Obstructive Sleep Apnea and Diabetic Neuropathy: a Novel Association in Patients with Type 2 Diabetes

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Online Supplement

Methods

Data collected

Data collected included demographics, anthropometrics, metabolic indices and renal function (estimated glomerular filtration rate (eGFR) using the MDRD equation). Sleep assessment included the use of sleep diaries and the Epworth sleepiness score (ESS).

The Michigan Neuropathy Screening Instrument (MNSI)

The MNSI is a validated, 2-component tool designed to facilitate the early diagnosis of DPN and has been used in several land mark epidemiological studies (1-5). The questionnaire component (MNSIq) comprises 15 questions seeking to characterize sensory disturbance, but also peripheral vascular disease and general asthenia **(Figure E1)**(1). The examination component (MNSIe) comprises a limited foot inspection to identify deformity, skin abnormalities, and ulceration, coupled with an assessment vibratory perception at the great toe (measured using a 128 Hz tuning fork) and ankle tendon reflexes **(Figure E1)**(1).

In the MNSIq, responses of “yes” to items 1-3, 5-6, 8-9, 11-12, 14-15 are each counted as one point. A “no” response on items 7 and 13 counts as 1 point. Item #4 is a measure of impaired circulation and item #10 is a measure of general aesthenia and are not included in scoring.

The MNSIe is scored as indicated in **Figure E1**. Foot Inspection includes looking for evidence of excessively dry skin, callous formation, fissures, frank ulceration, oedema or deformities. Deformities include flat feet, hammer toes, overlapping toes, halux valgus, joint subluxation, prominent metatarsal heads, medial convexity (Charcot foot), edema and amputation. Having any abnormalities on inspection is scored as 1, while 0 is given for normal feet. Vibration sensation and ankle reflexes are scored as 0 for absent, 0.5 for reduced and 1 for absent.

When validated against nerve conduction studies, MNSIe score > 2 had a sensitivity and specificity of at least 80% and 75% respectively (1,6,7). The MNSI also has been reported to have a high inter- and intra observer reproducibility (88.8% and 95% respectively) (6).

We used the MNSI in our study because it has been used in several landmark studies, It is easy to use and can be used to examine a large number of patients with little cost and little time. MNSI has also been validated against nerve conduction studies, and was shown to have a high inter- and intra observer reproducibility. In addition, we chose to use the MNSI (in concert with the 10g monofilament) since they offer the advantage of consisting of robust, meaningful, clinically detectable end-points, rather than just electrical neurophysiology.

Figure E1: The Michigan Neuropathy Screening Instrument.

Patient Version

MICHIGAN NEUROPATHY SCREENING INSTRUMENT

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

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

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

Total: _____

MICHIGAN NEUROPATHY SCREENING INSTRUMENT

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

1. Appearance of Feet

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

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

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

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

The monofilament test

We have also used the perception to a 10-g monofilament (applied to 10 positions, the tip of each toe, under 3 metatarsal heads, the plantar surface of the foot and the dorsal space between the first and second toe) as a test for foot insensitivity; an abnormal monofilament test was defined as < 8 correct responses (8). The sensitivity and specificity of the monofilament test to predict amputations or foot ulceration were 62% and 92% respectively (8). Monofilament insensitivity was found to be an independent predictor of foot ulceration and amputations (9-11).

OSA diagnosis

OSA was assessed by a single overnight home-based cardio-respiratory sleep study using a portable multi-channel device (Alice PDX, Philips Respironics) and scored in accordance with the American Academy of Sleep Medicine guidelines(12). Sleep studies with <4 hours of adequate recordings were repeated and excluded if the quality remained poor. Patients with predominantly central sleep apnea (CSA) were excluded (two patients). All sleep studies were double scored. An apnea hypopnea index (AHI) ≥ 5 events/hour was consistent with the diagnosis of OSA(13). OSA severity was assessed based on the AHI, oxygen desaturation index (ODI, the number of oxygen desaturations of ≥ 4% per hour), the time spent with oxygen saturations < 90% and <80% and the nadir oxygen levels during sleep.

Lipid peroxide

Samples were analysed for lipid peroxides using a modification of a method by el-Saadani et al (14).The principle of this assay is based on the ability of lipid peroxides to convert iodide to iodine, which can be then measured using a spectrophotometer.

Make up the reagent mix containing the following: Potassium Phosphate (0.2M, pH 6.2), Potassium Iodide (0.12M), Sodium Azide (0.15µm), Triton X (2g/ml), Alkylbenzyldimethylammonium Chloride (0.1g/ml), Ammonium Molybdate (10µM).

Add 200ul of sample/blank to a cuvette and 2000ul of reagent mix, incubated in the dark for 30 minutes at 25°C and read the cuvette at 365nm in a spectrophotometer.

The concentration of lipid peroxides was calculated using the Beer-Lambert Law using the extinction coefficient for iodine of 24600. All samples and blanks were analysed in duplicate.

Microvascular/endothelial assessment

Microvascular and endothelial assessment was performed on a casually chosen representative patient subset using Laser speckle contrast imaging (Moor Instruments Ltd, Devon, UK)(15). Microvascular blood flow (measured in arbitrary perfusion units (APU) or flux) was assessed at the left mid thigh level under standardized conditions(16). Imaging was performed over 20 minutes. Blood flow was measured at baseline and following heating to 44^0C and following the iontophoresis of 1% acetylcholine (Ach) and 2% sodium nitroprusside (SNP) (5 pulses over 5 minutes). Data are presented as absolute values, conductance (measured as flux divided by mean arterial pressure(MAP) in order to account for differences in BP), and as percentage of maximal dilatation flow as recommended by previous reports(16).

All microvascular function tests were performed between 10am and 4pm in the same room in our research centre. Room temperature was maintained at 22-24 C. The area of interest was exposed and left to adapt to room temperature for 15-20 minutes before starting the test. We have used the middle of the left thigh in all patients to keep consistency. All studies were performed by the same person following the same protocol.

Laser Speckle Contrast Imaging (LSCI) has been reported to have a mean day-to-day coefficient of variation (CV) of 8%, and intra-class correlation coefficients (ICC) of 0.76 (17). We have examined 4 subjects using the LSCI twice one week apart to assess the reliability and reproducibility of our protocol. The ICC for the baseline, heating, acetylcholine and sodium nitroprusside measurements was 0.9, 0.7, 0.9, and 0.8. The CV for the baseline, heating, acetylcholine and sodium nitroprusside measurements were of 7%, 6%, 14%, and 10% respectively. The ICC for the ratios of baseline, acetycholine and sodium nitroprusside responses to maximum vasodilatation were 0.7, 1.0 and 0.9. The CV for the ratios was 10%, 8% and 11% respectively. These results suggest a robust performance of our protocol which is in line of what is reported in the literature.

Statistical methods

Data analysis was performed using SPSS 15.0 software (SPSS Inc, Chicago, USA). Data are presented as mean (SD) or median (IQR) depending on data distribution. Normality testing was performed using histograms and the Shapiro-Wilk test. Independent continuous variables were compared using the Student's t-test or the Mann-Whitney test. Categorical variables were compared using the Chi-square test. The Bonferroni correction was applied to define statistical significance when comparing the components of the MNSIe and MNSIq between patients with and without OSA. Correlations between continuous variables were performed using the Pearson or Spearman tests. Differences between independent groups were assessed by analysis of variance (ANOVA) with post-hoc analysis. Analysis of covariance (ANCOVA), was used to assess the impact of covariates on the differences between several independent groups. To assess whether OSA status is an independent predictor of DPN, multiple logistic regression (forced entry method) was used. To assess the relation between OSA severity and DPN, AHI quartiles and nocturnal hypoxemia measures were used in the logistic regression models. To assess the independent predictors of continuous variables, multiple linear regression (forced entry method) was used. Non-normally distributed data was normalized by log or square root transformation. Variables included in the regression models were based on known outcome-related risk factors and/or variables that differed between patients with and without OSA. In order to further explore the impact of baseline differences on the associations observed, a sub-group of 70 patients with and 70 without OSA were group matched for a variety of risk factors. A p value < 0.05 was considered significant unless stated otherwise.

Multicollinearity and model checking:

We assessed multicollinearity in both multiple linear and logistic regression models using simple correlations between variables plus the tolerance values, and the condition indices. No tolerance values were < 0.1 and no variables had strong correlations ($r > 0.8$). Condition indices > 30 were taken to indicate multicollinearity problems with variances proportions > 0.5 to indicate the

variables involved. The results of these procedures were to identify evidence of collinearity, but sequentially removing variables involved had limited impact on models estimates for the main exposure.

To investigate and deal with collinearity, where condition indices >30, variables with variances proportions > 0.5 were removed individually and sequentially until no variances proportions > 0.5 remained. Overall, whilst collinearity problems were observed for a number of variables in most models, the impact on estimates for the main exposure variable (OSA) were minimal. For example, in the fully adjusted model (outcome: DPN; Predictors: OSA, age, ethnicity, gender, height, diabetes duration, BMI, alcohol intake, HbA1c, insulin use, blood pressure, eGFR, peripheral vascular disease, smoking, total cholesterol, triglycerides, HDL, oral anti-diabetes treatment, individual anti-hypertensive agents, lipid lowering therapy, anti-platelets and recruitment site) there was significant collinearity with 12 condition indicies > 30. After sequentially removing variables from the model and leaving only Ethnicity, Gender, Age, diabetes duration, OSA, eGFR, Insulin use, and BMI there was only one condition index above 30 and all the rest were below 15. This removal of variables from the model changed the odds ratios for having DPN in patients with OSA from 2.95 (95% CI 1.44-6.05) to 2.83 (95% CI 1.46-5.52). This suggests that the collinearity had had limited impact on models estimates for the main exposure (OSA).

Final models presented thus include variables based on the known outcome-related risk factors and/or possible confounders and/or variables that differed between patients with and without OSA , regardless of the presence of collinearity.

In multiple linear regression models, the residuals were examined. In all the models presented, residuals followed a normal distribution with uniform variance and there was no relationship between the residual and the predictor of interest (OSA, apnea hypopnea index or hypoxemia measures depending on the model).

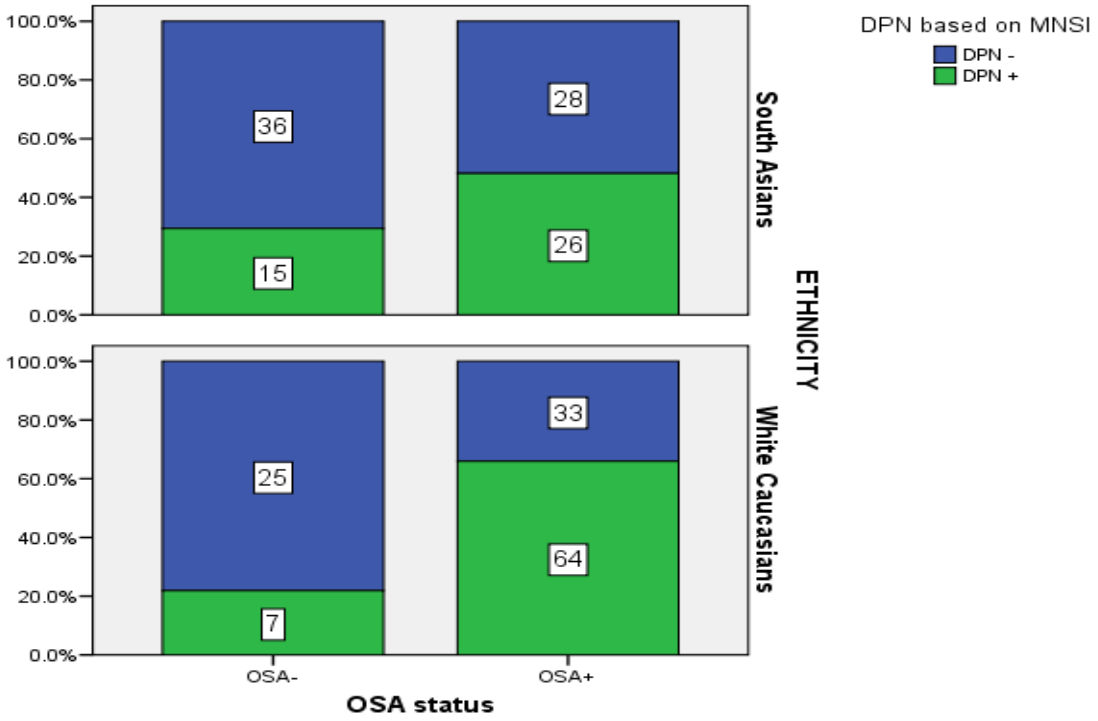
Results

The relationship between OSA and DPN by ethnicity subgroups

The overall prevalence of DPN was significantly higher in OSA+ compared to OSA- patients (60% vs. 27%, p<0.001, respectively). This relationship between OSA and DPN was present irrespective of ethnicity. The prevalence of DPN was higher in patients with OSA whether they were White Caucasians (66% vs. 22%, p<0.001) or South Asians (48% vs. 29%, p=0.049) (**Figure E2**).

The overall prevalence of foot insensitivity was 37%. Foot insensitivity was significantly higher in OSA+ compared to OSA- patients (50% vs. 15%, p<0.001, respectively). The prevalence of an abnormal monofilament test was also more common in patients with OSA whether they were White Caucasians (57% vs. 16%, p<0.001) or South Asians (37% vs. 14%, p=0.006).

Figure E2: The relationship between OSA and DP in ethnicity subgroups.



A multivariate analysis of the relationship between OSA, its severity and DPN:

To assess the relationship between OSA and DPN we used logistic regression models using the forced entry method. The results of the main analysis can be found in Table 3 in the main manuscript.

In order to assess whether any of the OSA metrics or nocturnal hypoxemia parameters are associated with DPN, we have repeated the logistic regression (as in model 1 in Table 3) after removing OSA and inserting AHI (as quartiles), ODI (as quartiles), time spent with oxygen saturation < 80% (as binary variable) and nadir nocturnal oxygen saturations separately into separate models using the forced entry method.

Using AHI quartile 1 (AHI<2.90) as the reference point showed that quartile 2 (AHI 2.90 to 7.59) (OR 2.56, 95% CI 1.10-6.04, p=0.03), quartile 3 (AHI 7.60-16.09) (OR 3.84, 95%CI 1.58-9.33, p=0.003) and quartile 4 (AHI ≥16.01) (OR 3.08, 95%CI 1.20-7.86, p=0.02) were independently associated with DPN. Nadir nocturnal oxygen saturation (OR 0.97, 95% CI 0.93-1.00, p=0.05) was of borderline significance. ODI quartile 3 (ODI 6.65-14.39) was independently associated with DPN when considering quartile 1 (ODI < 2.70) as the reference point (OR 3.03, 95% CI 1.27-7.25, p=0.01). Time spent with oxygen saturation <80% was not independently associated with DPN (OR 1.86, 95%CI 0.93-3.70, p=0.08).

AHI quartiles where also independently associated with abnormal 10g monofilament sensation (p=0.04); with quartile 1 as the reference, quartile 2 (OR 3.22, 95%CI 1.17-8.85, p=0.02), quartile 3 (OR 4.38, 95%CI 1.55-12.36, p=0.005), and quartile 4 (OR 3.16, 95%CI 1.10-9.05, p=0.03) were all independently associated with the “at risk foot”. Only ODI quartile 3 was independently associated with abnormal 10g monofilament perception (OR 3.27, 95% CI 1.23-8.67, p=0.02) when quartile 1 was taken as the reference point. Nadir nocturnal oxygen saturation and time spent with oxygen saturations < 80% were not independent predictors after adjustment.

The relationship between OSA and clinical signs of DPN

To assess the relationship between OSA and the clinical findings on foot examination (MNSIe components), we repeated the logistic regression models as performed in model 1 in **Table 3** , but changed the outcome measure to the aspect of clinical examination of interest.

Using the forced entry method, OSA was independently associated with reduced/absent ankle jerk reflex (OR 2.64, 95% CI 1.35-5.16, p=0.005). OSA was also independently associated with reduced/absent vibration sensation (OR 3.18, 95% CI 1.59-6.39, p=0.001). OSA was not independently associated with having an abnormality on foot inspection (OR 1.82, 95% CI 0.96-3.43, p=0.07). This is in addition to the relationship we described between OSA and the 10g monofilament test, which suggest that OSA is independently associated with different aspects of foot examination. In addition, with quartile 1 being the reference, AHI quartiles were independent predictors of reduced/absent ankle jerk reflex (p=0.03); quartile 2 (OR 2.33, 95%CI 1.01-5.38, p=0.047), quartile 3 (OR 1.94, 95%CI 0.82-4.58, p=0.13), quartile 4 (OR 4.10, 95%CI 1.62-10.31, p=0.003). AHI quartiles were also independent predictors of reduced/absent vibrations sensation (p=0.02); quartile 2 (OR 2.64, 95%CI 1.07-6.50, p=0.04), quartile 3 (OR 4.56, 95%CI 1.78-11.66, p=0.002) and quartile 4 (OR 2.65, 95%CI 1.01-6.94, p=0.048). AHI quartiles were not independently associated with abnormal foot inspection.

Clinical characteristics of patients according to the MNSIe groups

This is referring to **Table 4** in the main manuscript

Table E1: Participants characteristics in relation to MNSIe categories. Data presented as median (IQR) or mean(SD). GFR: Glomerular Filtration Rate.

	Group 1: < 2 (n=90)	Group 2: 2 - < 4 (n=100)	Group 3: ≥ 4 (n=44)	**P value**
Male	51%	54%	80%	0.005
Age (years)	55.0(12.9)	56.7±10.2	62.7±10.5	0.001
Diabetes Duration (years)	10.0 (6.0-12.0)	11.0 (6.0-16.0)	17.0 (11.0-24.7)	< 0.001
Body Mass Index (kg/m^2)	31.6 (27.9-36.4)	33.8 (30.0-38.3)	34.1 (29.2-40.5)	0.032
Alcohol (drinks alcohol)	23%	30.0%	31.8%	0.475
eGFR	90.1(27.1)	87.4±24.4	75.1±26.8	0.009

Clinical characteristics of matched sub-group

Table E2: The characteristics of patients in the matched subgroup in relation to OSA status. Data presented as median (IQR) or mean (SD). GFR: Glomerular Filtration Rate, PVD: Peripheral Vascular Disease. Variables that are matched are highlighted in red. The main aim for this subgroup is to match for BMI and diabetes duration.

	OSA- (n=70)	OSA+ (n=70)	P value
Male	47%	71%	0.003
Caucasians	31%	60%	0.001
Age (years)	55.1(12.2)	59.8(10.2)	0.02
Diabetes Duration (years)	10.0 (6.0-15.0)	10.0 (6.0-15.0)	0.88
Body Mass Index (kg/m^2)	30.0 (26.9-33.9)	31.3 (27.8-33.8)	0.39
Height (cm)	163.6(8.5)	167.7(9.1)	0.006
Systolic blood pressure (mmHg)	125.5 (115.4-136.0)	129.5 (121.5-137.1)	0.08
Diastolic blood pressure (mmHg)	78.5 (70.1-85.6)	78.0 (73.1-82.1)	0.88
HbA1c (%)	7.7 (7.1-8.7)	8.0 (7.0-9.1)	0.68

Total cholesterol (mmol/L)	3.7 (3.3-4.4)	3.6 (3.1-4.2)	0.32
Triglycerides (mmol/L)	1.6 (1.1-2.3)	1.7 (1.2-2.4)	0.76
HDL (mmol/L)	1.2 (1.0-1.4)	1.1 (0.9-1.2)	0.01
Estimated GFR (ml/min/1.73 m^2)	90.3(23.2)	84.7(24.1)	0.16
TSH	1.7 (1.1-2.2)	1.6 (1.0 vs. 2.1)	0.68
Epworth sleepiness score	5 (1-11)	8 (3-13)	0.03
Smoking (current or ex-smoker)	41%	41%	1.0
Alcohol (drinks alcohol)	11%	33%	0.002
Oral anti-diabetes treatment	97%	93%	0.25
Insulin	44%	54%	0.24
Lipid lowering therapy	84%	79%	0.39
Anti-hypertensives	73%	80%	0.32
PVD	1%	4%	0.31

Clinical characteristics of patients undergoing serum nitrotyrosine assessment

Table E3: The characteristics of patients who had undergone serum nitrotyrosine assessment in relation to OSA status. Data presented as median (IQR) or mean (SD). GFR: Glomerular Filtration Rate.

	OSA- (n=29)	OSA+ (n=73)	P value
Male	35%	66%	0.004
Caucasians	55%	67%	0.26
Age (years)	54.8(12.1)	59.0(11.0)	0.10
Diabetes Duration (years)	10.0 (4.5-12.0)	12.0 (6.0-20.0)	0.08
Body Mass Index (kg/m^2)	31.0 (28.1-35.2)	34.6 (31.1-39.8)	0.006
Systolic blood pressure (mmHg)	127.0 (120.5-137.5)	131.5 (123.7-140.5)	0.12

Diastolic blood pressure (mmHg)	78.3(8.9)	77.2(10.1)	0.63
HbA1c (%)	7.4 (6.8-8.5)	8.0 (7.1-9.1)	0.20
Total cholesterol (mmol/L)	3.8 (3.2-4.3)	3.7 (3.2-4.3)	0.77
Triglycerides (mmol/L)	1.6 (1.2-2.3)	1.8 (1.3-2.5)	0.66
HDL (mmol/L)	1.1 (0.9-1.5)	1.1 (0.9-1.2)	0.395
Estimated GFR (ml/min/1.73 m^2)	89.7(24.7)	82.2(27.6)	0.20
Epworth sleepiness score	7.0 (4.0-14.0)	8.0 (3.2-11.7)	1.0
Smoking (current or ex-smoker)	45%	41%	0.73
Alcohol (drinks alcohol)	17%	38%	0.04
Oral anti-diabetes treatment	97%	95%	0.67
Insulin	45%	59%	0.20
Lipid lowering therapy	83%	82.%	0.95
Anti-hypertensives	69%	89%	0.01

Table E4: Comparison of the characteristics of patients who had serum nitrotyrosine measured and those who did not. Data presented as median (IQR) or mean (SD). GFR: Glomerular Filtration Rate.

	Nitrotyrosine not measured (n=132)	Nitrotyrosine measured (n=102)	P value
Male	58%	57%	0.82
Caucasians	49%	64%	0.02
Age (years)	56.7 (11.8)	57.8 (11.4)	0.50
Diabetes Duration (years)	11.0 (6.0-16.0)	11.0 (6.0-16.5)	0.89
Body Mass Index (kg/m^2)	33.7 (7.9)	35.3 (8.8)	0.15
Waist circumference (cm)	112.6 (16.7)	115.2 (16.3)	0.24
Waist hip ratio	1.0 (0.1)	0.9 (0.1)	0.46

Neck circumference (cm)	41.1 (4.7)	41.8 (4.8)	0.25
Systolic blood pressure (mmHg)	128.9 (17.7)	131.7 (16.2)	0.22
Diastolic blood pressure (mmHg)	78.2 (10.4)	77.5 (9.8)	0.64
HbA1c (%)	8.4 (1.5)	8.1 (1.3)	0.07
Total cholesterol (mmol/L)	4.0 (1.1)	3.9 (0.9)	0.69
Triglycerides (mmol/L)	2.0 (1.3)	1.9 (1.1)	0.56
HDL (mmol/L)	1.1 (0.3)	1.1 (0.3)	0.75
Estimated GFR (ml/min/1.73 m^2)	87.5 (26.0)	84.3 (26.9)	0.36
Epworth sleepiness score	7.0 (3.0-13.0)	8.0 (4.0-12.5)	0.95
Smoking (current or ex-smoker)	39%	42%	0.59
Alcohol (drinks alcohol)	24%	32%	0.17
Oral anti-diabetes treatment	92%	95%	0.30
Insulin	52%	55%	0.69
Lipid lowering therapy	85%	82%	0.61
Anti-hypertensives	80%	83%	0.46
Anti-platelets	69%	64%	0.40

Results of lipid peroxide studies

Lipid peroxide levels were higher in patients with DPN (21.14 (3.86-42.48) vs. 12.20 (2.90-24.55), p=0.014). OSA was associated with higher lipid peroxide levels (µM/ml) compared to patients without OSA [18.39 (8.33-37.40) vs. 7.93 (0.81-22.76), p=0.014] which remained significant after adjusting for age, BMI and diabetes duration (p=0.02). Lipid peroxide levels correlated with ODI (r=0.225, p=0.025), time spent with oxygen saturations < 90% (r=0.263, p=0.008), time spent with oxygen saturations < 80% (r=0.229, p=0.022), and nadir nocturnal oxygen saturations (r= -0.236, p=0.019). The correlation with AHI was borderline (r= 0.188, p=0.062). After adjustment for age, BMI

and diabetes duration the correlations between lipid peroxide levels and ODI (r=0.221, p=0.031) and nadir nocturnal oxygen saturations (r=-0.220, p=0.032) remained significant**.** After adjustment for age, gender, ethnicity, diabetes duration, BMI, HbA1c, BMI and mean arterial pressure using multiple linear regression there was no independent association between OSA, OSA metrics or hypoxemia measures and lipid peroxide levels. Only HbA1c was independently associated with lipid peroxide (B=0.61, p=0.001).

Clinical characteristics of patients who had undergone microvascular assessment

Table E5: The characteristics of patients who had undergone microvascular assessment in relation to OSA status. Data presented as median (IQR) or mean (SD). GFR: Glomerular Filtration Rate.

	OSA- (n=24)	OSA+ (n=47)	P value
Male	38%	68%	0.01
Caucasians	33%	64%	0.02
Age (years)	56.0(10.1)	60.6(11.3)	0.09
Diabetes Duration (years)	10.0 (7.2-15.7)	14.0 (6.0-20.0)	0.27
Body Mass Index (kg/m^2)	30.7 (28.0-35.8)	34.4 (31.1-36.6)	0.06
Systolic blood pressure (mmHg)	125.5 (112.5-132.6)	132.0 (121.5-139.0)	0.08
Diastolic blood pressure (mmHg)	77.6(9.106)	76.9(9.5)	0.24
HbA1c (%)	7.4 (6.6-8.4)	7.6 (7.1-8.6)	0.38
Total cholesterol (mmol/L)	3.7 (3.3-4.4)	3.7 (3.1-4.1)	0.28
Triglycerides (mmol/L)	1.6 (1.0-2.7)	1.7 (1.2-2.2)	0.78
HDL (mmol/L)	1.1 (0.9-1.5)	1.1 (0.9-1.3)	0.45
Estimated GFR ($ml/min/1.73\ m^2$)	86.1(25.1)	78.8(24.7)	0.78
Epworth sleepiness score	7.0 (3.2-13.5)	8.5 (3.0-12.2)	0.66
Smoking (current or ex-smoker)	46%	34%	0.33

Alcohol (drinks alcohol)	13%	36%	0.04
Oral anti-diabetes treatment	96%	89%	0.35
Insulin	46%	53%	0.56
Lipid lowering therapy	88%	87%	0.98
Anti-hypertensive agents	75%	89%	0.11

Table E6: Comparison of the characteristics of patients who had Laser Speckle Contrast Imaging/microvascular regulation assessed and those who did not. Data presented as median (IQR) or mean (SD). GFR: Glomerular Filtration Rate.

	Microvascular assessment not preformed (n=156)	Microvascular assessment preformed (n=71)	P value
Male	58%	58%	0.93
Caucasians	55%	54%	0.89
Age (years)	56.1 (11.9)	59.1 (11.0)	0.08
Diabetes Duration (years)	10.0 (6.0-15.8)	11.5 (7.0-20.0)	0.08
Body Mass Index (kg/m^2)	33.8 (7.3)	34.6 (9.2)	0.51
Waist circumference (cm)	113.8 (17.0)	112.2 (14.8)	0.49
Waist hip ratio	1.0 (0.1)	1.0 (0.1)	0.15
Neck circumference (cm)	41.6 (4.9)	40.8 (4.3)	0.24
Systolic blood pressure (mmHg)	130.0 (16.6)	130.1 (18.7)	0.97
Diastolic blood pressure (mmHg)	78.1 (10.5)	77.2 (9.3)	0.51
HbA1c (%)	8.4 (1.4)	8.0 (1.4)	0.02
Total cholesterol (mmol/L)	3.9 (1.0)	3.8 (1.0)	0.39
Triglycerides (mmol/L)	2.1 (1.3)	1.9 (1.2)	0.34

HDL (mmol/L)	1.1 (0.3)	1.2 (0.33)	0.48
Estimated GFR (ml/min/1.73 m^2)	88.2 (27.1)	81.2 (24.9)	0.07
Epworth sleepiness score	7.0 (4.0-12.8)	8.0 (3.0-12.2)	0.77
Smoking (current or ex-smoker)	39.7%	38.0%	0.81
Alcohol (drinks alcohol)	27.6%	28.2%	0.93
Oral anti-diabetes treatment	93.6%	91.5%	0.58
Insulin	55.1%	50.7%	0.54
Lipid lowering therapy	81.4%	87.3%	0.27
Anti-hypertensives	78.8%	84.5%	0.32
Anti-platelets	64.7%	70.4%	0.40

The adjusted analysis for the relationship between microvascular function and OSA metrics:

Table E7: The adjusted analysis of the impact of OSA on microvascular blood flow and endothelial function in patients with type 2 diabetes. Data presented as B and p value. The analysis was performed using blood flow as the outcome and ethnicity, gender, age at diabetes diagnosis, diabetes duration, BMI and OSA (and its metrics) as the independent predictors. Blood flux was measured in arbitrary perfusion units (APU). Conductance is calculated by dividing flux by the mean arterial pressure. Multiple linear regression, the *forced entry method* was used to conduct this analysis. Ach: Acetylcholine, SNP: Sodium nitroprusside.

	OSA	AHI	ODI	Nadir nocturnal oxygen saturation
Conductance				
Baseline	B=-0.22, p <0.001	B= -0.21, p= 0.001	B= -0.14, p= 0.03	B= 0.12, p= 0.34
Heating	B= -0.04, p= 0.42	B= -0.05, p= 0.34	B=-0.03, p=0.49	B= 0.04, p= 0.66
Ach	B= -0.1, p= 0.10	B= -0.05, p= 0.46	B= -0.02, p= 0.80	B= -0.06, p= 0.65
SNP	B= -0.25, p <0.001	B= -0.21, p= 0.005	B= -0.20, p= 0.006	B= 0.29, p= 0.047
Flux in relation to maximum vasodilatation				
Baseline	B= -0.19, p= 0.002	B= -0.16, p= 0.008	B= -0.11, p= 0.09	B= 0.08, p= 0.51
Ach	B= -0.06, p= 0.26	B= -0.002, p= 0.97	B= 0.02, p= 0.78	B= -0.10, p= 0.40
SNP	B= -0.21, p= 0.001	B= -0.16, p= 0.01	B= -0.17, p= 0.009	B= 0.25, p= 0.05

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