruited and a total of 741 PSG were performed. Prevalence of OSA increases as age increases (AHI ≥10 events/hr). For the age group 20 and 44 years, OSA prevalence was 3.2% (95% CI: 1.6 to 6.4); for age group 45 to 64 years, prevalence was 18.8% (95% CI: 15.4 to 22.8) and age ≥65 years, prevalence was 24.8% (95% CI: 16.3 to 35.7). However, there seems to be a quadratic relationship between age and OSA syndrome. Prevalence of OSA syndrome was 1.2% (95% CI: 0.4 to 3.8), 4.7% (95% CI: 3.1 to 7.1) and 1.7% (95% CI: 0.3 to 9.1%) for age groups 20 to 44 years, 45 to 64 years and ≥65 years, respectively. In addition, the severity of hypoxaemia based on minimum oxygen saturation also shares the quadratic relationship as shown in Figure 2-1. The slope for AHI ≥20 events/hr was 0.22 (95% CI: 0.01 to 0.43) after adjusted for BMI, this suggests that younger men with OSA were more hypoxic compared to older men.

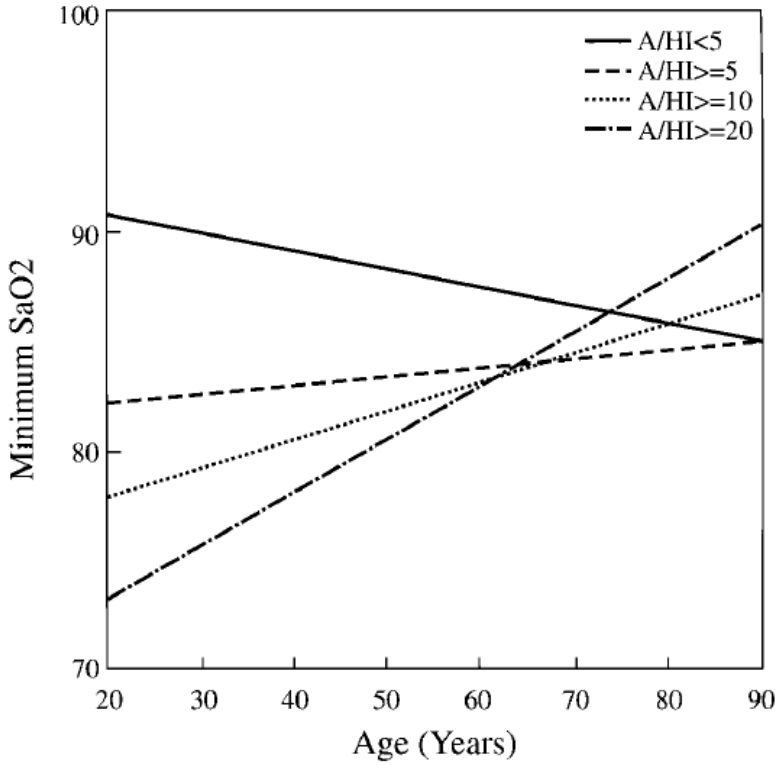

Figure 2-1: Relationship between minimum oxygen saturation (SaO2) and age based on 4 apnoea-hypopnoea indexes. AHI represents apnoea-hypopnoea index and lower minimum oxygen saturation indicates greater hypoxaemia. Reprinted with permission of the American Thoracic Society. Copyright © 2014 American Thoracic Society. Reference: Bixler EO, Vgontzas AN, Ten Have T, Tyson K, Kales A. 1998. Effects of age on sleep apnoea in men: I. Prevalence and severity. American Journal of Respiratory and Critical Care Medicine;157:144-8. Official Journal of the American Thoracic Society.

The mechanisms of the quadratic effect of age on OSA remain unclear. It may be that individuals with OSA suffered from premature mortality and those who live past 65 years might be a 'fitter population on the whole' based on Darwin's theory on survival of the fittest.[123] Also, it may be that the influence of obesity or weight gain in the elderly might be less significant compared to middle aged individuals.[137] Another possibility is that since hereditary diseases tend to be more severe in the young and in middle aged, OSA may be associated with some form of genetic predisposition.[123]

2.1.4 Prevalence of OSA in the obese populations

Unsurprisingly, the prevalence of OSA amongst obese individuals is much higher.[138,139] All epidemiological studies of OSA in this group of individuals were performed in a hospital/clinic setting using PSG. The largest study based on data collected from 22 European centres reported a prevalence of 79.4%[138] in individuals with a mean BMI of 31Kg/m^2 and mean age of 51.8 years.[138] The Sleep Ahead study reported a higher OSA prevalence of 86% in obese (mean BMI 36.5Kg/m^2), older (mean age 61years) DM patients.[139]

Amongst very severe obese individuals (BMI ≥40Kg/m^2), the prevalence of OSA has been found to be between 61 and 98%.[95,140-145] The majority of these studies were on patients who needed bariatric surgery.[95,143-145] The two largest studies were from the USA (349 patients)[145] and Singapore (350 patients).[144] They reported an OSA rate of 83%[145] and 65%[144], respectively. The discrepancy may be due to the latter population being generally young (9 year age difference) and less obese (10Kg/m^2 lower BMI)[144] compared to the US study.[145] Indeed, a stepwise relationship has been shown by Lopez and colleagues that the prevalence of OSA rises as BMI increases: 71% in those with severe obesity (BMI between 35 and 39.9Kg/m^2), 74% in those with BMI between 40 and 49.9Kg/m^2, 77% in those BMI between 50 and 59.9Kg/m^2 and 95% in those with BMI ≥60Kg/m^2.[146]

2.1.5 Prevalence of OSA in South Asians

Epidemiological studies performed in South Asians countries (predominantly India) have found a prevalence of OSA to be between 14 and 27%[96,102,147] which is consistent with the studies from Western countries. However, compared to white Europeans, South Asians (those of Indian/Pakistani/Bangladeshi/Sri Lankan origin) residing in Western countries, have a

higher prevalence of DM, dyslipidaemia, and CVD.[148] Considering these conditions commonly accompany OSA, it is possible that South Asians who live in Western countries are at greater risk of OSA compared to white Europeans. This potential ethnic difference has not, however, been comprehensively examined, particularly in very severely obese individuals. A recent UK study compared the prevalence of OSA in South Asians and white Europeans but found no difference between groups.[124] This study, however, characterised OSA based on subjective responses to the Berlin questionnaire, which has not previously demonstrated a high level of diagnostic specificity[149] and does not provide an objective measure of OSA.

We therefore sought to assess potential differences in objectively-determined OSA prevalence and severity between severely obese South Asians and white Europeans. We specifically assessed a severely obese patient population since this group is at increased risk of CVD. Based on the evidence that South Asians have a higher prevalence of metabolic and cardiovascular morbidities, we hypothesised that severely obese South Asians would have a higher prevalence and more severe OSA compared to white Europeans.

2.2 Aim and objectives

The aim of this chapter is to examine the prevalence and severity of OSA, co-morbidities and glycaemic control in two different ethnic groups with severe obesity. The following are the objectives of the study:

1. To estimate the prevalence of OSA in South Asians and white Europeans with very severe obesity;
2. To compare the severity of OSA amongst South Asians and white Europeans with very severe obesity;
3. To examine the range and number of co-morbidities and glycaemic control between South Asians and white Europeans with very severe obesity; and
4. To demonstrate potential differences in respiratory parameters between males and females.

2.3 Methods

2.3.1 Study design and study participants

Routine clinical data were prospectively collected from consecutive adults with age greater or equal to 18 years who were attending a West Midlands regional specialist weight management service from January 2009 to December 2011 at the Heart of England NHS Foundation Trust, Birmingham, UK. Over 10% of the West Midlands inhabitants with an estimated total of 5.5 million individuals are South Asians.[150] The majority of South Asian patients local to the specialist weight management service originate from the Mirpur region in Pakistan. All patients were referred to the service based on the following criteria:

1) BMI $\geq$35Kg/m^2 with at least one co-morbidity, or
2) BMI $\geq$40Kg/m^2 without a comorbidity.

The aim of the specialist service is to provide a comprehensive clinical assessment of patients with obesity and the associated co-morbidities, and the development of a therapeutic plan that may include bariatric surgery. Patients and referring physicians are informed of the pathways and processes within the service and the treatment options available.

In the specialist service, all patients are formally assessed for sleep-disordered breathing (SDB). Anonymised data from patients attending the service with OSA assessment were included. Data available included age, gender, ethnicity, objective anthropometry (height, weight, BMI), systolic and diastolic blood pressure (BP), and the presence of obesity-related co-morbidities. The co-morbidities recorded included presence or absence of type 2 diabetes (DM), hypertension and coronary artery disease (CAD).

2.3.2 Respiratory sleep monitoring

The Embletta^{TM} (Embla systems) home sleep portable monitoring device was employed as part of the trust's clinical service protocol. Patients were given a demonstration of the equipment by a trained sleep physiologist on the afternoon of the test night. Measurement channels included airflow using a nasal pressure sensor device, chest and abdominal movements (inductance plethysmography), oxygen saturation (O_2), and heart rate using a finger oximetry probe. The sleep physiologist observed the patient undergoing self-application of the equipment before leaving the sleep centre. Patients also completed a diary of sleep-wake times for the test night, which was completed the morning after the test night. This was used to determine sleep onset and wake times as well as subjectively confirming that the patient achieved at least four hours sleep duration. South Asian and white European patients did not differ in the interpretation of sleep diaries, which were in agreement with the respiratory data as seen by movement artefacts. Equipment and diaries were returned the next day, and data were downloaded and reviewed. Studies with at least 4 hours of good quality respiratory signals were considered acceptable. A retest was offered if data were inadequate. All respiratory data were manually scored by blinded trained sleep-respiratory physiologists and rescored by a sleep physician and author DB for confirmation. All scoring was blinded to patient characteristics including name and ethnicity.

All respiratory parameters were scored based on the standard American Academy of Sleep Medicine guidelines.[151] To summarise, an apnoea was defined as a complete cessation of airflow for at least 10 seconds while hypopnoea was defined as a reduction of at least 30% of

airflow accompanied by at least 4% reduction in oxygen saturation with the presence of thoracic and abdominal movement. Central events were characterised by the absence of thoracic and abdominal movements. The AHI was calculated as the average number of episodes of apnoea and hypopnoea per hour of sleep. AHI was calculated for the whole night and was not differentiated for supine and non-supine positions.

Respiratory parameter data collected included: AHI, mean and minimum oxygen saturation during sleep (%), and percentage of time spent under 90% oxygen saturation while asleep (%TST<90). OSA was defined using the standard AHI cut-point of at least 5 events/hr.[73] Mild, moderate and severe OSA were defined as AHI of between 5 and 15 events/hr, 15 and 30 events/hr, and ≥30 events/hr, respectively.

The anonymised data collected for analysis was conducted as part of service evaluation and did not require formal ethical committee approval, as recommended by the UK National Research Ethics Service.[152] This study used the current available database from the weight management service and was designed to evaluate the characteristics of the patients referred to our local service. The results of the study will provide valuable insights on current clinical need and care of very severe obese individuals. We also hope to provide more information to assist in the improvement and design of the weight management services within the NHS.

2.3.4 Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences version 19 (SPSS, Chicago, IL). Normality of continuous data was ascertained through visual inspection and the Kolmogorov-Smirnov test. Normally distributed data are reported as mean ± standard

deviation (SD), while non-normally distributed variables are reported as median with inter-quartile range (IQR). Mann Whitney U-tests and independent t-tests were used for non-parametric and normally distributed data, respectively. Differences between categorical variables were analysed using chi-square test. We ran a series of Mann Whitney U-tests to assess the potential difference in AHI, mean O_2 saturation, minimum O_2 saturation, and %TST<90 according to ethnicity.

Natural log transformation was performed for variable AHI to make it normally distributed prior to multivariate linear regression analysis. As some of the AHI results were zero, the natural log transformation was performed on AHI+1. As AHI was in the form of a continuous data, three models were explored using linear regression analysis. The base model was unadjusted; Model 1 adjusted for age, sex, ethnicity (white Europeans/South Asians) and/or BMI, as appropriate; Model 2 further adjusted for the presence/absence of co-morbidities (presence of type 2 diabetes, hypertension, CAD and insulin treatment). The factors adjusted included in the adjusted models were those that are considered to be clinically important and the rationale for including each factor is discussed elsewhere (see table 2-2). Clinically relevant results from log-transformed data were back-transformed and results are reported as unstandardised beta coefficients (β) along with 95% confidence intervals (CI), for AHI+1. Results were considered statistically significant when $p<0.05$. No subgroup or sensitivity analysis was carried out.

Table 2-2: Factors adjusted and the appropriate sections discussing the rationales behind

Factors	**Sections**
Age	Introduction of Chapter 2 (section 2.1.3)
Gender	Chapter 2's discussion (section 2.5.5)
Body mass index	Chapter 1 (section 1.3.4)
Type 2 diabetes	Chapter 3 (section 3.5.2)
Insulin treatment	Chapter 4 (section 4.5.5)
Hypertension	Chapter 1 (section 1.3.3) and chapter 4 (section 4.5.3)
Coronary artery disease	Chapter 1 (section 1.3.3)

2.4 Results

2.4.1 Study characteristics

A total of 343 consecutive patients referred to the Specialist Weight Management Clinic underwent overnight sleep assessment during the study period. Data from four patients were not analysable, seven patients had missing anthropometry data, and 24 patients were from another ethnic group and were excluded, leaving complete data on 308 patients for subsequent analyses (Figure 2-2).

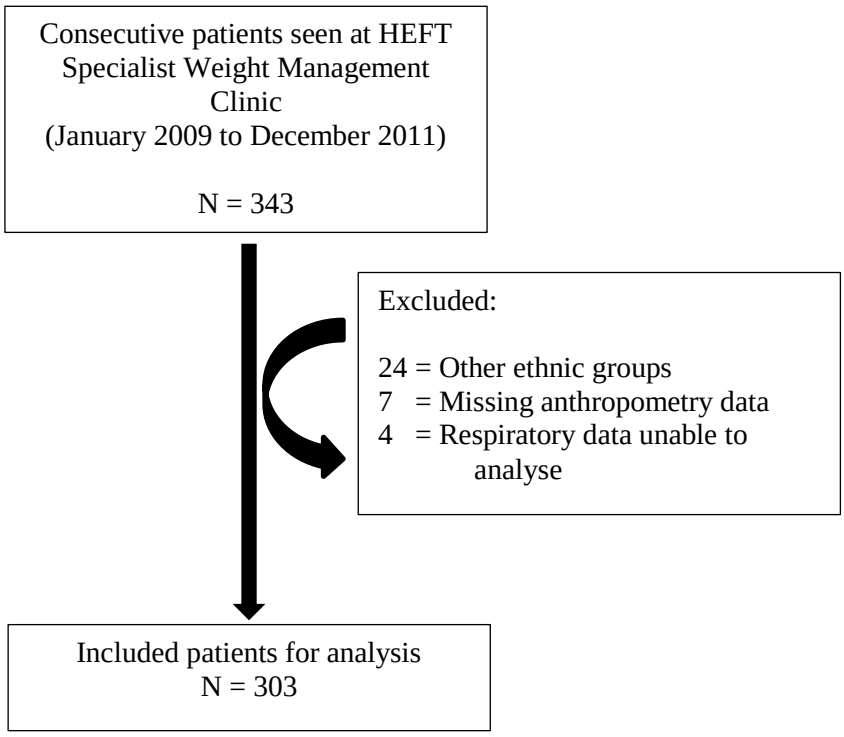

Figure 2-2: Flow diagram of the number of referrals seen at HEFT Specialist Weight Management Clinic, and those included in the study
HEFT – Heart of England NHS Foundation Trust

Table 2-3: Patient characteristics in 308 obese patients according to ethnic group

	White European (n=268)	**South Asians (n=40)**	**p value**
Age (years)	46.6 ± 12.2	43.8 ± 10.9	0.17
Sex n (%)			0.10
Male	75 (27.3)	16 (40.0)	
Female	200 (72.7)	24 (60.0)	
Weight (Kg)	133.2 ± 24.9	134.9 ± 30.9	0.71
BMI (Kg/m^2)	48.6 ± 8.0	49.8 ± 10.9	0.42
Systolic BP (mmHg)*	142 ± 18	142 ± 15	0.99
Diastolic BP (mmHg)*	87 ± 12	82 ± 14	0.09
Overall prevalence of OSA (AHI ≥5) n (%)	177 (66.0)	34 (85.0)	**0.017**
OSA severity n (%)			**0.015**
No OSA (AHI <5)	91 (34.0)	6 (15.0)	
Mild OSA (AHI 5-15)	77 (28.7)	10 (25.0)	
Moderate OSA (AHI 15-30)	42 (15.7)	7 (17.5)	
Severe OSA (AHI >30)	58 (21.6)	17 (42.5)	
AHI (per hour)	9.0 (3.4, 26.6)	24.0 (9.3, 57.6)	**<0.01**
Co-morbidities			
DM %	33.1	60.0	**<0.01**
HTN %	36.6	42.5	0.48
CAD %	7.3	7.5	0.96
Presence of co-morbidities (DM, HTN or CAD)			**0.02**
None %	50.9	27.5	
One %	28.7	37.5	
Two %	16.4	32.5	
Three %	4.0	2.5	
HbA_{1c} (%)	6.3 (5.8, 7.3)	7.4 (6.3, 8.6)	**<0.01**
HbA_{1c} (mmol/mol)	45 (40, 56)	57 (45, 71)	**<0.01**
% on glucose-lowering medication	25.1	50.0	**<0.01**
Number of DM medications			**<0.01**
None %	74.9	50.0	
One %	10.5	10.0	
Two %	10.2	30.0	
Three or more %	4.4	10.0	

Data are reported as mean ± standard deviation or median (IQR), unless otherwise stated.
BMI=body mass index; BP=blood pressure; AHI = apnoea-hypopnoea index; DM=diabetes mellitus; HTN=hypertension; CAD=coronary artery disease, HbA_{1c}=glycosylated haemoglobin A_{1c}.
* Blood pressure data presented were available in 212 patients.
p values were calculated using either independent t-test, Mann Whitney U-test or chi-square, as appropriate.

The study sample characteristics are shown in Table 2-3. The majority of the sample was white European (87.3%) reflecting the ethnic composition of the local Birmingham population.[150] The majority of patients were female (71.1%), in agreement with attendance at services catering for severely obese patients.[153] There was no statistical significant gender difference between the ethnic groups (60.0% females in South Asians vs. 72.2% in White Europeans, p=0.10). No significant differences were found between ethnic groups for age (mean age 43.8±10.9years for South Asians vs. 46.6±12.2years for white Europeans, p=0.17), BMI (49.8±10.9 vs. 48.6±8.0Kg/m^2, p=0.42) or body weight (134.9±30.9 vs. 133.2±24.9Kg, p=0.71). The measured BP in both groups was also similar: 142±15 vs. 142±18mmHg for systolic BP (p=0.42) and 82±14 vs. 87±12mmHg (p=0.09) for diastolic BP.

2.4.2 Co-morbidities and glycaemic results

The percentage of South Asians with type 2 diabetes mellitus was, however, almost double that observed in white Europeans (60.0% vs. 33.1%, p<0.01) as shown in Table 2-3. A greater proportion of the South Asian group also took glucose-lowering medication (50.0% vs. 25.1%, p<0.01) and had significantly worse glycaemic control compared to white Europeans (HbA_{1c} 57 vs. 45mmol/mol, p<0.01). Poor glycaemic control was also reflected in the total number of hypoglycaemic medications required in the South Asians group (p<0.01). Although the prevalence of hypertension (42.5% vs. 36.6%, p=0.48) or coronary artery disease (7.5% vs. 7.3%, p=0.96) alone were not significantly different, South Asians had a significantly greater prevalence of total number of co-morbidities compared to white Europeans (p=0.02).

2.4.3 Prevalence and severity of OSA

The prevalence of OSA (AHI ≥5 events/hr) in our study population was 69%. The South Asian group had a significantly higher AHI compared to white Europeans (24 [IQR: 9.3, 57.6] vs. 9 [IQR: 3.4, 26.6] events/hr, p<0.01). South Asians had significantly greater prevalence of OSA (85% vs. 66%, p=0.017) and more severe OSA (p=0.015) compared to white Europeans as shown in Figure 2-2. In those with severe OSA (AHI >30 events/hr), South Asians were significantly younger (45±13years) than white Europeans (52±11years), p=0.04.

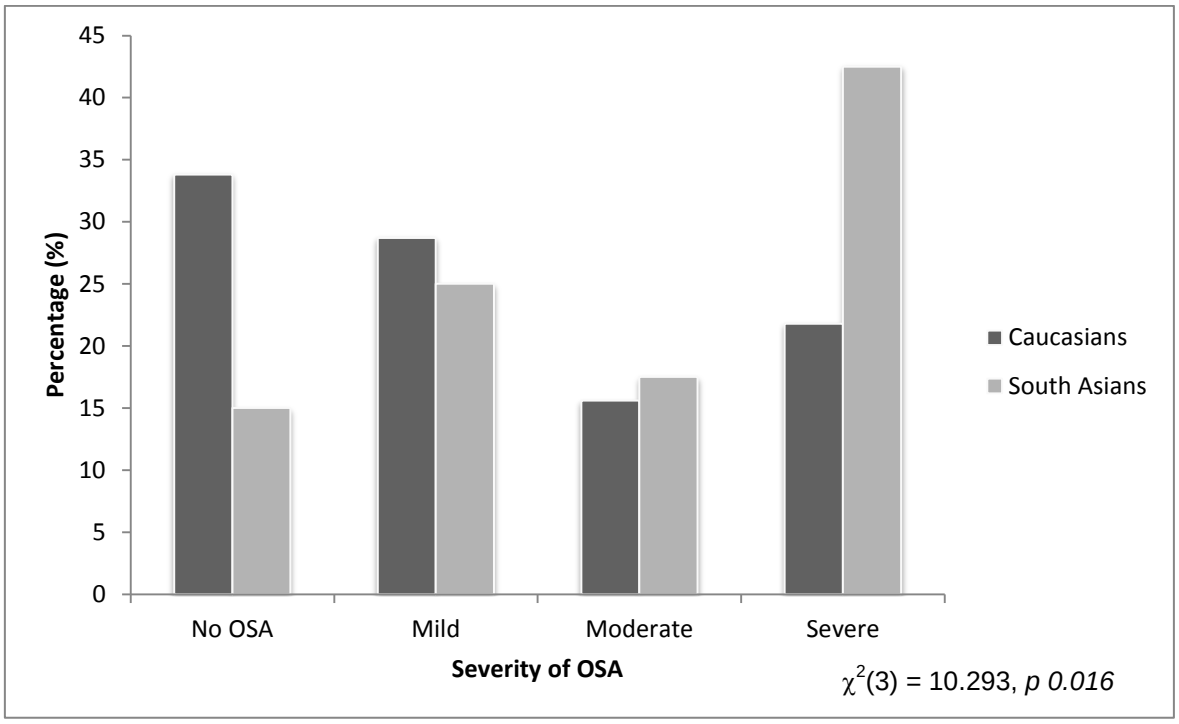

Figure 2-3: The prevalence and severity of obstructive sleep apnoea in South Asians and Caucasians.

Table 2-4 highlights a number of statistically significant differences according to ethnic group in relation to three of the four SDB measures. South Asians had a greater AHI (p<0.01). They also spent more time under 90% oxygen saturation (8.4% [IQR: 1.0, 24.3] vs. 2.4% [IQR: 0.2, 16.0], p=0.03) and had significantly lower minimum oxygen saturation (76% [IQR: 64, 84]

vs. 83% [IQR: 77, 87], ($p<0.01$) compared to white Europeans. The mean oxygen saturation was not significantly different between the two ethnic groups (93% [IQR: 91, 95] vs. 93% [IQR: 92, 95], p=0.41).

Table 2-4: Comparison of AHI, mean oxygen saturation, time under 90% oxygen saturation and minimum oxygen saturation between white Europeans and South Asians

	White European (n=268)	**South Asian (n=40)**	**p value**
AHI (events per hour)	9 (3.4, 26.6)	24 (9.3, 57.6)	**<0.01**
Mean O_2 saturation (%)	93 (92, 95)	93 (91, 95)	0.41
% time spent under 90% O_2 saturation	2.4 (0.2, 16.0)	8.4 (1.0, 24.3)	**0.03**
Minimum O_2 saturation (%)	83 (77, 87)	76 (64, 84)	**<0.01**

Data are presented as median (IQR).
IQR = inter-quartile range; AHI = apnoea-hypopnoea index; O_2 = oxygen, % = percentage.
p values were calculated using Mann Whitney U-test.

Table 2-5 demonstrates a significant positive association between South Asian ethnicity. Without any adjustments, regression analysis showed a 95% increase in AHI+1 (β=1.95, 95% CI: 1.29 to 2.95) in South Asians compared to white Europeans. This association remained after adjusted for age, sex and BMI (β=1.83, 95% CI: 1.28 to 2.63), and, after further adjustment for DM, insulin usage, hypertension and coronary artery disease was β=1.84 (95% CI: 1.27 to 2.65) compared to white Europeans. Thus, South Asian ethnicity was significantly associated with an 84% (95% CI: 27% to 165%) increase in AHI+1, after adjustment for potential confounders.

As expected, male gender was also an independent predictor of increasing AHI+1. The unadjusted model showed a 131% (95% CI: 72% to 211%) increase in AHI+1 for males compared to females. The association persisted after adjustment for age, BMI and ethnicity (β=2.38, 95% CI: 1.81 to 3.12) as well as with addition adjustment for comorbidities in Model 3 (β=2.42, 95% CI: 1.84 to 3.20).

Similarly, positive linear relationships were observed for age and BMI with AHI+1. In the unadjusted model, for every increment increase in age and BMI, there was an expected 3% (95% CI: 2% to 4%) and 3% (95% CI: 2% to 5%) increase in AHI+1, respectively. The associations persisted for both age and BMI after adjustment for sex and ethnicity: age (β=1.03, 95% CI: 1.02 to 1.04) and BMI (β=1.04, 95% CI: 1.03 to 1.06). Further adjustment for DM, hypertension, coronary artery disease and insulin as shown in model 3, illustrated the expected increase in AHI+1 was 3% (95% CI: 2% to 4%) for each unit increase in age (year) and 5% (95% CI: 3% to 6%) for BMI (Kg/m^2).

Table 2-5: Independent predictors of AHI+1 in 308 obese patients. Data were presented as unstandardised beta coefficients (95% CI) from multivariate linear regression analyses.

Predictor	**Models**		
	Unadjusted	**Model 1**	**Model 2**
South Asian	**1.95 (1.29-2.95)**	**1.83 (1.28-2.63)**	**1.84 (1.27-2.65)**
Age (years)	**1.03 (1.02-1.04)**	**1.03 (1.02-1.04)**	**1.03 (1.02-1.04)**
Males	**2.31 (1.72-3.11)**	**2.38 (1.81-3.12)**	**2.42 (1.84-3.20)**
BMI (Kg/m^2)	**1.03 (1.02-1.05)**	**1.04 (1.03-1.06)**	**1.05 (1.03-1.06)**
Diabetes Mellitus	**1.50 (1.13-2.00)**	Not applicable	0.83 (0.62-1.12)
Hypertension	**1.70 (1.28-2.26)**	Not applicable	1.21 (0.91-1.61)
Coronary Artery Disease	1.49 (0.87-2.55)	Not applicable	0.75 (0.46-1.23)
Insulin	**2.32 (1.47-3.68)**	Not applicable	1.51 (0.95-2.38)

Unadjusted: univariate models
Model : adjusted for age, sex, BMI, ethnicity, as appropriate.
Model 2: further adjusted for diabetes mellitus, hypertension, coronary artery disease, and insulin.
BMI = body mass index.

2.4.4 Gender differences

The differences in the patient characteristics between males and females were shown in Table 2-6. Male patients were slightly but not significantly older than females. There were no differences in ethnicity and systolic and diastolic BP between the genders. Females were heavier than males (BMI 49.9 vs. 46.3 Kg/m^2, p=0.001). A greater proportion of male patients suffered with DM as well as CAD with significant proportion of males having two or more co-morbidities compared to females. Males were also more likely to be on glucose-lowering medications compared to females (p=0.009).

A greater proportion of males have OSA compared to females (87% vs. 61%, p<0.001). Males were also more likely to have moderate and severe OSA compared to females (p<0.001). The AHI was significantly higher in males (19.1, IQR: 8.0, 55.0 vs. 7.9, IQR: 3.0, 20.0 events/hr, p<0.001) with a lower minimum oxygen saturation (80%, IQR: 71, 84 vs. 84%, IQR: 77, 88, p<0.001). The time spent under 90% oxygen saturation during sleep (p=0.002) as shown in Table 2-7.

Table 2-6: Patient characteristic differences between males and females with OSA

	Males N = 90	**Females N = 218**	**P value**
Age (years)	48 ±13	45 ±12	0.52
BMI (Kg/m^2)	46.3 ± 7.6	49.9 ± 8.5	**0.001**
Systolic BP (mmHg)	146 ± 15	141 ±19	0.83
Diastolic BP (mmHg)	86 ±9	86 ±13	0.85
Ethnicity (%)			
White Europeans	82.2%	90.0%	0.108
South Asians	17.8%	3.9%	
Diagnosis of OSA (%)	61%	87%	**<0.001**
Classification of OSA			
Mild	25.6%	29.4%	**<0.001**
Moderate	21.1%	13.8%	
Severe	40.0%	17.9%	
Co-morbidities (%)			
DM	53.9%	29.4%	**<0.001**
HTN	43.8%	24.8%	0.139
CAD	13.5%	4.8%	**0.008**
Presence of co-morbidities			
None	0%	0%	**0.001**
One	33.3%	53.2%	
Two	31.1%	29.4%	
Three	35.6%	17.4%	
% on DM Medications	42.2%	23.4%	**0.001**
Number of DM medications (%)	57.8%	76.6%	**0.009**
None	14.4%	9.2%	
One	18.9%	10.6%	
Two	8.9%	0.4%	
Three or more			

Data are reported as mean ± standard deviation, unless otherwise stated.
BMI=body mass index; BP=blood pressure; OSA = obstructive sleep apnoea; DM=diabetes mellitus; HTN=hypertension; CAD=coronary artery disease.
* Blood pressure data presented were available in 212 patients.
p values were calculated using either independent t-test, Mann Whitney U-test or chi-square, as appropriate.

Table 2-7: Comparison of apnoea-hypopnoea index, mean oxyhaemoglobin saturation, time under 90% saturation and minimum oxygen saturation between genders

		Median (IQR)	p value
Apnoea-hypopnoea Index (events/hour)		19.1 (8.0, 55.0)	**<0.001**
	Males		
	Females	7.9 (3.0, 20.0)	
Mean O_2 saturation (%)			
	Males	93.0 (91.0, 93.7)	**<0.001**
	Females	93.9 (92.0, 5.0)	
Time under 90% O_2 saturation (%)			
	Males	4.4 (1.0, 25.4)	**0.002**
	Females	1.8 (0.1, 16.0)	
Minimum O_2 saturation (%)			
	Males	80 (71, 84)	**<0.001**
	Females	84 (77, 88)	

Data are presented as median (IQR).
IQR = inter-quartile range; AHI = apnoea-hypopnoea index; O_2 = oxygen.
p values were calculated using Mann Whitney U-test. ** signify p value of < 0.01

2.5 Discussion

2.5.1 Patient demographics

In this study of very severe obese patients attending a specialist weight management clinic, majority of patients were females. This is consistent with the data observed in other weight management services which offer bariatric surgeries.[46,153-155] Indeed, the Health Survey for England reported that in 2011, there were at least twice as many females with Class III obesity compared to males (3.5% vs. 1.5%)[156] which is reflected in the higher female population seen in weight management services. An observation study of the Hospital Episode Statistics database from April 2000 to March 2008 on the laparoscopic bariatric surgery performed in England also found that the majority of the patients who had had bariatric surgery were females (80.8%).[157]

In our study, there were no difference in the weight and BMI in both our ethnic groups. The mean BMI was 48.8Kg/m^2 for our patients and this is at the higher end of the mean BMI range between 45 and 51Kg/m^2 found in most OSA prevalence studies in very severely obese individuals.[95,140-143] Although white Europeans were slightly older (47years) compared to South Asians (44years) in our study, this was not statistically significant. Other prevalence studies on OSA in very severely obese individuals have slightly younger patients with age ranging between 37 and 43 years.[95,140-143]

2.5.2 Prevalence of obstructive sleep apnoea

The general prevalence of OSA has been reported to be between 16 and 27%[94,123,131,134] and is significantly lower than our study (68.5%), which has only Class III obese patients. Moreover, the OSA prevalence for South Asians ethnic group in our study is 3 times higher

than the general population studies from India.[96,102,147] Studies by Reddy et al. and Sharma et al. generated a random list of participants using information from their respective electoral rolls in South Delhi.[96,102] Udvadia and colleagues recruited a random sample of healthy males who presented to Hinduja National Hospital for health check-ups.[147] All three Indian studies were conducted in a 2 stage process: Stage 1 consists of screening population to identify habitual and non-habitual snorers, and Stage 2 involves random selection of a sample from the screened population for PSG. A proportion of habitual snorers (between 15% and 100%) and non-habitual snorers (between 5% and 25%) were invited for PSG. Subsequently, overall population prevalence of OSA was estimated. Apart from the differences in recruitment, obesity levels between the Indian populations studies (mean BMI ranges from 24.3 to 27.0Kg/m^2)[96,102,147] and our South Asians sample, which only included vey severely obese patients, may partially explained the differences in OSA prevalence. The mean age of our patients (44years) is similar to the Indian studies which range from 41 to 48 years.[96,102,147] Our study also differed in gender distribution when compared to the Indian population studies as we have a greater proportion of females. The study by Udwadia and colleagues only consists of urban Indian males[147] while the other 2 Indian studies are slightly skewed towards higher proportions of males.[96,102]

OSA is increasingly appreciated as an important contributor for CVD[93,158,159] risk and mortality.[93,160,161] The prevalence and severity of OSA in the South Asian population, a group at increased risk of CVD, have not been comprehensively examined. Our study is the first to objectively investigate prevalence and severity of OSA in severely obese South Asians residing in the west. Our results demonstrated that South Asians have a significantly greater prevalence of OSA with more severe OSA compared to white Europeans. To date, only one

Western study has investigated ethnic differences in OSA, which reported no significant ethnic differences.[124] The UK study by Brady and colleagues recruited participants from a community setting consists of urban, suburban and rural areas. As the study by Brady and colleagues was a population-based study, obesity was not part of the inclusion criteria. Furthermore, diagnosis of OSA was determined through the Berlin questionnaire,[124] which is a screening, rather than a diagnostic tool. Participants who scored high risk in the Berlin questionnaire are considered likely to have OSA.[124] A community-based study on 100 participants showed that the Berlin questionnaire is very useful for OSA risk stratification with a sensitivity of 0.86 for those who have a high risk score and specificity of 0.77 for those with a low risk score.[72] However, a recent Chinese community study of 143 participants reported a sensitivity of 0.89 with a specificity of 0.35 for moderate-to-severe OSA using the Berlin questionnaire.[162] No study has investigated the performance of the Berlin questionnaire in South Asians population. Therefore, the study by Brady and colleagues[124] may not represent the true prevalence of OSA in South Asians and white Europeans residing in the UK. Given that our patient group was very severely obese, a greater prevalence of OSA was anticipated compared to the study by Brady and colleagues (69% vs. 28%).[124]

In very severely obese populations (BMI $\geq$40Kg/m^2), prevalence rates of OSA have been reported to be between 61% and 98%.[95,140-145] In our patients who were attending the regional specialist weight management service, the prevalence of OSA was comparable to these reports. This is in spite of our patients being slightly older (mean age 46years) compared to the other studies (mean age range between 37 and 43years).[95,140-143] The prevalence of OSA in obese populations is clearly elevated compared to the general population,[94,96] which is not surprising given the close association of obesity with OSA.[94,95,98] The Wisconsin Sleep Cohort

which performed PSG in 602 individuals between age 30 and 60 showed that for each standard deviation increase in BMI, there was a threefold increase in the risk of development of OSA.[94] Likewise, Bixler and colleagues assessed 1741 individuals in Pennsylvania and reported that obese individuals have a RR of 11.5 (95% CI: 3.3 to 33.8) in females and RR of 7.0 (95% CI: 3.4 to 13.9) in obese males compared to non-obese counterparts in developing OSA.[98]

2.5.3 Severity of obstructive sleep apnoea

We showed, after adjustment, that South Asian ethnicity was significantly associated with an 84% increase in AHI+1. While there were no significant differences in BMI between the South Asian and white European groups in our sample, South Asians have greater cardiovascular risk for an equivalent BMI.[163] This has resulted in adoption of lower BMI cut-points for overweight/obesity for South Asians by the WHO.[163] A marker for adiposity is visceral adipose tissue (VAT). The Multicultural Health Assessment Trial (M-CHAT) was a cross sectional study aimed to compare adiposity levels between 4 ethnicities: 201 Europeans, 195 Aboriginals, 219 Chinese and 207 South Asians.[164] The South Asians were from Bangladesh, India, Nepal, Pakistan and Sri Lanka. The participants were matched for ethnicity, sex and BMI range (≤24.9, between 25.0 and 29.9 and ≥30.0Kg/m^2). The results showed that South Asians have significantly greater VAT compared to Europeans (115.2, IQR: 87.2 to 159.0 vs. 100.8, IQR: 73.6 to 142.9 cm^2, p=0.009). After adjusting for age, sex, BMI, total body fat, ethnicity, education, smoking, physical activity and humeral breadth, South Asians with ≤37.4Kg total body fat has greater VAT compared to Europeans. In our study, both the age and BMI were similar in the two ethnic groups. Nevertheless, the South Asians in our study most likely have greater visceral fat for the given BMI.

Apart from acting as a marker for adiposity, visceral fat is also found to significantly correlate with apnoea index or AHI.[67,165,166] Deegan and colleagues assessed 140 males and found that waist circumference, as a surrogate marker for visceral adiposity, correlated significantly to AHI (r^2=0.125).[67] A group of Japanese researchers investigated 37 individuals with a mean BMI of 36.5Kg/m^2 and mean age of 46.5 years through computer tomography (CT) imaging to assess visceral fat.[166] They reported that all individuals with visceral fat greater than 220 cm^2 have OSA syndrome while those with visceral fat less than 120 cm^2 do not suffer with OSA syndrome. The correlation between visceral adiposity and AHI was found to be significant at 0.63 in this study.[166] Another small study by Schafer and colleagues assessed 81 patients with a mean BMI of 30Kg/m^2 and mean age of 55 years.[165] Nuclear magnetic resonance imaging (MRI) was used to assess abdominal fat in 60 patients and they found a statistical significant correlation between AHI and visceral fat (r=0.29).[165] This greater propensity to visceral adiposity and the association with AHI may explain the greater prevalence and severity of OSA in South Asians.

It was also observed that in those with moderate to severe OSA, 58% of OSA was attributable to excess body weight.[167] While this percentage is likely to be much higher in those with very severe obesity, other factors including craniofacial anatomy and airway structure[168] and tone[169] may also be important. Cephalometric features associated with OSA include inferior position of the Hyoid bone,[170,171] greater mandibular prognathism,[170-172] larger tongue[170,173] and longer soft palate.[172] A few studies have reported ethnic differences in craniofacial morphology between Chinese/Far East and Caucasians,[168,174] which may predispose to OSA, especially in very severe obese individuals. Lam and colleagues examined participants from 2

regional sleep disorder centres: 164 Chinese in Hong Kong and 74 white Caucasians from Vancouver, British Columbia. They found that Chinese participants have a greater thyromental angle and smaller thyromental distance compared to Caucasians ($p<0.05$). The researchers also performed multivariate analysis and reported that larger thyromental angle is associated with OSA diagnosis adjusted for ethnic group, BMI and neck circumference ($p<0.05$).[168] Li and colleagues compared Far East Asian (Chinese, Korean and Japanese) and Caucasian men craniofacial profile. Participants were recruited from Stamford University. The researchers found that in Far East Asians, crowding of the retropalatal region due to reduction in cranial base dimensions as compared to more influence from the retroglossal region in Caucasians may explain the differences in prevalence and severity of OSA between these two ethnicities.[174] No studies have compared or studied the craniofacial morphology in South Asians. However, if South Asians have a similar profile as those from the Far East, as they do with adiposity increased for a given BMI,[175] South Asians may also have increased fat depositions in the palatal or upper airway region that may predispose these individuals to OSA.

In the South Asian with diabetes, it is also plausible that airway tone could have been altered through greater severity of diabetes and potential diabetic autonomic neuropathy. In a cross sectional study of 25 diabetes patients with autonomic neuropathy (AN) and 23 diabetes patients without AN, the prevalence of OSA was 26% in AN group and 0% in non-AN group ($p<0.01$).[176] In another recent cross sectional study of diabetic participants recruited randomly from two UK Birmingham hospitals, the researchers assessed diabetic peripheral neuropathy (DPN) using the Michigan Neuropathy Screening Instrument questionnaire and 10g monofilament test. OSA was assessed using a home portable device. Logistic regression

analysis demonstrated that OSA was associated with diabetic neuropathy (OR 2.72, 95% CI: 1.44 to 5.52) after adjusting for recruitment site, age, sex, ethnicity, DM duration, alcohol and smoking status, lipid profiles and medications for diabetes, hypertension, lipids and antiplatelets.[177] This suggests that neuropathy may cause pharyngeal collapsibility. Future studies could incorporate assessment of visceral obesity, craniofacial and airway anatomy. Whether there is also a genetic basis for the observed ethnic differences in OSA also requires exploration.

2.5.4 Obstructive sleep apnoea and co-morbidities

2.5.4.1 Hypertension and obstructive sleep apnoea

Although no significant differences were found in systolic BP between South Asians and white Europeans, the mean systolic BP (142mmHg) in our study was above the threshold for further investigation based on NICE guidance on the Clinical Guidance 127: Clinical management of primary hypertension in adults.[178] There was also a non-significant 4mmHg difference in diastolic BP between the two ethnic groups. A not significant higher proportion of South Asians had a diagnosis of hypertension compared to white Europeans (42.5% vs. 36.6%, p=0.48). These results are unsurprising because OSA is well known to be independently associated with systemic hypertension.[81-83,88-91,158,179-181]

Several general population and hospital/clinic based studies have concluded that there is an association between hypertension and OSA[81-83,88,89,158,179-181] except one study by Stradling and colleagues.[182] The study by Stradling and colleagues was performed in Oxford using overnight pulse oximetry and not PSG on 752 patients.[182] They found no correlation between hypertension and overnight hypoxaemia.[182] This Oxford group later performed a small case

control study with 45 matched OSA and control patients.[90] Individuals were matched for age, BMI, alcohol and smoking status, history of ischaemic heart disease and treated hypertension.[90] They reported a higher day and night time diastolic BP, greater night-time systolic BP and smaller nocturnal BP reduction.[90] The Sleep Heart Health Study,[81] a multicentre cohort study based in the US examined 6135 middle and older age individuals using a portable home device. Individuals had a 37% increase in risk for hypertension if they had AHI of ≥30 events/hr compared to an AHI <1.5 events/hr.[81] They also found dose-response relationship between AHI and the risk of hypertension.[81] This dose-response relationship is echoed by the Wisconsin Sleep Cohort Study, which investigated 1060 individuals using questionnaire and subsequently performed PSG in 305 participants with a mean BMI 29Kg/m^2 and mean age of 45 years.[82] Another large community study based in Pennsylvania performed PSG on 1741 individuals concluded that there was a strong association between hypertension and OSA especially in younger subjects.[83]

Several clinic based studies have also reported an association between BP and AHI.[4,88,89,179,180] Lavie et al. examined 2677 patients with PSG, mean age and BMI of 48.6 years and 30.5Kg/m^2, respectively and found that every unit increase in AHI event was associated with a 1% increase in the risk of hypertension while every 10% decrease in oxygen saturation is associated with 13% increase in risk of hypertension.[4] These studies[4,81-83,88-90,181] all support the existence of an association between AHI and BP. Longitudinal studies have also reported on the effect of OSA on the risk of development of hypertension.[91,158] The Wisconsin Sleep[158] Cohort Study followed up 706 patients for 4 years with baseline mean age of 46 years and mean BMI 29Kg/m^2. Although mean BP was lower after 4 years from 125/82 to 123/79mmHg, the prevalence of hypertension increased from 28% to 31%. The proportion of

individuals needing antihypertensive medications are also increased from 10% to 17%.[158] The odds ratio for hypertension in those with an AHI ≥15 events/hr was 2.89 (95% CI: 1.46 to 5.64) after adjustment for baseline hypertension status, body habitus, non-modifiable risk factors, alcohol and smoking status.[158] Wright et al. followed up 82 patients for 5 years and reported that increase in AHI was associated with higher 24 hour mean BP, and systolic BP.[91] Our study showed that the patients had elevated mean clinic systolic BP in clinic and this could be related to a concomitant diagnosis of OSA.

2.5.4.2 Metabolic syndrome, glycaemic control and obstructive sleep apnoea

Studies in predominantly white populations have found that OSA is associated with premature mortality[85,93,160,161] and obese individuals have a lower life expectancy compared to normal BMI counterparts.[17,183] This study found that South Asians not only had an elevated risk of severe OSA, but that those with severe OSA were seven years younger compared to white Europeans. This suggests that earlier exposure to intermittent hypoxia may contribute to more severe CVD and premature mortality, particularly in obese South Asian populations. The mechanisms involved in the link between OSA and ethnicity are still unknown and require detailed investigation.

Several studies have suggested that the metabolic syndrome is associated with OSA.[184,185] Although our study did not assess metabolic syndrome per se, we demonstrated that South Asians had a significantly higher prevalence of DM, a major risk factor for CVD, compared to white Europeans. The Southall and Brent Study followed up 1787 white Europeans and 1420 South Asians in those residing in the UK over a median of 16 years.[186] There were significant differences in the demographic between the ethnic groups. The South Asian ethnic group was

2 years younger with a lower BMI (26.2 vs. 25.9Kg/m^2) and had better cardiovascular risk profile with 0.1mmol/l lower total cholesterol level and more than half were non-smokers when compared to white Europeans.[186] Despite these differences, the researchers found that the prevalence of the combination of existing and newly diagnosed DM was significantly greater in prevalence amongst South Asians (22.1%) compared to white Europeans (7.2%).[186] Moreover, HOMA-IR analysis also showed greater insulin resistance (2.9% vs. 1.8%) and a higher prevalence of metabolic syndrome (44.6% vs. 23.1%) in South Asians compared to white Europeans.[186] This was consistent with our study finding of a greater proportion of DM amongst South Asians.

Whilst OSA has been linked with increased risk of mortality[85,93,160,161] and CVD,[85,93,187] the majority of the studies have been performed in white Europeans, although not exclusively. A small study from India of 134 patients of whom 62 were obese individuals with concomitant OSA, 46 obese controls and 26 lean controls with no diagnosis of OSA.[187] Subjects were matched for age and the obese subjects were matched for BMI, body fat percentage, fasting and prandial blood glucose levels.[187] This study examined high sensitivity C-reactive protein (hsCRP) level,[187] which is a surrogate marker for increased CVD risk.[188-190] The study found that Indians with co-existing OSA had significantly higher hsCRP (3.6mg/dl) followed by obese individuals without OSA (1.4mg/dl) and lean subjects (0.93mg/dl).[187] The recent study from Brady and colleagues reported that South Asians had a 2.8% greater body fat percentage ($p=0.016$), 0.08mmol/l lower HDL-cholesterol level ($p=0.06$) and are 9 years younger ($p<0.0001$) upon initial presentation/investigations for OSA compared to white Europeans.[124] The study also found a 0.3% ($p=0.001$) higher in HbA_{1c} level in South Asians suggesting greater risk for the development of DM.[124] Our observations are also suggestive of greater

CVD risk in South Asians, which may be an important consideration in clinical practice.

A Norwegian primary care study involving 11 GP practices and a total of 1653 DM patients compared the quality of DM care received between ethnic groups. The study shown that age of DM diagnosis was significantly younger for South Asians (45years) compared to indigenous Norwegians (60years). South Asians living in Norway with DM also had greater adiposity as measured by BMI compared to Caucasian Norwegians (30.1 vs. 29.8Kg/m^2) and had the greatest risk of needing to be treated with either oral hypoglycaemic medications (OHA) or combination OHA treatment with insulin. A total of 25.8% of South Asians were on either OHA alone or OHA with insulin compared to 17.4% of Norwegians. The study also found that the risk of poor glycaemic control (defined as HbA_{1c} above 9.0%) was 3 fold higher in South Asians.[191] Allsworth and colleagues examined DM care in 50,427 nursing home residents in 5 US cities and found that the use of glucose-lowering medication was more prominent in Asians.[192] Our study is consistent with these findings, where DM prevalence was significantly higher in South Asians compared to white Europeans. Glycaemia was also less well controlled and more anti-diabetes medications were required for South Asians, suggesting that this population had significantly more severe and more challenging diabetes mellitus.

2.5.5 Gender and obstructive sleep apnoea

Several epidemiological studies have shown that male gender is a risk factor for OSA.[94,96-98,102] Although we have a higher proportion of females in our study, we found that male gender was associated with an increased severity of OSA. This is consistent with findings from general community and sleep clinic studies.[94,95,98,99,193-195] The Wisconsin Sleep Cohort

found that the male to female ratio for OSA diagnosis is 3:1.[94] A similar ratio was found by the Pennsylvania study (3.3:1),[98] which performed PSG on 1741 individuals. A study by the Mayo Clinic on 406 individuals referred for sleep studies found a lower 2:1 ratio for OSA diagnosis despite a higher proportion of obese females (64.5% vs. 74.8%).[99] Mean age was similar for both genders.[99] Males were more likely to consume alcohol (49.4% vs. 30.1%) and have a greater AHI (26.6 vs. 22.1 events/hr) than females.[99] Another sleep clinic study from Toronto with 839 patients reported the same ratio of 2:1.[195] However, the ratio for males to every female was a lot higher for severe OSA (ratio 7:1).[195] Although the males in this study are 2 years younger than the females, it was not statistically significant. Females had a higher BMI compared to males (35.1 vs. 32.1Kg/m^2) in this study.[195]

In very severely obese individuals the gender difference was reduced with a ratio of between 1.4 and 1.7 in males to every female.[95,194] A study from Brazil examined 132 patients with very severe obesity; OSA is diagnosed in 77.4% males and 55.7% females.[95] Despite higher proportion of males with OSA, they were younger (42 vs. 48years), had more severe OSA (AHI 66.1 vs. 30.5 events/hr) with greater visceral adiposity (waist circumference 137 cm vs. 121 cm) and higher neck circumference (48.5 vs. 40.8cm) compared to female counterparts.[95] Unsurprisingly, this study also reported a higher ratio for moderate and severe OSA (2.3:1.0).[95] A Singaporean study investigated 176 obese patients and found that 92.5% of males and 54.2% of females had OSA.[194] They also found that the a neck circumference greater than 43 cm had a 80% sensitivity, 83% specificity, 80% positive predictive value and 75% negative predictive value for a diagnosis of OSA.[194] Our study found that 61% of females and 87% of males were diagnosed with OSA giving a ratio of 1.4:1, which is consistent with the studies for Class III BMI individuals.[95,194]

The gender differences in OSA parameters could partly be explained by anatomical difference, discrepancies in airway responsiveness and hormonal influence.[98,196,197] Studies have found that men have a higher neck circumference when compared to females[193,198] and this could represent a higher fat deposition in the pharynx resulting in higher incidence of upper airway obstructions. Mohsenin[199] examined the effect of the differences in airway size and sleep positions between genders and found that men had a lower upper airway diameter and greater changes in mandibular movement. Our results also showed a lower minimum oxyhaemoglobin saturation in males as well as a twofold increase in time spent below 90% oxyhaemoglobin saturation compared to females. This may be partly due to variations in pharyngeal muscular tone between the sexes.[169]

Hormonal differences in gender may also influence airway responsiveness. The Pennsylvania study conducted PSG in 1000 women.[98] OSA was diagnosed if AHI was ≥15 events/hr. Interestingly, there was a significant difference in the risk of OSA between post-menopausal women who were taking hormonal replacement treatment (HRT) (RR 1.9, 95% CI: 0.4 to 10.1) and those who were not (RR 9.3, 95% CI: 2.6 to 25.8) when compared to pre-menopausal women. The effect remained in post-menopausal women without HRT (OR 4.3, 95% CI: 1.1 to 17.3) after adjustment for confounders. Studies also found that females with polycystic ovarian syndrome (PCOS) have a greater risk and severity of OSA. PCOS is a condition characterised by hyper-androgenism, menstrual disturbance and obesity. A study compared 53 females with PCOS to 452 healthy premenopausal females reported a 30-fold (OR 30.6, 95% CI: 7.2 to 139.4) higher OSA prevalence in PCOS group.[200] Another study compared age and weight matched 18 PCOS females with those without PCOS found that

PCOS females had greater AHI (22.5 vs. 6.7 events/hr, p=0.008) and AHI was also correlated with free testosterone (r=0.50, p<0.05).[201] Furthermore, excess androgens administration in either males or females results in worsening OSA.[202,203]

2.5.6 Limitations

Whilst our study is the first to report on the ethnic differences of OSA prevalence and severity in severely obese individuals, it is important to acknowledge the limitations. Our population was recruited from the specialist weight management service and may be subject to selection bias with findings only being representative of very severely obese individuals attending such a service. Furthermore, ethnic differences in those who initially present with obesity and those who are referred by physicians are unknown, and may have produced further selection bias in our sample.

Although all UK residents have access to the National Health Service, we cannot rule out the possibility of ethnic group differences for the presentation of obesity. However, as previously noted, the West Midlands South Asian population is approximately 10% of the total population.[152] Our patient population of South Asian's referred to our Specialist Weight Management Clinic was 13% and thus may be representative of this minority group in the West Midlands. Referral bias may explain the greater prevalence of co-morbidities and a younger age of presentation of South Asians. We acknowledge that referral bias may play a role, however a recent assessment that utilised the National Bariatric Surgery Registry and census data from the UK and Ireland, demonstrated that ethnic minority groups have equal access to this type of service/procedure.[204]

One further limitation is that sleep was not assessed using full polysomnography, which is impractical and not cost-effective in large clinical populations. Validated instruments for assessment of sleep apnoea were however used.[205] We did not assess or collect data for OSA symptoms as most of our patients are referred to us are for considerations of bariatric surgery. Since OSA increases the risks of peri-operative complications, prolonged apnoeic periods are associated with risk of respiratory arrest,[206] we routinely assess potential SDB prior to consideration for bariatric surgery. Moreover, Kapur et al. found that subjective sleepiness was not present in more than half of the individuals with moderate to severe OSA.[207] Carneiro et al.[95] also found the Epworth Sleepiness Score was not a useful predictor of OSA in such obese populations. Whilst our study benefitted from adjustment for a range of potential confounders, we did not consider smoking and alcohol consumption as this data was unavailable. Cigarette smoking had a positive correlation with OSA although no causal association has been established.[104]

2.6 Conclusions

In summary, OSA prevalence and comorbidities were greater in these severely obese South Asians compared with equivalent white Europeans. South Asians also had more severe OSA compared to BMI-matched white Europeans. Our data also support previously confirmed risk factors for AHI severity including higher BMI, older age and male gender. The precise mechanisms involved in these ethnic differences are still to be explored and understood although we hypothesise that visceral adiposity and different fat depositions play a role. Other potential contributory factors may be genetically mediated and include craniofacial structure and differences in pharyngeal muscular tone. Further exploration of mechanisms underlying ethnic differences in OSA severity is likely to extend the current understanding of OSA.

CHAPTER 3: OBSTRUCTIVE SLEEP APNOEA AND GLYCAEMIC CONTROL IN TYPE 2 DIABETES MELLITUS WITH EXTREME OBESITY

3.1 Introduction

3.1.1 Obesity and Type 2 diabetes mellitus (T2DM)

Obesity is a well-established risk factor for T2DM. A hospital-based study on 3637 UK patients with T2DM showed that 86% of the individuals are either overweight or obese.[208] The Health Professional Follow-up Study which consists of 51,529 male participants demonstrated that those with a BMI $\geq$35Kg/m^2 has a greater risk of T2DM (RR 42.1, 95% CI: 22.0 to 80.6) compared with those with a BMI <23.0Kg/m^2 over 5 years of follow-up.[209] Likewise, the Nurses Health Study with 114,281 participants with 1.49 million person-years follow-up ascertained that those who gained >5 Kg in weight had a higher risk of developing T2DM: weight gain between 5.0-7.9 Kg had RR 1.9 (95% CI: 1.5 to 2.3); and weight gain between 8.0-10.9Kg had RR 2.7 (95% CI: 2.1 to 3.3).[210]

Several intervention studies of obese individuals have shown that weight reduction improved insulin sensitivity and lower the risk of developing T2DM. The landmark Diabetes Prevention Programme with 2.8 years of follow-up established that in pre-diabetic individuals, lifestyle interventions with a 4Kg weight reduction significantly reduced the risk of T2DM incident (RR 0.42, 95% CI: 0.52 to 0.44) compared to placebo.[211] Additionally, the Look AHEAD (Action for Health and Diabetes) study showed that after 4 years of intensive lifestyle intervention, mean weight loss was 6.15% with a mean reduction in HbA_{1c} of 0.36%.[212] Additionally, a recent meta-analysis of 11 RCTs showed that bariatric surgery, a treatment for

obese T2DM patients, resulted in a 26Kg (95% CI: -31 to -21) weight reduction and a greater T2DM remission (RR 22.1, 95% CI: 3.2 to 154.3) compared to non-surgical treatment.[213]

3.1.2 Obstructive sleep apnoea and glycaemia

Both obesity and diabetes are related to multiple co-morbidities that may contribute to the perpetuation of obesity and deterioration of glycaemic control. A common co-morbidity that accompanies both obesity and DM is obstructive sleep apnoea (OSA).[80,139,142] Several studies have shown that OSA is associated with DM.[80,139,142] The Sleep AHEAD study reported that the prevalence of OSA (AHI ≥5 events/hr) can be as high as 86% amongst obese DM individuals.[139] A recent meta-analysis of 6 cohort studies over a follow-up period of between 2.7 to 16 years with 5653 participants and 332 incident cases of DM found that moderate-to-severe OSA conferred a relative risk of 1.63 (95% CI: 1.09 to 2.45) for DM.[80]

OSA has been found to affect insulin sensitivity and worsen glycaemia in non-DM individuals.[214,215] In a study of 52 young healthy lean individuals with similar characteristics (age, BMI, DM risk, family history of DM and physical activity levels) and normal blood pressure (BP) as well as lipid profile; individuals with OSA were noted to have greater insulin secretion suggesting lower insulin sensitivity.[214] The Sleep Heart Health Study (SHHS) also reported a significantly higher risk for impaired fasting glucose as well as impaired glucose tolerance in OSA compared to non-OSA participants independent of adiposity.[215] Another study of 98 non-DM obese Italian women demonstrated that insulin sensitivity and fasting glucose levels correlated with both nocturnal mean and minimum oxygen saturation saturations.[216] The Sleep Extension Study which consists of 96 participants, showed that the respiratory disturbances index is a predictor of fasting glucose concentration (mean 5.8, 95%

CI: 0.3 to 11.3) after adjusted for potential confounders.[217] The pathophysiological link between OSA and glucose regulation will be described in detail in the discussion section to minimise repetition.

Currently, the management of glycaemia mainly consists of lifestyle advice, and pharmacotherapy, and in those with extreme obesity, bariatric surgery is increasingly advocated. Although bariatric surgery has been shown to be very effective in treating DM,[213] not all severely obese individuals fulfil the criteria for bariatric surgery and these patients tend to be managed on high doses of insulin treatment in conjunction with oral therapies. As DM has been shown to be associated with OSA, the purpose of the current evaluation was to explore if OSA is an important factor for the management of challenging insulin resistance in individuals with extreme obesity. The impact of nocturnal hypoxia caused by OSA on glycosylated haemoglobin A_{1c} (HbA_{1c}) levels were investigated in this high risk patient group.

3.2 Aim and objectives

The aim of this chapter is to examine the effect of OSA on glycaemia amongst DM with severe obesity. The following are the objectives of the study:

1. To compare the prevalence of OSA in extremely obese individuals with and without diabetes, and
2. To explore the relationship between OSA and glycosylated haemoglobin A_{1c}

3.3 Methods

3.3.1 Study Design

The methodology had been described in Chapter 2. The participants; however, were consecutive patients from January 2009 to April 2013. The recruitment period was longer compared with chapter 2 as this study was carried out at a later date. Respiratory sleep monitoring was also described in Chapter 2.

3.3.2 Statistical analysis

All data (including those with and without DM) were anonymised prior to any statistical analysis. However, for the multivariate linear regression analysis, only individuals with DM were included. Distribution of the data was determined through visual inspection. Normally distributed data was reported as mean ± standard deviation whilst non-normally distributed data was reported as median with inter-quartile range (IQR). T-test and Mann Whitney U-tests were used for parametric and non-parametric data, respectively. Chi-squared test was utilised for categorical data.

The association between respiratory parameters and HbA_{1c} was carried out using multivariate linear regression analysis in DM patients only. The base model was unadjusted, Model 1 was adjusted for age, gender, BMI and ethnicity (white Europeans vs. non-white Europeans) while further adjustments for number of DM medications were performed in Model 2. The factors included in the adjusted models were those that are considered to be clinically important, and the rationale for including each factors is discussed elsewhere (see table 3- 1). Results are reported as beta coefficient with 95% confidence interval (95% CI). A p value of <0.05 was

considered significant. All statistical analyses were performed using Stata 13 (StataCorp LP, College Station, Texas).

Table 3-1: Factors adjusted and the appropriate sections discussing the rationales behind

Factors	**Sections / rationale**
Age	Introduction of Chapter 2 (section 2.1.3)
Gender	Chapter 2's discussion (section 2.5.5)
Ethnicity	Chapter 2's discussion (section 2.5.3)
Body mass index	Chapter 1 (section 1.3.4)
Number of anti-diabetes medications	Can influence HbA_{1c} level

3.4 Results

3.4.1 Study characteristics

There were a total of 433 patients. However, 145 patients had missing HbA_{1c} results, four patients did not have analysable results from the overnight sleep study, and one patient had missing BMI data. Therefore a total of 283 eligible patient records were examined, including 161 with DM (56.9%).

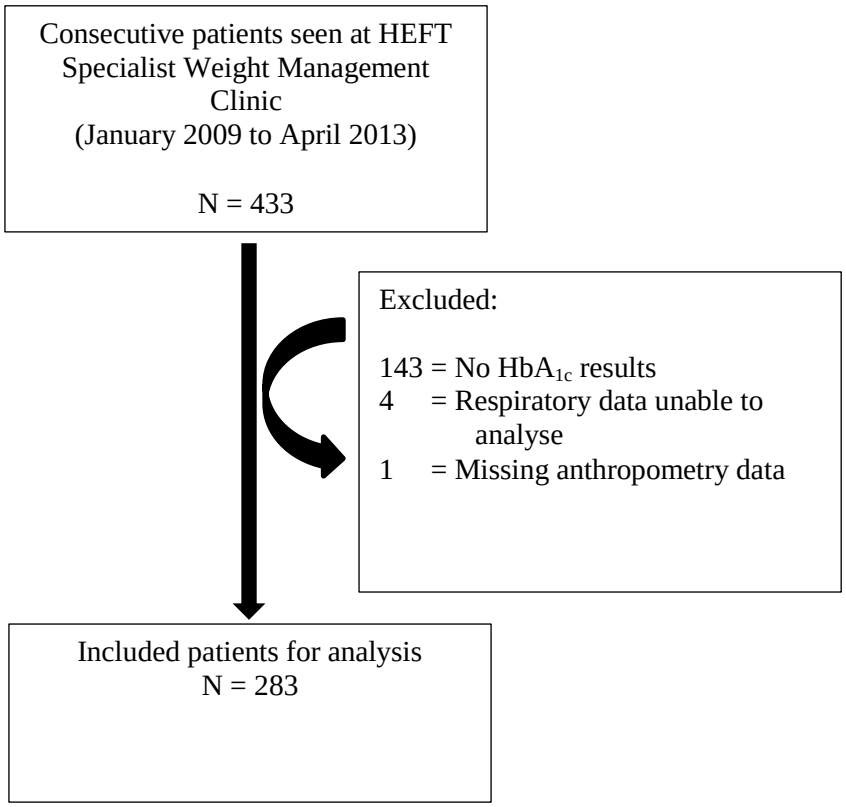

Figure 3-1: Flow diagram of referrals seen at the HEFT Specialist Weight Management Clinic, and those included in the study
HEFT – Heart of England NHS Foundation Trust

Table 3-2 describes the characteristics of non-DM and DM individuals in this study. DM patients were 10 years older compared to non-DM patients (51.1±10.2 vs. 41.8±12.5 years, p<0.0001). There were also more males (40.4% vs 23.8%, p=0.003) and a lower proportion of white Europeans (75.2% vs. 92.6%, p=0.010) amongst the DM patients. However, there were no significant differences in BMI (48.8±9.1 vs. 49.59±8.4Kg/m^2, p=0.47), systolic (145.4±20.1 vs. 141.1±18.0mmHg, p=0.12) and diastolic BP (87.0±11.1 vs. 86.6±12.6, p=0.84) between the two groups. As expected, there were greater proportions of DM patients with HTN (57.8% vs. 23.8%, p<0.001) and CAD (10.6% vs. 2.5%, p=0.008) when compared to non-DM individuals.

There were also significant differences in the respiratory parameters (p<0.05). The overall OSA prevalence (AHI ≥5 events/hr) was 72.8%; and it was significantly more common in DM (80.1%) compared with non–DM individuals (63.1%, p=0.001). There were also greater proportions of DM individuals with moderate and severe AHI (47.2% vs. 34.4%, p=0.015). DM individuals had higher median AHI (14.0 [IQR: 6.0, 36.0] vs. 8.0 [IQR: 3.5, 24.5] events/hr, p=0.009), lower median minimum O_2 saturation (82% [IQR: 74, 85] vs. 83% [IQR: 78, 88], p=0.01), mean oxygen saturation (93% [IQR: 91, 95] vs. 94% [IQR: 92, 95%], p=0.026) and significantly longer median time spent under 90% O_2 saturations (4.0% [IQR: 0.7, 15.8] vs. 1.0% [IQR: 0.1, 13.2], p=0.007) compared with non-DM patients.

Table 3-2: Characteristics of obese individuals with and without diabetes mellitus

	Overall N=283	**Non-DM N = 122**	**DM N = 161**	**P value**
Age (years)	47.1 ± 12.1	41.8 ± 12.5	51.1 ± 10.2	**<0.001**
Sex n (%)				
Females	189 (66.8%)	99 (81.1%)	96 (59.6%)	**0.003**
Males	93 (33.2%)	29 (23.8%)	65 (40.4%)	
Ethnicity				
White European	230 (81.3%)	113 (92.6%)	121 (75.2%)	**0.010**
South Asian	39 (13.8%)	10 (8.2%)	30 (18.6%)	
Afro-Caribbean	14 (4.9)	4 (3.2%)	10 (6.2%)	
BMI (Kg/m^2)	49.2 ± 8.8	49.59 ± 8.38	48.8 ± 9.1	0.467
Systolic BP (mmHg)	143.5 ± 19.3	141.1 ± 18.0	145.4 ± 20.1	0.123
Diastolic BP (mmHg)	86.8 ± 11.7	86.6 ± 12.6	87.0 ± 11.1	0.838
Co-morbidities				
Hypertension	122 (43.1%)	29 (23.8%)	93 (57.8%)	**<0.001**
Coronary artery disease	20 (7.1%)	3 (2.5%)	17 (10.6%)	**0.008**
Respiratory parameters				
AHI (events/hr)	11.8 (4.0, 31.8)	8.0 (3.5, 24.5)	14.0 (6.0, 36.0)	**0.009**
Mean O_2 sat (%)	93.4 (91.6, 95.0)	94.0 (92.0, 95.0)	93.0 (91.2, 95.0)	**0.026**
Min O_2 sat (%)	83.0 (76.0, 87.0)	83.0 (78.0, 88.0)	81.5 (74.0, 85.0)	**0.011**
%TST<90%	2.3 (0.2, 15.0)	1.0 (0.1, 13.2)	4.0 (0.7, 15.8)	**0.007**
OSA (AHI > 5/hr)	206 (72.8%)	77 (63.1%)	129 (80.1%)	**0.001**
Classification of OSA	87 (30.7%)	34 (26.6%)	53 (32.9%)	**0.015**
Mild	45 (15.9%)	17 (13.3%)	28 (17.4%)	
Moderate	74 (26.1%)	26 (21.1%)	48 (29.8%)	
Severe				

Data presented as mean ± standard deviation (SD), median (interquartile range) or number (%). DM = Type 2 diabetes mellitus, BMI = body mass index, BP = blood pressure, AHI = apnoea-hypopnoea index, O_2 sat = oxygen saturation, %TST<90% = % time spent below 90% oxygen saturation, OSA = obstructive sleep apnoea.

3.4.2 Respiratory parameters and glycaemia

The univariate model of linear regression showed that AHI was not associated with glycaemic levels (β=0.0018, 95% CI: -0.0079 to 0.0115) as shown in Table 3-3. The result remained non-significant after adjustment for age, gender, ethnicity and BMI (β=0.003, 95% CI: -

0.0099 to 0.0106) as well as further adjustment for the number of diabetic medications (β=0.0011, 95% CI: -0.0088 to 0.0109). Similarly, mean oxygen saturation did not show any significant association with HbA_{1c} in the univariate (β=-0.0577, 95% CI: -0.1441 to 0.0287), Model 1 (β=-0.0690, 95% CI: -0.1637 to 0.0256) and Model 2 (β=-0.0688, 95% CI: -0.1598 to 0.0221). Minimum oxygen saturation also was not associated with the level of glycaemia in the univariate model (β=-0.0267, 95% CI: -0.0542 to 0.0008). However, the association became significant after adjustment for age, gender, ethnicity and BMI (β=-0.0320, 95% CI: -0.0622 to -0.0018) and remained significant with further adjustments for the number of glucose-lowering medications (β=-0.0304, 95% CI: -0.0595 to -0.0013). The final model (Model 2) showed that each 1% increase in the minimum oxygen saturation was associated with 0.03% reduction in HbA_{1c}. In other words, a 0.3% increase in HbA_{1c} was associated with a 10% reduction in minimum O_2 saturation.

Likewise, univariate linear regression analysis showed a positive relationship between %TST<90 and HbA_{1c} levels (β=0.0155, 95% CI: 0.0026 to 0.0284). The association persisted after adjustment for age, gender, BMI and ethnicity (Model 1: β=0.0189, 95% CI: 0.0050 to 0.0237) and further adjustments for diabetic medication (Model 2: β=0.0177, 95% CI: 0.0043 to 0.0310). This demonstrated a 0.02% increase in HbA1c with each 1% increase in %TST<90. Non-significant associations with HbA_{1c} were found for AHI and mean O_2 saturations.

Table 3-3: Independent predictors of HbA1c in DM patients (n=161)

Predictors	Models		
	Beta coefficients (95% confidence intervals)		
	Univariate analyses	Model 1	Model 2
AHI	0.0018 (-0.0079 to 0.0115)	0.0003 (-0.0099 to 0.0106)	0.0011 (-0.0088 to 0.0109)
Mean O_2	-0.0577 (-0.1441 to 0.0287)	-0.0690 (-0.1637 to 0.0256)	-0.0688 (-0.1598 to 0.0221)
Min O_2	-0.0267 (-0.0542 to 0.0008)	**-0.0320 (-0.0622 to -0.0018)**	**-0.0304 (-0.0595 to -0.0013)**
%TST<90	**0.0155 (0.0026 to 0.0284)**	**0.0189 (0.0050 to 0.0327)**	**0.0177 (0.0043 to 0.0310)**
Age	-0.0219 (-0.0476 to 0.0038)	-0.0258 (-0.0520 to 0.0005)*	**-0.0258 (-0.0510 to -0.0005)***
Male	**0.6754 (0.1478 to 1.2031)**	**0.7586 (0.1837 to 1.3334)***	**0.6858 (0.1311 to 1.2404)***
BMI	-0.0043 (-0.3345 to 0.0250)	0.0047 (-0.0268 to 0.0362)*	0.0116 (-0.0190 to 0.0421)*
Non WE	0.0484 (-0.4055 to 0.5023)	-0.0051 (-0.4454 to 0.4461)*	-0.0491 (-0.4841 to 0.3858)*
No. DM medications	**0.4604 (0.2187 to 0.7021)**	Not applicable	**0.4487 (0.2063 to 0.6912)***

Analysis performed using linear regression. HbA_{1c} = glycosylated haemoglobin A_{1c}, AHI = apnoea-hypopnoea index, O_2 = oxygen saturation, Min O_2 = minimum oxygen saturation, %TST90 = % time spent below 90% oxygen saturation, BMI = body mass index, Non WE = Non White Europeans, No. DM medications = Number of diabetes mellitus medications

*Adjusted for apnoea-hypopnoea index only to avoid multi-collinearity

Univariate analyses = unadjusted models

Model 1 = adjusted for age, gender, body mass index and ethnicity

Model 2 = adjustments for gender, body mass index, ethnicity, number of glucose-lowering medications

3.5 Discussion

3.5.1 Prevalence of obstructive sleep apnoea in the diabetes mellitus populations

Our study showed that the prevalence of OSA was significantly higher in DM individuals with extreme obesity. As discussed in Chapter 2, the general prevalence of OSA has been reported to be between 16 and 27%[94,123,131,134] whilst in the extremely obese populations, between 61 and 98% had OSA.[95,140-145] However, one of the limitations of the prevalence studies in obese population is the underreporting of chronic diseases therefore inclusion of multiple co-morbidities including DM may lead to an overestimation of the prevalence of OSA.[95,140,144,145] In fact, our observations were comparable with the findings from several studies involving DM individuals. The Sleep Ahead study, which examined 305 obese DM individuals, reported an OSA rate of 86%.[139] In the Morbid Obesity treatment, Bariatric surgery versus Intensive lifestyle intervention (MOBIL) study, the prevalence of OSA was 78% amongst DM individuals, which was significantly higher than in patients with normal glucose tolerance (33%).[142]

Aronsohn and colleagues reported an OSA prevalence of 77% among 60 DM individuals.[218] However, the Sleep Heart Health Study (SHHS) found that only 58% of DM patients had OSA.[219] The lower prevalence rate reported by the SHHS study compared to our study may be partially due to the differences in adiposity levels (SHHS mean BMI was 31.3±6.0 Kg/m^2).[219] Also, our study included 'undiagnosed' DM individuals with HbA_{1c} results of >6.5% as well as known DM in the analysis and this is likely to represent a better OSA prevalence rate amongst DM whilst the SHHS study had only recorded self-reported DM patients.[207] Therefore, SHHS is likely to represent an underestimation of the reported OSA rate.

3.5.2 Obstructive sleep apnoea and glycaemic control

In the literature, limited studies have examined the relationship between OSA and glycaemic control amongst DM individuals.[218,220] A study from Oxford screened 938 T2DM men using the Berlin questionnaire and selected 240 (124 high risk and 116 low risk men) T2DM male individuals for overnight pulse oximetry investigation.[220] They first used the oxygen desaturation index (ODI), with an event defined as a drop of oxygen saturation of at least 5% and then used 10 events/hr to identify individuals with OSA. This was subsequently confirmed using a portable home device to diagnose OSA. Those who had an ODI of less than 10 events/hr but had a sleep tracing resembling OSA were also confirmed with the portable device for the diagnosis of OSA. The researchers found a correlation between pulse oximetry and HbA_{1c} ($r=0.2$, $p=0.0006$) in a subgroup analysis of their hospital-recruited DM individuals.[220] The strength of the relationship reduced (coefficient not reported) after adjusting for BMI ($p=0.03$). One limitation of the study is that the authors only adjusted for BMI and not other confounding factors such as DM duration, age and diabetic medication. Moreover, the positive correlation, was lost when a larger sample size of both community and hospital-recruited DM individuals were included[220] suggesting the correlation might be spurious.

Another study by Aronsohn and colleagues examined 60 DM individuals with overnight laboratory full PSG. Following a mean respiratory recording of 6 hours, mild OSA was associated with an increased HbA_{1c} of 1.49%, moderate OSA of 1.93% and severe OSA of 3.69%.[218] Aronsohn and colleagues used an AHI $\geq$5 events/hr for the diagnosis of OSA. In contrast, our results did not show any significant association between AHI and the level of

glycaemia in DM individuals. This could be due to the differences in the minimum duration of overnight sleep study recorded. We included all individuals with at least 4 hours of respiratory records. Aronsohn and colleagues found that when they used only the first 4 hours of sleep data, the association between OSA and HbA_{1c} was no longer significant. One hypothesis is that during REM sleep, there is a higher frequency of apnoea and hypopnoea episodes compared to other sleep stages[218] and REM sleep usually becomes more prominent as the night progresses.[221] From our experience, we find that it is difficult to achieve 6 hours of quality respiratory recording in our patients mainly due to the discomfort caused by the device and in other cases, the loss of connection with part of the device during sleep.

Although our results showed no significant association between AHI and levels of glycaemia in Type 2 diabetes mellitus individuals, we found a significant relationship between minimum O_2 saturation as well as %TST<90 and HbA_{1c} levels in these individuals. To our knowledge, no study has assessed the association between the levels and duration of hypoxia and glycaemic control in the obese DM population. In a non-DM study of 116 hypertensive individuals, a significant correlation was identified between minimum O_2 level and HbA_{1c} ($r=-0.25$, $p<0.001$)[222] as did another study from Japan non-DM individuals ($\beta=-0.29$, $p<0.001$).[223] This suggests that in DM individuals with limited respiratory recordings, nocturnal hypoxia, and not AHI, may be a useful indicator of greater insulin resistance and might contribute to poor glycaemic control. Moreover, our previous studies have also shown that the severity and duration of nocturnal hypoxaemia may be an important factor for DM micro-vascular complications, such as progression of diabetic retinal complication[224] (please refer to Chapter 4), and diabetic nephropathy[225] (please refer to Chapter 5).

This suggests that apart from using usual glucose-lowering medications, correcting nocturnal hypoxia may be beneficial in the reduction of HbA_{1c} notably amongst severely obese patients. The current CPAP trials on glycaemia have been inconclusive. A recent meta-analysis of 8 observational studies and 1 RCT found that CPAP treatment had no significant impact on glycaemia (mean change in HbA_{1c} was -0.08, 95% CI: -0.26 to 0.42%).[226] However, there were several limitations present in the meta-analysis especially in the quality of the included studies. Three of the observational studies included performed a repeated HbA_{1c} in less than 90 days: 1 after 41 days, 1 after 4 weeks, 1 after 2 months.[50,227,228] In vivo, lifespan of erythrocytes is between 2 to 3 months therefore the American Diabetes Association (ADA) has recommended that HbA_{1c} should be monitored approximately 3 monthly.[229]

Furthermore, some of the studies had problems with CPAP adherence. Since the development of CPAP, compliance with the device has been a major issue in the treatment of OSA. A study which examined the 'threshold' of CPAP usage which was likely to provide improvements for sleepiness symptoms suggested that at least 4 hours was needed.[230] This study examined 137 participants with complete baseline and 3 month follow-up data. Response was judged using the ESS, Multiple Sleep Latency Test (MSLT) and functional status using the Functional Outcomes of Sleep Questionnaire (FOSQ). The 'threshold' for improvement in sleep symptoms based on ESS, MSLT and FOSQ were 4 hours, 6 hours and 7.5 hours respectively at study end. Therefore, this suggests that at least a minimum duration of 4 hour CPAP usage is required to be beneficial. The only RCT[231] included in the meta-analysis had only 20 participants in the CPAP group and the mean CPAP usage was 3.6 hours compared with 22 in placebo group with 3.3 hours of CPAP per night. Unsurprisingly, the result from the RCT was not statistically significant for improvement in HbA_{1c} after 3 months

of treatment. Apart from the relatively small sample size, the minimum level of CPAP adherence was not achieved by West and associates (mean hours used 3.6±2.8 hours).

Finally, 4 small studies from the meta-analysis which provided at least 4 hours of compliance with CPAP showed statistically significant correlations between CPAP usage and HbA_{1c} reduction.[232] Babu et al compared 12 participants with <4 hours and 12 with >4 hours of CPAP usage for between 30 and 90 days and showed that those with >4 hours had significant correlation between glycaemia and days of CPAP use (r=0.74, p=0.006).[232-235] Another study from Greece had 21 non-DM individuals with CPAP use of 4.71±0.55 hours expressed a HbA_{1c} reduction from 5.55±0.4 to 5.38±0.45% (p=0.004) after 6 months of treatment.[233] A prospective cohort study found that out of 19 participants with severe OSA being followed up for between 3 to 5 months, 12 had a least 4 hours of CPAP usage and the mean HbA_{1c} decreased from 6.47±0.67% to 6.28±0.51% (p=0.038).[234] Moreover, a cross-sectional study of 29 patients with mean of 4±3 hours use per day demonstrated a drop of HbA_{1c} from 7.8±1.4% to 7.3±1.3% (p<0.001) at least 3 months apart.[235] Consequently, future well designed RCTs with appropriate sample size and good adherence of CPAP of at least 6 hours amongst DM individuals are required to enhance our understanding on the effect of treating nocturnal hypoxia.

3.5.2.1 Pathophysiology between OSA and glycaemia

The effect of OSA on glucose may be caused through several mechanisms as shown in Figure 3-2. Sleep disturbances (arousal, sleep fragmentation, sleep deprivation and napping), chronic intermittent hypoxaemia and hypercapnia are likely play a part in affecting insulin sensitivity and beta cell function. The elevated sympathetic drive[236-238] alters glucose and fat metabolism

causing an increase in hepatic gluconeogenesis, inhibition of insulin secretion hence lowering of glucose uptake by insulin-dependent tissue such as muscle.[239] Likewise, greater release of cortisol was demonstrated to have similar effects on hepatic glucose production (gluconeogenesis and glycogenolysis) and inhibition of insulin secretion.[238] OSA also activates the renin-angiotensin-aldosterone system (RAAS).[240] Recent emerging evidence suggests that RAAS plays a role in glucose metabolism via inhibition of insulin signally in skeletal muscles thus insulin resistance.[241] Additionally, angiotensin receptors were found in pancreatic islet cells and angiotensin II appears to suppress glucose-induced insulin secretion and lowers islet blood flow affecting insulin production.[241] In addition, drugs which inhibit the RAAS system seems to improve glucose tolerance and insulin sensitivity[242,243] in animal studies. Furthermore, a systematic review and meta-analysis of 11 RCTs on the effect of angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) demonstrated a reduction in risk of the development of T2DM (OR 0.78, 95% CI: 0.73 to 0.83) compared to placebo.[244]

Insulin resistance caused by OSA may also partly be explained through activation of oxidative stress pathways.[245] Excess of reactive oxygen species (ROS) either from overproduction or ineffective removal can alter cellular function and activates greater oxidative stress and inflammatory reactions resulting in endothelial dysfunction.[245] Indeed, several studies have demonstrated greater levels of inflammatory markers such as interleukin-6 (IL-6), tumour necrosis factor-alpha (TNFα) as well as other inflammatory markers in individuals with OSA.[246-248] In severely hypoxic OSA individuals, there seems to be an up-regulation of the hypoxia-inducible factor-1 alpha (HIF-1α) expression,[249] HIF-1a in turn has been found to down-regulate insulin signalling pathways and the synthesis of glucose transport proteins[250]

which will contribute to the fall in insulin sensitivity. Humoral modulations also occurred with OSA as leptin levels was found to be elevated while adiponectin levels were significantly reduced.[142,247] Leptin has been shown to inhibit glucose-induced insulin secretion from the pancreatic islet cells[251] whilst adiponectin has been found to stimulate insulin secretion, suppress hepatic gluconeogenesis and increase glucose uptake in skeletal muscles.[252]

Collectively, these mechanisms play a vital role in the impairment of pancreatic beta cell function and insulin sensitivity. The pancreatic beta cell is very sensitive to oxygen deprivation. Hypoxia in mice has been shown to result in impairment in beta cell function[253] and also cell apoptosis.[254] Indeed, it has been shown in several islet cells transplantation studies for type 1 diabetes mellitus that reduction in oxygenation is one of the main factors resulting in transplant failure.[255,256] Alternatively, it is plausible that DM may lead to the development of OSA. The possible pathophysiology of DM causing OSA will be discussed in detail in Chapter 4.

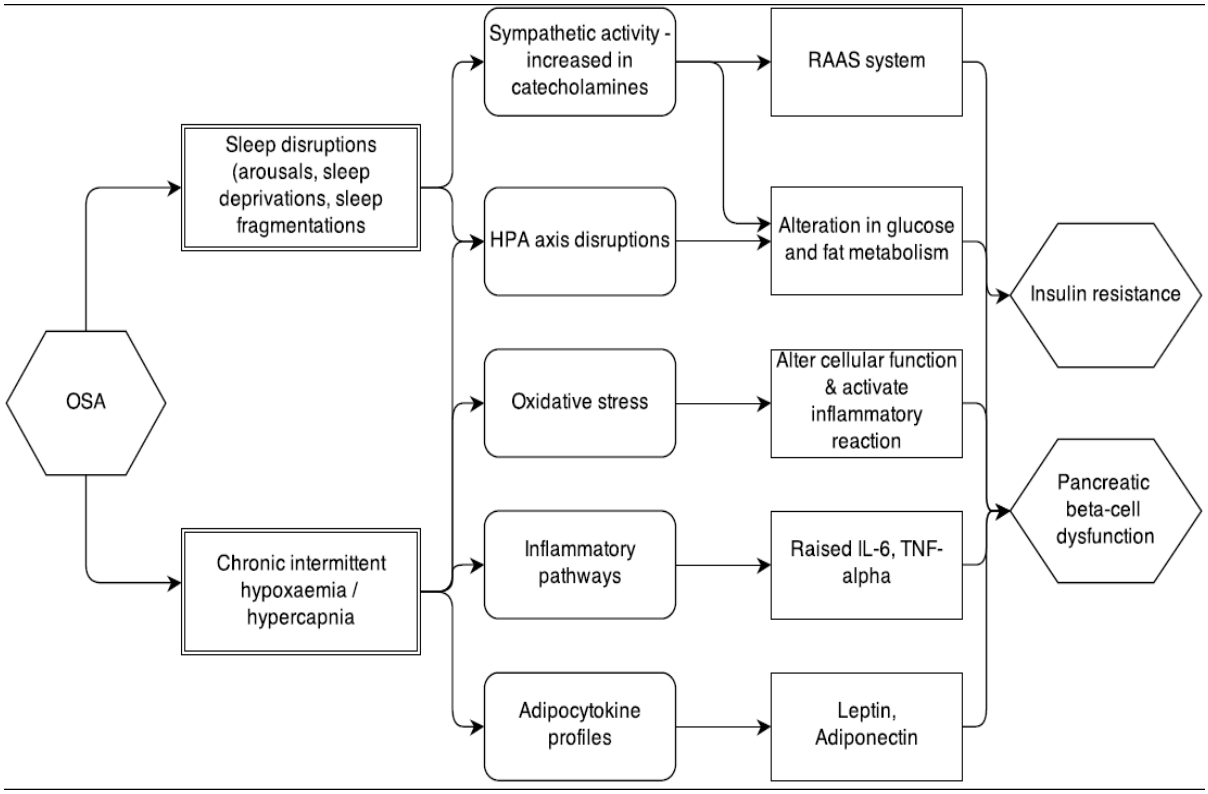

Figure 3-2: Pathogenic mechanisms linking OSA to diabetes mellitus. OSA=obstructive sleep apnoea, HPA=hypothalamic-pituitary-adrenal axis, RAAS=renin-angiotensin-aldosterone system, IL-6=interleukin-6, TNF-alpha= tumour necrosis factor alpha

3.5.3 Limitations and benefits of the study

There were several limitations to our study. Firstly, it only examined extremely obese individuals, which are an especially high-risk group of individuals for health complications and mortality. Secondly, the limitations of observational studies apply to our study and it is difficult to exclude reverse causality. Thirdly, there was no data on smoking and alcohol status. However, this study had a large number of patients and is representatives of the local population with extreme obesity. The analyses were also adjusted for multiple potential confounders in the regression analyses.

3.6 Conclusion

This study observed an inverse relationship between nocturnal hypoxaemia and glycaemia control among extremely obese DM individuals. It was also observed that the prevalence of OSA amongst DM individuals, especially in those who needed insulin therapy, was very high. This supports the notion that OSA, in particular hypoxaemia, may have an adverse effect on glucose metabolism. Further well-designed, adequately powered studies on the impact of correcting nocturnal hypoxaemia on glycaemia are needed.

CHAPTER 4 – THE POTENTIAL ASSOCIATION BETWEEN OBSTRUCTIVE SLEEP APNOEA AND DIABETIC RETINOPATHY IN SEVERE OBESITY

4.1 Introduction

4.1.1 Diabetic retinal disease

The chronic hyperglycaemic state is associated with both macro and micro-vascular complications. Macro-vascular complications of diabetes mellitus (DM) are mainly due to atherosclerosis of the arteries leading to myocardial infarction, coronary artery disease, stroke and peripheral vascular disease. DM micro-vascular complications include nephropathy, neuropathy and diabetic retinal disease. The most common diabetic retinal disease is diabetic retinopathy (DR). Stages of DR include background, pre-proliferative and proliferative retinopathy disease. The initial stage - background retinopathy involves presence of micro-aneurysm lesions whereby the lining of the retinal vessels weakened leading to leakage of lipoproteins (termed hard exudates) or blood. Presence of the blood in the retina has been described to be either dot (small dots of blood) or flame (larger blotches of blood) haemorrhages.[257]

The next stage - pre-proliferative DR involves large pools of blood leakage from the vessels causing large dark blot haemorrhages or multiple blood exudates. At this stage, disruption of the blood supply in some areas of the retina can occur leading to micro-infarctions of the nerve fibre. Such changes looked are termed ‘cotton-wool’ spots. In order to compensate blood supply to these ischaemic areas, abnormal branching or dilatation of retinal capillaries occur. These abnormal vessels are called intra-retinal micro-vascular abnormalities (IRMA).[257] The final stage - proliferative DR (PDR) occurs when abnormal new blood vessel

are present within the retina. These vessels are different from IRMA as they are finer and much more delicate hence easier to rupture resulting in vitreous haemorrhage.[257] A summary of the features of the DR stages are shown in Table 4-1.

Table 4-1: Stages of diabetic retinopathy

Stages	Features
Background diabetic retinopathy	Micro-aneurysms
	Hard exudates
	Flame, dot, blot haemorrhages
Pre-proliferative diabetic retinopathy	Large blot haemorrhages
	Cotton wool spots
	Intra-retinal microvascular abnormalities (IRMA)
	Venous beading
Proliferative diabetic retinopathy	Abnormal new vessel on optic disc or retina
	Vitreous haemorrhages

The WHO Global Burden of Disease 2000 Project reported that DR accounts for 15% to 17% of blindness in developed Western countries.[258] Although DR is the third most common threat to vision after age-related macular degeneration and glaucoma, it is the most common cause of blindness in people within the working age group,[259] making it an important cause for disability and morbidity. Apart from the retina, DM also affects the macula. Changes within the macula due to diabetes are termed diabetic maculopathy (DMac). DMac can occur at any stage of DR and is potentially very serious as it can threaten central vision. Presence of DMac include any exudative leak of proteins which causes macula oedema, micro-aneurysms or haemorrhages within the central fovea or ischaemic changes to the macula.[260] Other eye diseases associated with DM includes retinal detachment,[260] glaucoma[261] and cataract.[262]

4.1.2 Obesity, obstructive sleep apnoea and diabetic retinal disease

Both observational[263,264] and longitudinal studies[265,266] have demonstrated an association between obesity and diabetic retinal disease especially DR. The Diabetic Management Project based in Australia recruited 492 Type 1 and Type 2 DM participants with a median age of 65 years demonstrated that obesity with BMI ≥30Kg/m^2 had a 3-fold greater risk of DR (OR 3.12, 95% CI: 1.20 to 8.16) compared to normal BMI individuals after adjustment for a range of confounders.[263] Similarly, the Hoorn study,[264] a population study from the Netherlands also reported that DM patients with higher BMI were at increased risk of DR (OR 3.52, 95% CI: 1.05 to 11.8 in those with BMI between 28.4 and 45.7Kg/m^2 compared with BMI between 15.0 and 20.1Kg/m^2) after adjusted for age and gender. Longitudinal study from the Diabetes Incidence Study in Sweden (DISS)[265] followed up 627 participants for 10 years. The incidence of DR was 39% and BMI was found to be predictor for DR (HR 1.11, 95% CI: 1.04 to 1.18).

Frequent episodes of hypoxaemia during sleep in OSA have been reported to be associated with raised inflammatory mediators.[267] In DM, inflammatory mediators are also elevated,[268] and are contributory to diabetic micro-vascular complications such as diabetic retinal disease.[259] More recently, literature has emerged to suggest that concomitant OSA in DM patients might be associated with significantly more DR cases, and more advanced sight-threatening DMac, with the suggestion that OSA is an independent risk factor for diabetic retinal complications.[269,270] These studies used oxygenation as part of their definition of OSA, suggesting that the intermittent hypoxaemia and re-oxygenation episodes in OSA lead to a

greater oxidative stress and inflammatory responses, and hence deterioration of diabetic retinal disease.[271]

There is very little information, however, regarding the association between sleep-disordered breathing as defined by apnoea-hypopnoea frequency and diabetic retinal complications, or whether other hypoxaemia parameters are important, particularly the degree of hypoxaemia during sleep (e.g. minimum oxygen saturation (O_2) or time spent under 90% oxygen saturations [%TST<90]). Furthermore, little has been studied in patients with severe obesity, a high-risk group for OSA and DM. We hypothesised that DM patients with severe obesity and moderate-to-severe OSA (as defined by AHI) will have a higher prevalence of diabetic retinal disease compared to those without OSA and that OSA is associated with more severe diabetic retinal disease. We therefore undertook a cross-sectional evaluation of a cohort of consecutive severe obese patients with DM undergoing assessment with routine clinical retinal screening and overnight respiratory sleep monitoring.

4.2 Aim and objectives

The aim for this chapter is to examine the prevalence, risk factors and relationship between OSA and diabetic retinal disease in a severely obese population. The following are the objectives for the study:

1. To explore the prevalence of diabetic retinal disease amongst DM individuals who are very severely obese

2. To examine the relationship between nocturnal hypoxaemia and diabetic retinal disease in very severely obese DM participants, and

3. To investigate the risk factors for diabetic retinal disease amongst very severely obese DM individuals.

4.3 Methods

4.3.1 Study design and study participants

A cohort of consecutive patients attending the regional specialist weight management service at Birmingham Heartlands Hospital, UK between January 2009 and December 2011 was studied. This study was carried out at the same period as Chapter 2 hence the identical study period. Only DM individuals were included. Routine clinical data collected included age, gender, self-reported ethnicity, weight, BMI, cardiovascular co-morbidities (hypertension and coronary artery disease), duration of DM, DM medications, HbA_{1c} (DCCT-aligned and IFCC), and renal function (creatinine and estimated glomerular filtration rate (eGFR)).

4.3.2 Respiratory sleep monitoring methods

Overnight sleep recording had been described in detail in Chapter 2. OSA in this chapter was classified into participants 2 groups: those with AHI of <15 events/hr was regarded as the 'OSA– group' while those with the presence of AHI ≥15 events/hr was in the 'OSA+ group'. The cut-off point for AHI of 15 events/hr was selected as it signifies moderate-to-severe OSA and forms part of the clinical decision making determining whether or not to treat with continuous positive airway pressure (CPAP). The AHI was used as the diagnostic parameter for OSA and not oxygen desaturation index (ODI) as ODI was not measured in this study.

4.3.3 Retinal screening methods

DR data were obtained from routine local screening program based on the English National Screening Programme for Diabetic Retinopathy (ENSPDR) in Birmingham Heartlands Hospital. The ENSPDR was implemented from 2003.[156] The screening programme aims to

offer regular eye examinations in order to detect DR at an early stage so appropriate and effective eye treatment can be provided to diabetes patients.[156] Digital photographs of two 45º fields per eye (1 fovea-centre-ed, 1 disc-centre-ed) are obtained annually. ENSPDR also developed guidance on the classification of the severity of diabetic retinopathy in England and Wales.[272] All diabetes patients attending the clinic underwent annual retinal screening and data were acquired from the most recent screening assessment. Presence or absence of DR and DMac were recorded. DMac is defined as any evidence of exudate, micro-aneurysm or haemorrhage with 1 disc diameter of the centre of the fovea.[272]

4.3.4 Statistical analysis

All statistical analyses were carried out using the Statistical Package for Social Sciences, version 19 (SPSS, Chicago, IL). Data analysis was performed on anonymised patients as part of service evaluation. Normally distributed data are reported as mean±standard deviation (SD) and analysed using an independent t-test whilst skewed data are reported as median and interquartile range (IQR) and the Mann Whitney U-test was used for analysis. Categorical data was analysed using the chi-squared test. Statistical comparisons were made for patients with and without DR, DMac and OSA.

Univariate analyses were carried out to examine factors associated with diabetic retinal disease. Logistic regression analyses were conducted and three models constructed to examine associations between 1) AHI (continuous); 2) mean O_2; 3) minimum O_2; and 4) %TST<90; and DR as well as DMac. The base model is unadjusted, Model 1 was adjusted for age, gender, BMI and ethnicity (white Europeans vs. non-white Europeans), and Model 2 was further adjusted for DM duration, presence/absence of insulin treatment and HbA_{1c},

presence/absence of hypertension, and presence/absence of coronary artery disease. Logistic regression analyses were also performed to investigate the associations between other variables and DR as well as DMac. The models aimed to adjust factors which were considered to be clinically important. To avoid repetition, the influence of these factors has been or will be described elsewhere. For the effect of age on OSA, please refer to the introduction of chapter 2 (section 2.1.3), the effects of gender (section 2.4.4) and ethnicity (section 2.5.3) on OSA have been discussed in details in the chapter 2's discussion. Please refer to Chapter 1 (section 1.3.4) for the effect of body mass index on OSA. The effect of type 2 diabetes (HbA_{1c}) on OSA was discussed in chapter 3 (section 3.5.2). As for insulin treatment and DM duration, these signify severity of diabetes mellitus (please refer to section 4.5.5). Hypertension (section 1.3.3 and 4.5.3) and coronary artery disease (section 1.3.3) have been shown to be associated with OSA. Results were reported as odds ratio (OR) with 95% confidence interval (95% CI). A p value of <0.05 was considered statistically significant.

Table 4-2: Factors adjusted and the appropriate sections discussing the rationales behind

Factors	**Sections**
Age	Introduction of Chapter 2 (section 2.1.3)
Gender	Chapter 2's discussion (section 2.5.5)
Ethnicity	Chapter 2's discussion (section 2.5.3
Body mass index	Chapter 1 (section 1.3.4)
Type 2 diabetes	Chapter 3 (section 3.5.2)
Insulin treatment	Chapter 4 (section 4.5.5)
Hypertension	Chapter 1 (section 1.3.3) and chapter 4 (section 4.5.3)
Coronary artery disease	Chapter 1 (section 1.3.3)

4.4 Results

There were a total of 121 eligible DM patients, but two patients' sleep respiratory data were not analysable, and 26 patients did not have the complete retinopathy screening data available; therefore, a total of 93 eligible patients were included in the study (Figure 4-1). No patient had a diagnosis of central sleep apnoea.

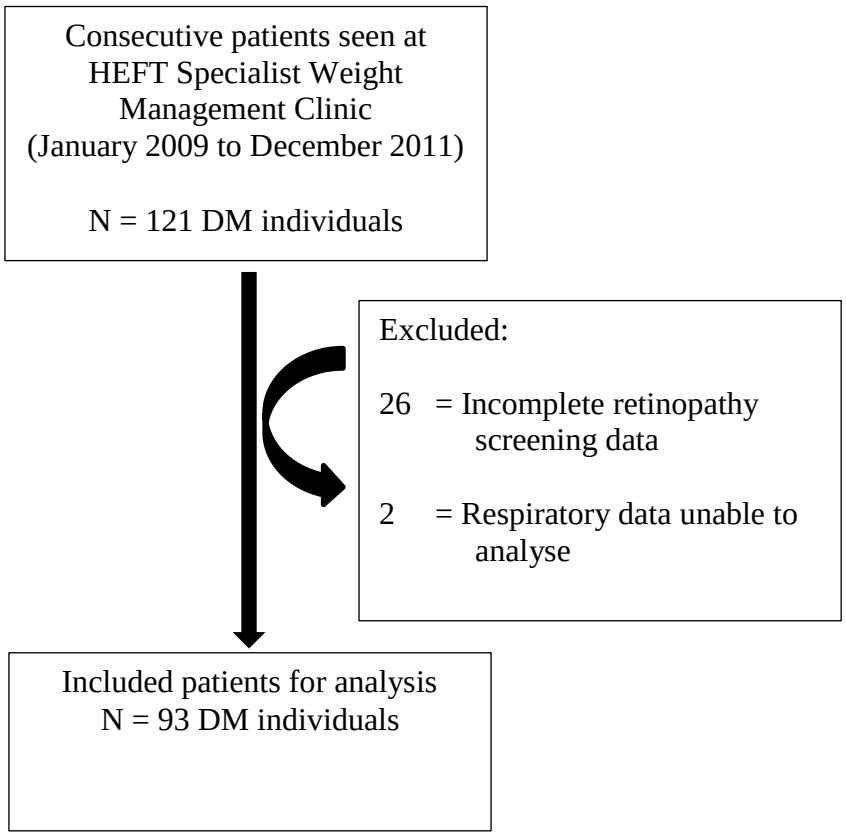

Figure 4-1: Flow diagram of referrals seen at the HEFT Specialist Weight Management Clinic, and those included in the study
HEFT – Heart of England NHS Foundation Trust
DM = Diabetes Mellitus

4.4.1 Study characteristics

The mean±SD age of the patients was 52±10 years and 59% of the patients were females. The majority of patients were of white European origin (69%) and 23% were of South Asian origin. The mean±SD for weight and BMI were 130.4±25.3Kg and 47.3±8.3Kg/m^2, respectively. Sixty-three per cent of patients suffered with hypertension. Median duration for DM was 6 years (IQR: 2, 10) with a median HbA_{1c} of 62mmol/mol (IQR: 51, 77) or 7.8% (IQR: 6.8, 9.2). The majority of patients were on diabetic lowering medications (81.7%) and 33% of the patients were on insulin treatment.

Median AHI was 14 events/hr (IQR: 7, 36) with a median minimum O_2 of 82% (IQR: 74, 85) and median %TST<90 of 3% (IQR: 1, 12) for the total sample. The mean O_2 was 93% (IQR: 92, 95). Results for creatinine and estimated GFR were 72micromol/l (IQR: 63, 87) and 86ml/min/1.73m^2 (IQR: 71, 90). The overall prevalence of DR and DMac were present in 38.7% (95% CI: 29.2 to 49.1) and 17.2% (95% CI: 10.7 to 26.5), respectively.

4.4.2 Diabetic retinopathy

A total of 36 patients had a diagnosis of DR. Characteristics of patients with and without DR was shown in Table 4-3 below. No significant differences were found for mean age (53±10 vs. 51±11years, p=0.228), BMI (46.2±7.8 vs. 48.1±8.5Kg/m^2, p=0.276), systolic BP (144±24 vs. 143±17mmHg, p=0.819) and diastolic BP (83±15 vs. 84±8mmHg, p=0.734) between those with and without DR. There were also no significant differences in gender distribution between the groups (p=0.759).

Significant differences were present for all diabetic parameters. Glycosylated haemoglobin A_{1c} for patients with DR were significant higher at 8.5% (IQR: 7.3, 9.9) when compared to those without the disease (7.5% IQR: 6.6, 8.7). The duration of DM was also significantly longer in the diseased group (10 [IQR: 5, 15] vs. 5 [IQR: 2, 9]years, p=0.003). Even though there was a slightly greater proportion of DM patients on medications for non-DR group (82.5% vs. 80.6%), majority of the patients were only on single DM medications (35.1%). In comparison, more than half of the DR patients received 2 or more DM medications (69.4%, p=0.012). Significant differences were also present for those treated with insulin between DR and non-DR groups (69.4% vs. 15.8%, p<0.001).

There were no significant differences found for all respiratory parameters between individuals with and without DR (p>0.05). For the renal parameters, both creatinine and eGFR reached statistical significance when compared in both groups. Both creatinine and eGFR had a 10µmol/l (p=0.013) and 10ml/min/1.73m^2 (p=0.006) difference respectively between the groups.

Table 4-3: Comparison of patients with and without diabetic retinopathy

	Diabetic retinopathy absence (n=57)	Diabetic retinopathy presence (n=36)	p value
Age (years)	51 ± 11	53 ± 10	0.228
Gender			0.759
Female	33 (57.9%)	22 (61.1%)	
Male	24 (42.1%)	14 (38.9%)	
Ethnicity			0.906
White Europeans	40 (70.2%)	24 (66.7%)	
South Asians	12 (21.1%)	9 (25.0%)	
Afro-Caribbean	5 (8.8%)	3 (8.3%)	
Weight (Kg)	134 ± 26	124 ± 24	
BMI (Kg/m^2)	48.1 ± 8.5	46.2 ± 7.8	0.276
Systolic BP (mmHg)	143 ± 17	144 ± 24	0.819
Diastolic BP (mmHg)	84 ± 8	83 ± 15	0.734
Diabetes parameters			
HbA_{1c} (%)	7.5 (6.6, 8.7)	8.5 (7.3, 9.9)	**0.020**
Insulin	9 (15.8%)	25 (69.4%)	**<0.001**
DM duration (years)	5 (2,9)	10 (5, 15)	**0.003**
DM medications			
None	10 (17.5%)	7 (19.4%)	**0.012**
1	20 (35.1%)	4 (11.1%)	
2	16 (28.1%)	21 (58.3%)	
>3	11 (19.3%)	4 (11.1%)	
Respiratory parameters			
AHI (events/hr)	14 (6, 30)	15 (7, 61)	0.493
Mean O2 sats (%)	93 (92, 95)	93 (91, 95)	0.200
Min O2 sats (%)	82 (77, 84)	81 (71, 85)	0.292
Time < 90% (%)	2 (1,9)	8 (1, 27)	0.117
Renal parameters			
Creatinine (µmol/l)	70 (60, 78)	80 (66, 98)	**0.013**
eGFR ($ml/min/1.73m^2$)	90 (78, 90)	80 (59, 90)	**0.006**

Data are presented as mean ± standard deviation, number (%), or median (interquartile range).
BMI=body mass index; CAD=coronary artery disease; DM=diabetes mellitus; BP=blood pressure; eGFR=estimated glomerular filtration rate, HbA_{1c}=glycosylated haemoglobin A_{1c}.
p values were calculated using either independent t-test, chi square or Mann Whitney U-test.

4.4.3 Diabetic maculopathy

A comparison was made between individuals with presence and absence of diabetic maculopathy as summarised in Table 4-4. A total of 16 patients had DMac. There were no statistical differences in the recorded age (53±10 vs. 51±11years, p=0.671), BMI (45.8±8.4 vs. 47.6±8.2Kg/m^2, p=0.420), systolic BP (139±24 vs. 144±20mmHg, p=0.403) and diastolic BP (84±21 vs. 84±9mmHg, p=1.000) between those with and without DMac. There was a greater proportion of males in DMac compared to those with no DMac (56.3% vs. 37.7%, p=0.777), however, it did not reached statistical significance. There were also almost equal proportions of White Europeans (62.5% vs. 70.1%), South Asians (25.0% vs. 22.1%) and Afro-Caribbean (12.5% vs. 7.8%) in both groups (p=0.777).

Similarly to DR, there were significant differences in diabetes parameters. DMac individuals were found to have higher HbA_{1c} (9.2% [IQR: 8.1, 10.3] vs. 7.6% [IQR: 6.7, 8.9], p=0.003) and double the duration of DM (12 [IQR: 7, 19] vs. 6 [IQR: 2, 9]years, p=0.003) compared to disease free individuals. Although there was a four-fold difference in insulin treatment for DMac patients (87.5% vs. 22.1%, p<0.001), no significance were found for the number of DM medications (p=0.172). For respiratory parameters, the only significant finding was on minimum O_2. There was almost a 10% reduction in minimum O_2 in DMac vs non-DMac patients (71% [IQR: 63, 84] vs. 82% [IQR: 77, 85], p=0.021). Even though there was almost 2 fold higher in the AHI (29 [IQR: 11, 69] vs. 14 [IQR: 6, 32] events/hr), this did not achieve statistical significance (p=0.074). Similarly, no significant difference were found for %TST<90 in spite of an almost 4 fold difference between the two groups (11% [IQR: 1, 27] vs. 3% [IQR: 1, 11], p=0.239). Creatinine was found to be significantly different between the

groups (88 [IQR: 67, 108] vs. 71 [IQR: 60, 84]µmol/l, p=0.042) while eGFR was not (83 [IQR: 60, 90] vs. 89 [IQR: 71, 90]ml/min/1.73m^2, p=0.140).

Table 4-4: Comparison of patients with and without diabetic maculopathy

	Diabetic maculopathy absence (n=77)	**Diabetic maculopathy presence (n=16)**	**P value**
Age (years)	51 ± 11	53 ± 10	0.671
Gender			0.169
Female	48 (62.3%)	7 (43.8%)	
Male	29 (37.7%)	9 (56.3%)	
Ethnicity			0.777
White Europeans	54 (70.1%)	10 (62.5%)	
South Asians	17 (22.1%)	4 (25.0%)	
Afro-Caribbean	6 (7.8%)	2 (12.5%)	
Weight (Kg)	131 ± 26	125 ± 21	
BMI (Kg/m^2)	47.6 ± 8.2	45.8 ± 8.4	0.420
Systolic BP (mmHg)	144 ± 20	139 ± 24	0.403
Diastolic BP (mmHg)	84 ± 9	84 ± 21	1.000
Diabetes parameters			
HbA_{1c} (%)	7.6 (6.7, 8.9)	9.2 (8.1, 10.3)	**0.003**
Insulin	17 (22.1%)	14 (87.5)	**<0.001**
DM duration (years)	6 (2, 9)	12 (7, 19)	**0.003**
DM medications			0.172
None	14 (22.1%)	3 (18.8%)	
1	22 (28.6%)	2 (11.1%)	
2	27 (35.1%)	10 (62.5%)	
>3	14 (18.2%)	1 (6.3%)	
Respiratory parameters	14 (6, 32)	29 (11, 69)	0.074
AHI (events/hr)	93 (92, 95)	93 (90, 95)	0.906
Mean O2 sats (%)	82 (77, 85)	71 (63, 84)	**0.021**
Min O2 sats (%)	3 (1, 11)	11 (1, 27)	0.239
Time < 90% (%)			
Renal parameters			
Creatinine (µmol/l)	71 (60, 84)	88 (67, 108)	**0.042**
eGFR (ml/min/1.73m^2)	89 (71, 90)	83 (60, 90)	0.140

Data are presented as mean±standard deviation, number (%), or median (interquartile range).
BMI=body mass index; CAD=coronary artery disease; DM=diabetes mellitus; BP=blood pressure; eGFR=estimated glomerular filtration rate, HbA_{1c}=glycosylated haemoglobin $A_{1c/}$

4.4.4 Diabetic retinal diseases (DR and DMac) and obstructive sleep apnoea

Table 4-5 shows the patient characteristics, according to the presence or absence of OSA, determined by AHI. Of the 93 patients, 46 (51%) had an AHI ≥15events/hour with a median (IQR) AHI of 37 (23, 74) events/hr (OSA+ group), compared to those with an AHI <15 events/hr; median (IQR) for AHI was 7 (4 to 11) events/hr (OSA- group). Both OSA+ and OSA- groups were well matched for ethnicity (p=0.716), weight (135±27 vs. 127±23Kg, p=0.142), BMI (47.6±8.5 vs. 47.1±8.1Kg/m^2, p=0.776), diastolic BP (85±12 vs. 83±10mmHg, p=0.446) and coronary artery disease (15% vs. 11%, p=0.510). Although not statistically significant, systolic BP (146±23 vs. 139±15mmHg, p=0.177) and hypertension (72% vs. 55%, p=0.100) higher in OSA+ group. The OSA+ group were slightly older than those without OSA (54±10 vs. 49±10years, p=0.01) with a greater proportion of males (66% vs. 25%, p=0.002).

No significant differences were found for diabetes parameters, which includes diabetes duration (6 [IQR: 2, 10] vs. 6 [IQR: 3, 11]years, p=0.423), HbA_{1c} (8.1% [IQR: 7.1, 9.2] vs. 7.7% [IQR: 6.7, 9.3], p=0.267], insulin requirement (41% vs. 26%, p=0.123) and the number of DM medications required (p=0.795). As expected, there were significant differences in all the respiratory parameters. The AHI was 37 (IQR 23, 74) events/hr for OSA+ group compared to 7 (IQR: 4, 11) events/hr for OSA- group. Mean and minimum O_2 were 93% (IQR: 91, 94) and 76% (IQR: 71, 82), respectively for OSA+ group and 94% (IQR: 92, 95, p=0.001) and 84% (IQR: 82 to 88, p<0.001) for OSA- group. Significant higher %TST<90 were found for OSA+ group (11% [IQR: 2, 25] vs. 1% (IQR: 0, 3], p<0.001). There was significant higher renal creatinine level in OSA+ group (78 [IQR: 66, 93]µmol/l) when

compared to disease free group (67 [IQR: 57, 79]µmol/l, p=0.002). Non statistical differences were reported for eGFR (83 [IQR: 61 to 90] vs. 90 [IQR: 77 to 90]ml/min/1.73m^2, p=0.068).

Table 4-5: Sample characteristics of 93 patients according to presence/absence of obstructive sleep apnoea

	OSA- (n=47)	**OSA+ (n=46)**	**p value**
Age (years)	49 ± 10	54 ± 10	**0.010**
Gender, n (%)			**0.002**
Female, n (%)	35 (75)	20 (43.5)	
Ethnicity, n (%)			0.716
White European	34 (72)	30 (65)	
South Asian	9 (19)	12 (26)	
Afro-Caribbean	4 (9)	4 (9)	
Weight (kg)	127 ± 23	135 ± 27	0.142
BMI (kg/m^2)	47.1 ± 8.1	47.6 ± 8.5	0.776
Systolic BP (mmHg)	139 ± 15	146 ± 23	0.177
Diastolic BP (mmHg)	83 ± 10	85 ± 12	0.446
Co-morbidities, n (%)			
Hypertension	26 (55)	33 (72)	0.100
CAD	5 (11)	7 (15)	0.510
Diabetes parameters			
DM duration (years)	6 (3-11)	6 (2-10)	0.423
DM medications, n (%)			0.795
None	9 (19)	8 (17)	
One	10 (21)	14 (30)	
Two	20 (43)	17 (37)	
Three or more	8 (17)	7 (15)	
HbA_{1c} (%)	7.7 (6.7-9.3)	8.1 (7.1-9.2)	0.267
Insulin treatment	12 (26)	19 (41)	0.123
Respiratory parameters			
AHI (/hour)	7 (4-11)	37 (23-74)	**<0.001**
Mean O2 saturation (%)	94 (92-95)	93 (91-94)	**0.001**
Minimum O2 saturation (%)	84 (82-88)	76 (71-82)	**<0.001**
% Time spent <90% saturation	1 (0-3)	11 (2-25)	**<0.001**
Renal parameters			
Creatinine (µmol/l)	67 (57-79)	78 (66-93)	**0.002**
eGFR (ml/min/1.73m^2)	90 (77-90)	83 (61-90)	0.068
Retinopathy outcome, n (%)			
Retinopathy present	18 (38)	18 (39)	0.934
Maculopathy present	6 (13)	10 (22)	0.252

Data are presented as mean±standard deviation, number (%), or median (interquartile range).
OSA=obstructive sleep apnoea; BMI=body mass index; CAD=coronary artery disease; DM=diabetes mellitus; BP=blood pressure; eGFR=estimated glomerular filtration rate, HbA_{1c}=glycosylated haemoglobin $A_{1c.}$
p values were calculated using either independent t-test, chi square or Mann Whitney U-test.

Table 4-6 shows the retinal screening results. Most of the patients did not have DR for those with or without OSA (60.9% vs. 61.7%, p=0.765). Background retinopathy was present in 13 OSA patients and 15 non-OSA patients. Three patients with OSA had pre-proliferative retinopathy compared to one in OSA- group. There were 2 patients each in both groups for proliferative DR. No significant differences were found between the presence and absence of OSA for DR (39% vs. 38%, p=0.765). However more OSA+ patients had DMac compared to OSA- patients (22% vs. 13%), but this did not reach statistical significance (p=0.252). A total of 5 patients had had photocoagulation therapy.

Table 4-6: Diabetic retinopathy in 93 patients according to presence/absence of obstructive sleep apnoea

	OSA- (n=47) n (%)	**OSA+ (n=46) n (%)**	**p value**
Either eye			
No retinopathy	29 (61.7)	28 (60.9)	0.765
Background retinopathy	15 (31.9)	13 (28.3)	
Pre-proliferative retinopathy	1 (2.1)	3 (6.5)	
Proliferative retinopathy	2 (4.3)	2 (4.3)	
Maculopathy	6 (12.8)	10 (21.7)	0.252
Photocoagulation	3 (6.4)	2 (4.3)	0.664
Right eye			
No retinopathy	32 (68.1)	30 (65.2)	0.943
Background retinopathy	12 (25.5)	12 (26.1)	
Pre-proliferative retinopathy	2 (4.3)	2 (4.3)	
Proliferative retinopathy	1 (2.1)	2 (4.3)	
Maculopathy	5 (10.6)	5 (10.9)	0.971
Photocoagulation	3 (6.4)	2 (4.3)	0.664
Left eye			
No retinopathy	33 (70.2)	31 (67.4)	0.777
Background retinopathy	11 (23.4)	10 (21.7)	
Pre-proliferative retinopathy	1 (2.1)	3 (6.5)	
Proliferative retinopathy	2 (4.3)	2 (4.3)	
Maculopathy	5 (10.6)	10 (21.7)	0.146
Photocoagulation	3 (6.4)	2 (4.3)	0.664

OSA=obstructive sleep apnoea.
p values derived from chi square test.

Univariate logistic regression (Table 4-7) identified factors significantly associated with diabetic retinal disease, which was minimum O_2 with both DR and DMac. The results of the multivariate logistic regression analyses are shown in Table 4-6. We observed, after adjustment, no relationship between DR and AHI, mean O_2, minimum O_2, and %TST<90. After adjustment, the presence of DMac was not associated with AHI, mean O_2, and %TST<90. Minimum O_2 was, however, after adjustment, an independent and significant predictor for the presence of DMac (OR 0.79 [95% CI: 0.65 to 0.95], $p<0.05$). This represents that each 1% increase in minimum oxygen saturation was associated with 21% reduction in the risk of DMac.

Table 4-7: Logistic regression analyses assessing the presence of diabetic retinopathy (DR) and maculopathy (DMac) with four respiratory parameters

Predictors of thepresence of retinopathy (DR)	**Unadjusted**	**Models Model 1**	**Model 2**
AHI	1.01 (0.99-1.02)	1.01 (0.99-1.02)	1.00 (0.98-1.02)
Mean oxygen saturation	0.89 (0.77-1.03)	0.90 (0.77-1.05)	0.83 (0.67-1.02)
Minimum oxygen saturation	**0.95* (0.90-1.00)**	0.95 (0.89-1.00)	0.93 (0.86-1.01)
% Time spent <90% oxygen saturation	1.03 (1.00-1.05)	1.03 (1.00-1.06)	1.03 (1.00-1.06)
Age	1.03 (0.98-1.07)	1.02 (0.98-1.07)*	1.01 (0.95-1.06)*
Male	0.88 (0.37-2.05)	0.54 (0.20-1.45)*	0.43 (0.12-1.54)*
BMI	0.97 (0.72-1.02)	0.95 (0.90-1.01)*	0.94 (0.87-1.01)*
Non WE	1.08 (0.56-2.06)	1.00 (0.60-1.98)*	1.08 (0.45-2.56)*
DM Duration	**1.12 (1.04-1.21)**	Not applicable	1.04 (0.94-1.15)*
Insulin	**8.21 (3.08-21.83)**	Not applicable	**6.55 (1.63-26.26)***
HbA_{1c}	**1.32 (1.01-1.73)**	Not applicable	1.36 (0.98-1.90)*
Hypertension	1.54 (0.63-3.72)	Not applicable	0.35 (0.09-1.32)*
CAD	1.70 (0.50-5.75)	Not applicable	3.01 (0.62-14.54)*
Predictors of the presence of maculopathy (DMac)	**Unadjusted**	**Models Model 1**	**Model 2**
AHI	1.01 (1.00-1.03)	1.01 (0.99-1.03)	1.01 (0.98-1.04)
Mean oxygen saturation	0.93 (0.79-1.08)	0.92 (0.77-1.09)	0.80 (0.59-1.07)
Minimum oxygen saturation	**0.91** (0.85-0.97)**	**0.90** (0.84-0.97)**	**0.79* (0.65-0.95)**
% Time spent <90% oxygen saturation	1.02 (0.99-1.04)	1.02 (0.99-1.04)	1.03 (0.99-1.08)
Age	1.01 (0.96-1.07)	1.00 (0.94-1.06)*	0.95 (0.87-1.04)*
Male	2.13 (0.72-6.33)	1.61 (0.49-5.33)*	1.49 (0.27-8.25)*
BMI	0.97 (0.91-1.04)	0.98 (0.91-1.06)*	0.95 (0.36-1.05)*
Non WE	1.32 (0.60-2.90)	1.18 (0.50-2.77)*	0.83 (0.23-2.97)*
DM Duration	**1.16 (1.06-1.26)**	Not applicable	1.09 (0.97-1.22)*
Insulin	**24.29 (5.02-117.57)**	Not applicable	**10.02 (1.49-67.59)***
HbA_{1c}	**1.49 (1.07-2.07)**	Not applicable	1.41 (0.90-2.20)*
Hypertension	**4.98 (1.06-23.44)**	Not applicable	3.94 (0.28-54.56)*
CAD	0.96 (0.19-4.85)	Not applicable	0.79 (0.08-8.11)*

Unadjusted: univariate models.
Model 1: adjusted for age, gender, body mass index, ethnicity.
Model 2: further adjusted for diabetes mellitus duration, insulin treatment, HbA_{1c}, hypertension, and coronary artery disease. * adjusted for AHI only to avoid multi-collinearity
AHI = apnoea-hypopnoea index, HbA_{1c}=glycosylated haemoglobin A_{1c}, BMI = body mass index, Non WE = non White Europeans, DM = Diabetes mellitus, CAD = coronary artery disease.

4.4.5 Factors associated with worsening of diabetic retinopathy and maculopathy

Apart from respiratory parameters, logistic regression analysis was also performed for other factors, namely gender, age, BMI, duration of DM, HbA_{1c} level, hypertension and insulin treatment. No associations were found for age, gender and BMI with DR or DMac. Univariate analysis showed that DM duration was associated with greater progression of both DR (OR 1.123, 95% CI: 1.042 to 1.211) and DMac (OR 1.156, 95% CI: 1.060 to 1.260). Similarly, DM patients who did not require treatment for insulin were protective of DR (OR 0.122, 95% CI: 0.046 to 0.324) and DMac (OR 0.041, 95% CI: 0.009 to 0.199). Greater level of HbA_{1c} was associated with presence of DMac (OR 1.481, 95% CI: 1.063 to 2.064) while absence of hypertension was protective of DMac (OR 0.201, 95% CI: 0.043 to 0.946).

In Model 1, adjustment for gender, age and BMI were performed. The protective association of the absence of insulin treatment with DR (OR 0.111, 95% CI: 0.038 to 0.323) and DMac (OR 0.035, 95% CI: 0.007 to 0.188) persisted. Longer duration of DM (OR 1.110, 95% CI: 1.028 to 1.199) and higher HbA_{1c} level (OR 1.333, 95% CI: 1.003 to 1.771) were associated with worsening of DR. The association between hypertension and DMac was no longer significant in Model 2. However, the associations between all variables with DR and DMac disappeared apart from treatment with insulin after adjustment in Model 2. Only absence of insulin treatment was found to be protective of DR (OR 0.163, 95% CI: 0.044 to 0.609) and DMac (OR 0.109, 95% CI: 0.018 to 0.668) as shown in Table 4-8.

Table 4-8: Logistic regression analyses assessing the presence of diabetic retinopathy (DR) and maculopathy (DMac) and other factors

Predictors of the presence of retinopathy (DR)	**Unadjusted**	**Models** **Model 1**	**Model 2**
Female	1.143 (0.488–2.679)		1.854(0.577-5.959)
Age	1.026(0.984-1.069)		1.011(0.960-1.066)
BMI	0.971(0.921-1.023)		0.952(0.890-1.018)
DM duration	**1.123(1.042-1.211)****	**1.110(1.028-1.199)****	1.039(0.942-1.147)
HbA_{1c}	1.291(0.982-1.699)	**1.333 (1.003-1.771)***	1.210(0.863-1.697)
No Insulin	**0.122(0.046-0.324)****	**0.111(0.038-0.323)****	**0.163(0.044-0.609)****
No hypertension	0.650 (0.268-1.576)	0.719(0.284-1.824)	2.150(0.624-7.405)
Predictors of the presence of maculopathy (DMac)	**Unadjusted**	**Models** **Model 1**	**Model 2**
Female	0.470(0.158-1.398)		0.474(0.107-2.103)
Age	1.011(0.960-1.066)		0.965(0.892-1.044)
BMI	0.972(0.907-1.041)		0.997(0.892-1.070)
DM duration	**1.156(1.060-1.260)****	0.427(0.119-1.534)	1.091 (0.974-1.222)
HbA_{1c}	**1.481(1.063-2.064)***	**1.153 (1.061-2.157)***	1.212(0.760-1.932)
No Insulin	**0.041 (0.009-0.199)****	**0.035 (0.007-0.188)****	**0.109(0.018-0.668)***
No hypertension	**0.201 (0.043-0.946)***	0.211 (0.043-1.029)	0.255 (0.022-2.953)

BMI = body mass index, DM = diabetes mellitus, HbA_{1c} = glycosylated haemoglobin A_{1c}
Unadjusted: univariate models
Model 1: adjusted for age, gender, BMI
Model 2: further adjusted for hypertension, diabetes mellitus duration, insulin treatment and HbA_{1c}
* $p<0.05$; ** $p<0.01$.

4.5 Discussion

4.5.1 Prevalence of diabetic retinal disease

Diabetic retinal disease is a well-recognised micro-vascular complication of DM.[273] There are several epidemiological studies on the prevalence of DR for both type 1 and type 2 DM.[274-277] However, the scope of the problem has not been studied in the extreme obese population, a high risk group of individuals. Our study showed that the overall prevalence of DR amongst extreme obese DM patients was 38.7% (95% CI: 29.2 to 19.1%) and for diabetic maculopathy was 17.2% (95% CI: 10.7 to 26.5%).

A study on the sample of National Health and Nutrition Examination Survey (NHANES) from 2005 to 2008 found that there were 1006 individuals aged 40 and above with DM including 795 self-reported DM patients and 211 undiagnosed patients ($HbA_{1c} \geq 6.5\%$).[276] The reported estimated crude prevalence for DR was 28.5% (95% CI: 24.9 to 32.5%) and the age standardised prevalence was 22.1% (95% CI: 18.4 to 26.35). DR prevalence was higher at 32.8% (95% CI: 28.6 to 37.2%) when the 211 undiagnosed DM individuals were excluded. In spite of this, the reported prevalence is still lower compared to our study. The estimated crude prevalence for diabetic macular oedema was 2.7% (95% CI: 1.8 – 4.0%). The NHANES study had a lower mean HbA_{1c} 7.9% (95% CI: 7.6 to 8.1%) for the DR group compared to our study of 8.5% and this may partially explain the differences in prevalence. However, the NHANES patients are 9 years older in age compared to those in our study - the mean ages for the NHANES study are 62 (95% CI: 60 to 63) years for DR patients and 60 (95% CI: 59 to 61) years for non-DR individuals.[276] The duration of DM was also 5 years longer for the NHANES DR individuals at 15.0 (95% CI: 13.4 to 16.5) years compared to our DR patients. One of the most likely factors contributing to the greater prevalence of DR in our study is the

level of adiposity. All our patients were very severely obese with mean BMI of 47.3±8.3Kg/m^2 while in the NHANES examined the general population as a whole and only 57.1% (95% CI: 48.5 to 65.2) with DR have a BMI≥30 Kg/m^2.[276]

4.5.1.1 Different era of diabetes care

Another report on a pool of 8 population studies of those aged 40 and above with both type 1 and type 2 DM. The study had 4440 individuals. The 8 studies included in the report were the Barbados Eye study,[278] Beaver Dam Eye Study,[279] Blue Mountains Eye Study,[280] Melbourne Visual Impairment Project,[281] Proyecto Vision Evaluation Research,[282] San Antonio Heart Study,[283] San Luis Valley Diabetes Study[284] and Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR).[285] The estimated overall crude prevalence for DR was 40.3% (95% CI: 38.8 to 41.7). The higher crude prevalence may be partially influenced by the WESDR prevalence rate (36.6%) as the WESDR study was conducted between 1980 and 1982.[285]

When the WESDR study was excluded from the analysis, the crude prevalence reported was 35.8%,[277] which is comparable to our study's figure. All the data from the pool of 8 studies were collected prior to year 2001 while our data was collected after 2009.[277] It may be that this represents the difference in the standard of care for DM in the 2 eras which consequently have an impact on the reported DR prevalence. Since the publication of the landmark studies such as Diabetes Control and Complications Trial (DCCT) in 1993 for type 1 DM[286] and the UK Prospective Diabetes Study (UKPDS) in 2001 for type 2 DM,[287] there has been significant focus on the importance of tight glycaemic and BP control to reduce the risk of the development and progression of DM micro-vascular complications. These trials have led to significant changes in clinical management and care for DM patients and these changes most

likely have contributed to the lower DR prevalence in our study despite the high level of adiposity.

A recent report by Yau and colleagues examined the global DR prevalence[274] from a pool of 35 studies including 22,896 individuals of both type 1 and type 2 DM patients. The mean age was 58.1 years with 52% female and median DM duration and HbA_{1c} of 7.9 (IQR 3-16) years and 8.0% (IQR 6.7-9.9), respectively. The overall age standardised prevalence was 34.6% (95% CI: 34.5 to 34.8) for DR and 6.81% (95% CI: 6.74 to 6.89) for macular oedema. The global DR rates were even lower when studies performed before the year 2000 were excluded in the subgroup analysis. The reported global age standardised prevalence rate for post year 2000 for DR was 24.79% (95% CI: 24.57 to 25.00) and 5.46% (95% CI: 5.35 to 5.56) for DMac. These figures are lower compared to our study's result especially for DMac, which is at least 3 fold lower.

In addition, despite having a higher prevalence, our patients were also 6 years younger in age and have shorter DM duration compared to the study by Yau et al. (median 7.9 with IQR 3 to 16years).[274] The study from Yau and colleagues did not report on the number of glucose-lowering medications or insulin treatment for their participants. Although DM duration may be an indicator of the severity of DM, glucose-lowering treatment especially insulin therapy[288] may be a better predictor. More than half of our study participants who had DR and DMac were on insulin treatment. Moreover, the majority of DR and DMac patients required at least 2 or more glucose-lowering medications indicating high level of insulin resistance. For our DMac patients, HbA_{1c} level was also significantly higher compared to Yau et al. (median HbA_{1c} 8.0% [IQR: 6.7 to 9.9]).[274] All these factors demonstrated that our patients may have

more severe DM and as a consequent of this, a greater prevalence of diabetic retinal disease. Both the greater severity of DM as well as the greater prevalence of DR and DMac may be a result from a combination of high level of adiposity and nocturnal hypoxia. To our knowledge, this is the first DR prevalence study on extreme obese individuals suitable for bariatric surgery.

4.5.2 Relationship between obstructive sleep apnoea and diabetic retinopathy

4.5.2.1 OSA and retinopathy in general population

There has been much interest in the role of OSA in the development of micro-vascular complications. The first study to examine the effect is the Sleep Heart Health Study (SHHS).[289] The SHHS examined a total of 2927 participants. Of those 2927 participants, 1867 participants were from the Atherosclerosis Risk in Communities (ARIC)[290] and 1060 participants were from the Cardiovascular Health Study (CHS).[291] All the participants received retinal photography as part of the primary study (within the study protocol of ARIC and CHS) while all patients had home sleep monitoring performed as part of SHHS.[289] The majority of the participants were white Caucasians (93%). The median AHI was 4.7 events/hr (IQR: 1.5, 11.6) and about 70% of the participants had AHI ≤10 events/hr.

The investigators also reported the median %TST<90 as 0.7% (IQR: 0.1, 6.6%). The presence of retinopathy was found in 6.6% of the participants. Unsurprisingly, the proportion of retinopathy was higher in DM individuals (19.4%) and hypertensive individuals (9.7%).[289] Both AHI and %TST<90 were categorised into four quartiles. Logistic regression was performed and no associations were found between retinopathy and AHI as well as %TST<90 quartiles after adjustment for age, gender, BMI, hypertension, systolic BP, smoking status,

DM and primary study.[289] Compared to the lowest quartile, the fourth quartile for AHI (OR 1.17, 95% CI: 0.73 to 1.88) and the 4th quartile for %TST<90 (OR 1.21, 95% CI: 0.75 to 1.94) did not show any significant increase in risk for any retinopathy. However, there are several limiting factors from the SHHS. Although the sample size was large, the numbers of diseased individuals for retinopathy (6.6%) were limited. There was a difference in age as ARIC age range between 51 to 71 years while CHS age range between 69 to 97 years old, this suggests heterogeneity within sample.[289] Due to these limitations for the SHHS study, even though no associations were found, more research is needed especially in the high risk individuals.

4.5.2.2 OSA and diabetic retinopathy

Limited studies are available in the literature to describe the relationship between OSA and DR.[270,271,292,293] Our study consists of very severely obese DM individuals and our result did not show any significant difference in the prevalence of diabetic retinal disease between those who had moderate-to-severe OSA compared to those who did not. We also examined the relationship between diabetic retinal disease and various respiratory parameters. After correcting for multiple confounders, there was no association found between mean oxygen saturation, and %TST<90 with DR as well as DMac. Our negative results were consistent with two Japanese studies, from the same investigators, which did not find any correlation with the above respiratory parameters.[270,271] The Japanese researchers examined DM inpatients who were admitted for retinal surgery such as vitreous surgery for macular oedema, cataract surgery, neovascular glaucoma, vitreous haemorrhage or retinal detachment surgery.[270,271] The investigators did not perform a full PSG, instead, they used pulse oximetry.[270,271] The first study had 116 inpatients in which 48 had a diagnosis of non-proliferative DR and 116 had proliferative-DR (PDR).[270] No significant association was found

between mean oxygen saturation and PDR after adjustment for age, DM duration, BMI, HbA_{1c}, hypertension, eGFR and other respiratory parameters.[270] For the second study, there were 219 inpatients of which 68 had non-PDR and 151 had PDR.[271] Logistic regression analysis was performed and again, no association was demonstrated between %TST<90 and PDR after adjustment for HbA_{1c}, age, minimum oxygen saturation, 4% oxygen desaturation index (ODI) and $HbA_{1c.}$[271]

Apart from mean oxygen saturation and %TST<90, our study also showed that OSA, when measured using AHI, has no association with retinal complications after adjustment for confounders. One study investigated the effect of AHI and DR in 98 veterans who were predominantly male.[292] The study used portable home sleep monitors and sleep was monitored for 3 nights.[292] All 98 participants had OSA and good DM control with HbA_{1c} levels of 6.5% and below.[292] The veteran study found a significant difference in the mean AHI between those with DR (AHI 44.2 events/hr) and those without DR (AHI 27.2 events/hr).[292] The discrepancy in the results between our and the veteran study may be partially explained by the differences in the characteristics of both patient groups. The mean age for the veteran participants was 61.1±8.8 years,[292] which was almost 10 years older than our cohort of individuals. Almost all the veteran participants suffered from hypertension (95%)[292] compared to less than three quarters in our patients. As expected, there was a great difference in the level of adiposity as the mean BMI was 33.7±5.0Kg/m^2 for the veteran study.[292] However, the main limitation factor was the absence of regression analysis performed to take in to account potential confounders.[292]

In a short report by Rudrappa and colleagues described the results of 31 DM individuals who received in-hospital PSG.[293] OSA was diagnosed based on AHI ≥5 events/hr and they found that 17 patients had OSA.[293] Regression analysis performed showed a significant association between OSA and total retinopathy score (p=0.008) after adjustment for confounders including serum biomarkers.[293] However, no dose response association was demonstrated between severity of OSA and retinopathy scoring.[293] The relatively small sample size is a major limitation for this study. Also, the 17 OSA positive patients probably had more severe DM compared to our study as the mean HbA_{1c} was 10.0%,[293] which is 2% higher than our study and mean DM duration of 12 years,[293] which is 2 fold higher than our OSA patients. Furthermore, all recruited patients were Caucasians therefore limiting the generalizability of this study.[293] Other studies have examined ODI instead of AHI.[269,270]

Although AHI is the recommended measurement for OSA, it measured both hypopnoea episodes which are associated with 4% oxygen desaturation and apnoea episodes[75] which may not include a dip in oxygen saturation. Therefore, one could argue ODI may be a better measure of hypoxia episodes instead of AHI. The Japanese group who compared 116 PDR and 48 non-PDR inpatients as described above reported a correlation between ODI and PDR (r=0.43, p=0.03).[270] They also performed regression analysis and found that ODI (β=0.20, t value=2.15, p=0.03) was one of the risk factor for PDR after adjustment for age, DM duration, BMI, HbA_{1c}, eGFR, hypertension and other respiratory parameters.[270] Our study was not designed to investigate the association between PDR and OSA as we only had 4 cases of PDR in our study while the Japanese study consists of DM individuals with either non-PDR or PDR.[270]

Another study from Oxford performed pulse oximetry on 140 high risk OSA individuals assessed using the Berlin questionnaire and 100 low risk OSA individuals between 2004 and 2005.[269] All 240 participants were men, of whom 118 had had a retinal screen. Twenty-four per-cent (n=28) had a diagnosis of OSA based on ODI.[269] The proportion of DR in OSA participants was 54%[269] which was significantly higher compared to our OSA+ individuals (39%). Multiple regression analysis confirms the association between ODI and retinopathy.[269] This study is not comparable to our study on several factors. First, it used ODI rather than AHI as a measure of OSA. The Oxford researchers defined ODI episode as having at least 4% reduction in oxygen saturation and OSA was diagnosed as having ODI of 10 or more events/hr.[269]

Second, the Oxford study recruited and performed pulse oximetry on their participants at a much earlier interval (between 2004 and 2005) while digital retinal photographs were most likely taken at a later interval. This is because the Oxfordshire Diabetic Eye Screening Service (ODESS) was launched only in January 2006[148] so there was a time lag between the 2 investigations which may lead to greater progression of DR. Third and finally, the Oxford study's participants were much older when pulse oximetry was first measured (at least 12 years older compared to our study's OSA+ group) with a much longer duration of DM (10.4±8.2years) and they also have greater proportion of individuals with DR in their OSA individuals (54%).[269] In addition, all the participants were male in the Oxford study[269] and it is well known that males have a greater risk of developing OSA compared to females[195] as discussed in Chapter 2.

Since our study was part of a service evaluation, and the current clinical recommendation[75] is the measurement of AHI, we do not have results for ODI. However, we attempted to compensate for the apnoea episodes by using AHI ≥15 events/hr for our OSA+ group. Although our study did not find any significant association between AHI and DR, we showed that the degree of hypoxaemia, determined by minimum oxygen saturation during sleep, was associated with the development of DMac. This association, however, was not seen with DR. As the OSA group was more hypoxaemic than the group without moderate-to-severe OSA, this may explain why the OSA+ had a higher prevalence of DMac. The relationship between OSA and diabetic retinal disease is important given the risk of blindness.[273] The Oxford study of 118 DM male patients reported several findings.[269] They reported that OSA individuals (mean ODI 20.9±16.6 events/hr vs. 2.8±2.1 events/hr in their control group), was associated with DMac (r^2=0.3, p<0.00001).[269] In another study by the Japanese researchers of 219 DM patients, it was demonstrated that, after adjusting for potential confounders, the lowest oxygen saturation and not ODI or %TST<90 significantly correlated with the development of PDR (OR: 0.93, 95% CI: 0.88 to 0.99; p=0.02).[271] It is of note that the diagnosis of OSA for both the studies was made on the basis of oxygenation (ODI) and not the recommended breathing parameters (AHI) and the severity of obesity as measured by BMI was significantly lower than our study.

The findings of our study show that minimum oxygen saturation may be associated with DMac supports the findings by Shiba et al. The Japanese researchers found that the risk for DR was 7% lower as the minimum oxygen saturation level increased.[271] Although our study measured AHI rather than ODI, the combination of results from our's and Shiba's[270] studies on oxygenation and diabetic eye micro-vascular disease, may indicate that intermittent

hypoxaemia and lowest oxygen saturation (and not apnoea/hypopnoea frequency) is independently associated with diabetic retinal disease especially DMac. OSA may impact on DR indirectly through its effect on glycaemia and other systemic diseases such as hypertension or directly from hypoxaemia.

4.5.2.3 Pathophysiology for OSA and diabetic retinopathy

As discussed in Chapter 3, OSA is associated with insulin resistance and glucose intolerance. Excess glucose in turn will cause overproduction of ROS, especially superoxide which has been shown to activate the polyol, AGE, protein kinase C and hexosamine pathways.[294] A cascade of oxidative stress and inflammatory processes occur from these pathways causing endothelial dysfunction.

The visual process is very energy demanding as evidenced by the greater consumption of oxygen by the retina compared to the brain[295] therefore the retina is very oxygen-sensitive. Both animal[296] and human[297] studies have shown that chronic intermittent hypoxia is linked to a reduction in endothelium relaxation and greater vasoconstriction. This impairs oxygenation to retina causing activation of inflammatory and oxidative stress markers. ROS were found to be elevated in pathological conditions of both OSA and DR.[298-303] Studies have found that there was a negative correlation between nitrite oxide (NO) levels[298-300] and OSA as well as a positive correlation with ROS such as superoxide[299,300] and urinary 8-hydroxy-2-deoxyguanosine (8-OHdG)[301] with OSA. Similarly in DR, there was a greater level of 8-OHdG in PDR patients compared to individuals with non-PDR or no DR.[302] These oxidative stress markers from OSA were likely to cause dysfunction in the NO activity and subsequently endothelial dysfunction.[303] The higher levels of ROS were found to induce

structural and functional changes in the retinal microvasculature such as thickening of basement membrane in the retina leading to alterations in retinal blood flow.[304] In addition, inflammatory markers such as vascular endothelial growth factor (VEGF) were also found to be elevated in hypoxic OSA patients[267] and VEGF has been shown to be associated with the neovascularisation process in ischaemic retinal disease.[268] Further longitudinal studies are required to examine the causal effect of hypoxia from OSA on diabetic retinal disease.

Apart from intermittent hypoxaemia, the level of hypoxia is also important. A Chinese study examined rats exposed to intermittent hypoxaemia (IH) at varying degrees of oxygenation.[247] Group 1 had blood oxygenation of 60%, group 2 was 71% and group 3 was 79%. The researchers documented greater levels of glucose, insulin and inflammatory makers in group 1 compared to groups 2 and 3. The levels of nuclear factor kappa B (NF-κB), a major activator of inflammatory markers, were highest in group 1, followed by group 2 and finally group 3 ($p<0.05$). Furthermore, hypoxia-inducible factor 1-alpha (HIF-1α), tumour necrosis factor alpha (TNFα) and interleukin-6 (IL-6) levels were also all significantly higher in group 1 compared to groups 2 and 3 ($p<0.05$). Another study compared IH rats treated with and without oxygen also demonstrated that NF-κB level was significantly greater in IH rats with hypoxia compared to IH treated with oxygen.[248] A recent human study compared skin biopsies of 12 OSA individuals with oxygen saturation of <75% with 12 OSA of ≥75%, showed a greater level of HIF-1α and VEGF gene expression levels suggesting greater inflammation in those with lower oxygenation.[249]

On the other hand, it is also plausible that diabetic microvascular complication causes the development of OSA. In the majority of the DM patients, microvascular complications occur

simultaneously[305] as DR individuals may also have diabetic neuropathy.[177] Diabetic neuropathy may affect the pharyngeal muscles increasing upper airway collapsibility. In a case of one family with Charcot-Marie-Tooth (CMT) disease, 11 out of the 14 family members were diagnosed to have CMT. In those with CMT, all had sleep apnoea (9 had OSA, 2 had central sleep apnoea). The researchers also found that CMT correlated highly with OSA ($r=0.69$, $p=0.029$).[306] Likewise, DM patients with autonomic neuropathy (AN) were found to have a greater risk of OSA compared to those without AN in several studies.[307,308] Therefore, the relationship between OSA and DR is likely to be bi-directional.[309]

4.5.3 Hypertension and diabetic retinopathy

OSA could also mediate the progression of DR through other systemic diseases such as hypertension.[158] Hypertension is strongly associated with OSA as shown in results of large epidemiology studies.[81,158] The Wisconsin Sleep Cohort Study examined 709 participants over 4 years and found a step wise increase in odds for hypertension for those with mild, moderate and severe OSA.[158] The SHHS examined a total of 6132 participants reported odds of 1.37 (95% CI: 1.03 to 1.83) for hypertension in those with AHI ≥30 events/hr compared to <1.5 events/hr.[81]

Although not statistically significant, systolic BP for our OSA+ patients was higher compared to OSA-. In the univariate analysis, the individuals with no previous history of hypertension had lower levels of DMac. This is unsurprising as it is well-recognised that BP plays an important role in DR progression as shown in the UKPDS studies.[287,310] In the UKPDS study, 1148 patients were randomised to receive tight BP control (BP ≤150/85mmHg) or non-tight BP control (BP <180/105mmHg) for the hypertension trial in 1987.[310] Two-thirds (n=758)

received tight BP control while 390 had lax BP control.[310] After a median follow-up of 7.4 years, the researchers found that there was a 34% (95% CI: 0.50 to 0.89) reduction in the risk of retinopathy as the number of patients requiring photocoagulation treatment decreased and a 47% (95% CI: 0.30 to 0.93) reduction in the risk of deterioration in vision (defined as ≥3 lines of the early treatment of diabetic retinopathy study chart) in in tight BP control group.[310] The researchers also examined retinal photography results and found that after 6 years of follow-up, there was a dose-response relationship between systolic BP and DR.[287] Those with systolic BP of between 125 and 139mmHg had a RR of 1.5 (95% CI: 1.2 to 2.6) while systolic BP ≥140mmHg had a RR 2.8 (95% CI: 2.2 to 3.5) for a new diagnosis of DR.[287]

Other cohort and observational studies have also demonstrated the relationship between hypertension and DR. The Hoorn Study that randomly selected individuals followed-up 233 DM and non-DM individuals over 9 years, reported that those who developed new onset DR had at least a 2-fold increase in risk (OR 2.36, 95% CI: 1.02 to 5.49) for hypertension.[311] The Barbados Eye study followed up 324 Africans with DM over 9 years and showed a reduction in DR risk in those with anti-hypertensive treatment (RR: 0.5, 95% CI: 0.3 to 0.9) and a 30% increase in risk (RR: 1.3, 95% CI: 1.1 to 1.4) for every 10mmHg increase in systolic BP.[312] The NHANES study was a cross sectional study of 1006 DM participants reported a 3% increase in risk (OR: 1.03, 95% CI: 1.02 to 1.03) for every 1mmHg increase in systolic BP[276] while the Singapore Malay Eye Study, another cross sectional observational study of 757 Malay DM patients reported OR of 1.85 (95% CI: 1.04 to 3.30) for DR in those with hypertension compared to disease free individuals.[313]

4.5.4 Glycaemic control and diabetic retinopathy

Other than hypertension, glycaemic control is also an important risk factor in the progression of DR. Our study reported a 33% increase in risk for DR for each 1% increase in HbA_{1c} after adjustment for age, gender and BMI. However, the relationship was lost after further adjustment for hypertension, insulin use and duration of DM. We also found significant relationship between HbA_{1c} and DMac. Similarly, the association became non-significant after further adjustment.

Major cohort studies have showed the link between glycaemia control and DR. The UKPDS researchers followed-up 3867 newly diagnosed DM individuals for 10 years and showed that intensive glycaemic control (HbA_{1c} 7.0%, 95% CI: 6.2 to 8.2) resulted in 25% (95% CI: 7 to 40) reduction in micro-vascular end points and a 21% reduction in DR compared to conventional control (HbA_{1c} 7.9%, 95% CI: 6.9 to 8.8 %).[314] The WESDR study followed-up 955 type 1 diabetes patients over 25 years and found that the risk for each unit increment in HbA_{1c} level was a 38% increase in risk for progression to proliferative DR (95% CI: 1.31 to 1.46),[315] 17% increase in risk for DMac (95% CI: 1.10 to 1.25)[316] and 28% increase in risk for visual impairment (95% CI: 1.04 to 2.83).[317] The Hoorn study demonstrated that for those with HbA_{1c} between 5.8 to 13.1%, the OR for DR was 2.36 (95% CI: 1.02 to 5.49) compared to those with HbA_{1c} <5.8%.[311] The Barbados Eye study reported a 3% increase in risk (95% CI: 1.2 to 1.5) for every 1% increase in $HbA_{1c.}$[312] The non-significant association from our study was likely due to insufficient sample size as this study was not powered to examine effects of glycaemia and diabetic retinal disease.

Studies have reported that some of the main risk factors for DR are duration of DM and insulin treatment.[276,318] Our study found an increase in risk of DR per year for DM diagnosis in the univariate analysis. It remained significant after adjustment for age, gender and BMI. However, the association diminished after further adjustment for hypertension, insulin treatment and HbA_{1c}. Other studies have shown a positive relationship between years of DM and DR. A study on 120 consecutive DM individuals with a median age of 74 years found that severity of DR was associated with duration of DM (OR: 3.37, 95% CI: 2.34 to 4.85) after adjustment for gender, age, HT and $HbA_{1c.}$[318] Similarly, the NHANES study also reported greater risk of DR with higher DM duration (OR: 1.06, 95% CI: 1.03 to 1.10 per year increase in DM).[276]

Insulin treatment is sometimes used as a surrogate marker for a greater severity of DM and has been found to have a significant impact on DR.[312] In our study, a greater proportion in OSA+ group was treated with insulin and no insulin treatment was protective of DR as well as DMac. This is unsurprising as there is an association between greater insulin resistance and impaired glucose metabolism with severity of OSA.[80,319] Both the Barbados Eye study[312] and the NHANES study[276] results reflect the significance of insulin treatment as an indication of greater severity of DM on diabetic retinal disease. The Barbados Eye study reported a 6 fold increase in risk (95% CI: 1.8 to 24.1)[312] while the NHANES study showed 3 fold (95% CI: 1.99 to 5.26) higher risk of retinal disease in diabetic patients who are on insulin treatment.[276] Admittedly our study did not demonstrate that HbA_{1c} or DM duration were associated with diabetic retinal disease, insulin treatment has been shown to be significant and this suggests that in obese DM patients, insulin requirement may be a better indicator of greater DM severity compared to HbA_{1c} or DM duration.

4.5.5 Benefits and limitations of the study

Apart from cardiovascular risk factors, our study also examined creatinine and eGFR levels. There was a significantly higher creatinine level and a non-significant lower eGFR in OSA+ group. These findings reassured that the greater risk in the early development of diabetic retinal disease as part of DM micro-vascular complications in our OSA+ group. The effect of OSA treatment on diabetes micro-vascular complications remains unknown but a study recently by Mason et al. examined the impact of CPAP on 35 OSA patients (defined as ODI >10 events/hr or AHI >15 events/hr) with macular oedema showing no improvement, but interestingly, some improvement in visual acuity occurred after 6 months of treatment in both high (p=0.0009) and low (p=0.0001) CPAP compliers.[320] Our study benefits from well-matched groups that are representative of the severely obese population and the ethnic diversity of our local population. The OSA+ and OSA- groups were well-matched for BMI, co-morbidities, diabetes duration, and glucose-lowering medications. The OSA+ group had more men and was older than the OSA- but these differences were accounted for when undergoing statistical analyses. Also, in our analyses, we observed the expected and previously reported factors associated with diabetic retinal disease.

The limitations of our study include its cross-sectional design and lack of information regarding other factors such as smoking although the association between DR and smoking is still unclear. A multicentre study of 636 Type 1 DM in Germany followed up for 6 years showed an association between DR and cigarette smoking during DM (OR 1.36, 95% CI: 1.02 to 1.81 for diabetes duration cigarette pack years).[321] The WESDR study (25 years follow-up) however, showed that there was no association between smoking and PDR[315] as

well as DMac.[316] However, WESDR study did find that cigarette smoking had an adverse effect on visual impairment (OR 1.63, 95% CI: 1.01 to 2.61).[317] On the other hand, the UKPDS study showed that smoking status was protective of the incidence (RR 0.63, 95% CI: 0.48 to 0.82) and progression (RR 0.50, 95% CI: 0.36 to 0.71) of DR.[287] These studies showed that the relationship between smoking and DR seems to be inconsistent therefore the lack of smoking information in our study may not have major impact on our results. Visual acuity data were also not available as our study aimed to assess impact of OSA on DR rather than visual impairment.

The major limiting factor for our study was the unavailability of information on anti-hypertensive and lipid-lowering treatment agents. Information on the use of angiotensin-converting enzyme (ACE) inhibitor and angiotensin receptor blocker (ARB) on the renin-angiotensin system (RAS) were also not available. The Eurodiab Controlled Trial of Lisinopril in Insulin-Dependent Diabetes (EUCLID) trial showed that lisinopil, a drug which affect the RAS system, seems to have a protective effect on PDR progression (OR 0.18, 95% CI: 0.04 to 0.82) independent of BP effect.[322] DIabetic REtinopathy Candesartan Trial (DIRECT-Protect-2) has also shown that candesartan may regress DR in type 2 DM (DR Improvement OR 1.17, 95%: 1.05 to 1.30).[323] Fenofibrate, a peroxisome proliferator-activated receptor alpha (PPAR-α) agonist, is a cholesterol-lowering agent. It has been shown in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) eye study group that fenofibrate in addition to simvastatin had significantly lower the risk for the progression of DR (OR 0.60, 95% CI: 0.42 to 0.87).[324] The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study also demonstrated a lower risk for laser treatment (HR 0.69, 95% CI:

0.54 to 0.84) in the fenofibrate-treated group.[325] These studies suggest a possible effect of these agents on DR.

Finally, an important limitation in this study was the relatively small number of outcome events. As there were only 36 participants with DR, the lack of association observed between OSA and DR may be attributed to lack of power.

4.6 Conclusions

Our study of severely obese patients with DM found no association between OSA and DR but there was a higher prevalence of DMac in those with moderate to severe OSA. Further analysis suggests that the severity of hypoxaemia, particularly minimum oxygen saturation has an independent association with the development of maculopathy but the exact mechanism for this remains unclear. As this OSA group was more hypoxaemic, it is hypothesised that hypoxaemia and not apnoea/hypopnoea frequency per se is the key factor in developing diabetic micro-vascular complications. The other likely potential mechanisms were levels of greater systemic hypertension and severity of DM in OSA. The impact of CPAP on micro-vascular retinal complications in OSA patients remains undetermined, but further RCTs may be warranted to specifically look at the hypoxaemic OSA group.

CHAPTER 5 – THE IMPACT OF OBSTRUCTIVE SLEEP APNOEA ON DIABETIC KIDNEY DISEASE IN EXTREMELY OBESE PATIENTS WITH TYPE 2 DIABETES MELLITUS

5.1 Introduction

The increasing worldwide prevalence of obesity has led to a parallel rise in type 2 diabetes mellitus (T2DM). T2DM is associated with micro and macro-vascular complications with significant impact on morbidity and mortality. A common micro-vascular T2DM complication is diabetic kidney disease (DKD). DKD is characterised by the presence of persistent albuminuria, elevation of arterial blood pressure and decline in renal function in DM individuals. Indeed, DM is a major risk factor for the development of chronic kidney disease (CKD) and subsequent end stage renal disease (ESRD) needing renal replacement treatment (RRT).[326] The UK Prospective Diabetes Study (UKPDS) found that in patients with T2DM, over 10 years, the cumulative-incidence of micro-albuminuria was 24.9%, macro-albuminuria prevalence was 5.3%, and the incidence of those with creatinine above 175µmol/l or needing RRT was 0.8%.[327] DKD is also especially important because of the association with excess mortality risk.[328] A recent NHANES III study on the 10 year cumulative standardised all-cause mortality for DM and non-DM was 19.1% (95% CI: 15.5 to 22.7) and 8.6% (95% CI: 7.9 to 9.3), respectively.[328] The study also found that DM individuals without DKD had similar mortality compared to non-DM individuals (absolute risk difference was 3.4% (95 CI: -0.3 to 0.7). However, in DM with DKD, the absolute risk difference compared to non-DM was 23.4% (95% CI: 17.2 to 29.6) after adjusted for confounders. Therefore, one can argue that the excess mortality risk in DM may be associated with DKD (through its effects in the cardiovascular system).

5.1.1 The natural history of diabetic nephropathy

It is useful to understand the natural history of DKD to help to tackle the problem of excess mortality in this group. The classic text book description of the natural history of diabetic nephropathy (DN) can be characterised based on its structural or functional changes. Table 5-1 summarises the stages of DN. Structurally, a diabetic kidney begins with hypertrophy of the glomeruli followed by thickening of the glomerular basement membrane (GBM).[329] GBM thickening is caused by the accumulation of fibronectin, laminin and collagen types IV. The thickness of GBM increases with DM duration. Subsequently, mesangial expansion or diabetic glomerulosclerosis occur and this can be mild or severe. Mesangial expansion is described as an enlarged mesangium with a diameter exceeding 2 mesangial cell nuclei. The expansion occurs following the accumulation of extracellular proteins and other materials in the mesangium. Kimmelsteil-Wilson lesion is the next stage representing nodular sclerosis and the final stage of DN is advanced diabetic glomerulosclerosis.[329]

Table 5-1: Stages of diabetic nephropathy

Stage	Structural change	Functional change
Pre	Glomerular hypertrophy	Increased in GFR
I	GBM thickening	Micro-albuminuria
II	Mesangial expansion	Micro-albuminuria
III	Nodular sclerosis	Proteinuria
IV	Advanced diabetic glomerulosclerosis	End stage renal failure

Functionally, DN begins with hyperfiltration due to hypertrophy of the glomeruli.[330] Glomerular filtration rate is increased at this stage. Next, incipient DN with micro-albuminuria develops. Pathological findings which accompany this stage are thickened GBM and mesangial expansion. Consequently, overt proteinuria occurs at the Kimmelsteil-Wilson stage. Finally, end stage renal failure develops.[330] The changes in the glomerular filtration function (GFR) of the hyperfiltration, micro-albuminuria and proteinuria stage was demonstrated by Nelson and colleagues.[331] They followed-up 194 Pima Indians at different stages of T2DM: normal glucose tolerance, impaired glucose tolerance, newly-diagnosed T2DM, T2DM with normo-albuminuria, T2DM with micro-albuminuria; and T2DM with macro-albuminuria for four years. The researchers found that in the newly-diagnosed T2DM group, GFR increased by 18% after 4 years ($p=0.008$). The GFR in T2DM with normo-albuminuria and micro-albuminuria remained stable over similar time period. However, in those with macro-albuminuria, there was a decline in renal function by 35% ($p<0.001$). The mean change in GFR from baseline in those with newly-diagnosed T2DM, micro-albuminuria and macro-albuminuria groups over 4 years were illustrated in Figure 5-1.[331]

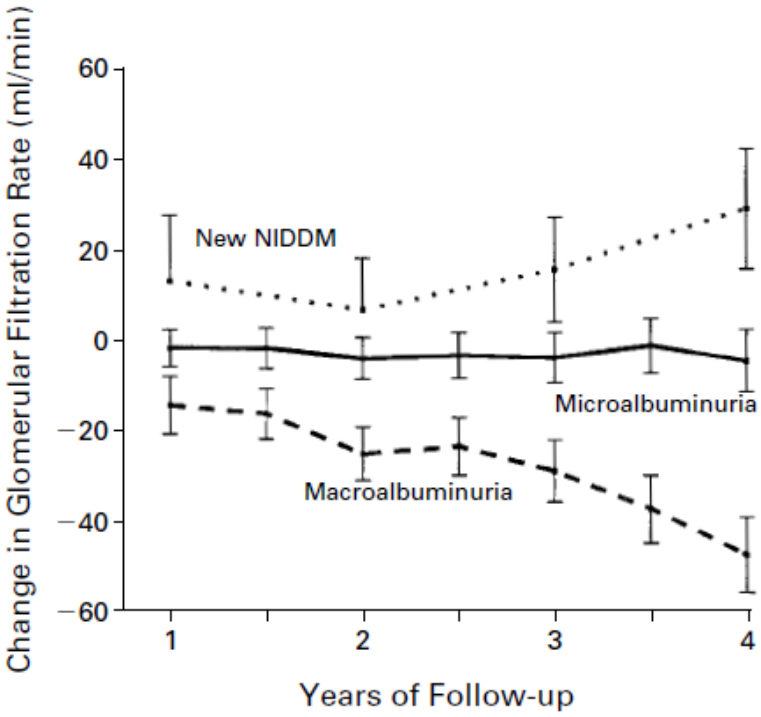

Figure 5-1: Mean (± standard error) change in the glomerular filtration rate from baseline during four years of follow-up in participants with newly-diagnosed diabetes mellitus (DM), DM with micro-albuminuria and DM with macro-albuminuria [331]. Reproduced with permission from Nelson RG et al. N Engl J Med 1996;335:1636-1642, Copyright Massachusetts Medical Society

In reality, the progression of DKD does not necessary follow the stages described above. Patients with a decline in GFR may not necessarily have albuminuria. For example, the UKPDS found that after 15 years of follow-up, 28% of participants had an estimated GFR (eGFR) <60ml/min/1.73m^2 or chronic kidney disease (CKD) Stage 3 and above.[332] However, over half (51%) of the UKPDS participants with CKD Stage 3 and above did not develop albuminuria. Similarly, the Diabetes Control and Complications Trial (DCCT) reported that 6.2% of type 1 diabetes mellitus (T1DM) individuals developed CKD Stage 3 and above but 24% of these participants did not develop albuminuria after 19 years of follow-up.[333] The ADVANCE (Action in Diabetes and Vascular disease: preterAx and diamicroN modified

release Controlled Evaluation) trial had 10,640 participants and reported that 19% of the participants had eGFR <60ml/min/1.73m^2 but only 38% had albuminuria.[334] This may be because, apart from hyperglycaemia, atherosclerosis may also play a role. This is evidenced by the study of 608 Japanese DM individuals followed-up over 7.5 years. At baseline, the participants had magnetic resonance imaging (MRI) to assess for cerebral infracts. Those with subclinical cerebral infarcts, defined as infarcts detected by MRI without a history of stroke or transient ischaemic attack (n=177), had a greater risk of needing RRT or doubling of serum creatinine (26.0%) compared with those without subclinical infarcts (5.6%, HR 4.79, 95% CI: 2.72 to 8.46) after 7.5 years after adjustment for confounders.[335] However, the subclinical cerebral infarct group had no increase in the risk of progression to albuminuria at study end (39.5% vs. 27.1%, HR 1.23, 95% CI: 0.88 to 1.73).

5.1.2 Obesity, obstructive sleep apnoea and diabetic kidney disease

Apart from ischaemic nephropathy, obesity is also a risk factor for renal disease. The Kaiser Permanente Study consisted of 320,252 participants who had health check-ups performed between 1946 and 1985.[336] The researchers matched the participants to the U.S. Renal Data Registry for ESRD until year 2000. In 8,347,955 person years of follow-up, there were a total of 1471 ESRD cases. After correcting for confounding factors, the researchers found a step-wise increased in the risk of ESRD as BMI increased. The risks reported compared to normal BMI (between 18.5 and 24.9 Kg/m^2) was RR 1.87 (95% CI: 1.64 to 2.14) for overweight individuals; RR 3.57 (95% CI: 3.05 to 4.18) for those with BMI between 30 and 34.9Kg/m^2; RR 6.12 (95% CI: 4.97 to 7.54) for those with BMI between 35 and 39.9Kg/m^2 and RR 7.07 (95% CI: 5.37 to 9.31) for those above BMI 40Kg/m^2.

The effect of obesity on CKD is further confirmed by a systematic review and meta-analysis of 3 cohort studies in the general population reported a pooled-RR of 1.26 (95% CI: 1.10 to 1.45) for overweight individuals and a pooled-RR 1.34 (95% CI: 0.86 to 2.09) in obese individuals for CKD when compared with individuals with normal BMI.[337] For ESRD, there were only 2 cohort studies available for the meta-analysis and the pooled-RR was 1.68 (95% CI: 1.49 to 1.90) in overweight and 4.07 (95% CI: 2.87 to 5.76) in obese individuals compared with normal BMI individuals. Furthermore, a study by researchers at the Cleveland Clinic Bariatric and Metabolic Institute examined T2DM patients after median of 6 years follow-up post Roux-en-Y gastric bypass surgery (RYGB), a type of bariatric surgery.[338] A total of 59 patients had both baseline and follow-up results on creatinine and urine albumin creatinine ratio (ACR). Nineteen patients had albuminuria at baseline and improvement occurred in 10 (53%) with the remaining 9 (47%) patients' ACR status remaining stable without any progression at study end. This suggests that correcting obesity may lead to improvement in renal function.

OSA may also play a role in DKD. Several studies have attempted to examine the relationship between OSA and renal impairment.[339,340] The Cleveland Family Study observed 496 middle aged adults with a mean age of 44.5±17.3 years.[339] Renal function was assessed using urine albumin creatinine ratio (ACR). The log ACR was found to have a positive association with severe OSA (β=0.308, standard error [SE]: 0.152, p=0.033) after adjusting for confounders (age, gender, ethnicity, BMI, GFR, hypertension and diabetes). Another observational study analysed the data on 1,377,427 adult members of the Kaiser Permanente Southern California health care system.[340] This study demonstrated that those with eGFR of >45ml/min/1.73m^2 (CKD stage 3a) had increased risk of OSA when compared to participants with eGFR

>90ml/min/1.73m^2 (CKD stage 1, normal renal function). They reported a risk of OR 1.21 (95% CI: 1.17 to 1.25) for eGFR between 75 and 89ml/min/1.73m^2; OR 1.25 (95% CI: 1.21 to 1.30) for eGFR between 60 and 74ml/min/1.73m^2; and OR 1.18 (95% CI: 1.12 to 1.24) for eGFR between 45 and 50ml/min/1.73m^2. Another Japanese study consisting of 119 ESRD patients on RRT found that AHI correlated with urea ($r=0.490$, $p<0.01$), creatinine ($r=0.418$, $p<0.05$) and BMI ($r=0.489$, $p<0.01$).[341] These studies showed that OSA is associated with greater renal impairment and may be a risk factor for CKD.

Currently, there is no information on the impact of OSA on renal function in T2DM individuals with extreme obesity (BMI≥40Kg/m^2), an increasingly important clinical population. As shown in Chapter 4, nocturnal hypoxaemia parameters other than the AHI may also play an important role in diabetic maculopathy, a well-known diabetic microvascular complication, amongst extreme obese individuals. We therefore hypothesised that OSA will also be associated with greater DKD severity, another well-recognised diabetic microvascular complication.

5.2 Aim and objectives

The aim of this chapter is to examine the prevalence of diabetic kidney disease (DKD) and the relationship between OSA and DKD in a severely obese population. The following are the study's objectives:

1. To describe the prevalence of DKD amongst DM individuals who are very severely obese using 2 estimated glomerular filtration rate (eGFR) equations and,
2. To examine the relationship between nocturnal hypoxaemia and nephropathy in very severely obese DM participants.

5.3 Methods

5.3.1 Study design and study participants

The methodology has been described in Chapter 2. In brief, anonymised data were collected between January 2009 and December 2011 of prospective DM patients. This study was carried out over the same time period as those included in Chapters 2 and 4. Data collected included: demographic, anthropometry, DM history and co-morbidities. The duration of DM, anti-diabetes medication and HbA_{1c} (DCCT-aligned and IFCC) and serum creatinine were also obtained. Data on treatment with either angiotensin-converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) were also collected.

5.3.2 Measurement for chronic kidney disease

The estimated glomerular filtration rate (eGFR) was calculated based on recommendations from both the National Institute for Health and Clinical Excellence (NICE)[342] and the National Kidney Foundation Kidney Disease outcome Quality Initiative Clinical Practice Guidelines[343] using the abbreviated 4-variable Modification of Diet in Renal Disease (MDRD) equation. The MDRD equation used for calculating the eGFR in this study based on serum creatinine (S. Creat) (μmol/l) was as follows (S. Creat=serum creatinine):

$$eGFR=32788 \times S.\,Creat^{-1.154} \times Age^{-0.203} \times (1.212 \text{ if Afro-Caribbean}) \times (0.742 \text{ if female})$$

In addition, we also used the CKD Epidemiology Collaboration (CKD-EPI) equation for regression analysis as it may provide better accuracy compared to the MDRD formula.[344] The comparison between the MDRD and the CKD-EPI formulae will be elaborated further in the discussion section. The equations for the CKD-EPI according to gender were as follows:[344,345]

a. Females

If S. Creat ≤62µmol/l: $eGFR=144 \times (S.Creat/88.4/0.7^{-0.329}) \times 0.993^{age}$

If S. Creat >62µmol/l: $eGFR=144 \times (S.Creat/88.4/0.7^{-1.209}) \times 0.993^{age}$

b. Males

If S. Creat ≤80µmol/l: $eGFR=144 \times (S.Creat/88.4/0.9^{-0.411}) \times 0.993^{age}$

If S. Creat >80µmol/l: $eGFR=144 \times (S.Creat/88.4/0.9^{-1.209}) \times 0.993^{age}$

Staging of chronic kidney disease (CKD) was based on eGFR values. Patients with an eGFR of >90.0ml/min/1.73m^2 had CKD stage 1; eGFR of between 60.0 and 89.9ml/min/1.73m^2 was classified as stage 2; eGFR 30.0 to 59.9ml/min/1.73m^2 was stage 3; eGFR 15.0 to 29.9ml/min/1.73m^2 was stage 4; and eGFR <14.9ml/min/1.73m^2 was stage 5. Patients with eGFR of <60ml/min/1.73m^2 (CKD stage 3 and above) were grouped as having CKD (CKD+) while those with eGFR >60ml/min/1.73m^2 (CKD stage 1 and 2) were categorised as having no CKD (CKD-) using both the MDRD and CKD-EPI calculations.

5.3.3 Respiratory sleep monitoring methods

The respiratory monitoring methods were described in Chapter 2.

5.3.4 Statistical analyses

Statistical analyses were carried out using Stata 13 (StataCorp LP, College Station, Texas). Distribution of data was determined using visual inspection and Shapiro Wilk test. When eGFR was calculated using CKD-EPI formula, the data was skewed therefore log transformation was performed prior to regression analysis. Mean and standard deviation (SD) were used to report parametric data while median and interquartile ranges (IQR) were used for non-parametric data. Patients were categorised into eGFR of >60ml/min/1.73m^2 (CKD- group) and <60ml/min/1.73m^2 (CKD+ group). Independent t test, Mann Whitney U test and chi square test were used for analysis of normal distributed data, skewed and categorical data respectively. Linear regression analysis was used for further analyses. The base model is unadjusted, Model 1 was adjusted for age, gender and BMI and Model 2 was adjusted for age, gender, BMI, presence/absence of hypertension, presence/absence of CAD, duration of DM, presence/absence of insulin treatment and presence/absence of treatment on ACE inhibitor or ARB. The factors included in the adjusted models were those that are considered to be clinically important, and the rationale for including each factor is discussed elsewhere (see table 5-2 below). The results for linear regression analyses using the MDRD eGFR equation were reported as beta coefficients with 95% confidence intervals (95% CI). Regression results for log transformed eGFR based on CKD-EPI formula were back transformed and were reported as percentage change with 95% CI. A p value of less than 0.05 was considered statistically significant.

Table 5-2: Factors adjusted and the appropriate sections discussing the rationales behind

Factors	**Sections**
Age	Introduction of Chapter 2 (section 2.1.3)
Gender	Chapter 2's discussion (section 2.5.5)
Body mass index	Chapter 1 (section 1.3.4)
Type 2 diabetes	Chapter 3 (section 3.5.2)
Insulin treatment	Chapter 4 (section 4.5.5)
Hypertension	Chapter 1 (section 1.3.3) and chapter 4 (section 4.5.3)
Coronary artery disease	Chapter 1 (section 1.3.3)
Angiotensin converting enzyme inhibitor or Angiotensin receptor blocker	Sections 5.5.2 and 5.5.3

5.4 Results

5.4.1 Study characteristics

A total of 121 consecutive obese DM patients were seen at the service between 2009 and 2011. However, 12 did not have available renal function measures, and respiratory parameter measures were not available for 19 patients (Figure 5-2). This resulted in data from 90 eligible patients for analysis. None of the patients had a diagnosis of central sleep apnoea.

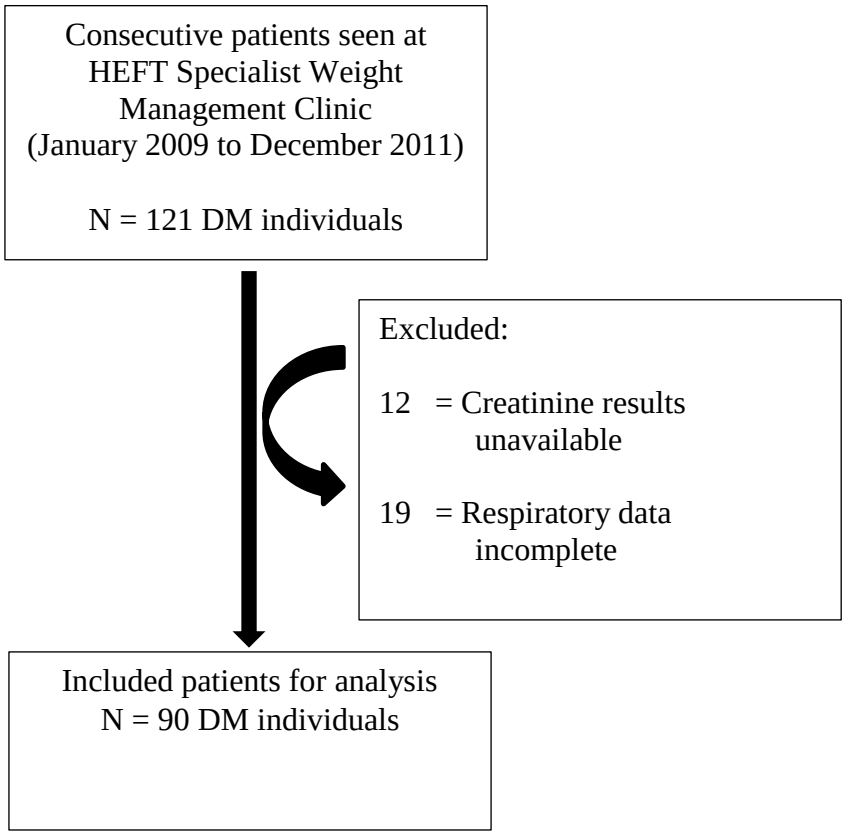

Figure 5-2: Flow diagram of referrals seen at the HEFT Specialist Weight Management Clinic, and those included in the study
HEFT – Heart of England NHS Foundation Trust
DM = Diabetes Mellitus

The mean age was 51±10 years with slightly more females (57%), and the majority were white Europeans (91%). The mean BMI was 46.8±7.7Kg/m^2 with 61% of patients having concomitant hypertension and 10% diagnosed with CAD. Median duration of DM was 6 years (IQR 2, 10) with HbA_{1c} of 64mmol/mol (IQR 51, 80) or 8.0% (IQR 6.8, 9.5). The majority of the patients required at least 2 glucose-lowering medications (53%) and 31% were on insulin treatment. The patients had a mean MDRD and CKD-EPI eGFR of 78.3±25.1 and 89.9±21.6 ml/min/1.73m^2, respectively. The median AHI was 15 events/hr (IQR 7, 37), mean oxygen saturations of 93% (IQR 92, 95), minimum oxygen saturations 82% (IQR 74, 85), and the % of time spent below 90% oxygen saturation (%TST<90%) of 2.9% (IQR 0.7, 15.0). The prevalence of OSA based on AHI ≥5 events/hr was 83% (95% CI: 74 to 90) and the proportion with moderate-to-severe OSA (AHI ≥15 events/hr) was 51% (95% CI: 41 to 61). Of those with a diagnosis of OSA, 22.7% and 13.3% had MDRD and CKD-EPI eGFR of ≤60ml/min/173m^2, respectively.

5.4.2 Prevalence of chronic kidney disease

Twenty-nine percent of the DM individuals (n=26) did not suffer with CKD (MDRD eGFR >90ml/min/1.73m^2) while 49% (n=44) had CKD stage 2. A total of 20 patients had MDRD eGFR <60ml/min/1.73m^2 (22%, 95% CI: 15 to 32). None of the patients had CKD stage 5 and only 1 had stage 4 CKD. Using the CKD-EPI equation, a lower proportion (28.9%, n=26) had CKD stage 2, 10% had stage 3 (n=9) whilst 1 patient had stage 4, and none had stage 5 CKD. A summary table of the characteristics of 90 DM patients is shown in Table 5-3.

5.4.3 Comparisons of CKD and non-CKD patients based on MDRD eGFR

Please refer to Table 5-4 for a summary of the results. Patients in the MDRD CKD+ group were older with a mean age of 57±11 years compared to those in the MDRD CKD- group (50±9years, p=0.003). No significant differences were found in ethnicity between the groups (p=0.207). There were also significantly more females (80%) in CKD+ group compared to CKD- (50%, p=0.017) and greater adiposity in CKD+ group with BMI of 50.6±8.7Kg/m^2 compared to 45.7±7.1Kg/m^2 in CKD- group (p=0.012). Systolic BP was significantly higher in CKD+ group (p=0.037) but this was not the case for diastolic BP. Although no difference was found in the proportion with hypertension between the two groups, there was at least a 3 fold higher CAD in the MDRD CKD+ group (25% vs. 7%, p=0.025).

There were no significant differences in DM duration and HbA_{1c} levels between the two groups. Insulin usage was at least 2-fold higher in the MDRD CKD+ group (p=0.009). A greater proportion of patients were treated with either ACE inhibitor or ARB in the MDRD CKD+ group (p=0.021). No significant differences were found in OSA prevalence based on AHI ≥5 events/hr or in the proportion of moderate-to-severe OSA. AHI and minimum oxygen saturation levels were also not significantly different between the groups. However, there was a significant difference in the mean oxygen saturation (p=0.043) and at least 3-times higher in %TST<90% in the MDRD CKD+ patients (p=0.046).

Table 5-3: Characteristics of 90 Type 2 diabetes mellitus patients

	N	Mean ± SD or Median (IQR)
Age (years)	90	51 ± 10
Females (%)	90	57%
Ethnicity (%)		
White Europeans	64	91%
South Asians	19	27%
Afro-Caribbean	7	8%
Body Mass Index (Kg/m^2)	90	46.8 ± 7.7
Systolic BP (mmHg)	67	143 ± 20
Diastolic BP (mmHg)	68	84 ± 11
Co-morbidities		
Hypertension (%)	55	61%
Coronary artery disease (%)	10	10%
DM parameters		
DM Duration (years)	87	6 (2, 10)
Insulin (%)	28	31%
HbA_{1c} (%)	88	8.0 (6.8, 9.5)
HbA_{1c} (mmol/mol)	88	64 (51, 80)
Number of DM Medications (%)		
0	18	20%
1	24	27%
2	37	41%
>3	11	12%
AHI (events/hour)	90	15 (7, 37)
Mean O_2 sats (%)	90	93 (92, 95)
Minimum O_2 sats (%)	90	82 (74, 85)
Time under 90% sats (%)	83	2.9 (0.7, 15.0)
MDRD eGFR (ml/min/1.73m^2)	90	78.3 ± 25.1
MDRD eGFR < 60 ml/min/1.73m^2	20	22.2%
CKD stages		
1	26	29%
2	44	49%
3	19	21%
4	1	1%
CKD-EPI eGFR (ml/min/1.73m^2)	90	89.9 ± 21.6
CKD-EPI eGFR < 60 ml/min/1.73m^2	10	11.1%
CKD stages		
1	54	60%
2	26	29%
3	9	10%
4	1	1%

BP = blood pressure, DM = diabetes mellitus, AHI = apnoea-hypopnoea index, O_2 = oxygen, sats = saturations, MDRD = Modification of Diet in Renal Disease, eGFR = estimated glomerular filtration rate, CKD = Chronic Kidney Disease, CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration, HbA_{1c}=glycosylated haemoglobin A_{1c}

Table 5-4: Sample characteristics of patients with and without at least chronic kidney disease stage 3 (CKD 3) based on Modification of Diet in Renal Disease (MDRD) formula

	N	**CKD+ N=20**	**N**	**CKD- N=70**	**p value**
Age (years)	20	57 ± 11	70	50 ± 9	**0.003**
Female	16	80%	35	50%	**0.017**
Ethnicity					0.207
White Europeans	17	85%	47	67%	
South Asians	3	15%	16	23%	
Afro-Caribbean	0	0%	7	10%	
BMI (kg/m^2)	20	50.6 ± 8.7	70	45.7 ± 7.1	**0.012**
Systolic BP (mmHg)	17	152 ± 26	50	140 ± 17	**0.037**
Diastolic BP (mmHg)	17	88 ± 17	50	83 ± 8	0.0954
Co-morbidities					
Hypertension	15	75%	40	57%	0.149
Coronary artery disease	5	25%	5	7%	**0.025**
ACE-I or ARB	15	75.0%	32	57.5%	**0.021**
ACE inhibitor	6	30.0%	23	18.6%	0.809
ARB	9	45.0%	13	18.6%	**0.015**
DM parameters					
DM Duration	20	9 (4, 13)	67	6 (2, 10)	0.149
HbA_{1c} (%)	19	7.5 (6.8, 9)	69	8.1 (6.9, 9.7)	0.503
HbA_{1c} (mmol/mol)	19	58 (51, 75)	69	65 (52, 83)	0.503
Insulin	11	55%	17	24%	**0.009**
Number of DM medications					0.814
None	3	15%	15	21%	
1	5	25%	19	34%	
2	10	50%	27	39%	
>3	2	10%	9	13%	
Respiratory parameters					
OSA (AHI ≥ 5)	17	85%	58	83%	0.821
AHI ≥ 15	10	50%	36	51%	0.910
AHI (events/hr)	20	17 (7, 63)	70	15 (7, 31)	0.600
Mean O_2 sats (%)	20	92 (91, 94)	70	93 (92, 95)	**0.043**
Minimum O_2 sats (%)	20	81 (73, 84)	70	83 (74, 85)	0.268
Time under 90% sats (%)	19	8.6 (1.1, 33.3)	64	2.5 (0.5, 12.1)	**0.046**

Data are presented as mean ± standard deviation, percentage or median (interquartile range).
CKD+ = presence of chronic kidney disease stage 3 and below; CKD- = absence of chronic kidney disease stage 3 and below; BMI = body mass index; BP = blood pressure; DM = type 2 diabetes mellitus; AHI = apnoea-hypopnoea index; O_2 = oxygen; sats = saturation, HbA_{1c}=glycosylated haemoglobin A_{1c}. p values were calculated using either independent t test, Mann Whitney U test or chi square test.

5.4.4 Comparisons of CKD and non-CKD patients based on CKD-EPI eGFR

Please refer to Table 5-5 for a summary of the results. Comparisons were also made between the CKD+ and CKD- using the CKD-EPI formulae. Similar to the MDRD equation, there was a significant difference in age between the CKD+ (63.3±9.2years) compared to the CKD- (49.9±9.5years, p<0.0001) group. No significant differences were found for gender, ethnicity and BMI. Although systolic BP was 12mmHg higher in the CKD+ compared to the CKD-, this was not statistically significant. Similarly, there was no difference in diastolic BP (3mmHg difference), proportion of hypertensive individuals (80.0% vs, 58.8%) and coronary artery disease (20.0% vs. 10.0%), HbA_{1c} level (1.3% or 14mmol/mol higher in the CKD+ group) as well as number of DM medications used. The duration of DM was significantly longer for CKD+ (10 [IQR: 7, 14] vs. 6 [IQR: 2, 10] years, p=0.042) and proportion needed for insulin was also significantly higher (80.0% vs. 25.0%, p<0.001). The proportions of individuals receiving either ACE inhibitor or ARB were not statistical significant (p=0.062).

There was no difference in the proportion of individuals with OSA between the CKD groups (p=0.134). However, the proportion of individuals with moderate-to-severe OSA almost reached statistical significance and was greater in the CKD+ group (80.0% vs. 47.5%, p=0.053). Therefore, unsurprisingly, there was a significant difference in AHI. Median AHI for the CKD+ group was 45 events/hr (IQR: 19, 74) compared to 14 events/hr (IQR: 6, 31) in the CKD- group (p=0.033). The CKD+ group also spent at least 5 fold greater in % time spent below 90% oxygen saturation (%TST<90) (13.8% [IQR: 2.0, 91.0] vs. 2.7% [IQR: 0.7, 12.0], p=0.046) whilst there were no significant difference in mean and minimum oxygen saturations between the 2 groups.

Table 5-5: Sample characteristics of patients with and without at least chronic kidney disease stage 3 (CKD 3) based on Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula

	N	**CKD3+ N=10**	**N**	**CKD3- N=80**	**P value**
Age (years)	10	63.3 ± 9.2	80	49.9 ± 9.5	**<0.0001**
Female	6	60.0%	51	63.8%	0.821
Ethnicity					0.652
White Europeans	8	80.0%	56	70.0%	
South Asians	1	10.0%	18	22.5%	
Afro-Caribbean	1	10.0%	6	0.08%	
BMI (Kg/m^2)	10	49.6 ± 9.1	80	46.5 ± 7.5	0.2337
Systolic BP (mmHg)	8	154 ± 26	59	142 ± 19	0.1060
Diastolic BP (mmHg)	8	87 ± 23	59	84 ± 9	0.4716
Co-morbidities					
Hypertension	8	80.0%	47	58.8%	0.1940
Coronary artery disease	2	20.0%	8	10.0%	0.3430
ACE-I or ARB	8	80.0%	39	48.8%	0.062
ACE inhibitor	3	30.0%	26	32.5%	0.873
ARB	5	50.0%	17	21.3%	**0.046**
DM parameters					
DM Duration	10	10 (7, 14)	77	6 (2,10)	**0.0418**
HbA_{1c} (%)	9	9.2 (8.1, 10.1)	79	7.9 (6.7, 9.4)	0.0619
HbA_{1c} (mmol/mol)	9	77 (65, 87)	79	63 (50, 79)	0.0619
Insulin	8	80.0%	20	25.0%	**<0.001**
Number of DM medications					0.398
None	1	10.0%	17	21.3%	
1	3	30.0%	21	26.3%	
2	6	60.0%	31	38.8%	
>3	0	0.0%	11	13.8%	
Respiratory parameters					
AHI (events/hr)	10	45 (19, 74)	80	14 (6, 31)	**0.0325**
Mean O_2 sats (%)	10	92 (85, 95)	80	93 (92, 95)	0.2878
Minimum O_2 sats (%)	10	78 (71, 83)	80	82 (76, 86)	0.1300
Time under 90% sats (%)	10	13.8 (2.0, 91.0)	80	2.7 (0.7, 12.0)	**0.0460**
OSA (AHI ≥ 5)	10	100%	65	81.3%	0.134
AHI ≥ 15	8	80.0%	38	47.5%	0.053

Data are presented as mean ± standard deviation, percentage or median (interquartile range).
CKD+ = presence of chronic kidney disease stage 3 and below; CKD- = absence of chronic kidney disease stage 3 and below; BMI = body mass index; BP = blood pressure; DM = type 2 diabetes mellitus; AHI = apnoea-hypopnoea index; O_2 = oxygen; sats = saturation, HbA_{1c}=glycosylated haemoglobin A_{1c}. p values were calculated using either independent t test, Mann Whitney U test or chi square test.

5.4.5 Multivariate regression analysis

Pearson correlation analyses found no significant correlations between AHI, mean and minimum oxygen saturations and the MDRD eGFR. However, there was a significant correlation between the MDRD eGFR and %TST<90% (r=-0.24). This was confirmed with univariate linear regression analyses with a 0.27% reduction in the MDRD eGFR for every 1% increase in %TST<90% (95% CI: -0.51 to -0.03). No significant results were found for AHI (β=-0.06, 95% CI: -0.25 to 0.13), minimum (β=-0.01, 95% CI: -0.54 to 0.52) and mean oxygen saturations (β=1.41, 95% CI: -0.06 to 2.89) with the MDRD eGFR as shown in Table 5-6. However, using the CKD-EPI calculation for eGFR, all 4 respiratory parameters were significantly associated with the CKD-EPI eGFR as shown in Table 5-7. A unit increase in AHI resulted in 30% (95% CI: -0.52 to -0.08) whilst %TST<90 52% (95% CI: -0.79 to -0.25) reduction in the CKD-EPI eGFR. Similarly, higher mean (β=3.15, 95% CI: 15.26 to 4.90) and minimum (β=0.74, 95 CI: 0.13 to 1.36) oxygen saturations resulted in a greater CKD-EPI eGFR.

In Model 1 of the multivariate linear regression analyses using the MDRD eGFR as the outcome, the inverse association between %TST<90% remained significant (β=-0.25, 95% CI: -0.45 to -0.06) after adjusting for age, gender and BMI. Similarly, an increase in the AHI also resulted in a reduction in renal function (β=-0.21, 95% CI: -0.35 to -0.05) and a higher mean oxygen saturation was associated with better renal function (β=1.49, 95% CI: 0.25 to 2.74) in Model 1 based on the MDRD formula. No significant association was found for minimum oxygen saturations and the MDRD eGFR (β=0.20, 95% CI: -0.24 to 0.65). The CKD-EPI eGFR regression models revealed similar results as for the MDRD eGFR and this is

shown in Table 5-6. Apart from minimum oxygen saturation (β=0.44, 95% CI: -0.16 to 1.05), AHI (β=-0.27, 95% CI: -0.47 to -0.67), mean oxygen saturation (β=2.10, 95% CI: 0.38 to 3.86) and %TST<90 (β=-0.38, 95% CI: -0.65 to -0.11) maintained statistical significance.

Table 5-6: Linear regression analysis assessing associations between Modification of Diet in Renal Disease (MDRD) eGFR and respiratory parameters

Predictors	**Models** Reported as beta coefficients (95% Confidence Intervals)		
	Unadjusted	**Model 1**	**Model 2**
AHI	-0.058 (-0.247 to 0.130)	**-0.207 (-0.353 to -0.061)**	**-0.170 (-0.316 to -0.024)**
Min O_2 sat	-0.011 (-0.539 to 0.516)	0.202 (-0.241 to 0.645)	0.053 (-0.388 to 0.495)
Mean O_2	1.413 (-0.0638 to 2.890)	**1.494 (0.247 to 2.741)**	1.191 (-0.039 to 2.420)
%TST<90%	**-0.272 (-0.512 to -0.032)**	**-0.254 (-0.453 to -0.055)**	**-0.215 (-0.406 to -0.023)**
Age*	**-0.848 (-1.332 to -0.363)**	**-0.937 (-1.305 to -0.569)**	**-0.821 (-1.199 to -0.442)**
Male*	**28.149 (19.324 to 36.975)**	**35.307 (26.703 to 43.911)**	**36.904 (28.434 to 45.375)**
BMI*	-0.606 (-1.285 to 0.073)	0.254 (-0.278 to 0.787)	0.279 (-0.244 to 0.803)
Hypertension*	-6.358 (-17.101 to 4.385)	Not applicable	3.204 (-6.316 to 12.723)
CAD*	-10.670 (-27.312 to 5.972)	Not applicable	-6.580 (-18.953 to 5.792)
DM duration*	-0.819 (-1.668 to 0.029)	Not applicable	0.259 (-0.467 to 0.986)
Insulin*	**-17.954 (-28.702 to -7.206)**	Not applicable	**-17.309 (-27.273 to -7.344)**
ACE/ARB*	-6.524 (-16.999 to 3.952)	Not applicable	1.421 (-7.657 to 10.498)

Unadjusted: univariate models
Model 1: adjusted for age, gender, body mass index
Model 2: further adjusted for hypertension, coronary artery disease and duration of type 2 diabetes mellitus, insulin treatment and angiotensin-converting enzyme inhibitor or angiotensin receptor blocker treatment.
*Adjusted for apnoea-hypopnoea index only to avoid multi-collinearity
eGFR = estimated glomerular filtration rate; AHI = apnoea-hypopnoea index; Min O_2 sat = minimum oxygen saturation; Mean O_2 = mean oxygen saturation; %TST<90% = time spent under 90% oxygen saturation, BMI = Body mass index, CAD = coronary artery disease, DM = type 2 diabetes, ACE/ARB = angiotensin-converting enzyme inhibitor or angiotensin receptor blocker treatment

In Model 2, the associations remained significant after further adjustment for hypertension, CAD, DM duration, insulin treatment and treatment for ACE inhibitor or ARB. For AHI, there was a 0.17% reduction in the MDRD eGFR (95% CI: -0.32 to -0.02) for each increase in event/hr and a 0.22% reduction in the MDRD eGFR for each increase in % of %TST<90% (95% CI: -0.41 to -0.02). As with Model 1, there were no significant association between minimum oxygen saturations and the MDRD eGFR. The mean oxygen saturation and the MDRD eGFR was no longer significant (β=1.19, 95% CI: -0.04 to 2.42) after adjustment for the additional variables. Regression models based on the CKD-EPI calculation revealed that only %TST<90 (β=-0.30, 95% CI: -0.56 to -0.03) was significantly associated with a reduction in renal function as shown in Table 5-7. Model 3 also showed that AHI (β=-0.19, 95% CI: -0.04 to 0.01) was approaching statistical significance based on the CKD-EPI calculation. Both minimum (β=0.16, 95% CI: -0.44 to 0.76) and mean (β=1.50, 95% CI: -0.21 to 3.22) oxygen saturations were not significant.

Table 5-7: Linear regression analysis assessing associations between Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) eGFR and respiratory parameters

Predictors	**Models**		
	Reported as percentage change (95% Confidence Intervals)		
	Univariate models	**Model 1**	**Model 2**
AHI	**-0.300 (-0.517 to -0.082) ***	**-0.268 (-0.468 to -0.669) ***	-0.189 (-0.391 to 0.013)
Min O_2 sat	**0.744 (0.128 to 1.363) ***	0.439 (-0.164 to 1.046)	0.159 (-0.443 to 0.764)
Mean O_2	**3.152 (15.257 to 4.903) ***	**2.105 (0.377 to 3.862) ***	1.495 (-0.206 to 3.225)
%TST<90%	**-0.524 (-0.794 to -0.254) ***	**-0.381 (-0.651 to -0.111) ***	**-0.296 (-0.562 to -0.029) ***
Age*	**1.600 (1.090 to 2.108)**	**1.540 (1.040 to 2.037)**	**1.540 (1.040 to 2.037)**
Male*	-3.924 (-18.049 to 8.511)	**-12.614 (-26.776 to -0.034)**	**-13.889 (-28.061 to -1.285)**
BMI*	0.408 (-0.416 to 1.225)	0.051 (-0.685 to 0.781)	0.093 (-0.634 to 0.814)
Hypertension*	**15.248 (3.965 to 25.206)**	Not applicable	2.397 (-11.355 to 14.451)
CAD*	**23.998 (7.839 to 37.324)**	Not applicable	11.488 (-5.052 to 25.424)
DM Duration*	**1.174 (0.174 to 2.164)**	Not applicable	-0.297 (-1.311 to 0.707)
Insulin*	**25.574 (15.957 to 34.091)**	Not applicable	**20.291 (8.499 to 30.563)**
ACE/ARB*	11.071 (-0.691 to 21.459)	Not applicable	-5.020 (-19.086 to 7.384)

Univariate models: unadjusted
Model 1: adjusted for age, gender, body mass index
Model 2: further adjusted for hypertension, coronary artery disease and duration of type 2 diabetes mellitus, insulin treatment and angiotensin-converting enzyme inhibitor or angiotensin receptor blocker treatment
*Adjusted for apnoea-hypopnoea index only to avoid multi-collinearity
eGFR = estimated glomerular filtration rate; AHI = apnoea-hypopnoea index; Min O_2 sat = minimum oxygen saturation; Mean O_2 = mean oxygen saturation; %TST<90% = time spent under 90% oxygen saturation, BMI = Body mass index, CAD = coronary artery disease, DM = type 2 diabetes, ACE/ARB = angiotensin-converting enzyme inhibitor or angiotensin receptor blocker treatment

5.5 Discussion

5.5.1 Prevalence of diabetic kidney disease (DKD)

Our study found that the proportion of DM with diabetic kidney disease was 22% based on the MDRD or 10% based on the CKD-EPI calculations. Several factors can influence the prevalence rates. These include population selection, characteristics of the population as well as the definition of CKD.

5.5.1.1 Prevalence of diabetic kidney disease (DKD) based on the MDRD eGFR

Several UK studies have reported that the prevalence of DKD was between 18% and 31.3% based on the MDRD criteria for CKD Stage 3 and below in people with diabetes.[346-349] A lower prevalence rate was reported by Dreyer and colleagues;[349] and Tahrani and colleagues.[348] Dreyer and colleagues extracted data from 148 GP practices of three deprived areas in Britain: Newham, Tower Hamlets and City & Hackney.[349] These are all socially deprived areas located in London. The crude prevalence of DKD was reported as 18%. Tahrani and colleagues reported a prevalence of 16.5% based on the MDRD eGFR <60ml/min/1.73m^2. The socio-economic status (SES) of our study population was similar to that study of Dreyer and colleagues[349] and was almost identical with the study by Tahrani and colleagues.[348] This was because one of the 2 centres of recruitment by Tahrani and colleagues was HEFT (our centre).

About 40% of the Birmingham's population was living in the most deprived 10% area in England; additionally, part of HEFT (our service) also covers the area within the most deprived 1% in the UK.[150] Deprivation and low SES have been shown to be associated with late and more severe presentations of CKD.[350] Therefore, a greater number of patients with

DKD might present to the combined DM-renal or the renal clinics instead of the weight management service meaning our observation might have underestimated the true prevalence of DKD for our population. Furthermore, selection bias may occur in our patient population. HEFT specialist weight management service offers bariatric surgery, and bariatric surgery has been shown to result in remission of DM especially in 'mild' DM patients.[154] This may have resulted in selective referral of patients who were considered to have 'less severe' DM by GPs in order to achieve greatest clinical success, as well as being cost effective for the NHS.

The higher DKD prevalence was reported by the NEw Opportunities for Early Renal Intervention by Computerised Assessment (NEOERICA) study.[346] The study compiled databases of 17 general practice (GP) practices in Kent, Greater Manchester and West Surrey over 13 years. Of a total of 4139 DM patients with eGFR results, 31.3% had DKD. This was much greater than our observation. This may be partly because of the clinical management in GP practices as general practitioners may have a tendency to assess renal function more frequently in older patients (age >65years).[351] Indeed, Kent, a part of south-east England, has a greater proportion of older adults (13.8%) compared with the UK national average (11.5%).[351] Age is a well-known risk factor for CKD[352] therefore the study may have identified a greater prevalence rate compared to our result. Apart from this, no research has validated the MDRD equation in older adult (>70 years) populations. Therefore, reliability and accuracy of the DKD diagnosis in the NEOERICA study might be an issue. The age influence on DKD was also demonstrated by Middleton and colleagues, who analysed the Electronic Patient Record of DM individuals (GP practices) from Salford, Greater Manchester.[347] Although they found that 27.5% had DKD, the prevalence was only 16% in those <70 years compared to 49% in patients >70 years ($p>0.05$).

5.5.1.2 Prevalence of diabetic kidney disease (DKD) based on the CKD-EPI eGFR

The CKD-EPI is a relatively new estimation of the GFR and to date, has not been widely used in the UK. Therefore, the majority of the prevalence studies based on the CKD-EPI formula were results from the US. Three studies using the National Health And Nutrition Examination Survey (NHANES) data reported that the prevalence of DKD was between 13.8% and 17.7%.[353,354] The NHANES is a programme of studies conducted in non-institutionalised adults by the National Centre for Health Statistics. It aims to assess the health and nutritional status of about 5000 US citizens each year as a representative sample of the US population. The US and UK has different population characteristics therefore, it is more difficult to compare our results with the NHANES result.

DKD in the NHANES studies was defined as the presence of urinary albuminuria of >30mg/g or eGFR <60ml/min/1.73m^2. Looking at the temporal trend of DKD, de Boer and colleagues examined NHANES results from three different time periods: from 1988 to 1994, 1999 to 2004 and 2005 to 2008.[353] Excluding albuminuria, the prevalence of CKD Stage 3 and above was 14.9% (95% CI: 12.1 to 17.8) in 1988 to 1994, 16.7% (95% CI: 14.6 to 18.9) in 1999 to 2004 and 17.7% (95% CI: 15.2% to 20.2) in 2005 to 2008. The second NHANES study by Plantinga and colleagues used data from 1999 to 2006 and compare the prevalence between participants with diagnosed and undiagnosed DM.[354] The researchers found a similar prevalence for diagnosed (14.0%, 95% CI: 10.5 to 18.4) and undiagnosed DM (13.8%, 95% CI: 10.1 to 18.6) in those with eGFR<60ml/min/1.73m^2. Based on the NHANES results, the prevalence of DKD seems to increase with time and this may be due to the parallel increase in the prevalence of diabetes.

The slightly greater rates reported by the NHANES studies compared to our result can partially be explained by the selection bias in our study as discussed above, and the differences in the characteristics of the two populations such as age. The prevalence of DKD seems to increase with age. In a subgroup analysis of the NHANES study, de Boer and colleagues[353] reported that the prevalence of CKD ≥Stage 3 in those between the age of 20 and 65 years was 4.5% (95% CI: 2.0 to 7.1) for the period 1988 to 1994; 5.8% (95% CI: 3.9 to 7.6) for period 1999 to 2004 and 6.0% (95% CI: 3.9 to 8.2) for period 2005 to 2008.[353] For those age >65 years, the prevalence was 31.2% (95% CI: 27.3 to 35.1) for period 1988 to 1994; 35.5% (95% CI: 31.4 to 39.4%) for period 1999 to 2004 and 37.4% (95% CI: 31.5 to 43.3%) for period 2005 to 2008.

Both the NHANES and our study reported the crude prevalence rates for DKD. However, the subgroup analysis from the NHANES result suggests that the prevalence of DKD increases with age. Apart from the age influence, the NHANES studies consist of Hispanics (8%) and a greater proportion of Afro-Caribbeans (17%). Studies comparing the renal function between these two ethnicities and white Caucasians have shown that white Caucasians have a slower decline in renal function.[355,356] Therefore, the discrepancies in ethnicities will also contribute to the difference in prevalence rates.

5.5.1.3 Comparison between the MDRD and CKD-EPI eGFR prevalence

In comparison to the MDRD formula, utilising the CKD-EPI equations revealed lower prevalence rates in the current study. The MDRD formula was first developed in 2006 based on a population of individuals with renal impairment only.[344] Subsequently, the CKD-EPI was developed in 2009 to provide a better prediction in individuals with GFR >60ml/min/1.73m^2

compared to the MDRD formula.[344] This is because the CKD-EPI research development group included both individuals with and without renal impairment. A total of 8254 participants' data were used: 5504 participants' data were used for the development of the formula and 2750 for internal validation. All the participants had results on urinary clearance of iothalamate to measure actual GFR. An additional 3896 participants' data from 16 other studies were used for external validation of the CKD-EPI eGFR formula. During the external validation process, the accuracy of the CKD-EPI and MDRD equations within 30% of the measured GFR (P30) was found to be 84.1% and 80.6%, respectively. The study also found that the CKD-EPI formula performed as well as the MDRD equation in those with eGFR <60ml/min/1.73m^2 and is more accurate in the subgroup with eGFR >60ml/min/1.73m^2 (please refer to Figure 5-2).[344]

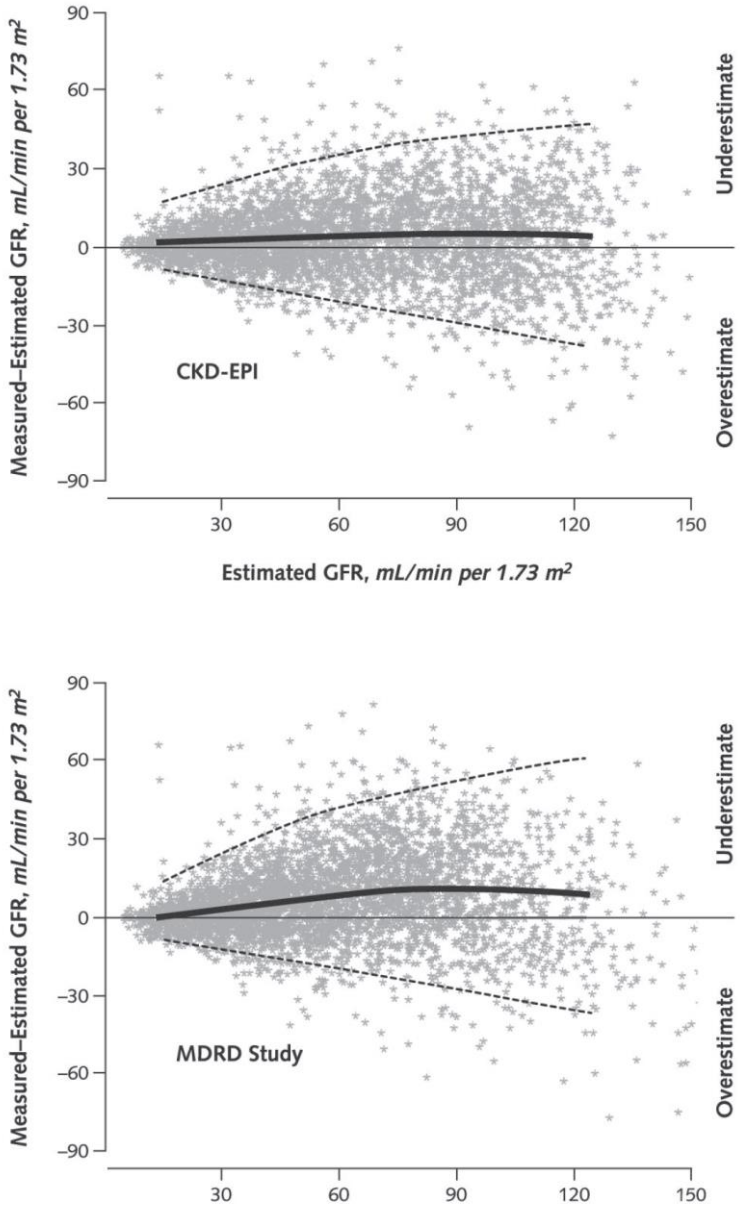

Figure 5-3: The accuracy of the MDRD and CKD-EPI equations in estimating the measured glomerular filtration rate. Reproduced with permission from Levey AS et al. Annals of Internal Medicine 2009;150:604-612, Copyright American College of Physicians.

As mentioned above, the majority of our patients were referred to be considered for bariatric surgery may have 'mild' DM without renal complications, so our patients may be more likely to have an eGFR >60ml/min/1.73m^2. If so, does this may mean that using the CKD-EPI formula is more accurate in estimating the DKD prevalence for our population? Two other

studies compared the formulae in other diabetic populations and did not report any differences in the performance of both the formulae.[357,358] Silveiro and colleagues used ^{51}Cr-EDTA to measure the isotope GFR (iGFR), which provides an accurate value of GFR for DM individuals with GFR >60ml/min/1.73m^2.[357] Compared to the measured iGFR of 103±23ml/min/1.73m^2, CKD-EPI calculated eGFR was 83±15ml/min/1.73m^2 and MDRD eGFR was 78±17ml/min/1.73m^2. The P30 accuracy of CKD-EPI (67%, 95% CI: 58-74) was found to be similar to the MDRD eGFR (64%, 95% CI: 56-75).

Similarly, Camargo and co-workers used ^{51}Cr-EDTA to measure iGFR in 52 T2DM patients.[358] The bias rate was similar for the CKD-EPI and MDRD equations (24ml/min/1.73m^2 vs. 26ml/min/1.73m^2), as was the P30 accuracy rate (66% vs. 64%). Due to the inconsistencies in the performance of both formulae in the DM populations compared to the general populations, more researches are needed before conclusion can be drawn in identifying a superior formula for the DM population. Additionally, both formulae have not been validated in extreme obese populations.

5.5.2 Obstructive sleep apnoea and diabetic kidney disease

5.5.2.1 OSA (defined by AHI/ODI) and DKD

The association between chronic nocturnal hypoxemia and renal function has been examined by several studies with contradictory results.[340,359-364] In a small study of 52 DM patients with sleep symptoms, the authors found no correlation between urinary ACR and AHI.[359] The small sample of patients and lack of control group were the main limitations of the study. Another study from Germany assessed 58 Type 1 diabetes and 498 Type 2 diabetic patients and reported a significant difference in the proportions of DKD based on the severity of AHI

(<5 events/hr, between 5 and 14.9 events/hr and those >15 events/hr) (p=0.001).[361] Although the study recruited 715 participants, only 556 individuals' data was used (30% excluded) due to issues with sleep recording and missing data. Another limitation was that researchers used a 2 hour sleep recordings rather than the commonly used minimum of 4 hours for sleep analysis. As mentioned in the discussion of Chapter 3, longer sleep recordings are better at diagnosing OSA because apnoea and hypopnoea episodes are higher during REM sleep.[218] The study also did not adjust for confounders and used a Level IV sleep device (ApneaLink) which lacks information on respiratory effort. Furthermore, the study's definition of OSA was AHI >15 events/hr. If, rather than comparing those with no, mild and moderate-to-severe OSA (as reported by the researchers); we used the study's conservative definition for the diagnosis of OSA, the association was no longer found to be significant (unadjusted OR 1.25, 95% CI: 0.72 to 2.14, own statistical calculation).

In contrast, an observational study in 513 diabetic patients by Furukawa and colleagues using pulse oximetry reported significant associations between ODI and albuminuria. The researchers employed ODI as a diagnosis of OSA (defined as ≥5 events/hr based on at least 3% ODI). This Japanese group found that those with OSA had up to a threefold increase in the risk for macro-albuminuria (OR 2.97, 95% CI: 1.36 to 6.9) and almost twofold increased risk for micro-albuminuria (OR 1.84, 95% CI: 1.16 to 2.96) after adjusting for confounders (age, gender, BMI, hypertension, dyslipidaemia, smoking, alcohol, cardiovascular medications, DM duration and HbA_{1c} level).[360] This study used a screening device (pulse oximetry) rather than polysomnography to diagnose OSA. Additionally, the researchers used the proprietary software accompanying the oximetry to analyse the oximetry data and this was not confirmed by a sleep physiologist or sleep expert. This suggests that artefacts and other

situations such as disconnection might not have been excluded from the analysis implying potential issues in the validity of the oximetry analysis.

Another study of 224 diabetic participants by Tahrani and colleagues demonstrated an almost three-fold higher risk of diabetic nephropathy in patients with OSA compared to non-OSA individuals (OR: 2.64, 95% CI: 1.13 to 6.16) after adjusting for multiple confounders.[348] In addition, they followed-up the individuals for 2.5±0.7 years. At the end of the study, 196 individuals had complete baseline and follow-up results. Regression analysis performed confirmed that OSA was a significant risk factor for study end MDRD eGFR (β=-3.8, p=0.04) after adjusting for age, DM duration, ethnicity, BMI, sex, smoking, mean arterial pressure, HbA_{1c}, insulin, total cholesterol, triglyceride, lipid-lowering, anti-diabetic, anti-hypertensive and anti-platelet medications. Using AHI instead of OSA in the analysis also showed a similar finding (β=-4.6, p=0.02). The researchers also did a sensitivity analysis. They replaced the lost to follow-up study-end MDRD eGFR values with baseline values; and the results remained similar for both OSA (β=-3.89, p=0.02) and AHI (β=-3.45, p=0.04) as an independent predictor for MDRD eGFR decline at the end of the study. The cross-sectional analyses of the data make it difficult to infer causality. Although the short-term follow-up may suggest a potential causal effect, this need to be confirmed in future prospective controlled interventional trials.

Our initial analysis did not find any significant differences in the AHI, as well as the prevalence and classification of OSA between CKD+ and CKD- individuals. However, after adjusting for potential confounders, we found that the AHI was inversely associated with eGFR based on the MDRD calculation. This association almost reached statistical

significance using the CKD-EPI formula. We postulated that the negative impact of the AHI on the glomerular function is mediated through episodic nocturnal intermittent hypoxemia. The AHI captures disordered breathing events with and without desaturation. Since this was a service evaluation and our local protocol was to evaluate AHI, we do not have data on ODI. Although AHI is not a measurement of nocturnal intermittent hypoxaemia, the repetitive long-term apnoeic and hypopnoeic episodes are significantly correlated with oxygen desaturation.[365] A recent Canadian study on 475 pre-surgical patients with a mean BMI of 31±7Kg/m^2 reported a correlation coefficient of 0.886 (95% CI: 0.865 to 0.904) between AHI (using an Embletta device) and ODI (using a PULSOX-300i device).[365] Another small study of 38 Mexican reported a correlation coefficient of 0.89 ($p<0.05$),[366] whilst a third study of 18 participants showed a correlation coefficient of 0.78 ($p<0.0001$) between AHI and ODI.[367]

5.5.2.2 Duration of nocturnal hypoxaemia and DKD

Apart from the AHI, we also found that the %TST<90% had an inverse impact on the renal function of DM patients based on both the MDRD and CKD-EPI formulae. Only a few studies have explored the relationship between the %TST<90% and renal function.[363,364,368] Unruh and colleagues compared 46 dialysis patients matched with 137 individuals (matched for gender, ethnicity, BMI and age) with normal renal function from the SHHS study.[363] They found that the renal patients were 4 times more likely to have nocturnal hypoxemia compared to matched individuals (%TST<90 7.2% vs. 1.8%, $p<0.0001$).[363] A major limitation for the SHHS study is that dialysis patients are frequently volume overloaded and nocturnal rostral fluid shift possibly to the pharyngeal area has been shown to worsen OSA in ESRD patients.[369] Furthermore, the SHHS study did not perform further adjustment for any cardiovascular confounders.[363] Another study by Nicholl and colleagues used the CKD-EPI

equation to calculate eGFR.[368] Participants were classified into 3 groups: 55 non-CKD (eGFR >60ml/min/1.73m^2), 124 CKD (eGFR ≤60ml/min/1.73m^2); and 75 ESRD patients, who were those on RRT. A total of 254 participants were recruited with at least 6 hours of sleep recording and 4% ODI ≥15 events/hr were used to diagnose OSA. Sleep analysis was performed by a sleep physician blinded on the participant's renal function. Nocturnal hypoxia, defined as %TST<90 ≥12% were demonstrated to be associated with CKD (OR 3.37, 95% CI: 1.04 to 10.90) after adjusting for age, sex, BMI, neck circumference, congestive cardiac failure, CAD, stroke, DM, sedatives, anti-depressants, narcotics and ODI. This study consisted of ESRD patients and carried out a sleep study on dialysis-free day representing a higher risk of fluid accumulation therefore the issue of rostral fluid shifts remained with the potential reverse causation of renal failure leading to longer duration of nocturnal hypoxia.

A recent study by Ahmed and colleagues evaluated the rate of renal decline in 858 participants in the general population. Two creatinine results were used: a) one year prior to the sleep study; and b) at the same time of the sleep study. The MDRD equation was used to calculate the eGFR. Accelerated decline in renal function was defined as a rate of decline >4ml/min/1.73m^2 per year. Ahmed and colleagues reported that the %TST<90% of more than 12% per night increased the risk for accelerated renal decline by threefold (OR 2.89, 95% CI: 1.25 to 6.67) after adjusting for AHI, age, BMI, DM and heart failure.[364] The study did not report on the duration of the sleep study. Additionally, the three studies above[363,364,368] included both DM and non-DM participants. The majority of the participants in the study by Ahmed and colleagues were non-DM individuals (80.4%)[364] while the SHHS only had 14.8% of DM participants[363] and Nicholl et al.[368] had 37% DM individuals. Therefore, it is difficult to generalise to the DM populations. All three studies also did not adjust for hypertension, a

well-known risk factor for CKD and well-recognised to be associated with OSA. Long-term cohort studies are needed to explore if prolonged exposure to nocturnal hypoxaemia results in accelerated progression of DKD.

5.5.2.3 Pathophysiology of OSA and DKD

Case reports have documented that OSA may cause focal glomerulosclerosis[370,371] and in one case, complete recovery of proteinuria after bi-level positive airway pressure treatment despite the patient remaining morbidly obese.[370] It is therefore unsurprising that our results showed that apnoeic and hypopnoeic episodes as well as the duration of nocturnal hypoxemia were associated with renal function in diabetic patients. Several factors may explain the association between AHI, as well as %TST<90%, and eGFR. It has been shown in animal models that prolonged exposure to intermittent hypoxemia results in the activation of the renin-angiotensin-aldosterone receptor system (RAAS).[372] In addition, intermittent awakening and sleep fragmentation from OSA activates the sympathetic nervous system leading to higher levels of adrenaline and noradrenaline.[136,214,373] Both the RAAS and elevation in stress hormones ultimately cause a rise in BP, which can have an adverse impact on renal function. We attempted to correct for these effects by adjusting for hypertension as well as treatment with ACE inhibitors and ARB. The results for the association between AHI and %TST<90% with eGFR remained significant. This suggests the possibility of other mediators being involved such as inflammation and oxidative stress although it is also possible that we might not have fully adjusted for the effects of RAAS.

Both OSA and diabetic nephropathy have been shown to be associated with increased inflammatory markers such as interleukin-6 (IL-6) and tumour necrosis factor-alpha

(TNFα).[374-377] These markers are not only associated with cardiovascular disease,[378] they also have an impact on the renal vasculature through the activation of oxidative stress pathways. Reactive oxygen species (ROS) affects the bioavailability of nitric oxide (NO)[379] causing vasoconstriction and reduction in renal blood flow affecting renal function. Vasoconstriction also impacts on BP and eventually systemic hypertension develops and exerts further adverse effects on the kidneys. Additionally, hypoxaemia from OSA leads to the activation of hypoxia-inducible factor-1 (HIF-1)[380] and nuclear factor-kappa B (NK-κB).[381,382] Both the HIF-1 and NK-κB control a huge number of processes including the expression for adhesion molecules.[383,384] These adhesion molecules have been shown to cause endothelial dysfunction and ultimately atherosclerosis[385] therefore causing ischaemia to the highly vascular renal organ.

Advanced glycation end products (AGEs) are well known to be elevated in DM individuals.[386] Interestingly, individuals with OSA also have elevated AGE levels.[386] This is important because AGE formation has been associated with alterations in the renal architecture as well as the decline in renal function.[387,388] In addition, OSA has also been found to up-regulate the protein kinase C (PKC) pathway. PKC is associated with the activation of transforming growth factor-beta (TGFβ) that promotes glomerulosclerosis and subsequently DKD.[389] Apart from AGEs and PKC, humoral factors such as leptin may also contribute to the deterioration of renal function. It has been found that patients with diabetic nephropathy have higher levels of leptin after adjusting for important confounders including obesity (waist-hip-ratio),[390] as do those with OSA.[391] The combined effect of both OSA and DM on inflammation, oxidative stress, AGEs, PKC and the endocrine system however, has not been comprehensively examined and may have a synergistic detrimental effect on renal

function. On the other hand, as discussed in Chapter 4, it is also plausible that diabetic microvascular complications in particular, diabetes neuropathy which can occur alongside DKD may lead to the development of OSA.

5.5.2.4 CPAP treatment and DKD

Only the study by Tahrani and colleagues assessed effect of CPAP on DKD after 2.5±0.7 years of follow-up.[348] A subgroup of 47 (81%) patients with moderate-to-severe OSA were treated with CPAP but only 16 were compliant with using more than 4 hours per night for at least 70% of the days (34% in total). The authors found that CPAP-treated patients have a significantly slower decline in eGFR. The eGFR results reported were as follows: -1.4% (-7.7% to 5.2%) in non-OSA group vs. -5.3% (16.5% to 2.7%) in mild OSA vs. -7.7% (-15.9% to -1.8%) in moderate-to-severe OSA compliant to CPAP treatment vs. -10.0% (-17.2% to 2.3%) in moderate-to-severe OSA non-compliant to CPAP (p=0.01 for trend). The study has several limitations. Apart from a small sample size and has no control group, the researchers also did not adjust for important confounders such as reno-protective treatment with ACE inhibitors or ARB. The participants who were CPAP-compliant might also be more likely to be compliant to other diabetic management such as better dietary adherence, self-monitoring of glucose levels and compliant with glucose-lowering, as well as other medications. These measures can curtail the progression of renal decline and was not assessed in the study. Due to the limited studies available describing the effect of CPAP in DKD, well-designed adequately powered RCTs are needed to establish the benefits of CPAP treatment.

5.5.3 Limitations and benefits of the study

There are several limitations in our study. This was a cross-sectional study, which showed a significant association between hypoxemia and diabetic nephropathy, but cannot infer causality. Additionally, we reported the crude prevalence DKD rate in an extreme obese population and it is difficult to compare prevalence rates from other populations due to the variations in the populations' characteristics. Although we adjusted for several important confounders, we did not take into account others such as smoking and alcohol status. Smoking has been found to be associated with greater progression of nephropathy in DM patients compared to non-smoker.[127,392] Alcohol has been found to have an U shape association with DKD in Type 1 diabetes patients.[393] Therefore, future studies should take into account both the effect from smoking and alcohol.

Using validated formulae to calculate estimated GFR are advantageous because it is non-invasive and inexpensive in comparison with other methods. However, both the MDRD and CKD-EPI equations have not been validated in the extremely obese population. Due to the nature of this study, which was a part of a service evaluation, more intrusive tests have not been carried out. Nevertheless, it is important to point out that all of the renal function tests were undertaken by HEFT biochemistry department and calibration of equipment is performed regularly. Creatinine results for our study were therefore reliable.

Data on albuminuria was also not included in the study, which may indicate that we missed a greater number of patients with diabetic nephropathy. We also did not assess volume status as this is difficult to assess accurately based on clinical examination for extremely obese

individuals. As our population only includes extremely obese individuals, the results should be interpreted with caution in the general DM population without extreme obesity.

Our study adjusted for multiple confounders; in particular, we adjusted for drugs affecting the RAAS system. A recent systematic review and meta-analysis examined 28 studies with 134,912 DM participants compared the treatment of using either ACE inhibitor or ARB with other anti-hypertensive drugs.[394] It showed that the RAAS drugs reduced serum creatinine with pooled relative risk (RR) of 0.66 (95% CI: 0.52 to 0.83). This suggests that reno-protective effect of the RAAS drugs is not only on BP-lowering. In fact, the effects of RAAS inhibition are likely to be complex as it caused a reduction in GFR initially through haemodynamic changes, however, in the long-term, RAAS drugs may protect the kidney through restriction of angiotensin activity which is associated with the prevention of renal parenchymal injury.[395]

5.6 Conclusion

This study showed that not only are apnoea and hypopnoea episodes potentially important in DKD, the duration of exposure of nocturnal hypoxemia also appears to play a significant role in the decline in renal function amongst extreme obese DM patients. Further studies should examine the factors mediating the effect of hypoxemia on renal function and the long-term impact of nocturnal hypoxemia on the progression on DM nephropathy, as well as the treatment of OSA on diabetic nephropathy still warrants further investigation.

CHAPTER 6 – THE EFFECT OF OBSTRUCTIVE SLEEP APNOEA ON DIABETIC RETINAL AND KIDNEY DISEASES: A SYSTEMATIC REVIEW AND META-ANALYSIS

6.1 Introduction

Obstructive sleep apnoea (OSA) is a chronic sleep disorder characterised by recurrent complete or partial episodic collapse of the upper airway resulting in chronic intermittent nocturnal hypoxemia (CIH), arousal, and sleep fragmentations. OSA is prevalent among people with diabetes and has been proposed to alter glycaemic control. A recent meta-analysis of cohort studies reported that moderate to severe OSA has a 63% increase in risk for diabetes [DM] (RR 1.63, 95% confidence interval [CI]: 1.09 to 2.45).[80] Additionally, the CIH that accompanies OSA results in the activation of oxidative stress and inflammatory pathways such as increased advanced glycation end products (AGEs)[386] that have also been implicated in the pathogenesis of diabetes vascular complications.[396]

As illustrated in Chapters 4 and 5, there were inconsistencies in the results for both diabetic retinal and kidney diseases. The differences may be due to methodological variations, such as the definition of OSA used, or the population under study, or may be masking small effects that are not identified within single studies.

6.2 Aim and objectives

Due to the inconsistency in the literature, we conducted a systematic review to summarise the association between chronic nocturnal hypoxemia caused by OSA and the risk of diabetes retinal disease (retinopathy and maculopathy) and diabetic kidney disease (DKD). The measures employed to assess OSA can include the apnoea-hypopnoea index (AHI), the

oxygen desaturation index (ODI), time spent below 90% oxygen saturations at night (%TST<90), mean oxygen saturation, and minimum oxygen saturation. By using the different measures to assess OSA, we aimed to further understand the pathophysiological pathways mediating the impact of OSA on diabetic retinopathy (DR), diabetic maculopathy and DKD.

6.3 Methods

The systematic review was conducted according to the recommended Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) statement[397] and the Meta-analysis of Observational Studies in Epidemiology (MOOSE) statement.[398] The protocol is registered in Prospero database (registration number was CRD42014008757).

6.3.1 Eligibility criteria

The full inclusion and exclusion criteria for the systematic review are summarised in Table 6-1. The review had no language restrictions and the following inclusion criteria were used:

Participants: Adults with either type 1 or type 2 diabetes mellitus (DM) are included. Therefore, non DM individuals, children and adolescents with DM, pregnant females, gestational DM or DM secondary to other causes such as maturity onset diabetes of the young (MODY) and latent autoimmune diabetes of adulthood (LADA) were excluded.

Exposure: DM individuals must have had a diagnosis of obstructive sleep apnoea. The diagnosis may or may not have been based on the American Academy of Sleep Medicine (AASM) guidance[75] of using AHI obtained from polysomnography. We also included studies

which used oxygen desaturation index (ODI) from pulse oximetry as a diagnostic measure of OSA. We excluded studies, which used screening questionnaires such as the Berlin questionnaire or Epworth Sleepiness Score as a diagnosis of OSA. Other respiratory disorders, which may induce hypoxia or hypoxaemia including central sleep apnoea, heart failure, chronic obstructive pulmonary disease or pulmonary fibrosis, were also excluded.

Comparator: People with no OSA were the comparator.

Outcomes: The primary outcome was the risk of development or progression of diabetic retinopathy (DR), diabetic maculopathy (DMac) and diabetic kidney disease (DKD). DR has been categorised into overall DR and advanced DR. Advanced DR is defined as either pre-proliferative or proliferative DR. DMac consists of both macular oedema and ischemia. For DKD, renal function assessed using either estimated glomerular filtration rate (eGFR), serum creatinine or albuminuria, which included both micro- and macro-albuminuria were included. Any studies reporting other diabetic eye diseases or eye diseases from other causes such as glaucoma, cataract, retinal vein or artery occlusion, were excluded. Furthermore, chronic renal failure or nephropathy secondary to causes other than DM was also excluded.

Study design: Only observational studies, which consisted of either cross-sectional, cohort or case-control studies, were included. This excluded all intervention studies such as randomised controlled trials, non-human studies, experimental studies, case reports, and studies with less than 10 participants. Due to the small number of studies available, which examine the relationship between OSA and diabetes retinal disease, we also included OSA prevalence studies (n=6) if data on diabetes microvascular complications were available.[292,320,399-402]

Table 6-1: Eligibility criteria for the systematic review

Inclusion criteria	Exclusion criteria
Participants: All adults with type 1 or type 2 diabetes mellitus (DM)	Children and adolescents (age below 18 years) Pregnant females Gestational diabetes mellitus Maturity onset diabetes of the young Latent autoimmune diabetes of adulthood No type 1 or type 2 DM
Exposure: Those with obstructive sleep apnoea (OSA) or chronic intermittent hypoxemia (CIH)	Hypoxemia secondary to other respiratory disorders Chronic obstructive pulmonary disease Central sleep apnoea Heart failure Asthma
Comparator: Adults without OSA or CIH	
Outcomes: Diabetic retinopathy (background, pre-proliferative and proliferative) Diabetic maculopathy or diabetic macular oedema	Retinopathy from non DM causes Glaucoma, cataract, retinitis, retinal vein occlusion, retinal artery occlusion Optic neuritis
Diabetic kidney disease (micro- and macro-albuminuria and chronic kidney disease assessed using glomerular filtration rate)	Renal impairment secondary to non DM causes
Study designs: Cross sectional study Cohort study Case series	Non-human studies Single case reports Sample size < 10 participants Randomised controlled trials Intervention studies Experimental studies Systematic reviews/reviews Editorials Protocol papers Letters Guidelines/consensus statements
Language: All	None

6.3.2 Search strategy

Both indexed term such as medical subject headings (MeSH) and free text were used to capture a wider aspect of the literature. The MeSH terms used for the searches are described in Table 6-2. Furthermore, no restriction was applied to any of the database searches. Searches were performed from the inception until January 2014 of the following electronic databases: MEDLINE, Excerpta Medica DataBase (EMBASE) and Cochrane Central Register of Controlled Trials. In addition, OpenGrey and Zetoc were also searched for grey literature such as conference abstracts. For OpenGrey, the following free text was used: “obstructive sleep apnoea” or “obstructive sleep apnea”. The search term for Zetoc included “obstructive sleep apnoea/apnea and diabetes” as well as “apnoea/apnea and diabetes”. Finally, we hand searched citations from included papers.

Table 6-2: The search terms for MEDLINE, EMBASE and Cochrane Database

Search terms for diabetic retinopathy
1. Diabet$.mp.
2. Respiratory system/ or breathing disorder/ or snoring/ or hypoventilation/ or sleep/ or polysomnography/ or sleep apnea syndrome/ or breathing/ or sleep disordered breathing/ or sleep disorder/
3. Hypoxemia/ or respiratory function disorder/ or anoxia/ or hypoxia/
4. Somnolence/ or apnea/ or apnea hypopnea index/ or hypopnea.mp.
5. 2 or 3 or 4
6. 1 and 5
7. Diabetic retinopathy/ or retinopathy/ proliferative retinopathy/ or retinopathy.mp.
8. Maculopathy.mp. or retina maculopathy/
9. Macular edema.mp. or retina macula edema/
10. Eye disease.mp. or eye disease/
11. 7 or 8 or 9 or 10
12. 6 and 11

Search terms for diabetic kidney disease
1. Diabet$.mp.
2. Respiratory system/ or breathing disorder/ or snoring/ or hypoventilation/ or sleep/ or polysomnography/ or sleep apnea syndrome/ or breathing/ or sleep disordered breathing/ or sleep disorder/
3. Hypoxemia/ or respiratory function disorder/ or anoxia/ or hypoxia/
4. Somnolence/ or apnea/ or apnea hypopnea index/ or hypopnea.mp.
5. 2 or 3 or 4
6. 1 and 5
7. Nephropathy.mp. or kidney disease/
8. Albuminuria.mp. or proteinuria/ or albuminuria/
9. $albuminuria.mp.
10. Renal failure.mp.
11. Kidney function/
12. Diabetic nephropathy/ or nephropa$.mp. or chronic kidney failure/
13. 7 or 8 or 9 or 10 or 11 or 12
14. 6 and 13

6.3.3 Study selection

Two authors independently reviewed the titles and abstracts to select eligible studies. This was followed by the review of full texts of the eligible studies. This process was accomplished independently by two authors again. Disagreements were resolved through discussion and if no consensus reached, a third author arbitrated. In cases whereby several duplicate or 'kin' studies were found, we chose the most recent study with the most complete data (especially results on adjusted odds ratios) available.

6.3.4 Data extraction

Prior to formal data extraction, a standardised form was designed based on the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement.[403] The standardised data extraction form was piloted on a sample of five studies and improvements were made before formal use. Data extraction was performed by one author and checked by other authors. When additional information was missing or needed, authors from the studies were contacted for unreported adjusted odds ratios with 95% confidence intervals (CI) or frequency data. A sample of the data extraction form is available in the appendix section.

6.3.5 Assessment of the quality of the study

A revised quality assessment form to assess the risk of bias for individual studies for both DR and DKD studies has been devised based on the Newcastle Ottawa Scale for nonrandomised studies in meta-analyses.[404] The quality of the study was assessed according to five components and they were study methods, selection bias, respiratory measurement, blinding of the assessor performing DR or sleep analyses and statistical analysis (Table 6-3). Each component had a set of criteria and each criterion was rated 'yes', 'no' or 'unclear'. An

overall judgement was made for each component to assess if the study was either 'weak', 'moderate' or 'strong'. Subsequently, an overall judgement was rated again with either as 'weak', 'moderate' or 'strong'. This is in accordance with the recommendations by the Cochrane Collaboration.[405] This was carried out independently by two reviewers, and any discrepancies were resolved by discussion. The form was piloted on known studies and necessary modifications were made. A copy of the quality assessment form is available in the appendix section.

Table 6-3: Criteria to assess risk of bias in individual studies

Selection bias
1. Does the study address an appropriate and clearly focused question?
2. Was consecutive patients recruited?
3. Was the cases and controls taken from comparable populations?
4. Were the exclusion criteria the same for both cases and controls?
5. Was the participant representative of the patient population?
6. If applicable, was the control group comparable to cases (consider suitability, recruitment and baseline characteristics)?
7. Was it clear that controls are not cases (in case control)?
Overall Judgment for selection bias (Weak / Moderate /Strong)
Respiratory measurement
1. Was a suitable measurement for OSA used? (PSG/oximetry measured in standard, valid and reliable way)
2. Was the scoring of the respiratory measures based on guidelines / consensus guidelines eg. AASM guidelines?
3. Was there a clear definition of OSA used?
Overall Judgment for measurement (Weak / Moderate / Strong)
Blinding
1. Blinding of assessor performing sleep analysis
2. Blinding of assessor of retinal photographs
Overall Judgment for measurement (Weak / Moderate / Strong)
Study methods
1. Were participant attrition rates /missing data documented?
2. Were reasons given for drop outs / missing data?
3. Is this retrospective / prospective design?
Overall Judgment for study design (Weak / Moderate / Strong)
Analysis
1. Were all outcomes reported?
2. Were confounding variables adjusted for?
Overall Judgment for analysis (Weak / Moderate /Strong)

6.3.6 Data synthesis and analysis

After data extraction and quality assessment, the findings were synthesised and summarised according to studies which reported on overall DR, advanced DR, DMac and DKD. Statistical analysis was carried out using Stata 13 (StataCorp LP, College station Texas). For the review on diabetic retinopathy, we performed meta-analyses on adjusted odd ratios (OR) for the effect of OSA on advanced DR, effect of ODI, and the effect of minimum oxygen saturation on DR. For the review on DKD, we performed a meta-analysis on studies which reported unadjusted OR on the effect of OSA on DKD. We also meta-analysed studies, which only reported adjusted ORs to minimise the influence of potential confounders. All meta-analyses were performed using both random and fixed effects model analyses.

For DR, due to the limited number of studies, no meta-analysis was carried out for mean oxygen saturation and %TST<90. Similarly, for DKD, meta-analysis was not performed for effect of mean and minimum oxygen saturation as well as percentage of time spent below 90% oxygen saturation (%TST<90) on DKD because only a limited number of studies were available. Funnel plots were utilised to assess publication bias and small study effects for meta-analysis which contained >5 studies.

We also carried out kappa statistics for to measure the agreement between the two authors. For DR, the kappa statistics were 0.95 (98% agreement, $p<0.001$) for study selection and 0.79 (88% agreement, $p<0.0001$) for quality assessment. For DN, the kappa statistics were 0.71 (87% agreement, $p<0.0001$) for study selection and 0.80 (89% agreement, $p<0.0004$) for quality assessment.

6.4 Results

6.4.1 Diabetic retinopathy and maculopathy

6.4.1.1 Study characteristics

The initial search identified 851 studies (814 from databases and 37 from grey literature). After the removal of duplicates, there were 731 records and 678 were excluded based on title and abstract screening. A total of 53 full-text articles were obtained and 37 were excluded. Of those excluded, 15 studies were duplicates or 'kin' studies, 11 did not have OSA as exposure, two studies did not assess DR or DMac as outcomes, one study did not have DM as the population, and eight were studies with other study designs. Therefore, 16 studies were included in the systematic review (please refer to Figure 6-1 for PRISMA flow chart).

The characteristics of the included studies, study population, methods of assessing OSA and the outcome measures used are summarised in Table 6-4. Out of the 16 included studies, four were conference abstracts.[406-409] Only one study was longitudinal,[406] and the remainder was cross-sectional. The majority of the studies were from European countries (10 studies) with three studies from Japan, one from India and two studies from the US. There are a total of 2731 DM participants (2636 with T2DM). Only two studies recruited community DM participants.[400,410] Gender proportions,[292,407,408,411] mean BMI[406-408,411] and the mean duration of DM[292,406-408] were not reported in 4 studies while mean age and HbA_{1c}, were not reported in 3 studies.[406-408] The studies included were predominantly males except the study by Banerjee et al.[224] Mean ages for the studies ranged between 43 and 67 years. One study only included extreme obese individuals with a mean BMI of 47.3Kg/m^2 [224] whilst the mean BMI from other studies ranged from 24.2 to 35.5Kg/m^2. Mean HbA_{1c} levels and DM duration ranged from 6.5% to 9.2% and 7.6 to 23.0 years, respectively.

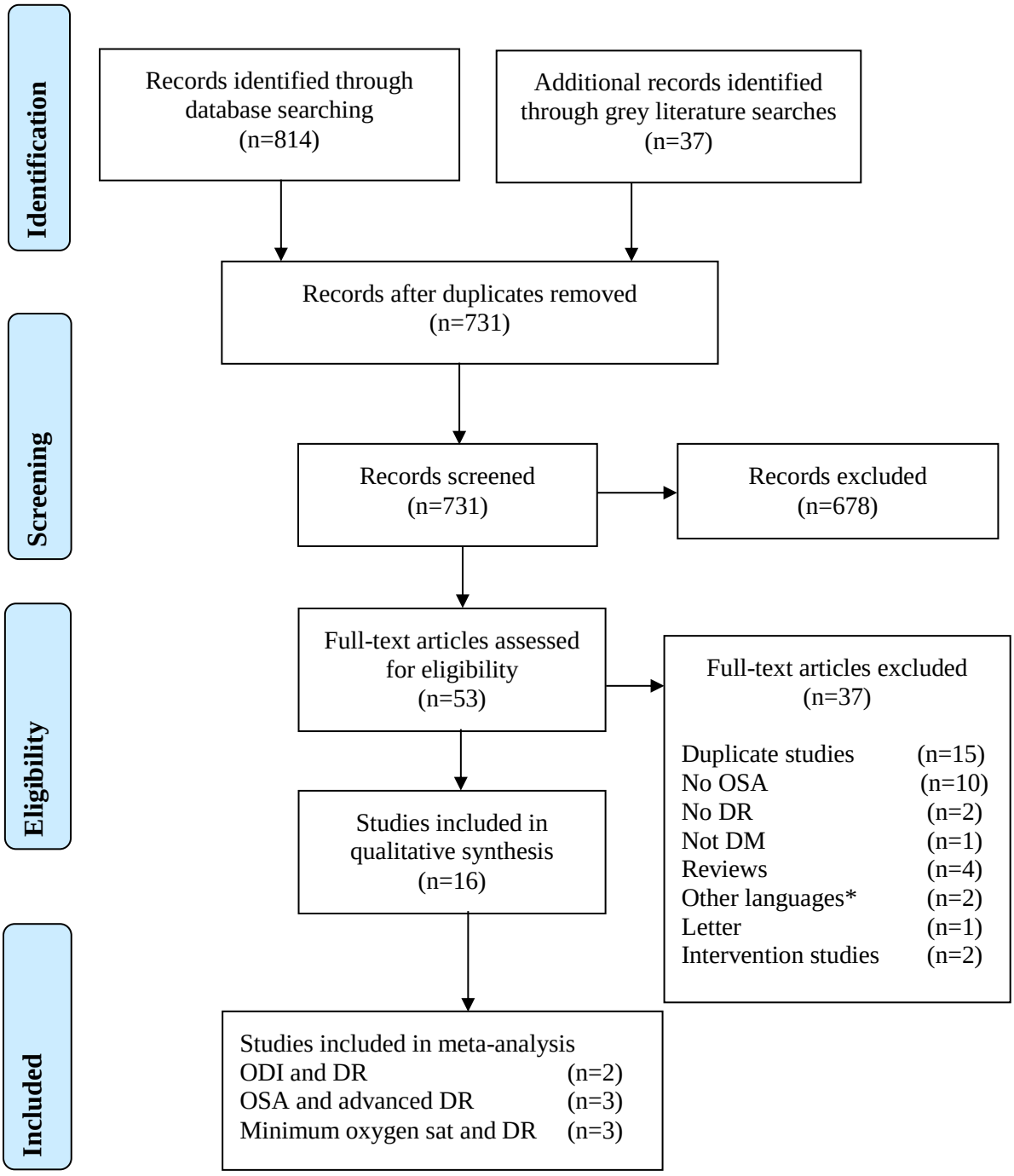

Figure 6-1: PRISMA flow chart on study selection for diabetic retinopathy.
OSA=obstructive sleep apnoea; DR=diabetic retinopathy; DM=diabetes mellitus; sat=saturation; DMac=diabetic maculopathy
*Other languages=1 study in Russian and 1 study in Czech language

Table 6-4: Characteristics and results of included studies for diabetic retinopathy and diabetic maculopathy

Study ID (country)	Study design	Patients	Demographics	OSA assessment	Results
Storgaard 2014[l] (Denmark)	CS	180 T2DM	Setting = Diabetes outpatient Mean age = 59 years* Females = 42%* Mean HbA_{1c} = 7.4%* Mean DM duration = 8.2 years* Mean BMI = 32.2 Kg/m^2 *	Berlin Q then Level III sleep test (ApneaLink) +/- Level IV sleep test (Embletta)[#]	*Univariate analysis for DR* - No difference in DR between OSA (26.4%) vs. non-OSA (19.4%) (p>0.05) - No difference in AHI between DR 17±20 vs. non-DR 17±25 events/hr (p>0.05)
Nishimura 2013[a] (Japan)	CS	136 T2DM	Setting = Unclear Mean age = NA Females = NA Mean HbA_{1c} = NA Mean DM duration = NA Mean BMI = NA	NA	*Univariate analysis for DR* - No significant difference in ODI for DR 17.1±15.2 vs. non-DR 15.4±14.7 events/hr (p=0.54). - No significant difference in AHI for DR 17.8±15.1 vs. non-DR 15.2±13.6 events/hr (p=0.33) *Multivariate analysis for DR* **- Association between min O_2 sat and DR (OR 0.89, 95% CI: 0.83 to 0.95).** Adjusted for HbA_{1c}, DM duration, history of CVD.
Furukawa 2013 (Japan)	CS	513 T2DM	Setting = Multicentre diabetes outpatients Mean age = 62 years Females: 43% Mean HbA_{1c} = 7.3% Mean DM duration = 11.7 years Mean BM = 25.2 Kg/m^2	Oximetry (PULSOX-3Si)[#]	*Multivariate analysis for DR* - No association between OSA and DR (OR 1.00, 95% CI: 0.60 to 1.68) Adjusted for age, sex, BMI, hypertension, hyperlipidaemia, smoking status, alcohol (regular or not), current medications for stroke, ischaemic heart disease, DM duration and HbA_{1c}.

Banerjee 2013 (UK)	CS	93 T2DM	Setting = Specialist weight management clinic Mean age = 52 years Females = 59% Mean HbA_{1c} = 8.1% Mean DM duration = 7.6 years Mean BMI = 47.3 Kg/m^2	Level III sleep test (Embletta)[#]~	*Multivariate analysis for DR* - No association between OSA and DR (OR 1.00, 95% CI: 0.98 to 1.02) - No association between min O_2 sat and DR (OR 0.93, 95% CI: 0.67 to 1.02) - No association between mean O_2 sat and DR (OR 0.93, 95% CI: 0.86 to 1.01) - No association between %TST<90 and DR (OR 1.03, 95% CI: 1.00 to 0.06) - No association between OSA and advanced DR (OR 1.01, 95% CI: 0.97 to 1.04 – unpublished data) Adjusted for age, sex, BMI, ethnicity, DM duration, insulin, HbA_{1c}, hypertension and coronary artery disease. *Multivariate analysis for DMac* - No association between OSA and DMac (OR 1.01, 95% CI: 0.98 to 1.04) - No association between mean O_2 sat and DMac (OR 0.80, 95% CI: 0.59 to 1.07) - **Association between min O_2 sat and Dmac (OR 0.79, 95% CI: 0.65 to 0.95)** - No association between %TST<90 and DMac (OR 1.03, 95% CI: 1.03, 95% CI: 0.99 to 1.08) Adjusted for age, sex, BMI, ethnicity, DM duration, insulin, HbA_{1c}, hypertension and coronary artery disease.
Altaf 2013[a] (UK)	Cohort	199 T2DM	Setting = Diabetes outpatient Mean age = NA Females = 43% Mean HbA_{1c} = NA Mean DM duration = NA Mean BMI = NA	Level III sleep test (Alice PDX)[#]~	*Multivariate analysis for advanced DR* - **Association between OSA and advanced DR (OR 3.90, 95% CI: 1.02 to 15.30)** - **Followed-up for 4.4 years, OSA independently associated with progression of advanced DR (OR 6.60, 95% CI: 1.20 to 13.10)** Adjusted for age, gender, ethnicity, BP, HbA1c, total cholesterol, triglycerides, diabetes duration, smoking,

					alcohol, renal function, WC, anti-platelet, anti-diabetes, lipid-lowering and anti-hypertensive treatments *Multivariate analysis for DMac* - **Association between OSA and DMac (OR 4.5, 95% CI: 1.8 to 11.4)** - Follow-up 4.4 years, OSA not associated with progression of DMac (effect size not reported) Adjusted for age, gender, ethnicity, BP, HbA1c, total cholesterol, triglycerides, diabetes duration, smoking, alcohol, renal function, WC, anti-platelet, anti-diabetes, lipid-lowering and anti-hypertensive treatments
Rudrappa 2012 (UK)	CS	31 T2DM	Setting = Diabetes outpatient Mean age = 55 years Females = 0% Mean HbA_{1c} = 9.2% Mean DM duration = 11.0 years Mean BMI = 35.3 Kg/m^2	Inpatient PSG (Visilab)	*Multivariate analysis* - **Association between total retinopathy scores and OSA (p=0.008)** - No association between OSA and advanced DR (OR 12.6, 95% CI: 0.62 to 255.76) - No association between OSA and DMac (effect size not reported) Adjusted for potential confounders including novel serum biomarkers for diabetic retinopathy and inflammation
Mehta 2012[a] (India)	CS	80 T2DM	Setting = Outpatient retinal clinic Mean age = NA Females = NA Mean HbA_{1c} = NA Mean DM duration = NA Mean BMI = NA	Inpatient PSG	*Multivariate analysis for DR* - No association between OSA and DR (effect size not reported) Did not report on factors adjusted
Mason 2012	CS	80 T2DM with	Setting = Macular oedema outpatient	Level IV sleep testing	*Univariate analysis for DR* - No associated between OSA and DR (p=0.32)

(UK)		macular oedema	Mean age = 65 years Females = 50% Mean HbA_{1c} = 7.8% Mean DM duration = 15.0 years Mean BMI = 30.2 Kg/m^2	(Apnealink)[‡]~	*Univariate analysis for DMac* - No association between macular thickness and AHI - No association between macular thickness and %TST<90 - No dose response association between severity of ODI and macular thickness
Kosseifi 2012[I] (US)	CS	98 T2DM	Setting = Outpatient sleep clinic Mean age = 61 Females = NA Mean HbA_{1c} = 6.5% Mean DM duration = NA Mean BM = 33.7 Kg/m^2	Level III sleep test (NovaSom QSG)	*Univariate analysis for DR* **- AHI in DR group (44.2) higher than non-DR group (22.7) ($p<0.05$)**
West 2010 (UK)	CS	118 T2DM	Setting = 1 diabetes outpatient and 5 primary care centers Mean age = 67 years* Females = 0% Mean HbA_{1c} = 8.0%* Mean DM duration = 10.0 years* Mean BMI = 30.2 Kg/m^2*	Oximetry and in some level III sleep test (Embletta)~	*Multivariate analysis* **- Retinopathy scores associated with OSA ($r^2=0.19$, $p<0.0001$)** **- OSA associated with DMac ($r^2=0.30$, $p<0.0001$)** Did not report on factors adjusted
Shiba 2010 (Japan)	CS	219 T2DM	Setting = Hospital inpatient (eye disease) Mean age = 63 years* Females = 43% Mean HbA_{1c} = 7.3% Mean DM duration = 11.5 years Mean BMI = 24.2 Kg/m^2	Oximetry (PMP-200G)	*Multivariate analysis for advanced DR* - **Association between advanced DR with neovascularisation and OSA (OR 1.09, 95% CI: 1.01 to 1.16)** Adjusted for insulin treatment, ODI, DM duration, sex, mean oxygen saturation and %TST<90 - **Association between min O_2 sat and advanced DR (OR 0.93, 95% CI: 0.88 to 0.99)** Adjusted for age, HbA_{1c}, hypertension, ODI and %TST<90

					- No association between %TST<90 and advanced DR (effect size not reported) - No difference in mean O_2 sat between OSA (97.6%±1.2) and non-OSA (97.3%±1.5) (p=0.14)
Schober 2010[I] (Germany)	CS	58 T1DM & 498 T2DM	Setting = 14 primary care centres Mean age = 60 years Females = 48% Mean HbA_{1c} = 7.6% Mean DM duration = 9.3 years Mean BMI = 31.9 Kg/m^2	Level IV sleep test (ApneaLink)[‡]~	Univariate analysis for DR - No difference in DR between OSA (13.9%) vs. non-OSA (11.5%) (p=0.45)
Borel 2010[I] (France)	CS	37 T1DM	Setting = Diabetes outpatient Mean age = 43 years Females = 32% Mean HbA_{1c} = 7.8% Mean DM Duration = 23.0 years Mean BMI = 24.7 Kg/m^2	Oximetry	*Univariate analysis for DR* **- Significant difference in DR between pathological recording (73.3%) vs. borderline recording (11.1%) (p<0.05)**
Unver 2009 (US)	CS	44 DM	Setting = Outpatient retinal clinic Mean age = 60 years* Females = NA Mean HbA_{1c} = 7.5%* Mean DM duration = 16.3 years* Mean BMI = NA	NA	*Univariate analysis for DR* **- Significant difference between DR and OSA (OR 143.18, 95% CI: 7.41 to 2767.79)** **- 10 eyes had pan-retinal photocoagulation in OSA group vs. 1 in non-OSA group** *Univariate analysis for DMac* **- Association between OSA and DMac (OR 14.44, 95% CI; 2.34 to 147.60)** - 7 OSA patients had laser treatment for macular edema and most worsened after treatment with some needing ≥2 treatment. 9 non-OSA patients had laser treatment for macular oedema and majority resolved without further

					progression
Laaban 2009[I] (France)	CS	303 T2DM	Setting = Hospital inpatient (DM) Mean age = 61 years Females = 49% Mean HbA_{1c} = 9.2% Mean DM duration = 14.5 years Mean BMI = 32.0 Kg/m^2	Level IV sleep test (CID 102)~	*Univariate analysis for DR* - **Significant association between OSA and DR (OR 0.56, 95% CI: 0.35 to 0.92)**
Merritt 2007[a] (UK)	CS	44 T2DM	Setting = Diabetes outpatient Mean age = 61 years Females = 32% Mean HbA_{1c} = 8.0%* Mean DM Duration = 16.5 years* Mean BMI = 30.4 Kg/m^2*	Oximetry (Minolta 300i®)	*Univariate analysis for advanced DR* - No significant difference in ODI between advanced DR (15.4±14.7) vs. non-advanced DR (17.1±15.2) (p=0.54) - **Significant difference in %TST>90% between advanced DR (12.6%±19.7) and non-advanced DR (1.8%±2.6) (p=0.03)**

CS = cross-sectional study, NA = information not available, HbA_{1c} = glycosylated haemoglobin A_{1c}, BMI = body mass index, T1DM = Type 1 diabetes mellitus, T2DM = Type 2 diabetes mellitus, OSA = obstructive sleep apnoea, PSG = polysomnography, Berlin Q = Berlin questionnaire, AHI = apnoea-hypopnoea index, ODI = oxygen desaturation index, O_2 = oxygen saturation, %TST<90 = percentage time spent under 90% oxygen saturation. DR = diabetic retinopathy, DMac = diabetic maculopathy, OR = odds ratio, 95% CI = 95% confidence intervals. Bolded texts indicate significant results.
[#]Minimum 4 hours recording; [$]Veteran study with low female participants; *Average values of the 2 groups; [a] Conference abstracts; [I] Prevalence studies

6.4.1.2 Respiratory methodology and OSA diagnosis

Only two studies carried out an in-hospital overnight sleep study.[407,412] Six studies conducted portable home sleep studies[224,292,320,400,401,406] while four studies used pulse oximetry.[360,399,409,413] Of those that utilised portable sleep studies, 3 studies used a level III device with data on respiratory effort, air flow, oxygen saturation and heart rate; 3 studies used a level IV device with no data on respiratory effort. Two studies used a mixed method: one utilised oximetry followed by a portable sleep study in a subset the participants[410] and another study identified at risk individuals using the Berlin questionnaire followed by a portable sleep study.[402] Two studies did not report on the equipment used for OSA assessment[408,411] as shown in Table 6-4.

There was a high level of heterogeneity in the sleep studies and diagnosis of OSA (Table 6-5). Of the 14 studies using either a pulse oximetry, an overnight sleep device or a mixed method,[224,320,360,399-402,406,407,409,410,412-414] ten studies[224,320,360,399-401,406,409,410,413] provided information on the definitions of apnoea, hypopnoea or oxygen desaturation. Of those 10 studies, only 2 studies were in line with the AASM criteria.[224,406] AASM guidelines[75] define apnoea as complete cessation of airflow for at least 10 seconds whilst hypopnoea as at least 30% reduction in airflow for at least 10 seconds accompanied by at least a 4% oxygen desaturation. One study[400] defined apnoea as at least 80% reduction in airflow for at least 10 seconds and hypopnoea as 50-80% reduction in airflow for at least 10 seconds with a 4% oxygen desaturation. Another study[401] that carried out sleep study using a Level IV device defined apnoea as an increased variation in suprasternal pressure with no airflow for at least 10 seconds. Two studies defined hypopnoea as a 50% reduction in airflow accompanied by ≥4% oxygen desaturation for ≥10 seconds.[320,401] Five studies performed pulse oximetry; one

study used 3% desaturation[360] while the other 4 studies used 4% oxygen desaturation[320,409,410,413] as the cut-point.

Based on the AASM guidelines,[75] an AHI of ≥5 events/hr is diagnostic of OSA. Mild, moderate and severe OSA are defined as AHI between 5.0 and 14.9 events/hr, between 15.0 and 29.9 events/hr and >30.0 events/hr, respectively.[75] The definition for OSA diagnosis was available in 10 studies.[224,320,360,400-402,406,410,412,413] Of the six studies which used AHI to diagnose OSA, four used an AHI cut-point of ≥5 events/hr[401,402,406,412] while 2 used AHI ≥15 events/hr.[224,400] In addition, Storgaard and co-workers defined OSA as AHI ≥5 events/hr with the presence of sleep symptoms.[402] Two studies used an ODI cut point of ≥5 events/hr[360,413] and another study utilised ODI ≥10 events/hr as OSA diagnosis.[410] Mason et al. defined OSA using both ODI ≥10 events/hr and AHI ≥15 events/hr.[320] The remainder of the studies (n=6) did not report on the criterion used for OSA diagnosis.[292,399,407-409,411]

6.4.1.3 Definition of diabetic retinopathy

As for the diagnosis of DR, the 4 studies from the UK used information from the English National Screening Program for Diabetic Retinopahty (ENSDPR).[224,406,410,412] The study by West et al. (UK study) had two independent trained ophthalmology graders to review the digital retinal photographs of all the participants.[410] Merritt et al.,[409] Storgaard et al.,[402] and Mason et al.[320] also performed digital retinal photography. In addition, Mason et al.[320] measured macula thickness using dual laser spectral-domain optical coherence tomography and had an ophthalmologist with a special interest in the retina to interpret the data. Furukawa et al.[360] carried out florescence fundoscopy performed by ophthalmology specialists in all the

participants. Two studies obtained information on DR from medical records.[407,413] The remaining six studies did not quote their source for the diagnosis of DR.[292,399-401,408,411]

Table 6-5: Criteria used for the diagnosis of obstructive sleep apnoea and diabetic retinopathy assessment as reported by the included studies

Study	Definition of apnoea/hypopnoea or oxygen desaturation (OD)	OSA diagnosis	Min recording	DR assessment
Storgaard 2014	NA	AHI ≥5 events/hr with sleep symptoms	4 hours	Digital photograph
Nishimura 2013	NA	NA	NA	NA
Furukawa 2013	OD ≥3%	ODI 3% ≥5 events/hr	4 hours	Florescence fundoscopy
Banerjee 2013	AASM criteria*	AHI ≥15 events/hr	4 hours	Digital photograph
Altaf 2013	AASM criteria*	AHI ≥5 events/hr	4 hours	Digital photograph
Rudrappa 2012	NA	AHI ≥5 events/hr	NA	Digital photograph
Mehta 2012	NA	NA	NA	Medical records
Mason 2012	Apnoea = complete cessation in airflow ≥10 seconds; hypopnoea = 50% reduction in airflow with ≥4% desaturations for ≥10 seconds; and OD ≥4%	ODI 4% ≥10 events/hr and AHI ≥15 events/hr	NA	Digital photograph + spectral domain optical coherence tomography
Kosseifi 2012	NA	NA	NA	NA
West 2010	OD ≥4%	ODI 4% ≥10 events/hr	NA	Digital photograph
Shiba 2010	OD ≥4%	ODI 4% ≥5 events/hr	Mean ≥6 hr	Medical records
Schober 2010	Apnoea = 80% reduction in airflow ≥10 seconds; hypopnoea = 50-80% reduction in airflow with ≥4% desaturations for ≥10seconds	AHI ≥15 events/hr	NA	NA
Borel 2010	Normal = no oxygen fluctuations; borderline = ‘oxygen saturation fluctuations limited in amplitude / appeared during limited periods of time; pathological = fluctuations in oxygen desaturation and re-saturation	Normal vs. borderline vs. pathological	NA	NA
Unver 2009	NA	NA	NA	NA
Laaban 2009	Apnoea = increased variation in suprasternal pressure with no airflow for ≥10 seconds; Hypopnoea = ≥50% reduction in airflow with ≥4% desaturation for ≥10 seconds	AHI ≥5 events/hr	NA	NA
Merritt 2007	OD ≥4%	NA	NA	Digital photograph

OD = oxygen desaturation, min = minimum, OSA = obstructive sleep apnoea, hr = hour, NA = not available, *AASM = American Academy of Sleep Medicine criteria: apnoea = complete cessation of airflow for ≥10 seconds; hypopnoea = ≥30% reduction in airflow ≥4% drop in oxygen desaturation for ≥10 seconds.

6.4.1.4 Quality assessment for studies on diabetic retinopathy

Table 6-6 summarises the quality assessment for the 16 studies. The overall quality of the studies were low-to-moderate (n=14). Only two studies were rated as high quality. The majority of studies were rated as either moderate or high for study methods (n=11). Selection bias was an issue in the majority of the studies (n=10 rated as low). Methods of recording sleep data were not presented in several studies (n=7 were weak), in particular, the criterion used for the diagnosis of OSA was poorly described. Only two studies blinded the sleep record assessor, while seven studies blinded the DR assessment.

Table 6-6: Quality assessment of included studies for diabetic retinopathy

Study	Components					
	Selection bias	**Methods and measurement**	**Blinding**	**Study design**	**Analysis**	**Overall**
Storgaard 2014	Weak	Moderate	Weak	Strong	Weak	Weak
Nishimura 2013	Moderate	Weak	Weak	Moderate	Moderate	Moderate
Furukawa 2013	Weak	Moderate	Moderate	Weak	Strong	Weak
Banerjee 2013	Strong	Strong	Strong	Moderate	Strong	Strong
Altaf 2013	Weak	Strong	Weak	Weak	Moderate	Moderate
Rudrappa 2012	Weak	Weak	Moderate	Strong	Moderate	Moderate
Mehta 2012	Weak	Weak	Weak	Moderate	Moderate	Weak
Mason 2012	Weak	Moderate	Moderate	Strong	Weak	Moderate
Kosseifi 2012	Weak	Weak	Weak	Moderate	Weak	Weak
West 2010	Moderate	Moderate	Strong	Strong	Strong	Strong
Shiba 2010	Moderate	Moderate	Moderate	Weak	Strong	Moderate
Schober 2010	Weak	Moderate	Weak	Strong	Weak	Weak
Borel 2010	Weak	Weak	Weak	Moderate	Weak	Weak
Unver 2009	Moderate	Weak	Weak	Weak	Weak	Weak
Laaban 2009	Moderate	Strong	Moderate	Moderate	Weak	Moderate
Merritt 2007	Weak	Weak	Moderate	Weak	Weak	Weak

6.4.1.5 The association between OSA and DR

The results from the studies are detailed in Table 6-4 and the overall summary of the results for all outcomes is presented in Table 6-7. Eight studies (n=1134) reported on the effect of OSA on overall DR;[224,320,400-402,407,411,414] six studies carried out univariate analysis[320,400-402,411,414] and two studies performed multivariate regression analysis.[224,407] The majority of studies, which carried out univariate analysis, were prevalence studies.[292,320,399-401] Schober and colleagues recruited 715 diabetic participants in Germany.[400] However, 217 participants (30%) were excluded due to issues with sleep recording and missing data. Therefore, only 556 (51 T1DM and 498 T2DM) participants' data were analysed. Apart from the high attrition rate, the portable sleep study (Apnealink) performed was missing data on respiratory effort and the researchers used a minimum of 2 hours recording instead of the commonly used 4 hours sleep recording. The diagnosis of apnoea and hypopnoea were also not in line with AASM. OSA was diagnosed if AHI was ≥15 events/hr. DR was present in 13.9% of OSA individuals and 11.3% in non-OSA participants (p=0.45). Mason et al.[320] also used the Apnealink device (no effort data) on their participants. The minimum sleep recording time was not reported and the participants were recruited from the Oxford Eye Hospital (Oxford, UK) database. All participants had had laser treatment for macular oedema. Of a total of 208 possible participants, 80 were recruited. No significant difference in DR between OSA and non-OSA groups was found (p=0.32).

Table 6-7: Summary of the results categorised according to diabetic retinopathy outcomes

	Overall Diabetic retinopathy (DR)	**Retinopathy score / Advanced DR**	**Diabetic maculopathy**
OSA (based on AHI)	8 studies (n=1134) Adjusted: (+) 0 studies; (-) 2 studies Unadjusted : (+) 2 studies; (-) 4 studies Pooled OR not calculated^ (I^2=79%) (5 studies, see Figure 6-2)	Retinopathy score (2 studies, n=149) Adjusted: (+) 2 studies; (-) 0 studies Advanced DR (4 studies, n=367) Adjusted: (+) 1 study; (-) 2 studies Pooled OR (2 studies – see Figure 6-7) 1.05, 95% CI: 0.95 to 1.16, I^2= 73.2% Unadjusted: (+) 1 study; (-) 0 studies	6 studies (n=565) Adjusted: (+) 1 study; (-) 1 study Pooled OR not calculated^ (I^2=90%) (2 studies, see Figure 6-8) Unadjusted : (+) 2 studies; (-) 4studies
OSA (based on ODI)	5 studies (n=949) Adjusted: (+) 1 study; (-) 1 study Pooled OR (2 studies – see Figure 6-4) 1.09. 95% CI: 1.02 to 1.17 I^2= 0.0% Pooled OR (4 studies, combined AHI & ODI as OSA diagnosis – see Figure 6-5) 1.05, 95% CI: 0.95 to 1.16, I^2= 65.9% Unadjusted: (+) 1 study; (-) 2 studies		1 study (n=80) Adjusted: (+) 0 study; (-) 1 study Unadjusted: (+) 0 study; (-) 1 study

%TST<90	3 studies (n=356) Adjusted: (+) 0 study; (-) 2 studies Unadjusted: (+) 1 study; (-) 0 study		2 studies (n=173) Adjusted: (+) 0 study; (-) 1 study Unadjusted: (+) 0 study; (-) 1 study
Mean O_2	2 studies (n=312) Adjusted: (+) 0 study; (-) 1 study Unadjusted: (+) 0 study; (-) 1 study		1 study (n=93) Adjusted: (+) 1 study; (-) 0 study OR 0.80, 95% CI: 0.59 to 1.07 Unadjusted: no data
Minimum O_2	3 studies (n=448) Adjusted: (+) 2 studies; (-) 1 study Pooled OR (3 studies – Figure 6-6): 0.91, 0.87 to 0.95, I^2=0% Unadjusted: no data		1 study (n=93) Adjusted: (+) 1 study;(-) 0 study OR 0.79, 95% CI: 0.65 to 0.95 Unadjusted: no data

(+): reported significant association; (-): reported no significant associations; shaded cells indicate that no study reported data for the exposure-outcome combination. DR = diabetic retinopathy; OSA = obstructive sleep apnoea; AHI = apnoea-hypopnea index; ODI = oxygen desaturation index; O_2 = oxygen saturation, n = total number of participants
^Due to high level of heterogeneity
*Only one study provided quantitative data

A prevalence study from Denmark recruited 180 T2DM participants.[402] The patients were first screened using the Berlin questionnaire and high-risk individuals were investigated using ApneaLink device with a minimum of 4 hour respiratory recording. A subset of participants had confirmatory portable sleep study using an Embletta device. No significant difference in DR was found between OSA (26.4%) and non-OSA (19.4%) individuals ($p>0.05$). Furthermore, the AHI was similar in DR (17±20 events/hr, n=74) compared to non-DR (17±25 events/hr, n=25) individuals. Another European study by Laaban and colleagues[401] recruited poorly controlled French T2DM inpatients over a 6-month period. An overnight sleep study was performed and diagnosis of OSA was in accordance with AASM guidelines. However, the minimum required sleep recording for analysis was not reported. Out of a total of 362 participants, 191 had OSA. Sixty-six per-cent of non-OSA had DR compared to 48% with mild OSA, 53% with moderate OSA and 68% in the severe OSA group. No statistical significant difference was found between non-OSA group and mild, moderate as well as severe OSA group ($p>0.05$). Overall, 52% of OSA individuals had DR.

A U.S. veteran study recruited 98 well-controlled T2DM ($HbA_{1c}<6.5\%$) with OSA.[292] All participants underwent three consecutive overnight sleep studies. Neither the minimum sleep recording time nor the criteria used for OSA diagnosis was reported. Also, the criteria used for the diagnosis of OSA were not reported. The study demonstrated a higher mean AHI in OSA participants (44.2 events/hr) with DR compared to those without DR (AHI 27.2 events/hr, $p<0.05$). Another U.S. study was conducted on 22 obese individuals with OSA matched with 22 obese T2DM for age, weight and duration of DM.[411] The participants were recruited consecutively from a retinal referral practice. The methods used

for sleep study and the diagnosis of OSA were not reported. The study found that all OSA individuals had DR with multiple nerve-fibre-layer infarcts compared with 14 participants in the non-OSA group (unadjusted RR 4.4, 95% CI: 2.04 to 9.51). Also, fluorescein angiography was performed and OSA participants were more likely to have widespread areas of capillary non-perfusion compared to non-OSA individuals (effect size not reported).

Mehta and co-workers[407] observed 80 T2DM patients presented to their eye clinic in India. They performed in-hospital sleep study. Fifty DR individuals were compared to 30 non-DR participants. Greater risk of OSA was observed in DR group compared to non-DR group (OR 2.91, 95% CI: 1.14 to 7.42). However, this did not remain significant after adjustment for confounders, although the confounders examined were not detailed. In addition, the study did not report the minimum sleep recording time as well as the criteria used for OSA diagnosis.

Banerjee and co-workers[224] reported on consecutive participants from the specialist weight management clinic in Birmingham, UK. Portable sleep studies were carried out as part of routine patient assessment. The AASM guidelines were followed for sleep analysis. OSA was diagnosed if AHI was ≥15 events/hr. At least 4 hours of sleep recording were available for all participants. A total of 93 individuals' sleep data was analysed after excluding participants with incomplete data. No significant association was reported in the unadjusted and adjusted regression models. The final model, which was adjusted for age, gender, ethnicity, DM duration, insulin treatment, hypertension, HbA_{1c} and coronary artery disease, reported OR 1.00 (95% CI: 0.98 to 1.02). However, the researchers could not

adjust for smoking, a potential important factor. The study also only included an extreme obese population, which is difficult to generalise to the wider populations.

6.4.1.5.1 The association between OSA and DR: summary and meta-analysis

Overall, the majority of the studies, which carried out univariate analysis, did not report any significant association between OSA and DR (n=4). Only two studies from the U.S.[292,411] demonstrated significant associations. However, these were unadjusted models and therefore exposed to multiple confounders such as gender and BMI. Two studies, which performed multivariate regression analysis, reported no significant association between OSA and DR. A meta-analysis was performed on six studies[224,400-402,407,411] that reported on the effect of OSA on overall DR in unadjusted models. Due to a significant level of heterogeneity (I^2=79%), pooled results were not presented as the results may be misleading. We, however, presented a forest plot of the reported ORs on all seven six as shown in Figure 6-2. A funnel plot was also carried out as shown in Figure 6-3. The funnel plot suggests an imbalance in small sample studies with negative results. Therefore, overall, there was no convincing evidence that OSA is associated with DR in T2DM populations. No study had examined the association between OSA and DR in T1DM.

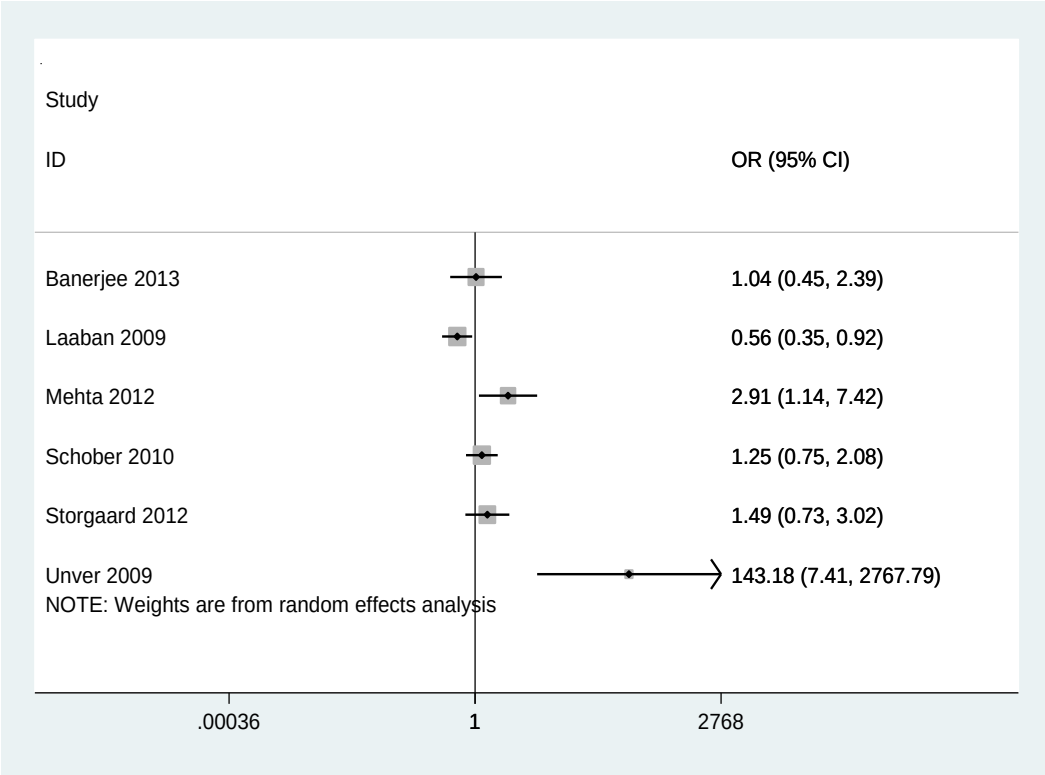

Figure 6-2: Forest plot on studies which reported unadjusted odds ratios and 95% confidence intervals on the effect of obstructive sleep apnoea on overall diabetic retinopathy.

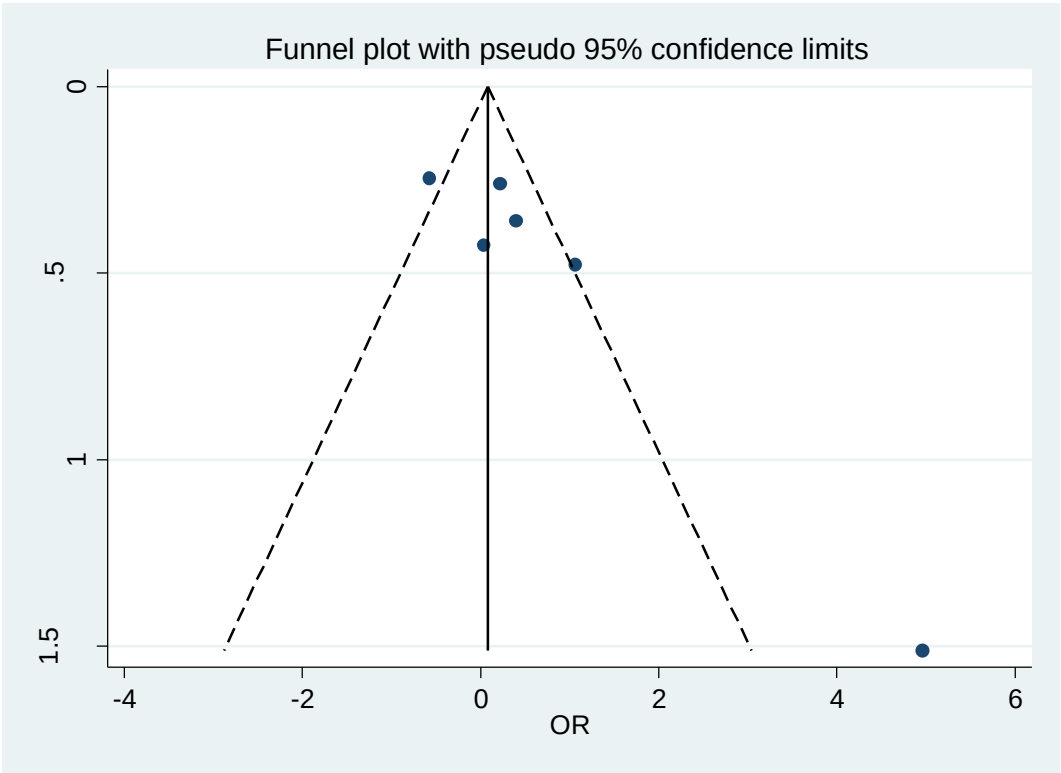

Figure 6-3: Funnel plot on studies which reported effect of obstructive sleep apnoea on overall diabetic retinopathy.

6.4.1.6 The association between oxygen desaturation index and diabetic retinopathy

Five studies (n=949) utilised oximetry to assess the effect on DR. A French study recruited T1DM (n=37) and participants were classified into normal, borderline and pathological recording groups.[399] Normal recording was defined as no oxygen fluctuations; borderline was defined as 'oxygen saturation fluctuations were limited in amplitude or appeared during limited periods of time'; and pathological was defined as fluctuations in oxygen desaturation and re-oxygenation.[399] Minimum sleep recording was not reported. Of those with pathological recordings, 73% had DR compared to 11% in borderline group and 33% in normal oximetry group. Comparing the three groups, significant difference was only detected between pathological and borderline groups ($p<0.05$) and no difference was found between pathological and the normal recording groups.

Merritt and colleagues[409] reported a significant difference in ODI between those with advanced DR (mean 13.6 events/hr) and those without advanced DR (mean 3.8 events/hr, $p=0.03$). Merritt and co-workers recruited overweight and obese participants from the UK with T2DM diagnosed >5 years. Overnight oximetry recording was carried out. No information was available for the minimum required sleep recording and no regression analysis was performed.

A Japanese study that recruited consecutive T2DM who had had a sleep study did not observe any significant difference in AHI for DR and non DR participants.[408] They excluded participants with heart failure, chronic pulmonary disease and those on haemodialysis; therefore 136 participants' data were analysed. The researchers did not report on the methods used for sleep study as well as the minimum sleep recording time.

No significant difference in ODI (17.1 events/hr vs. 15.4 events/hr, p=0.544) as well as AHI (17.8 events/hr vs. 15.1 events/hr, p=0.325) between DR and non-DR participants was reported.

Similarly, another multi-centred Japanese study (513 T2DM) also did not observe significant association between ODI and DR.[360] All the participants had a minimum of 4 hours sleep recording. OSA was diagnosed when 3% ODI occurred at least 5 times per hour. Both univariate and multivariate (OR 1.00, 95% CI: 0.60 to 1.68) regression analyses were not significant. Factors adjusted for in the multivariate analysis were age, gender, BMI, hypertension, hyperlipidaemia, smoking and alcohol status, medications for stroke and heart disease, DM duration and HbA_{1c} levels. The researchers, however, did not adjust for the usage of any DM medications especially insulin treatment.

Another Japanese study by Shiba et al.[413] recruited consecutive 219 T2DM Japanese inpatients who underwent eye surgery (cataract, glaucoma, retinal detachment or vitreous surgery) in a single centre. Sixty-eight had non-proliferative-DR (NPDR) and 151 had proliferative-DR (PDR). Pulse oximetry was utilised and OSA was diagnosed if 4% ODI >5 events/hr was present. The mean sleep recording time was at least 6 hours. No significant associations were found between OSA and PDR with multivariate logistic regression analysis (effect size not reported). Factors adjusted for included age, HbA_{1c}, hypertension, 4% ODI, minimum oxygen saturation and % time spent under 90% oxygen saturation.

However, in a subgroup analysis of 151 PDR individuals, 37 had iris and/or angle neovascularisation whilst 114 did not;[415] the researchers demonstrated a significant association between ODI and neovascularisation group (OR 1.09, 95% CI: 1.01 to 1.16) after adjusting for confounders (gender, DM duration, insulin treatment, mean oxygen saturation, 4%ODI, minimum oxygen saturation and percentage time spent under 90% oxygen saturation). Important factors such as age, BMI and smoking status were not included in the regression model.

6.4.1.6.1 The association between oxygen desaturation index and diabetic retinopathy: summary and meta-analysis

In summary, one study consists of T1DM[399] and another study in a T2DM population[415] reported significant associations between ODI and DR. We undertook a meta-analysis on the two studies,[360,415] which reported on the adjusted ORs of the effect of OSA diagnosed using ODI criteria on DR. The results showed a significant association (pooled-OR 1.09, 95% CI: 1.02 to 1.17, I^2=0.0%, Figure 6-4). However, the majority of the analytical weight was derived from the study by Shiba and colleagues[415] (97%). To address this that, we combined the studies that reported adjusted ORs using either AHI[224,406] or ODI[360,413] as criteria for OSA diagnosis. The result became non-significant (pooled-OR 1.05, 95% CI: 0.95 to 1.16) with a significant level of heterogeneity (I^2=65.9%, Figure 6-5). Therefore, the evidence on the effect of ODI on DR was inconclusive in T2DM, and there was insufficient evidence of the effect in T1DM as only one study was available.[399]

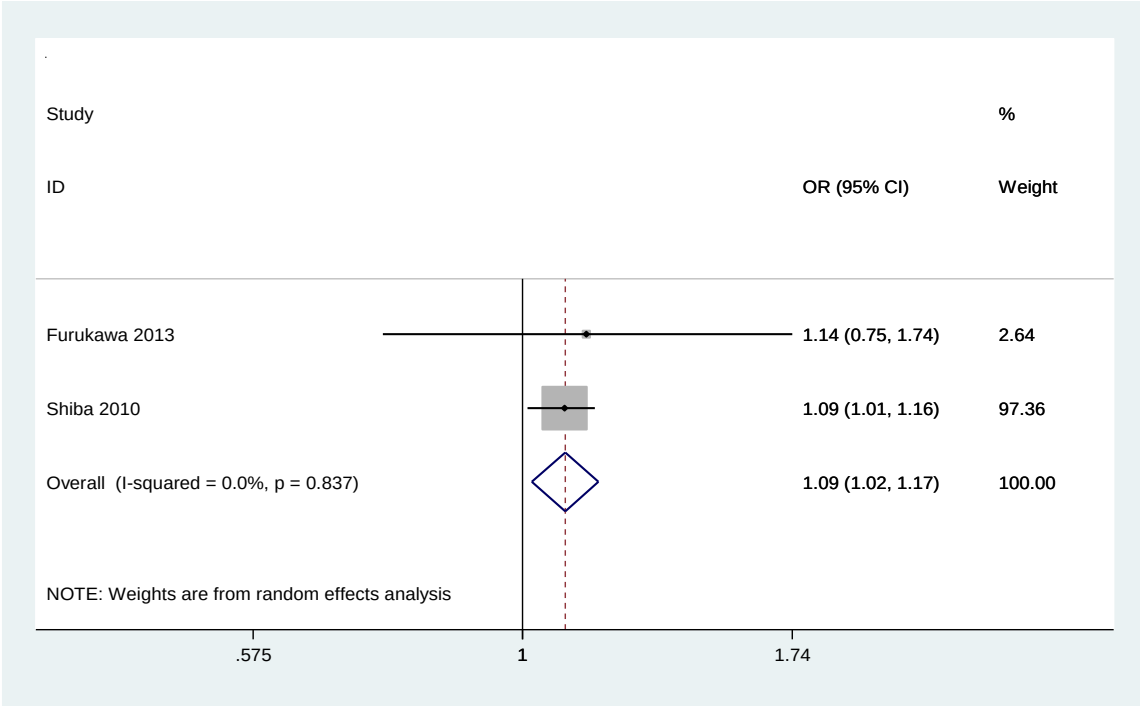

Figure 6-4: Forest plot on the effects of obstructive sleep apnoea diagnosed using oxygen desaturation index on diabetic retinopathy using results from studies which reported adjusted odds ratios only.

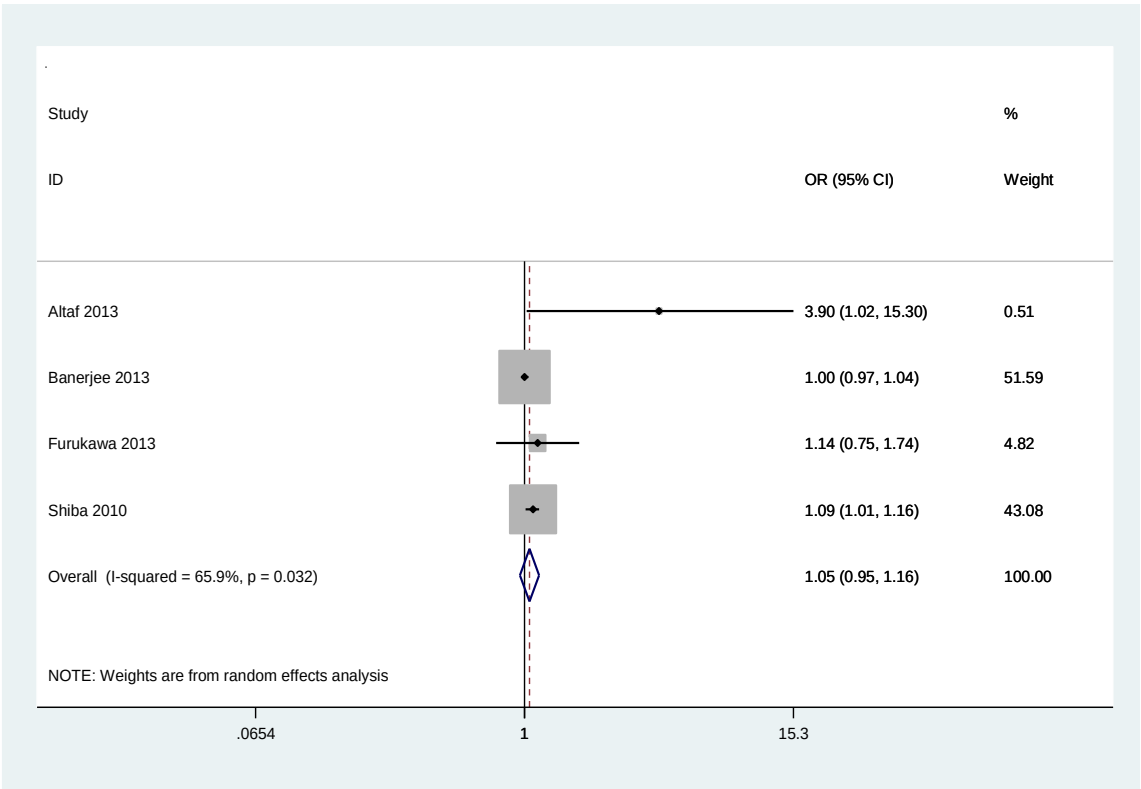

Figure 6-5: Forest plot on the effects of obstructive sleep apnoea diagnosed using oxygen desaturation index or apnoea-hypopnoea index on diabetic retinopathy using results from studies which reported adjusted odds ratios only.

6.4.1.7 The association between diabetic retinopathy and other respiratory parameters

Apart from ODI and AHI, sleep studies also recorded % time spent under 90% oxygen saturation (%TST<90), mean and minimum oxygen saturation levels. Only four studies reported the effect of these sleep parameters on DR.[224,408,409,413] However, not all the studies reported on all these three parameters. Two studies (n=312) reported on the effect of mean oxygen saturation.[224,415] Banerjee et al.[224] found no association between mean oxygen saturation using a multivariate logistic regression (OR 0.83, 95% CI: 0.67 to 1.02) nor did Shiba et al.[413] who performed univariate analyses between advanced DR (97.3%±1.5) and non-advanced DR group (97.6%±1.2, p=0.14).

Three studies (n=356) reported results on %TST<90.[224,409,413] Merritt and colleagues[409] found a significant difference between advanced DR and non-advanced DR (mean %TST<90 12.6% vs. 1.8%, p=0.03). Two studies, which carried out regression analysis, did not find any significant association after adjusting for potential confounders,[224,413] with only one study reported the effect size (OR 1.03, 95% CI: 1.00 to 1.06).[224] The factors adjusted in the respective models were described above.

In total, three studies (n=448) examined the effect of minimum oxygen saturation on DR using multivariate regression analysis.[224,408,413] Banerjee et al.[224] reported no association (OR 0.93, 95% CI: 0.67 to 1.02). On the other hand, Nishimura et al. adjusting for HbA_{1c} levels, DM duration and cardiovascular disease demonstrated significant association between minimum oxygen saturation and DR (OR 0.89, 95% CI: 0.83 to 0.95).[408] Shiba et

al. also found significant results between minimum oxygen saturation and advanced DR (OR 0.93, 95% CI: 0.88 to 0.99).[413]

6.4.1.7.1 The association between diabetic retinopathy and other respiratory parameters: summary and meta-analysis

A meta-analysis was also performed on the effect of minimum oxygen saturation on DR using multivariate regression analysis. The result was significant with low level of heterogeneity (pooled-OR 0.91, 95% CI: 0.87 to 0.95, I^2=0.0%) as shown in Figure 6-6. In summary, there was no evidence that mean oxygen saturation or %TST<90 were associated with DR. However, there was evidence from both our narrative synthesis and meta-analysis that minimum oxygen saturation had an impact on DR. Due to the limited available studies which reported on the effects of minimum oxygen saturation on DR, publication bias may be present.

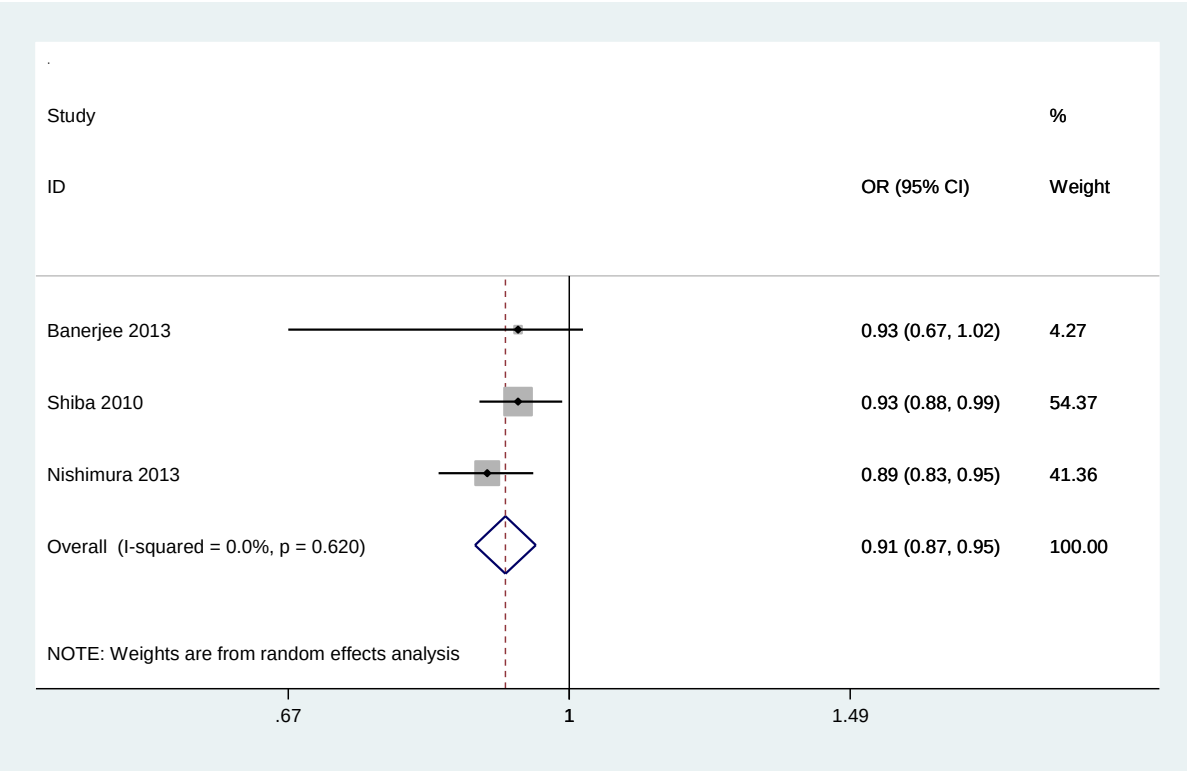

Figure 6-6: Forest plot on the pooled estimates of the effect of minimum oxygen saturation on diabetic retinopathy using results from studies which reported adjusted odds ratios only.

6.4.1.8 The association between OSA and retinopathy scores/advanced DR

Five studies examined the association between OSA and retinopathy scores including advanced DR.[224,406,410-412] To recap, advanced DR is defined as pre-proliferative or proliferative DR. Two studies[410,412] (n=149) reported a significant association between OSA and total retinopathy scores through multivariate regression analysis in males (both studies only recruited males) while four studies[224,293,406,411] (n=367) examined the relationship between OSA and advanced DR. West and co-workers[410] carried out overnight oximetry studies on 118 men. Information on minimum required sleep recording and the criteria used for diagnosis of OSA were not described. The retinopathy scores explained 19% of OSA variance (r^2=0.19, p<0.0001) in a multivariate-linear regression analysis.

Rudrappa and colleagues[412] performed overnight in-hospital sleep studies on 31 T2DM obese males, and OSA was diagnosed if AHI was ≥5 events/hr. In a multiple regression analysis, OSA was found to be a risk factor for retinopathy scores (p=0.008). Rudrappa also reported a significantly higher prevalence of advanced DR cases (n=4) in the OSA group compared to the non-OSA (n=0) group. However, in an unadjusted model, no significant association was found between OSA and advanced DR (OR 12.6, 95% CI: 0.62 to 255.76). The study is clearly underpowered thus the observations should be treated with caution. Banerjee et al.[224] also did not demonstrate any significant association in their univariate and multivariate analyses (unpublished data - OR 1.01, 95% CI: 0.97 to 1.04).

On the other hand, Unver and colleagues[411] described a difference in advanced DR between OSA and non-OSA individuals. They found that 10 of the eyes of the participants with OSA needed pan-retinal photocoagulation for proliferative-DR compared to one individual in the matched non-OSA group. Furthermore, the OSA participants who had photocoagulation, neovascularisation of vessels persisted despite treatment compared with resolution in non-OSA group. In addition, a UK study carried out by Altaf et al.[406] on T2DM reported that OSA was a risk factor for advanced DR (OR 3.9, 95% CI: 1.02 to 15.3) using multivariate regression analysis. The factors adjusted in the model were age, gender, ethnicity, smoking, alcohol, waist circumference, blood pressure, HbA_{1c}, DM duration, total cholesterol, triglycerides, renal function, anti-platelets agents, lipid lowering agents and medications for DM and hypertension. Participants had overnight portable sleep monitoring performed, diagnosis of OSA was in accordance with AASM criteria and the minimum period of sleep recording was 4 hours. An AHI ≥5 events/hr was considered diagnostic of OSA.

Moreover, Altaf and co-workers[406] followed-up their participants for 4.4±1 years and found that OSA participants were more likely to progress from no or background DR status to advanced DR state (15%) compared to non-OSA participants (3%, p=0.01). They also reported that OSA predicted the development of advanced DR (OR 6.6, 95% CI: 1.2 to 35.1, p=0.03) after adjusting for confounders (age, gender, ethnicity, smoking, alcohol, waist circumference, blood pressure, HbA_{1c}, DM duration, total cholesterol, triglycerides, renal function, anti-platelets, anti-diabetes, lipid lowering and anti-hypertensive treatments).

6.4.1.8.1 The association between OSA and retinopathy scores/advanced DR: summary and meta-analysis

A meta-analysis on three studies[224,406,415] that reported on adjusted ORs for advanced DR (one study used ODI as OSA diagnosis[415]), the pooled estimates did not show a significant association between advanced DR and OSA (pooled-OR 1.05, 95% CI: 0.95 to 1.16) with a significant level of heterogeneity (I^2=73.2%, Figure 6-7). In conclusion, from our narrative review, there was some evidence that OSA was associated with greater severity of DR as well as advanced DR amongst T2DM populations. The meta-analysis performed for the cross-sectional studies, however, did not support this notion. However, a significant level of heterogeneity as well as a small number of studies mean that the pooled results may not be reliable and needed to be interpreted with caution. No study has explored the association between OSA and severity of DR as well as advanced DR in T1DM populations.

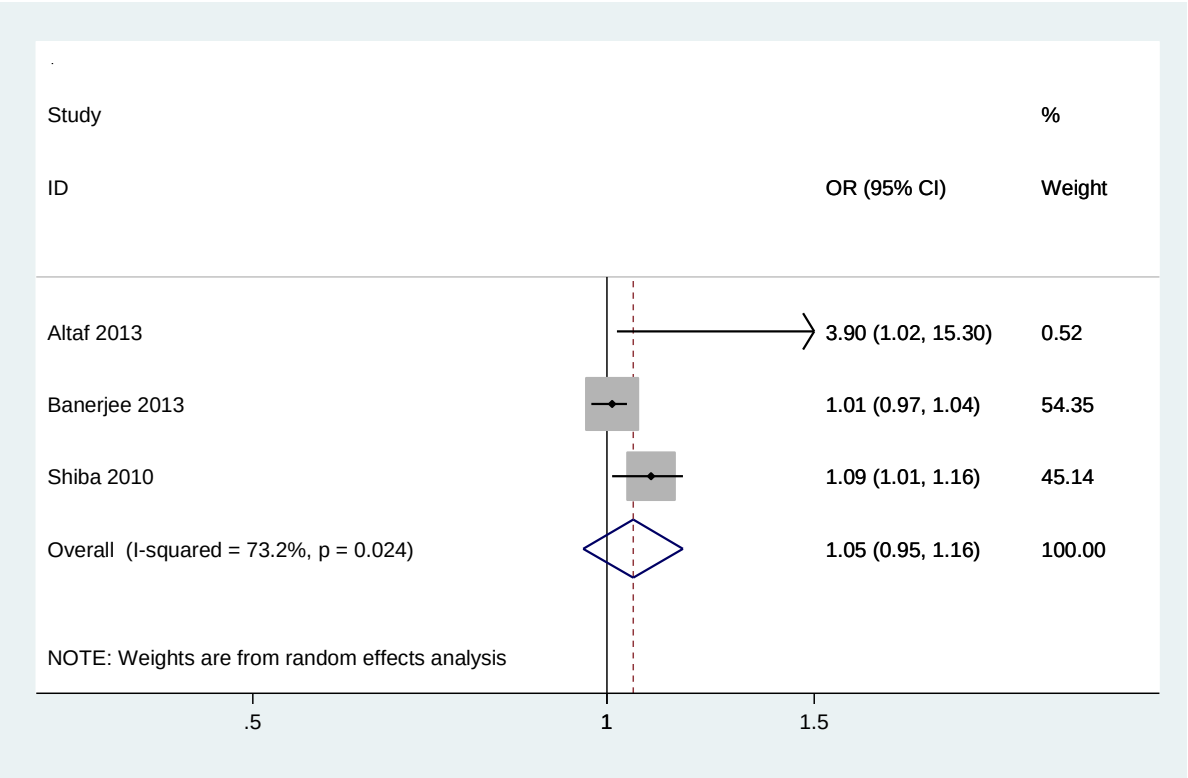

Figure 6-7: Forest plot on the effects of obstructive sleep apnoea on advanced diabetic retinopathy using results from studies which reported adjusted odds ratios only.

6.4.1.9 Association between diabetic maculopathy (DMac) and other respiratory parameters

Limited studies have explored the impact of OSA and DMac (n=949).[224,320,406,410-412] Three studies performed univariate analyses[320,411,412] and the remaining carried out multivariate analyses.[224,406,410] Mason et al.[320] did not find significant relationship between macula thickness with AHI (effect size not reported). They also did not report any dose response relationship between severity of OSA and macula thickness. Similarly, no significant association was reported by Rudrappa et al. (effect size not reported).[412] On the other hand, Unver and colleagues[411] described that out of 22 obese OSA individuals, 20 had diffused oedema alongside focal areas of non-perfusion in the participants' macula. In the matched

obese non-OSA group, only nine had macular oedema without evidence of capillary non-perfusion (unadjusted OR 14.44, 95% CI: 2.34 to 147.60). In addition, seven of the participants in the OSA required laser treatments for macular oedema and the majority of the participants' oedema worsened with greater leakage and infarcts despite laser treatment. In the non-OSA group, nine participants required laser treatment and the majority (n=8) resolved without further progression.

Three studies (n=410) carried out multivariate regression analysis.[224,406,410] Banerjee et al.[224] did not observed that AHI was a risk factor for DMac (OR 1.01, 95% CI: 0.98 to 1.04). In contrast, West et al.[410] reported that OSA explained 30% of the variance of DMac ($p<0.0001$). The final study by Altaf et al.[406] found that OSA (AHI $\geq$5 events/hr) was significantly associated with DMac (OR 4.5, 95% CI: 1.8 to 11.4, p=0.002). However, after 4 years of follow-up, OSA was not found to be a risk factor for the development of DMac (effect size not reported). A meta-analysis was performed for the two studies[224,406] which reported adjusted odd ratios. There was a significant level of heterogeneity (I^2=90.1%) therefore, pooled estimates were not presented (Figure 6-8).

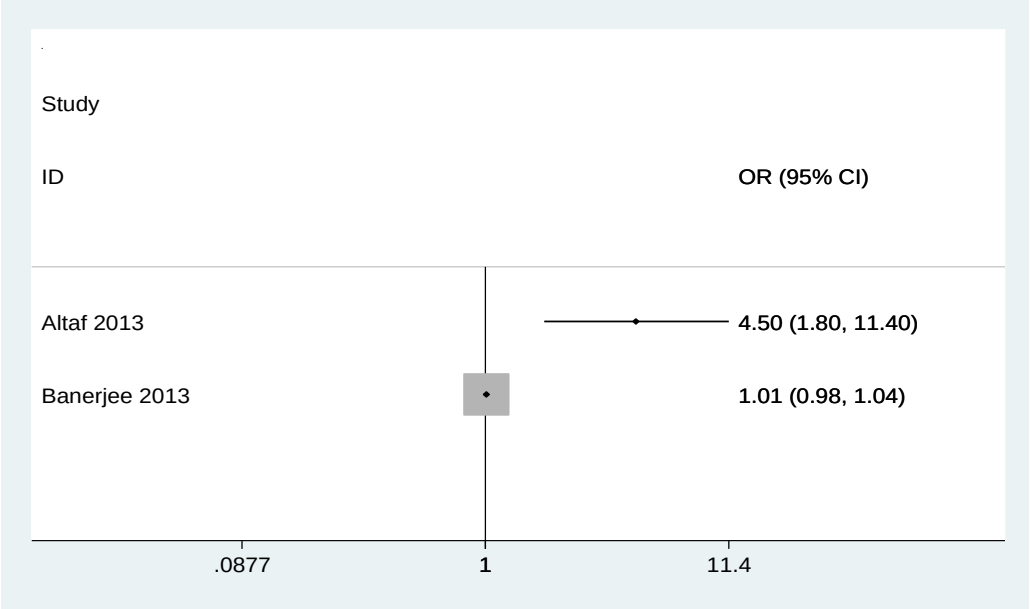

Figure 6-8: Forest plot on studies which reported adjusted odds ratios on the effect of obstructive sleep apnoea on diabetic maculopathy.

Two studies (n=173) examined the effect of %TST<90 on DMac.[224,320] Mason et al.[320] performed univariate analysis and did not find any significant relationship between macula thickness with %TST<90 (effect size not reported). Similarly, Banerjee et al. reported no association between %TST<90 and DMac (OR 1.03, 95% CI: 0.99 to 1.08) in their regression model. In addition, Banerjee and co-workers also reported non-significant findings on the effect of mean oxygen saturation on DMac (OR 0.80, 95% CI: 0.59 to 1.07). However, minimum oxygen saturation was observed to be an independent predictor of DMac (OR 0.79, 95% CI: 0.65 to 0.95). Only the study by Mason and colleagues examined the effect of ODI on DMac and this was found to be non-significant (effect size not reported). In conclusion, there was some evidence that OSA appeared to be associated with DMac. However, there was insufficient evidence to draw conclusions on the effect of mean oxygen saturation, %TST<90, minimum oxygen saturation and ODI on DMac.

6.4.2 Diabetic kidney disease

6.4.2.1 Study characteristics

Our initial searches identified a total of 1163 studies (1129 from databases and 34 from grey literature). After excluding obvious duplicates, there were 1062 studies and 1030 were subsequently removed after title and abstract screening. Thirty-two full text articles were retrieved and 9 studies were included in our narrative synthesis. We excluded 23 articles because of the following: ten studies did not include DM populations, nine were duplicate studies, two did not include participants with OSA, one study did not have DKD as an outcome and one study was a review article. Figure 6-9 showed the PRISMA flow chart for study selection.

The majority of the studies had a cross-sectional study design except the study by Tahrani and colleagues[348] which included a follow-up component. Most studies were from European countries (6 studies), two studies from Japan and one from the US. There were a total of 2920 DM participants (2862 T2DM) across all studies. The mean age of participants ranged from 51 to 62 years and proportion for females ranged from 33% to 73%. The study by Kosseifi and co-workers[414] did not report on gender distribution but it described US veterans therefore consisted of a high proportion of males. The mean HbA_{1c} and DM duration ranged between 6.5 and 9.2% and between 7.5 and 14.5 years, respectively. One study only included extreme obese individuals with a mean BMI of 46.8Kg/m^2.[225] The remainder of the studies mean BMI ranged from 25.2 to 33.7Kg/m^2.

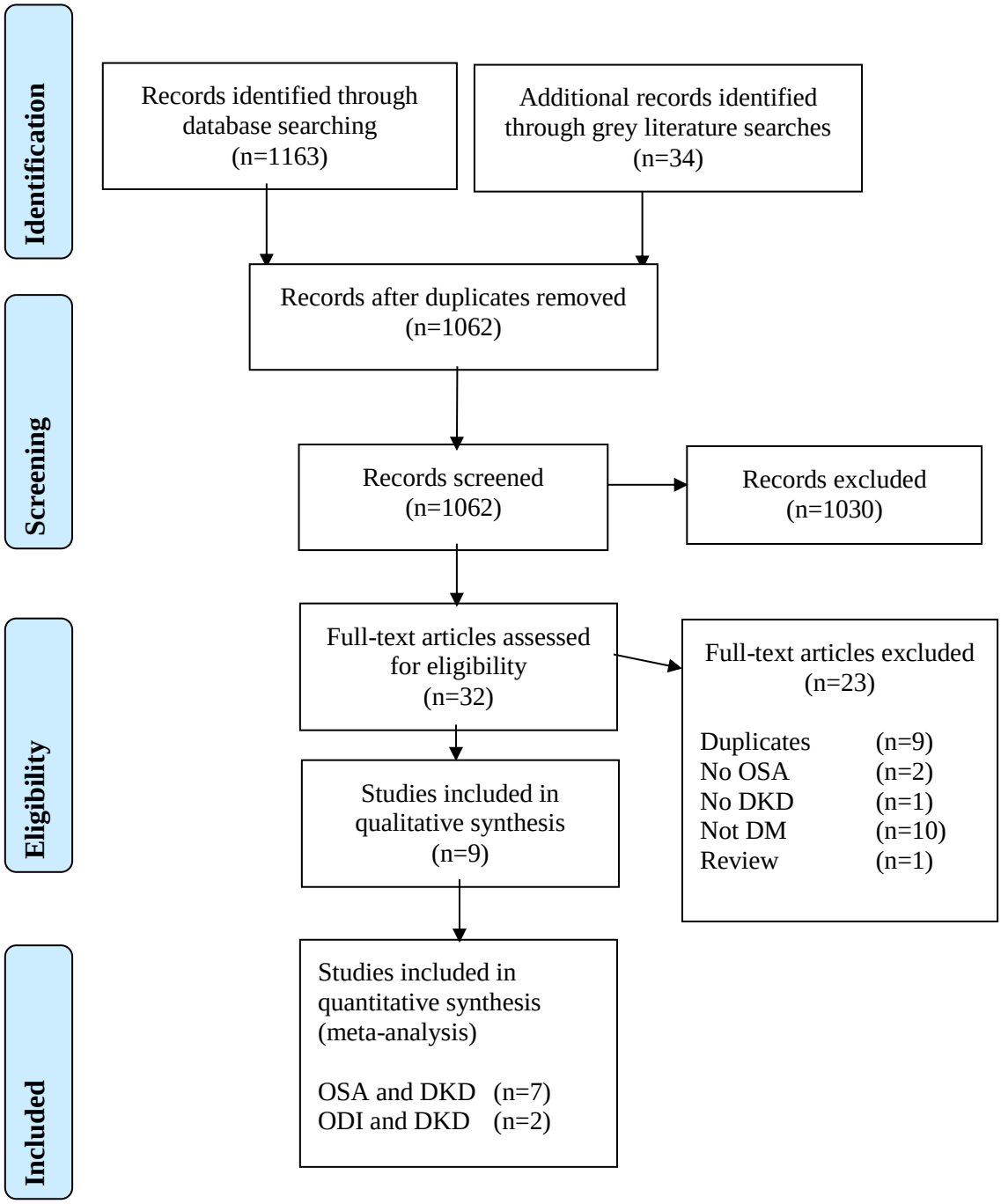

Figure 6-9: PRISMA flow chart on study selection for diabetic kidney disease.
OSA = obstructive sleep apnoea; DKD = diabetes kidney disease; DM = diabetes mellitus; ODI = oxygen desaturation index

Table 6-8: Characteristics and results of included studies for systemic review on diabetic kidney diseases

Study ID (country)	Study design	Participants	Demographics	OSA assessment	Results
Buyukaydin 2012 (Turkey)	CS	52 T2DM	Setting = Diabetes clinic Mean age = 56 years Females = 73% Mean HbA_{1c} = 7.9% Mean DM Duration = 12.8 years Mean BMI = 32.4 Kg/m^2	Inpatient PSG (Compumedics E 3142)	*Univariate analysis* - No difference in albuminuria between OSA (21.9%) and non-OSA (29.4%) groups. - No difference in AHI between micro-albuminuria 9.0±14.6 vs. no micro-albuminuria 10.5±10.0 events/hr (p>0.05)
Furukawa 2013 (Japan)	CS	513 T2DM	Setting = Multi-centre diabetes clinics Mean age = 62 years Females = 43% Mean HbA_{1c} = 7.3% Mean DM Duration = 11.7 years Mean BMI = 25.2 Kg/m^2	Oximetry (PULSOX-3Si) [#]	*Multivariate analysis* **- Association between ODI and micro-albuminuria (OR 1.84, 95% CI: 1.16 to 2.96)** **- Association between ODI and macro-albuminuria (OR 2.97, 95% CI: 1.36 to 6.90)** Adjusted for age, sex, BMI, hypertension, hyperlipidemia, smoking, alcohol, medications for stroke and IHD, duration of DM and HbA_{1c}
Kosseifi 2012[I] (US)	CS	98 T2DM	Setting = Sleep clinic Mean age = 61 years Females = NA[$] Mean HbA_{1c} = 6.5% Mean DM Duration = NA Mean BMI = 33.7 Kg/m^2	Level III sleep test (NovaSom QSG)	*Univariate analysis* - No difference in AHI between micro-albuminuria 33.5 vs. no micro-albuminuria 28.0 events/hr (p>0.05) **- Significant difference in oxygen saturation in micro-albuminuria 73.0% vs. no micro-albuminuria 77.4% (p<0.01)**
Laaban 2009[I] (France)	CS	303 T2DM	Setting = Hospital inpatient Mean age = 61 years Females = 49% Mean HbA_{1c} = 9.2% Mean DM Duration = 14.5 years Mean BMI = 32.0 Kg/m^2	Level IV sleep test (CID 102) [‡~]	*Univariate analysis* - No difference in albuminuria between groups: non-OSA (25%), mild OSA (34%), moderate OSA (38%) or severe OSA (39%). **- Association between OSA and albuminuria (unadjusted OR 1.70, 95% CI: 1.01 to 2.85).**

Leong 2014 (UK)	CS	90 T2DM	Setting = Specialist weight management clinic Mean age = 51 years Females = 57% Mean HbA_{1c} = 8.3% Mean DM Duration = 7.5 years Mean BMI = 46.8 Kg/m^2	Level III sleep test (Embletta) [#]~	*Multivariate analysis (linear regression)* **- Association between AHI and MDRD eGFR(β=-0.17, 95% CI: -0.32 to -0.02).** **- Association between %TST<90 and MDRD eGFR (β=-0.22 to 95% CI: -0.41 to -0.02).** - No association between mean O_2 and MDRD eGFR (β=1.91, 95% CI: -0.04 to 2.42). - No association between minimum O_2 and MDRD eGFR (β=0.05, 95% CI: -0.39 to 0.50) - No association between AHI and CKD-EPI eGFR (β=-0.19, 95% CI: -0.39 to 0.01) **- Association between %TST<90 and CKD-EPI eGFR (β=-0.30, 95% CI: -0.56 to -0.03)** - No association between minimum O_2 and CKD-EPI (β=0.16, 95% CI: -0.44 to 0.76) - No association between mean O_2 and CKD-EPI (β=1.50, 95% CI: -0.21 to 3.22) Adjusted for age, gender, BMI, hypertension, IHD, DM duration, insulin treatment, RAS treatment.
Schober 2010[ǁ] (Germany)	CS	58 T1DM & 498 T2DM	Setting = 14 primary care centres Mean age = 60 years Females = 48% Mean HbA_{1c} = 7.6% Mean DM Duration = 9.3 years Mean BMI = 31.9 Kg/m^2	Level IV sleep test (ApneaLink) [‡]~	*Univariate analysis* **- Significant difference between groups: AHI<5/hr 13 patients, AHI 5-14/hr 46 patients, AHI≥15/hr 42 patients (p=0.001).** - No association between OSA (AHI ≥15/hr) and nephropathy (unadjusted OR 1.24, 95% CI: 0.80 to 1.92).

Storgaard 2014[I] (Denmark)	CS	180 T2DM	Setting = Diabetes clinic Mean age = 59 years* Females = 42% Mean HbA_{1c} = 7.4%* Mean DM Duration = 8.2 years* Mean BMI = 32.2 Kg/m^2*	Berlin Q and ApneaLink +/- level III sleep test (Embletta) [#]	*Univariate analysis* - No association between OSA and albuminuria (OR 1.38, 95% CI: 0.76 to 2.53).
Tahrani 2013 (UK)	Cohort	224 T2DM	Setting = 2 diabetes clinics Mean age = 57 years* Females = 42% Mean HbA_{1c} = 8.2%* Mean DM Duration = 10.0 years* Mean BMI = 33.5 Kg/m^2*	Level III sleep test (Alice PDX) [#]~	*Multivariate analysis* **- Association between OSA and nephropathy (OR 2.64, 95% CI: 1.13 to 6.16)** - No association between minimum O_2 and nephropathy (OR 0.96, 95% CI: 0.93 to 1.00). Adjusted for age, sex, ethnicity, DM duration, BMI, mean arterial pressure, HbA_{1c}, triglycerides, insulin, GLP-1, anti-hypertensive, total cholesterol, high-density-lipoprotein cholesterol, lipid-lowering treatment, anti-platelets, oral DM agents, alcohol and smoking - After 2.5 years follow-up: **linear regression showed OSA predict study end MDRD eGFR (β=-3.8, p=0.04) and AHI associated with study end MDRD eGFR (β=-4.6, p=0.02).** Adjusted for age, DM duration, ethnicity, sex, BMI, mean arterial pressure, anti-hypertensive agent, HbA_{1c}, insulin, oral DM agents, total cholesterol, lipid lowering treatment, antiplatelet agents and smoking. - No association between minimum O_2 and study end eGFR (effect size not reported)

Tanaka 2009[a] [l] (Japan)	CS	904 T2DM	Setting = Multi-centre diabetes clinics Mean age = 62 years* Females = 33% Mean HbA_{1c} = 6.9% Mean DM Duration = 10.3 years* Mean BMI = 25.3 Kg/m^2	Oximetry	*Multivariate analysis* **- Association between OSA and creatinine (OR 2.37, 95% CI: 1.21 to 4.65)**

NA=information not available, HbA_{1c}=glycosylated haemoglobin A_{1c}, DM=Diabetes mellitus, BMI=body mass index, CS = cross-sectional study, T1DM=Type 1 diabetes mellitus, T2DM=Type 2 diabetes mellitus, OSA=obstructive sleep apnoea, PSG=polysomnography, ODI=oxygen desaturation index, AHI=apnoea-hypopnoea index, O_2=oxygen saturation, eGFR=estimated glomerular filtration rate, MDRD=modification of Diet in Renal Disease, CKD-EPI=chronic kidney disease Epidemiology Collaboration, IHD=ischemic heart disease, RAS=renin-angiotensin system, GLP-1=glucagon-like peptide-1

[ǂ]PSG data did not have data on effort; [#]Minimum 4 hours recording; [$]Veteran study with low female participants; *Average values from 2 groups;
[a] Conference abstracts; [l] Prevalence studies; ~Portable home sleep study

6.4.2.2 Respiratory methodologies and OSA diagnosis

Most of the studies used a 3-channeled ambulatory sleep device (oximetry, air-flow and respiratory effort) to assess OSA[225,348,414] while one performed full polysomnography (PSG) with cardiovascular and neurological channels in addition to the 3 respiratory channels.[359] Two studies used a device without data on respiratory effort to diagnose OSA (a two-channelled device).[400,401] One study used a mixed method:[402] the Berlin questionnaire was used to screen participants and high-risk individuals were offer a 2 channel portable device (pulse oximetry and air-flow using ApneaLink device). A subset of the participants also had a 3 channel device (Embletta) sleep study. Finally, two studies utilised pulse oximetry for sleep assessment.[360,416] The characteristics of the studies are summarised in Table 6-8.

A small degree of heterogeneity was present for the definition of apnoea and hypopnoea. Four studies utilised the AASM criteria for apnoea and hypopnoea.[225,348,359,401] One study which utilised a 2 channel device[400] defined apnoea as an 80% reduction in airflow for at least 10 seconds and hypopnoea as a 50-80% reduction in airflow with a 4% oxygen desaturation for more than 10 seconds. Two studies[360,416] which used pulse oximetry employed a 3% drop in oxygen saturation for oxygen desaturation, whilst the other two studies[402,414] did not describe the definition. For diagnosis of OSA, four studies utilised an AHI ≥5 events/hr as the diagnosis criterion[225,348,359,401] and one study employed AHI ≥15 events/hr.[400] Two studies used 3%ODI ≥5 events/hr[360,416] whilst the remaining two studies did not report on the criteria used to diagnose the condition.[402,414]

Table 6-9: Criteria used for the diagnosis of obstructive sleep apnoea and diabetic kidney disease assessment as reported by the included studies

Study	Definition of apnoea/hypopnoea or oxygen desaturation (OD)	OSA diagnosis	Min recording	DKD test	DKD diagnosis
Buyukaydin 2012	AASM criteria*	AHI≥5 events/hr	NA	24 hour urine albumin	Albuminuria ≥ 30mg/day
Furukawa 2013	OD ≥3%	ODI≥5 events/hr	4 hours	Early morning urine ACR	Micro-albuminuria ≥3.4mg/mmol Macro-albuminuria ≥ 34.0 mg/mmol
Kosseifi 2012	NA	NA	NA	NA	Micro-albuminuria
Laaban 2009	Apnoea = increased variation in suprasternal pressure with no airflow for ≥10 seconds; hypopnoea = ≥50% reduction in airflow with ≥4% desaturation for ≥ 10 seconds	AHI≥5 events/hr	NA	24 hour urine albumin	Micro-albuminuria ≥ 30mg/day
Leong 2014	AASM criteria*	AHI≥5 events/hr	4 hours	eGFR	eGFR <60min/ml/1.73m^2 based on MDRD and CKD-EPI equations
Schober 2010	Apnoea = 80% reduction in airflow ≥ 10 seconds; hypopnoea = 50-80% reduction in airflow with ≥ 4% desaturations for ≥ 10 seconds	AHI≥15 events/hr	NA	NA	NA
Storgaard 2014	NA	AHI≥5 events/hr	4 hours	24 hour urine albumin	Micro-albuminuria 30-300 mg/day Macro-albuminuria > 300mg/day (2 out of 3 samples
Tahrani 2013	AASM criteria*	AHI≥5 events/hr	4 hours	eGFR & urine ACR	MDRD eGFR <60min/ml/1.73m^2 ACR >3.4 mg/mmol
Tanaka 2009	OD ≥3%	ODI≥5 events/hr	NA	Creatinine (mg/dl)	NA

OD = oxygen desaturation, min = minimum, OSA = obstructive sleep apnoea, hr = hour, NA = not available, DKD = diabetic kidney disease, eGFR = estimated glomerular filtration rate, MDRD = Modification of Diet in Renal Disease, CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration, ACR = albumin-creatinine ratio.*AASM = American Academy of Sleep Medicine criteria: apnoea = complete cessation of airflow for ≥10 seconds; hypopnoea = ≥30% reduction in airflow ≥4% drop in oxygen desaturation for ≥10 seconds.

6.4.2.3 Definition for diabetic kidney disease

As expected, there were variations in the diagnoses of DKD. Three studies reported 24 hour urinary albumin results and diagnosed micro-albuminuria as a urine albumin concentration of >30mg/day.[359,401,402] One Japanese study utilised early morning urine ACR and diagnosed micro-albuminuria as an ACR>3.4mg/mmol creatinine with macro-albuminuria as an ACR >34mg/mmol creatinine.[360] The veteran study reported on micro-albuminuria but did not describe the diagnosis criteria.[414] Tahrani and colleagues reported on both urinary ACR and eGFR results.[348] Micro-albuminuria was diagnosed as an ACR >3.4mg/mmol with macro-albuminuria ≥30mg/mmol. Estimated GFR was calculated based on the Modification of Diet in Renal Disease (MDRD) equation. DKD was diagnosed as either presence of micro- or macro-albuminuria or eGFR <60ml/min/1.73m^2. Leong et al.[225] only used eGFR and this was calculated using the MDRD and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formulae. DKD was defined as an eGFR <60ml/min/1.73m^2. Schober and co-workers[400] used the term 'nephropathy' but did not report on the test or criteria used to diagnose DKD. Finally, Tanaka and colleagues[416] utilised serum creatinine levels (mg/dl) as a continuous variable. Please refer to Table 6-9 for further details.

Table 6-10: Summary of the results for diabetic kidney disease

	Diabetic kidney disease
OSA (based on AHI)	7 studies (n=1503) Adjusted: (+) 2 studies; (-) 0 studies Unadjusted : (+) 1 studies; (-) 4 studies Pooled OR (6 studies – see Figure 6-10) 1.56. 95% CI: 1.11 to 2.21 I^2= 37.3%
OSA (based on ODI)	2 studies (n=1317) Adjusted: (+) 2 study; (-) 0 studies Pooled OR (2 studies – see Figure 6-12) 2.00. 95% CI: 1.36 to 2.94 I^2= 0.0% Pooled OR (3 studies, combined AHI & ODI as OSA diagnosis – see Figure 6-13) 2.10, 95% CI: 1.48 to 2.97, I^2= 0.0% Unadjusted: No data
%TST<90	1 study (n=90) Adjusted: (+) 1 study; (-) 0 study Unadjusted: No data
Mean O_2	1 study (n=90) Adjusted: (+) 0 study; (-) 1 study Unadjusted: No data
Minimum O_2	3 studies (n=491) Adjusted: (+) 0 study; (-) 2 studies Unadjusted: (+) 1 study; (-) 0 study

(+): reported significant association; (-): reported no significant associations; shaded cells indicate that no study reported data for the exposure-outcome combination. DR = diabetic retinopathy; OSA = obstructive sleep apnoea; AHI = apnoea-hypopnoea index; ODI = oxygen desaturation index; %TST<90 = percentage time spent under 90% oxygen saturation; O_2 = oxygen saturation, n = total number of participants

6.4.2.4 Quality assessment

The quality of the studies was summarised in Table 6-11. Overall, the studies were of moderate quality (n=4).[360,400,401,416] Only 2 studies were rated high quality[225,348] and 3 were of low quality.[359,402,414] Majority of the studies were at risk of selection bias with six studies being rated as weak.[348,359,360,400,402,414] This was because several studies did not perform consecutive participant recruitment nor did they report if the study populations were representative of the local patient populations. In addition, only one study performed respiratory scoring with an assessor blinded of the participants clinical characteristics.[225] Several studies were rated at least moderate for methods of sleep assessment (n=7) in particular, many studies did not perform sleep analysis according to the AASM recommendations.[225,348,359,360,400,401,416] The majority of the studies scored reasonably well for study methods (n=6) as reasons were provided for any excluded or missing data.[225,348,360,400,401,414]

Table 6-11: Quality assessment of included studies for diabetic kidney disease

Study	Components					
	Selection bias	**Methods and measurement**	**Blinding**	**Study design**	**Analysis**	**Overall**
Buyukaydin 2012	Weak	Strong	Weak	Weak	Weak	Weak
Furukawa 2013	Weak	Moderate	Weak	Weak	Strong	Weak
Kosseifi 2012	Weak	Weak	Weak	Moderate	Weak	Weak
Laaban 2009	Moderate	Strong	Weak	Moderate	Weak	Moderate
Leong 2014	Strong	Strong	Strong	Moderate	Strong	Strong
Schober 2010	Weak	Moderate	Weak	Strong	Weak	Weak
Storgaard 2014	Weak	Moderate	Weak	Strong	Weak	Weak
Tahrani 2013	Weak	Strong	Weak	Strong	Strong	Strong
Tanaka 2009	Moderate	Moderate	Weak	Weak	Strong	Moderate

6.4.2.5 Association between OSA and DKD

The results from the studies are detailed in Table 6-8 and the overall summary of the results for all outcomes is presented in Table 6-10. A total of seven studies[225,348,359,361,402,414,417] (n=1503) examined the effect of OSA on DKD. Five studies (n-1189) performed only univariate analysis,[359,400-402,414] four of which demonstrated no significant associations, although the direction of effect for all but one study was of higher risk of DKD among patients who have OSA.[359,401,402,414] Buyukaydin and colleagues[359] recruited 52 Turkish T2DM participants from their clinic. Full inpatient PSG was performed and the respiratory recording was scored according to AASM criteria.[75] Minimum duration of sleep recording was described. Twenty-four hour urinary albumin excretion was used to assess DKD. The researchers did not find any significant correlation between urine albumin excretion and AHI (r=0.91, p=0.362) nor any significant difference in albuminuria between OSA and non-OSA group (OR 0.67, 95% CI: 0.15 to 3.31). Similarly, a French study recruited 303 poorly-controlled T2DM inpatients over a 6 month period showed no significant difference in micro-albuminuria based on 24 hour urinary albumin excretion results between those with no OSA (25%) compared to those with mild (34%), moderate (38%) or severe OSA (39%).[401] Comparing OSA and non-OSA individuals made no difference to the result (OR 1.70, 95% CI: 0.98 to 2.97). OSA was diagnosed using a level IV sleep device with no data on effort nor any information on minimum sleep recording.

A Danish study[402] recruited 183 participants who were first screened using the Berlin questionnaire. High risk individuals were invited for a portable home study using a two-channel sleep device with minimum of 4 hours respiratory recording. OSA was diagnosed

when AHI ≥5 events/hr was present alongside sleep symptoms. Again, 24 hour urine albumin excretion was used for the diagnosis of DKD. There were no significant difference in micro-albuminuria between OSA (36%) and non-OSA (28%) individuals ($p>0.05$). Likewise, no significant difference in macro-albuminuria was found between the two groups (10% vs. 9%, $p=0.2$). A U.S. veteran study on 98 OSA individuals with well-controlled T2DM ($HbA_{1c}<6.5\%$) who also suffered with OSA observed a non-significant difference in the AHI between those with micro-albuminuria (28.0 events/hr) and those without micro-albuminuria (33.5 events/hr, $p>0.05$).[414] Participants were predominantly males and had a level III device sleep test carried out for three consecutively nights. Neither the minimum sleep recording nor criteria used for albuminuria were reported.

Only one study demonstrated a significant difference in diabetic nephropathy between OSA and non-OSA individuals.[400] The study was designed as a prevalence study and was sponsored by a pharmaceutical company which provided support on data collection and analysis. Although 715 DM participants were recruited from 14 primary care centres in Germany, 217 participants was excluded (30%) due to missing sleep or participants' data. Therefore a total of 556 eligible participants (58 T1DM and 498 T2DM). A 2-channel ambulatory sleep study was performed with no data on respiratory effort making it difficult to distinguish obstructive from central sleep apnoea. In addition, the study adopted a minimum of 2 hours sleep recording and not the commonly used 4 hours. On top of that, the researchers utilised a conservative AHI ≥15 events/hr as the diagnosis of OSA. Method used for the diagnosis of nephropathy was not reported. A significant difference ($p=0.001$) in nephropathy between those without AHI <5evernts/hr (n=13), AHI between 5-14 events/hr (n=46) and AHI ≥15 events/hr (n=42) was recorded. However, when an AHI ≥15

events/hr was used (as per the German study's definition of OSA), the difference was no longer significant (OR 1.24, 95% CI: 0.78 to 1.97).

Univariate and multivariate analyses were carried out by two UK-based studies.[225,348] Leong et al. recruited consecutive extremely obese individuals with T2DM from our specialist weight management clinic.[225] Estimated GFR was calculated using MDRD and CKD-EPI formulae. Sleep study was performed using a level III sleep device and recording were scored by sleep physiologists and confirmed by a sleep physician blinded of the renal results. Scoring was done according to the AASM recommendations with a minimum of 4 hours sleep recording. Multivariate linear regression analysis demonstrated a linear association between AHI and MDRD eGFR results (β=-0.170, 95% CI: -0.316 to -0.024). However, this was not confirmed using the CKD-EPI formula (β=-0.19, 95% CI: -0.39 to 0.01). Factors adjusted included age, gender, BMI, hypertension, coronary artery disease, DM duration, insulin treatment and drugs for renin-angiotensin-aldosterone system.

Tahrani and colleagues[348] conducted a cohort study on 224 T2DM participants. DKD was assessed using both early morning urine ACR (>3.4 mg/mmol) and eGFR (<60ml/min/1.73m^2). MDRD equation was used to calculate eGFR. OSA assessment was carried out using a level III device. Analysis of sleep study was performed in accordance with the AASM guidelines with at least 4-hour sleep recording. Results demonstrated that OSA was an independent predictor for DKD (adjusted OR 2.64, 95% CI: 1.13 to 6.16) after adjusted for age, sex, ethnicity, BMI, DM duration, smoking and alcohol status, mean arterial pressure (MAP), HbA_{1c}, triglycerides, total cholesterol, high-density-lipoprotein

(HDL) cholesterol, insulin, hypoglycaemic agents, anti-hypertensive, lipid lowering agent. Moreover, 196 participants were followed-up for 2.5±0.7 years and linear regression analysis confirmed that OSA was associated with study-end eGFR result (β=-3.8, p=0.04) after adjusting for age, sex, ethnicity, smoking, BMI, DM duration, MAP, total cholesterol, triglycerides, anti-hypertensive and lipid lowering agents, HbA_{1c}, insulin and oral diabetes medications, antiplatelet agents and baseline eGFR. Substituting OSA with AHI revealed similar results (β=-4.6, p=0.02). As for albuminuria, 163 participants' data were available. Excluding 107 who had albuminuria at baseline, a non-significant difference was found between OSA (22.6%) and non-OSA (13.3%, p=0.23) group. Combining eGFR and albuminuria for the diagnosis of DKD, 169 patients had follow-up results and 76 patients' data were excluded due to known DKD at baseline; 23.1% OSA participants compared to 12.2% non-OSA participants developed DKD (p=0.18).

A meta-analysis was carried out for six studies which reported unadjusted ORs on the effect of OSA on DKD.[225,348,359,400-402] The pooled estimates showed a small, but significant association, with higher risk of DKD in those who have OSA (pooled OR 1.56, 95% CI: 1.11 to 2.21, I^2=37.3%, Figure 6-10). Funnel plot of these studies suggest an imbalance of small studies with positive results (Figure 6-11).

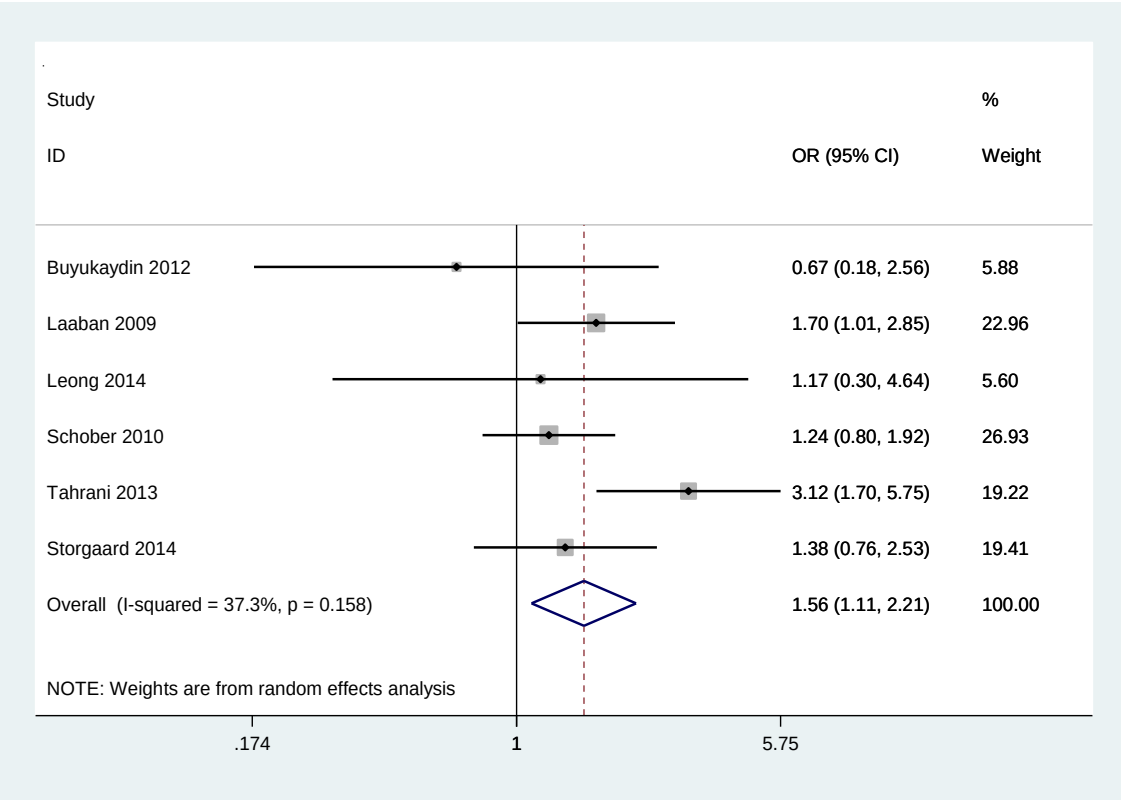

Figure 6-10: Forest plot on the effects of obstructive sleep apnoea on diabetic kidney disease using results from studies which reported unadjusted odds.

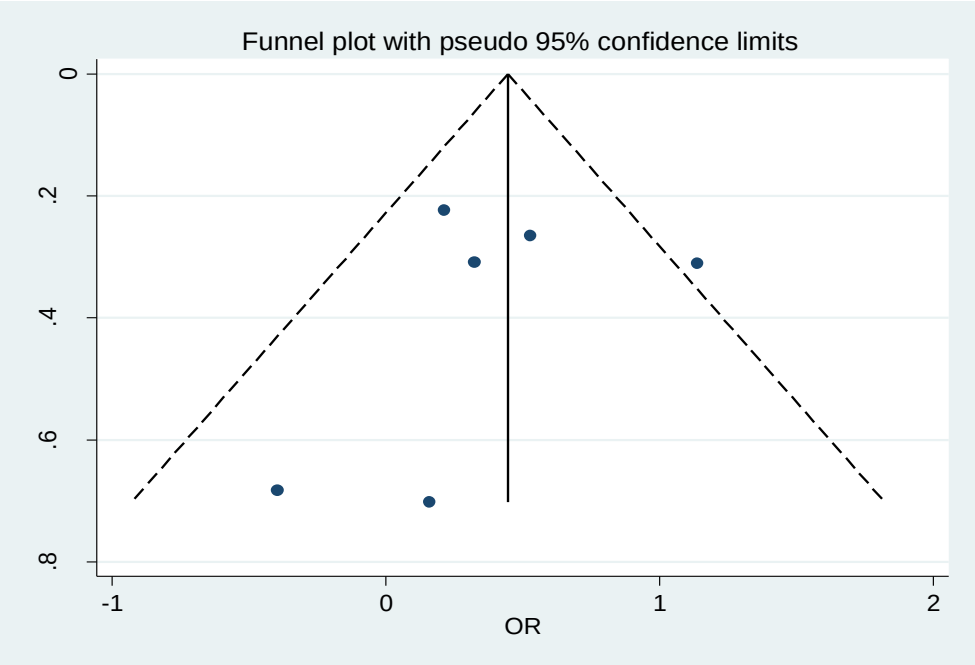

Figure 6-11: Funnel plot on studies which reported unadjusted odds ratios on the effect of obstructive sleep apnoea on diabetic kidney disease.

6.4.2.5.1 Association between OSA and DKD: summary

In summary, although there was no significant association between DKD and OSA in studies that used only the univariate analysis, pooling of these studies suggests that there is a significant association. The studies that did undertake multivariate analysis, and the one study which followed up patients longitudinally, all showed that patients with DM who have OSA had a higher risk of DKD.

6.4.2.6 Association between DKD and other respiratory parameters

Two studies (n=1317) from Japan utilised drop in oxygen levels during sleep (3%ODI ≥5 events/hr) as the diagnostic criterion for OSA.[360,416] Both reported a significant association between ODI and DKD. The Dogo Study was a multi-centre study consisted of 513 Japanese T2DM.[360] All participants had overnight pulse oximetry, data was analysed using a proprietary software with a minimum of 4 hours sleep recording. DKD was diagnosed as either micro-albuminuria (≥3.4mg/mmol) or macro-albuminuria (≥34 mg/mmol) based on early morning urine ACR. Multivariate analysis demonstrated that OSA was associated with micro-albuminuria (adjusted OR 1.84, 95% CI: 1.16 to 2.69) as well as macro-albuminuria (adjusted OR 2.97, 95% CI: 1.36 to 6.90). The confounders adjusted for were made for age, sex, BMI, hypertension, hyperlipidaemia, smoking and alcohol status, DM duration, HbA_{1c}, medications for stroke and ischemic heart disease.

Another multi-centred Japanese study recruited consecutive 904 T2DM individuals.[416] DKD was assessed based on creatinine levels. Minimum sleep recording was not reported. The researchers reported a significant association between OSA and creatinine levels (adjusted OR 2.37, 95% CI: 1.21 to 4.65) after adjusting for age, sex, BMI, heart rate and

hypertension. The researchers also observed that a stepwise increase in ODI severity (ODI <5 events/hr, ODI between 5 and 15 events/hr and ODI >15 events/hr) was associated with a 0.5mg/dl increase in creatinine (adjusted OR 1.54, 95% CI: 1.10 to 2.16) after adjusted for similar factors.

We also performed a meta-analysis on these two studies which used ODI criteria for the OSA diagnosis.[360,416] The results were significant (pooled OR 2.00, 95% CI; 1.36 to 2.94, I^2=0.0%, Figure 6-12). Combining the study by Tahrani and colleagues[348] which used an AHI criterion for OSA diagnosis into the model showed similar results (pooled OR 2.10, 95% CI: 1.48 to 2.97, I^2=0.0%) as shown in Figure 6-13. However, as all three studies reported positive results therefore publication bias cannot be ruled out.

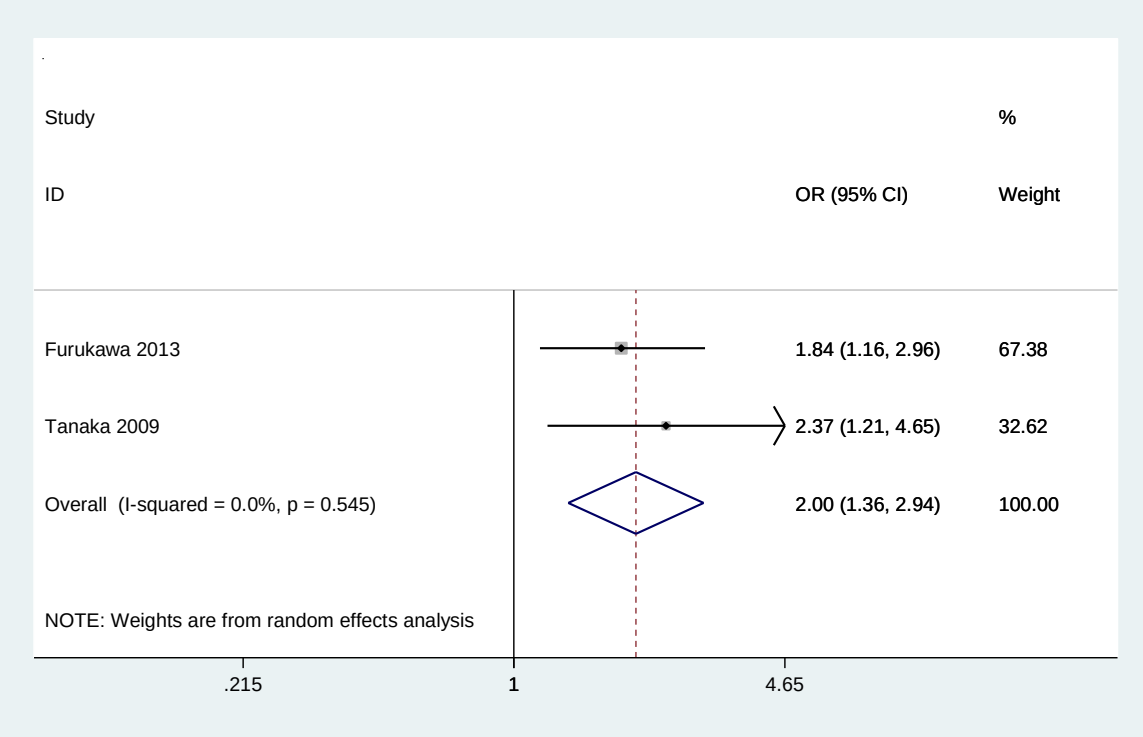

Figure 6-12: Forest plot on the effects of oxygen desaturation index on diabetic kidney disease using results from studies which reported adjusted odds ratios only.

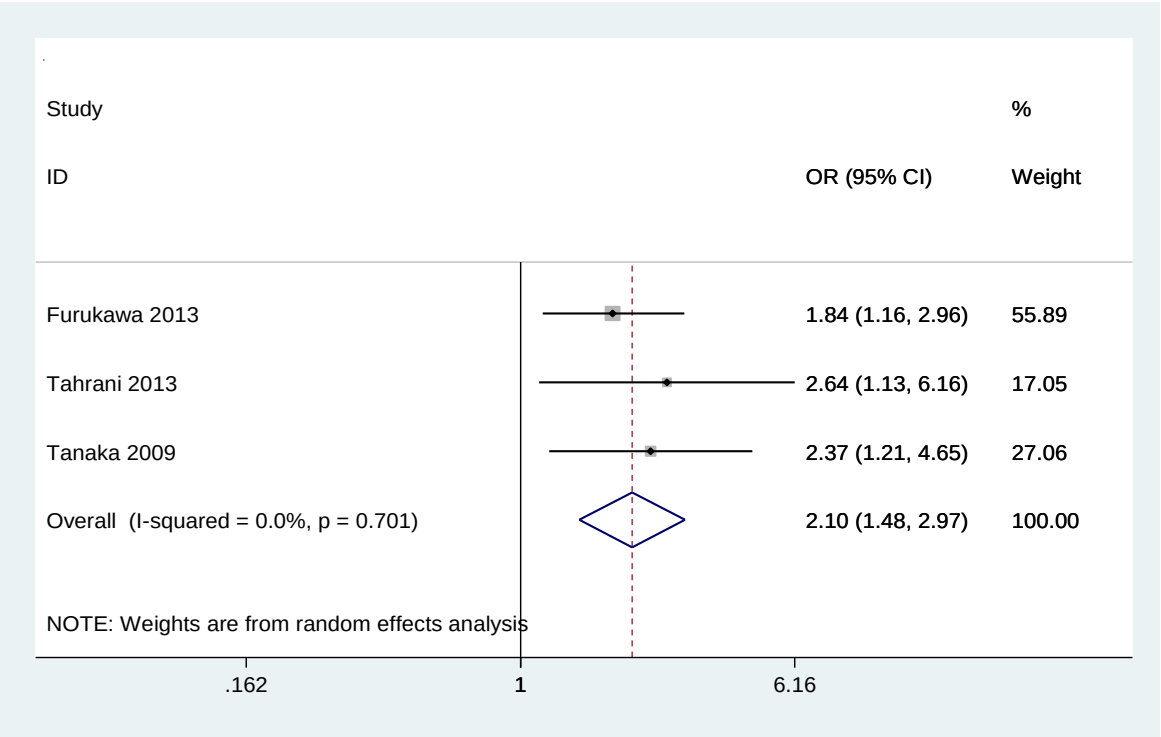

Figure 6-13: Forest plot on the effects of obstructive sleep apnoea on diabetic kidney disease using results from studies which reported adjusted odd ratios only. Note: Furukawa 2013 and Tanaka 2009 diagnosed OSA using 3% ODI ≥5 events/hr whilst Tahrani 2013 diagnosed OSA based on AHI ≥5 events/hr.

Three studies (n=491) examined the effect of minimum oxygen saturation on DKD,[225,348,414] with only one reporting a significant association.[414] Leong and co-workers[225] did not report any significant association (β=0.053, 95% CI: -0.388 to 0.495) after adjusted for confounding factors listed above. This was confirmed by the study by Tahrani and co-workers[348] (adjusted OR 0.96, 95% CI: 0.93 to 1.00). Leong and colleagues (n=93) also examined the effect of mean oxygen saturation which was not significant (β=1.191, 95% CI: -0.039 to 2.420),[225] whereas the association was significant between %TST<90 and eGFR using both MDRD (β=-0.215, 95% CI: -0.406 to -0.023) and CKD-EPI (β=-0.30, 95% CI: -0.56 to -0.03) equations.

6.4.2.6.1 Association between DKD and other respiratory parameters: summary

In summary, our narrative synthesis outlined a likely effect of sleep apnoea as measured by ODI on DKD in patients with T2DM, and this was confirmed by our meta-analysis which had a low level of heterogeneity. There was insufficient evidence that the level of hypoxaemia, %TST<90 and mean oxygen saturation have an impact on the risk of DKD.

6.5 Discussion

6.5.1 Diabetic retinal disease

There was no convincing evidence from our narrative synthesis that OSA is associated with DR especially for non-advanced DR in T2DM populations. The association between OSA and DR amongst T1DM was still unclear as only one study was available and it used oximetry rather than AHI as diagnosis of OSA. From the narrative synthesis, there appeared to be some evidence that OSA was associated with greater severity of DR as well as advanced DR amongst T2DM. The majority of the studies were cross-sectional except one cohort study, which reported significant association between OSA and the development of advanced DR. The meta-analysis performed for cross sectional studies, however, did not show any association between advanced DR and T2DM with a significant level of heterogeneity. Therefore, the pooled results might not be accurate. No study has explored the association between OSA and severity of DR as well as advanced DR in T1DM populations. Data on DMac was more limited and inconclusive. Whilst overall, larger cross-sectional studies suggested an association with OSA, this was not confirmed in a longitudinal study.[406] This may be due to the relatively short duration of follow-up. Therefore, well-designed with good sample size long-term case control or cohort studies are required to establish if OSA leads to greater progression of advanced DR as well as DMac in both T1DM and T2DM populations.

6.5.1.1 Other respiratory parameters and DR

The evidence for the effect of ODI on DR was inconclusive from the narrative synthesis as only 4 studies were available whereby one of the studies examined effect on advanced DR. Although the meta-analysis showed significant association between ODI and OSA, the

results were mainly from one study and addition of another study which used AHI as OSA diagnosis yielded a non-significant results with significant level of heterogeneity. There was insufficient evidence to conclude on the effect of ODI on DMac. There was no evidence that mean oxygen saturation or the duration of nocturnal hypoxemia was associated with DR. There was also insufficient evidence to conclude on the effect of both mean oxygen saturation and %TST<90 on DMac. However, there was evidence from both the narrative synthesis and meta-analysis that the level of nocturnal hypoxemia had an impact on DR. One study[224] also found that minimum oxygen saturation was associated with DMac. Due to the limited available studies, which report on the effects of minimum oxygen saturation on DR, publication bias of positive results might be present. Therefore, more studies are needed to examine the effect of minimum oxygen saturation especially with DMac. It will be of interest to know if low oxygen saturation in those with mild OSA (AHI between 5 and <15 events/hr) has any impact on DR as well as DMac in the long term given that this group of individuals are not likely to be offered CPAP treatment according to current guidelines and clinical practice.

6.5.1.2 Heterogeneity in respiratory assessment

There was a high degree of heterogeneity in the studies especially in the devices used to record sleep parameters. Majority of the studies used portable home sleep monitoring devices. The gold standard for the diagnosis of OSA is the overnight sleep laboratory polysomnography with a technician in attendance. Full PSG captures the most complete pathophysiological state of OSA using respiratory, cardiovascular, neurological, and muscle recordings. However, due to the increasing demand of sleep studies and the limited availability of facilities and technicians, portable devices are a useful cost-effective

alternative. A recent meta-analysis compared in-hospital PSG and portable sleep devices demonstrated that portable devices had good diagnostic performance with the area under the curve (AUC) to be between 0.85 and 0.99 depending on the severity of OSA.[418] In our review, the majority of the portable devices used by the researchers of included studies had 3 channels (airflow, oximetry and respiratory effort) except three studies, which utilised a device with only 2 channels (no data on effort).

Apart from portable home sleep device, some studies used pulse oximetry to diagnose OSA. A recent study of 475 participants who underwent simultaneous portable PSG (Embletta) and pulse oximetry (PULSOX-300i) found that ODI correlated highly with AHI ($r=0.886$, 95% CI: 0.865 to 0.904, $p<0.001$).[365] Furthermore, the AUC for ODI for the diagnosis of mild, moderate and severe OSA were 0.908 (95% CI: 0.880 to 0.936), 0.931 (95% CI: 0.909 to 0.952) and 0.958 (95% CI: 0.937 to 0.979), respectively.[365] Another study of 275 participants with OSA reported the accuracy for of using ODI >5 events/hr as the basis for diagnosis of OSA was 0.81.[419] Studies also differed tremendously in the definition for apnoea/hypopnoea as well as oxygen desaturation, and the diagnostic criteria used for of OSA. The information regarding the minimum sleep recording period was also generally unavailable.

Since the majority of the study designs were cross-sectional, it is difficult to infer causality. However, the study by Altaf et al. was prospective and they reported significant worsening of DR after 4 years of follow-up.[406] It is likely that OSA resulted in the development and greater progression of DR. The possible mechanisms of OSA causing DR had been discussed in detailed in Chapter 4. Likewise, plausibility of diabetic microvascular

complication leading to the development of OSA had also been covered in Chapter 4. Therefore, the association between OSA and DR is likely bi-directional.[309]

6.5.2 Diabetic kidney disease

Our narrative synthesis demonstrated evidence of a possible association between OSA (diagnosed using AHI) and DKD among T2DM populations. This was confirmed by a meta-analysis on the studies that carried out univariate analysis. Nevertheless, crude ORs are subjected to multiple confounders such as age, gender and BMI and the results may not be accurate. Studies which performed multivariate analysis adjusted for important confounders such as BMI, gender and DM duration, also demonstrated a significant association between OSA and DKD. Additionally, the only longitudinal study with medium-term follow-up also showed a significant worsening of eGFR in individuals with T2DM and OSA.[348] In that study, 47 participants were offered continuous positive airway pressure (CPAP) treatment, 16 of whom were compliant with CPAP. The eGFR decline was slower in the CPAP-compliant group (-7.7%, 95% CI: -15.9% to -1.8%) compared to non-compliant group (-10.0%, 95% CI: -17.2% to 2.3%). Although the CPAP results were not adjusted for important confounders and has no control group for comparison, it suggests that reversing sleep apnoea using CPAP may contribute to decelerating the decline in renal function.

One of the excluded studies, the Nutritional Health And Nutrition Examination Survey (NHANES) study was published as a conference abstract.[420] Participants were recruited between 2005 and 2008. The study was excluded because only 9.5% of the participants were diabetic and it was unclear whether the self-reported sleep apnoea was central or

OSA (present in 4.6% of the population). The NHANES researchers carried out multivariate regression analysis and demonstrated that when DM and sleep apnoea coexist, the risk of micro-albuminuria was three-fold higher (OR 3.4, 95% CI: 1.80, 6.39) and the risk for macro-albuminuria was 11-times greater (OR 11.39, 95% CI: 4.60 to 28.42) after adjusting for demographics, cardiovascular disease, blood pressure and BMI. Assuming the diagnosis of sleep apnoea was OSA in the majority of the participants, this research strengthens the evidence of the association between OSA and DKD in T2DM. Currently, there is a dearth of information on the effect of OSA on DKD in T1DM populations.

6.5.2.1 Other respiratory parameters and DKD

We also outlined a likely effect of ODI on DKD in T2DM populations and this was confirmed by our meta-analysis with low level of heterogeneity. However, publication bias may occur as no 'neutral result' study was available. It appears that there was no association between the level of hypoxemia and DKD although only two studies were available. There was insufficient evidence to conclude on the effect of mean oxygen saturation and %TST<90 on DKD. Currently, there is a dearth of information on the effect of these respiratory parameters in T1DM populations.

A small degree of heterogeneity was identified in the methods and the criteria used for OSA diagnosis. The majority of the studies utilised either a level III or level IV portable device probably because it is less labour intensive and less costly as discussed in the above section. Differences also occur with DKD diagnosis. Some studies used albuminuria whilst others used either eGFR or creatinine level. Only Tahrani and colleagues[348] used both albuminuria and eGFR as a diagnosis of DKD and is likely to provide a better overall

assessment of DKD. However, several studies, which used either eGFR or creatinine level, still demonstrated a significant association[225,360,416].

6.5.2.2 Pathophysiology of OSA and DKD

Almost all the studies were cross-sectional in design and therefore unable to demonstrate causality, with only the study by Tahrani and co-workers[348] longitudinal that reported a significant worsening of eGFR after 2.5 years of follow-up. OSA is likely to impact on both the development and progression of DKD directly through intermittent hypoxemia (IH) and sleep fragmentations, or indirect via insulin resistance and other systemic diseases. IH caused by OSA had been documented to cause greater level of oxidative stress response, activation of inflammatory pathways leading to endothelial dysfunction.[247,248] Additionally, the intermittent intra-renal hemodynamic changes from recurrent sympathetic overdrive secondary to sleep fragmentations can cause ischemia with intra-renal reperfusion injury leading to intrinsic renal injury.[421] Case reports on participants with OSA have shown secondary focal glomerulosclerosis[370,371] and in one case, complete resolution of proteinuria after bi-level positive airway pressure treatment.[370]

In addition, studies have shown a dose-response relationship between the severity of OSA and level of glycaemia.[218] This is likely to be caused by excess sympathetic activity, activation of the hypothalamic-pituitary-adrenal axis, direct insult to beta-cell function and activation of deleterious molecular pathways adversely affecting insulin sensitivity.[396] Collectively, these mechanisms result in greater insulin resistance among DM participants and greater insulin resistance amongst those with T2DM that has been shown to be a predictor of the development of micro-albuminuria irrespective of other metabolic

profiles.[422] Furthermore, OSA is a known cause of resistance hypertension,[423] and hypertension is a major risk factor for renal damage.[424] On the other hand, it is also plausible that diabetes microvascular complication causes OSA. This has been illustrated in Chapter 5. Therefore, there is likely to be a two-way association between OSA and DKD.[309]

6.5.3 Limitations

Our systematic review has limitations. The reliance on the results from cross-sectional studies is a major concern. It is well-documented in the literature that cross-sectional studies are usually subject to selection bias.[425] This was certainly reflected in our quality assessment in both selection bias. Most of the studies did not blind either the DKD or DR outcome from the assessor who scored the sleep recording reflecting possible measurement bias. However, several studies were conference abstracts and some were in the form of short reports for the review of DR. Due to the word limit, only limited information was available for these studies. Our quality assessment tool may not have completely assessed the rigour of these studies. Although several studies adjusted for confounders, the issue with residual confounders remained. We also did not examine the possible association between sleep apnoea related arousals and DR. The majority of the included studies were from European countries, there were a few from Asia and some small studies from North America. There were no large studies from the North and South American, African as well as Asia-pacific populations. However, the underlying mechanistic effects between OSA and diabetic microvascular complications should not differ in other populations therefore our results should be generalisable in all T2DM populations.

6.6 Conclusion

6.6.1 Diabetic retinal disease

Although our review explored the effects of OSA on DR, it is important to note that glycaemic control and hypertension are still the greatest risk factors for DR. This systematic review revealed key shortfalls in the reporting of the methodology used for sleep studies. We recommend that future studies should report the device used for sleep assessment, minimum sleep recording time, definition for apnoea/hypopnoea and the criteria used for the diagnosis of OSA as a minimum. In addition, several of the respiratory sleep parameter results were missing in majority of the studies. This could perhaps be avoided if core outcome reporting for sleep studies is introduced for observational studies in addition to intervention trials. Another important consideration is blind scoring of sleep/retinopathy data. Statistical analyses of such data should also report effect sizes after adjustment of major potential confounders. Given the high prevalence of OSA in patients with T2DM, the potential association between OSA, intermittent hypoxemia and diabetes complications, and plausibility of shared pathophysiological mechanisms between OSA and DM, there is a need for future large prospective studies with long term follow-up data to examine the long term effects of OSA including sleep apnoea related arousals as well as other respiratory sleep parameters on non-advanced as well as advanced DR in both T1DM and T2DM populations. Likewise, it is also important to follow-up OSA-free DR individuals to determine if DM micro-vascular complications contribute to OSA development.

6.6.2 Diabetic kidney disease

Our systematic review demonstrated that OSA was associated with DKD in T2DM populations. There is a dearth of information on the effect of OSA on DKD amongst

T1DM. In light of the plausible bi-directional mechanisms between OSA and DKD, there is a need for large prospective studies with long term follow-up to determine the long term effects of AHI as well as other respiratory parameters on both albuminuria and GFR in both T1DM and T2DM populations in the future. Similarly, it is also vital to monitor disease free DKD individuals to examine if micro-vascular complications play any role in the development of OSA.

CHAPTER 7 – CONCLUSIONS

In summary, this thesis demonstrated that the duration and the level of nocturnal hypoxaemia are associated with glycaemic control in patients with type 2 diabetes mellitus (T2DM). We also reported relationships between the duration of hypoxaemia and the risk of diabetic kidney disease (DKD); and the level of nocturnal hypoxaemia and the risk of diabetic maculopathy. The latter findings were further confirmed by the systematic review in which showed an inverse relationship between minimum oxygen saturation and the risk of diabetic retinopathy. Although we could not conclude on the effect of the duration of hypoxaemia on DKD, our systematic review demonstrated that the presence of obstructive sleep apnoea (OSA) does seem to be associated with the risk of DKD. We also showed that OSA is not only more prevalent but also more severe in South Asians compared to white Europeans.

The findings of this thesis have important implications in the management of extreme obese individuals as well as raising several issues for future researches. Some of the key implications include: 1) obesity is a complex disease, 2) the importance of individualised care especially in the management of OSA in South Asians, and 3) the reliance of using apnoea-hypopnoea index (AHI) in the management of OSA.

7.1 Findings in the context of obesity as a complex disease

It is well established that obesity is associated with both OSA (chapter 1, section 1.3.4) and diabetes mellitus (DM) (chapter 3, section 3.1.1). Other chronic diseases associated with obesity are cardiovascular disease, stroke, hypertension, dyslipidaemia and several

malignancies (chapter 1, section 1.1.5). Therefore, obesity is a complex disease affecting the functions of several organ systems. Our significant findings on the association between glycaemic control as well as diabetic microvascular complications and OSA amongst extreme obese individuals provide further evidence to support this notion.

Although future longitudinal research is needed to address the causal relationship between OSA and microvascular complications, it is likely that obesity will have, at least in part, an indirect influence through the effect of OSA (please refer to chapter 4, section 4.5.2 and chapter 5, section 5.5.2.3) or DM, on the development and progression of diabetic microvascular complications. Therefore, weight management strategies are likely to be beneficial in treating OSA, DM and possibly microvascular complications. The 'Action on obesity: comprehensive care for all' report published by the Royal College of Physicians recommended a multidisciplinary team (MDT) approach to weight management.[34] The MDT should be supported by policy makers, acting as the driver for good practice and involved in the development and implementation of local and national guidelines. Additionally, the team have a role in evaluating and improving their clinical practice as well as participation in future research to continually improve care.

7.2 Findings in the context of personalised care

Most importantly, the team will have the role of optimising individual patient care. The National Institute for Health and Care Excellence (NICE) clinical guidance 138: Patient experience in adult NHS services: improving the experience of care for people using adult NHS services, recommends individualisation of patient care, taking into account the variations in which a condition may impact on individuals, and tailoring treatment

accordingly.[426] Each patient is different and one distinct characteristic that must be considered is ethnic background. Our study found that South Asians, a relatively understudies sub-population, suffer from more severe OSA compared with white Europeans with extreme obesity.

Future studies are needed to explore the interactions between metabolic risk factors and quality of life in these group. There is also a need to have improved insight into the clinical presentations of OSA among South Asians patients. The Sleep Heart Health Study (SHHS) which consisted of 13,194 participants, demonstrated a difference in OSA symptomology amongst Hispanic, Afro-Caribbean and white Caucasians.[427] However, objective assessment of sleep symptoms has not been performed in South Asians populations in the UK. In addition, longitudinal studies on the health consequences of OSA among South Asians are needed to identify if this ethnic group faces similar risk as their white European counterparts. Finally, the effect of OSA treatment whether it is with continuous positive airway pressure (CPAP) or with weight reduction strategies, on various health outcomes, as well as adherence to treatment, should also be studied by ethnic group.

7.3 Findings in the context of respiratory parameters

Currently, the American Academy of Sleep Medicine (AASM) recommended that those with apnoea-hypopnoea index (AHI) ≥15 events/hr should be considered for CPAP regardless of the level of obesity.[75] This recommendation ignores individuals with AHI <15 events/hr, who may have low nocturnal oxygen saturation or those who have long durations of nocturnal hypoxaemia. Our results suggests that minimum oxygen saturation and percentage of time spent under 90% oxygen saturation (%TST<90) also may have a

pivotal role in both glycaemic control and diabetic microvascular complications in DM individuals with extreme obesity.

Although our study cannot infer causality, our findings raise questions on the reliance of the AHI as a sole criterion for the treatment of OSA. We therefore suggest that further research is needed to clarify this. The first step is to confirm if the level and duration of nocturnal hypoxaemia are better predictors compared to the AHI for the development and/or progression of diabetic microvascular complications in well-designed longitudinal studies. If this is true then the second step is to identify if there is a minimum threshold which may contribute to the glycaemic control and the development and progression of diabetic microvascular complications for both the minimum oxygen saturation and %TST<90 regardless of the AHI. Then the third step is to carry out a well-designed prospective randomised controlled trial to establish if correcting the level and/or duration of hypoxaemia has any beneficial effects on glucose levels and disease progression in diabetes mellitus. This is especially important in individuals with AHI <15 events/hr (not qualified for CPAP). It is also important to establish if there is any difference in the response to the treatment of hypoxaemia according to different ethnic groups such as South Asians.

One of the limitations of our study is that we only examined a severely obese T2DM population and the effect of OSA on Type 1 diabetes mellitus (T1DM) is not studied. Although we did not investigate the effect in T1DM, it is likely that the underlying mechanisms will not differ between these two groups. However, studies still needed to address if the impact of OSA on vascular complications in T1DM populations is similar to

those in T2DM. Future work should also examine if the factors which mediates the effect of hypoxaemia on glycaemic control, retinal and renal function in this population.

Our study identifies several gaps in our understanding of the effect of OSA on disease outcomes such as whether effects differ by ethnicity (South Asians). Although AHI is the recommended tool for diagnosis and treatment of OSA, we demonstrated that other nocturnal hypoxaemia parameters may also play a role in glycaemic control and diabetic microvascular complications. However, future research is needed to confirm this. Our findings also support the current available evidence that obesity is a complex disease.

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APPENDICES

Appendix 1: Data extraction form for systematic review chapter (Chapter 6)

Study identifications	Study No First author Year of publication Reference Country	
Publication characteristics	Source of Funding Role of funder Study design	
Methodology	Consecutive recruitment? Summary of study aims Setting Selected population Excluded population	
Respiratory methods	Sleep study (method, device) Definition of apnea/hypopnea/oxygen desaturation Definition for OSA diagnosis Minimum duration of recording	
Outcome measures (eg. retinopathy score, ACR)	Data source Scoring methods (eg. For retinopathy)	
Sample size	Total Number Attrition/missing data OSA Non OSA	

Demographics	Age Female (%) BMI HbA1c DM duration	
Outcome results **OSA vs. non-OSA**	Outcome 1 Outcome 2 Outcome 3 Outcome 4 Outcome 5 Outcome 6 Outcome 7	
Analysis	Method (?multivariate) Result outcome 1 Result outcome 2 Result outcome 3 Result outcome 4 Result outcome 5 Confounders adjusted	

Appendix 2: Quality assessment form for systematic review chapter (Chapter 6)

Author: **Year:** **Study ID:**
Reviewer initials:

	Yes/No/Unclear	Supporting evidence
Selection bias		
8. Does the study address an appropriate and clearly focused question?		
9. Was consecutive patients recruited?		
10. Was the cases and controls taken from comparable populations?		
11. Were the exclusion criteria the same for both cases and controls?		
12. Was the participant representative of the patient population?		
13. If applicable, was the control group comparable to cases (consider suitability, recruitment and baseline characteristics)?		
14. Was it clear that controls are not cases (in case control)?		
Overall Judgment for selection bias (Weak / Moderate /Strong)		
Respiratory measurement		
4. Was a suitable measurement for OSA used? (PSG/oximetry measured in standard, valid and reliable way)		
5. Was the scoring of the respiratory measures based on guidelines / consensus guidelines eg. AASM guidelines?		
6. Was there a clear definition of OSA used?		
Overall Judgment for measurement (Weak / Moderate / Strong)		
Blinding		
3. Blinding of assessor performing sleep analysis		
4. Blinding of assessor of retinal photographs		
Overall Judgment for measurement (Weak / Moderate / Strong)		
Study methods		
4. Were participant attrition rates /missing data documented?		
5. Were reasons given for drop outs / missing data?		
6. Is this retrospective / prospective design?		
Overall Judgment for study design (Weak / Moderate / Strong)		
Analysis		
3. Were all outcomes reported?		
4. Were confounding variables adjusted for?		
Overall Judgment for analysis (Weak / Moderate /Strong)		
Overall judgment for the study (Weak, Moderate, Strong)		

Appendix 3: MOOSE checklist for the systematic review on the effect of obstructive sleep apnoea on diabetic retinopathy

Criteria		Brief description of how the criteria were handled in the meta-analysis
Reporting of background should include		
√	Problem definition	Obstructive sleep apnoea (OSA) has been found to be associated with diabetes mellitus and worsening insulin resistance, and thus is likely to be associated with a greater risk of development or progression of diabetic microvascular complications. However, the current evidence on the associations between OSA or chronic intermittent hypoxaemia (CIH) and diabetic retinopathy (DR) and maculopathy (DMac) has been inconclusive.
√	Hypothesis statement	OSA increases the risk of DR and DMac
√	Description of study outcomes	Effects on DR and DMac
√	Type of exposure or intervention used	Presence or absence of OSA/CIH
√	Type of study designs used	We included prospective cohort and cross-sectional studies. We excluded intervention studies.
√	Study population	Type 1 or Type 2 diabetes mellitus. Please refer to Table 6-1 for full eligibility criteria.
Reporting of search strategy should include		
√	Qualifications of searchers	WBL - MBChB, MRCP
√	Search strategy, including time period included in the synthesis and keywords	MedLine from inception until January 2014 EMBASE from inception until January 2014 Cochrane Database from inception until January 2014 OpenGrey from Inception until January 2014 Zetoc from Inception until January 2014 We used both MeSH/Emtree Headings and free text for the search. Please refer to Table 6-2 for search terms.
√	Databases and registries searched	MedLine, EMBASE, Cochrane Database Grey literature: OpenGrey and Zetoc
√	Search software used, name and version, including special features	OvidSP was used for MEDLINE and EMBASE searches. EndNote X5 was employed to combine citations and eliminate duplications
√	Use of hand searching	We hand-searched citations of all included papers.
√	List of citations located and those excluded, including justifications	Please refer to Figure 6-1 of the PRISMA flow chart for study selection process. The full citation list is available upon request.
√	Method of addressing articles published in languages other than English	We included English, Chinese and French language papers. Unfortunately, due to limitation on skills and budget, we were unable to translate 2 papers (1 Russian and 1 Czech language).

√	Method of handling abstracts and unpublished studies	We contacted the authors of relevant unpublished studies (mainly conference abstracts) for additional data.
√	Description of any contact with authors	We contacted authors on number of DR in OSA and non OSA groups. When appropriate, we also sought data on diabetes maculopathy.
Reporting of methods should include		
√	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Eligibility criteria are described in the methods section of Chapter 6 and summarised in Table 6-1.
√	Rationale for the selection and coding of data	Prior to formal data extraction, a standardised form was designed based on the STROBE. The standardised data extraction form was piloted on a sample of 5 studies and improvements were made before formal use for the systematic review. Data extracted include study identification, study design, measures for OSA or CIH, characteristics of participants, funding, results on DR and DMac.
√	Assessment of confounding	We meta-analysed results from studies which performed multivariate analysis (adjusted ORs and 95% CI) to provide a least bias estimate.
√	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	A revised quality assessment form was devised based on the Newcastle Ottawa Scale. An overall judgment of either 'weak', 'moderate' or 'strong' were given to study based on the rating of 5 components: study design, selection bias, measurement and methods, blinding of the assessor performing DR or sleep analysis on participants' characteristics, and analysis. Each components had a set of criteria and each criterion was rated as 'yes', 'no' or 'unclear'.
√	Assessment of heterogeneity	The I^2 statistic was employed to assess heterogeneity.
√	Description of statistical methods in sufficient detail to be replicated	Stata 13 was used for meta-analysis. Adjusted ORs and 95% CI were log transformed followed by both fixed and random effects models analyses. Pooled results were back-transformed and only results from random effects model were presented.
√	Provision of appropriate tables and graphics	We included the following in Chapter 6: 1. Characteristics and results of included studies 2. Quality assessment of included studies 3. Figure 6-1: PRISMA flow chart on study selection 4. Forest plots of pooled estimates 5. Funnel plot for publication bias. We also included: 1. Eligibility criteria for the systematic review 2. Search terms for the systematic review 3. Quality assessment form 4. Criteria used for OSA and DKD assessment as

		reported by included studies
Reporting of results should include		
√	Graph summarizing individual study estimates and overall estimate	Please refer to Figure 6-2, 6-4, 6-5, 6-6, 6-7 and 6-8 for details.
√	Table giving descriptive information for each study included	Please refer to Table 6-3 in the manuscript
√	Results of sensitivity testing	Please refer to Figure 6-8.
√	Indication of statistical uncertainty of findings	Pooled-odds ratios with 95% confidence intervals and I^2 values were presented for all meta-analyses
Reporting of discussion should include		
√	Quantitative assessment of bias	Funnel plot was employed and risk of publication bias was discussed. Limitations of observational studies on residual bias was discussed
√	Justification for exclusion	We excluded studies that had not reported on our outcome of interests or not exposed to OSA.
√	Assessment of quality of included studies	Implications on the assessment of conference abstracts/short reports on the quality of studies was discussed.
Reporting of conclusions should include		
√	Consideration of alternative explanations for observed results	We discussed a possible bi-directional association between OSA and DR/DMac (Chapter 4) as well as the possibility of residual confounders.
√	Generalization of the conclusions	We noted a lack of studies from the North and South American, African as well as Asia-pacific populations. However, the underlying mechanistic effects between OSA and DR/DMac should not differ in other populations therefore our results should be generalisable in all T2DM populations.
√	Guidelines for future research	This systematic review found: a. Key shortfalls in the reporting of methodology used for sleep studies and that this could perhaps be avoided if core outcome reporting is introduced for observational studies. b. Future large prospective studies with long term follow-up data to examine the effects of OSA on DR/DMac in T1DM and T2DM populations are needed. c. Likewise, prospective studies to follow up OSA-

		free DR/DMac individuals to determine effect of DM micro-vascular complication on OSA development.
√	Disclosure of funding source	No funding

Appendix 4: MOOSE checklist for the systematic review on the effect of obstructive sleep apnoea on diabetic kidney disease

Criteria		Brief description of how the criteria were handled in the meta-analysis
Reporting of background should include		
√	Problem definition	Obstructive sleep apnoea (OSA) has been found to be associated with diabetes mellitus and worsening insulin resistance, and thus is likely to be associated with a greater risk of development or progression of diabetic microvascular complications. However, the current evidence on the associations between OSA or chronic intermittent hypoxemia (CIH) and diabetic kidney disease (DKD) has been inconclusive.
√	Hypothesis statement	OSA increases the risk of DKD
√	Description of study outcomes	Effects on DKD (renal function or albuminuria)
√	Type of exposure or intervention used	Presence or absence of OSA/CIH
√	Type of study designs used	We included prospective cohort and cross-sectional studies. We excluded intervention studies.
√	Study population	Type 1 or Type 2 diabetes mellitus. Please refer to Table 6-1 for full eligibility criteria.
Reporting of search strategy should include		
√	Qualifications of searchers	WBL - MBChB, MRCP
√	Search strategy, including time period included in the synthesis and keywords	MedLine from inception until January 2014 EMBASE from inception until January 2014 Cochrane Database from inception until January 2014 OpenGrey from Inception until January 2014 Zetoc from Inception until January 2014 We used both MeSH/Emtree Headings and free text for the search. Please refer to Table 6-2 for search terms.
√	Databases and registries searched	MedLine, EMBASE, Cochrane Database Grey literature: OpenGrey and Zetoc
√	Search software used, name and version, including special features	OvidSP was used for MEDLINE and EMBASE searches. EndNote X5 was employed to combine citations and eliminate duplications
√	Use of hand searching	We hand-searched citations of all included papers.
√	List of citations located and those excluded, including justifications	Please refer to Figure 6-9 of the PRISMA flow chart for study selection process. The full citation list is available upon request
√	Method of addressing articles published in languages other than English	We included English, Chinese and French language papers. Unfortunately, due to limitation on skills and budget, we were unable to translate 1 Czech paper
√	Method of handling abstracts	We contacted the authors of relevant unpublished studies

	and unpublished studies	(mainly conference abstracts) for additional data.
√	Description of any contact with authors	We contacted authors on frequency and adjusted odd ratios of DKD in OSA and non OSA groups.
Reporting of methods should include		
√	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Eligibility criteria are described in the methods section and summarised in Table 1 in supplementary materials.
√	Rationale for the selection and coding of data	Prior to formal data extraction, a standardised form was designed based on the STROBE. The standardised data extraction form was piloted on a sample of 5 studies and improvements were made before formal use for the systematic review. Data extracted include study identification, study design, measures for OSA or CIH, characteristics of participants, funding and results on DKD.
√	Assessment of confounding	We meta-analysed results from studies which performed multivariate analysis (adjusted ORs and 95% CI) to provide a least bias estimate.
√	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	A revised quality assessment form was devised based on the Newcastle Ottawa Scale. An overall judgment of either 'weak', 'moderate' or 'strong' were given to study based on the rating of 5 components: study design, selection bias, measurement and methods, blinding of the assessor performing sleep analysis on participants' characteristics, and analysis. Each components had a set of criteria and each criterion was rated as 'yes', 'no' or 'unclear'.
√	Assessment of heterogeneity	The I^2 statistic was employed to assess heterogeneity.
√	Description of statistical methods in sufficient detail to be replicated	Stata 13 was used for meta-analysis. Adjusted ORs and 95% CI were log transformed followed by both fixed and random effects models analyses. Pooled results were back-transformed and only results from random effects model were presented.
√	Provision of appropriate tables and graphics	We included the following in the manuscript: 5. Table 6-6: Characteristics and results of included studies 6. Table 6-8: Quality assessment of included studies 7. PRISMA flow chart on study selection 8. Forest plot for pooled estimates 9. Funnel plot for publication bias We also included: 10. Eligibility criteria for the systematic review 11. Search terms for the systematic review 12. Quality assessment form 13. Criteria used for OSA and DKD assessment as

		reported by included studies
Reporting of results should include		
√	Graph summarizing individual study estimates and overall estimate	Please refer to Figure 6-10, 6-12, 13
√	Table giving descriptive information for each study included	Please refer to Table 6-6
√	Results of sensitivity testing	Figure 6-12
√	Indication of statistical uncertainty of findings	Pooled-odds ratios with 95% confidence intervals and I^2 values were presented for all meta-analyses
Reporting of discussion should include		
√	Quantitative assessment of bias	Funnel plot was employed and risk of publication bias was discussed. Limitations of observational studies on residual bias was discussed
√	Justification for exclusion	We excluded studies that had not reported on our outcome of interests or not exposed to OSA.
√	Assessment of quality of included studies	Implications on the assessment of conference abstracts/short reports on the quality of studies were discussed.
Reporting of conclusions should include		
√	Consideration of alternative explanations for observed results	We discussed a possible bi-directional association between OSA and DKD (Chapter 5) as well as the possibility of residual confounders.
√	Generalization of the conclusions	We noted a lack of studies from the North and South American, African as well as Asia-pacific regions. However, the underlying mechanistic effects between OSA and DKD should not differ in other populations therefore our results should be generalisable in all T2DM populations.
√	Guidelines for future research	This systematic review found: d. OSA was associated with DKD in T2DM population e. Dearth of information on the effect of OSA on DKD in T1DM population f. Future prospective studies up to determine the long term effects of OSA on DKD in T1DM and T2DM populations.
√	Disclosure of funding source	No funding