

# openheart Subclinical sleep apnoea and plasma levels of endothelin-1 among young and healthy adults

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## ABSTRACT

**Objective:** Obstructive sleep apnoea (OSA) is a risk factor for vascular disease and other adverse outcomes. These associations may be at least partly due to early endothelin-1 (ET-1)-mediated endothelial dysfunction (ED). Therefore, we assessed the relationships between subclinical sleep apnoea and plasma levels of ET-1.

**Methods:** We performed a population-based study among 1255 young and healthy adults aged 25–41 years. Cardiovascular disease, diabetes or a body mass index >35 kg/m<sup>2</sup> were exclusion criteria. Plasma levels of ET-1 were measured using a high-sensitivity, single-molecule counting technology. The relationships between subclinical sleep apnoea (OSA indices: respiratory event index (REI), oxygen desaturation index (ODI), mean night-time blood oxygen saturation (SpO<sub>2</sub>)) and ET-1 levels were assessed by multivariable linear regression analysis.

**Results:** Median age of the cohort was 35 years. Median ET-1 levels were 2.9 (IQR 2.4–3.6) and 2.5 pg/mL (IQR 2.1–3.0) among patients with (n=105; 8%) and without subclinical sleep apnoea (REI 5–14), respectively. After multivariable adjustment, subclinical sleep apnoea remained significantly associated with plasma levels of ET-1 ( $\beta=0.13$  (95% CI 0.06 to 0.20)  $p=0.0002$  for a REI 5–14;  $\beta=0.10$  (95% CI 0.03 to 0.16)  $p=0.003$  for an ODI $\geq 5$ ). Every 1% decrease in mean night-time SpO<sub>2</sub> increased ET-1 levels by 0.1 pg/mL, an association that remained significant after multivariable adjustment ( $\beta=0.02$  (95% CI 0.003 to 0.033)  $p=0.02$ ).

**Conclusions:** In this study of young and healthy adults, we found that participants with subclinical sleep apnoea had elevated plasma ET-1 levels, an association that was due to night-time hypoxaemia. Our results suggest that ED may already be an important consequence of subclinical sleep apnoea.

## INTRODUCTION

Obstructive sleep apnoea (OSA) is a highly prevalent disorder, often underdiagnosed and significantly associated with adverse outcomes, including hypertension,<sup>1–5</sup> stroke,<sup>6</sup>

## KEY QUESTIONS

### What is already known about this subject?

► Prior research has demonstrated a tight relationship of sleep apnoea with hypertension and other cardiovascular events among patients with clinical sleep apnoea syndrome.

### What does this study add?

► This is the largest population-based study among young and healthy adults investigating the relationship of subclinical sleep apnoea and plasma endothelin-1 (ET-1) levels. This study demonstrates that apnoea-induced hypoxaemia is significantly related to increased ET-1 levels, suggesting that endothelial dysfunction may be an important consequence of obstructive sleep apnoea already at a subclinical stage.

### How might this impact on clinical practice?

► This association is independent of other important cardiovascular risk factors (eg, smoking, body mass index, blood pressure, renal function, high-sensitivity C reactive protein, glycated haemoglobin, low-density lipoprotein, high-density lipoprotein) and supports the importance of even subclinical sleep apnoea in the development of cardiovascular disease.

coronary artery disease<sup>7–8</sup> and sudden death.<sup>9–10</sup>

Although the precise pathophysiology linking OSA with vascular disease remains to be delineated, endothelial dysfunction (ED) may play a central role in this association.<sup>11–12</sup> ED is characterised by reduced vasodilatation and enhanced vasoconstriction, as well as by increased prothrombotic and inflammatory activity.<sup>13</sup> In this context, endothelin-1 (ET-1) has been implicated as an important factor in the development of vascular dysfunction and cardiovascular disease,<sup>14–17</sup> suggesting that ET-1 may be an easily measurable surrogate for endothelial function. ET-1 is released by endothelial cells and increases



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vascular tone by acting on underlying smooth muscle cells in a paracrine manner.

The resulting imbalance between vasodilator and vasoconstrictor endothelial mediators with overproduction of ET-1 increases vascular permeability and decreases antithrombotic activity, thereby promoting the occurrence of vascular disease.<sup>18 19</sup> ED is therefore considered an early state of atherosclerosis and cardiovascular disease<sup>20</sup> and may constitute a potential link that relates OSA with adverse cardiovascular outcomes. Preliminary evidence from animal models of sleep apnoea and data from patients with OSA support this hypothesis, but data from large-scale population-based studies are currently unavailable.<sup>21</sup> In addition, it remains unclear whether impaired vascular function is an early manifestation of OSA, or whether it may be a consequence of OSA-related comorbidities such as hypertension or obesity. Finally, quantification of endothelial function has been difficult and time-consuming, particularly in large-scale population-based studies. The emergence of new ET-1 assays facilitates its measurement and enables easier ET-1 quantification in large populations.<sup>22</sup>

To address some of these issues, we assessed the relationships between subclinical sleep apnoea (respiratory event index (REI)=5–14, oxygen desaturation index (ODI)  $\geq 5$ , mean night-time blood oxygen saturation (SpO<sub>2</sub>), and per cent of sleeping time with SpO<sub>2</sub><90%) and plasma levels of ET-1 in a large cohort of young and healthy adults.

## METHODS

### Study participants

The genetic and phenotypic determinants of blood pressure (BP) and other cardiovascular risk factors (GAPP) study is an ongoing prospective population-based cohort study in the Principality of Liechtenstein. Details about study design and methods have been described previously.<sup>23</sup> Briefly, between May 2010 and December 2013, all inhabitants of the Principality of Liechtenstein aged 25–41 years were invited to participate in GAPP. Exclusion criteria were a body mass index (BMI)  $>35$  kg/m<sup>2</sup>, prevalent cardiovascular disease, known and treated sleep apnoea syndrome, renal failure, current intake of antidiabetic drugs or any other severe illnesses.

Of the 2170 included participants, 1415 (65%) participants underwent night-time pulse oximetry with nasal flow measurement. To investigate effects of subclinical sleep apnoea, participants with a REI $\geq 15$  per hour (n=25) were excluded to minimise confounding due to potentially undiagnosed OSA syndrome. Further, we excluded 135 participants due to missing or incomplete sleep study parameters (n=57), missing ambulatory BP recordings (n=57) or other missing covariates (n=21), such that 1255 participants remained for the current analyses. Written informed consent was obtained from each participant. The local ethics committee approved the study protocol.

### Night-time pulse oximetry

Night-time pulse oximetry with nasal flow measurement was performed using the validated ApneaLink (ResMed, San Diego, California, USA) device<sup>24 25</sup> to obtain information on night-time oxygen saturation, apnoeas and hypopnoeas. Sleep study analyses were based on automatic scoring analysis. The sleep study was performed at the patient's home. Individuals were instructed by a trained study nurse on how to put on the nasal cannula and the oximetry probe. An apnoea was defined as a reduction of nasal airflow of  $\geq 80\%$  compared with baseline for  $\geq 10$  s. Hypopnoea was defined as a reduction of nasal airflow of  $\geq 30\%$  compared with baseline followed by a simultaneous decrease in oxygen saturation  $\geq 4\%$ . The apnoea index was defined as the number of apnoeas per hour. The REI was defined as the number of apnoeas and hypopnoeas per hour of sleep according to the 2015 updated version of the American Academy of Sleep Medicine (AASM) criteria.<sup>26–28</sup> Subclinical sleep apnoea was defined to be present when the REI was 5, but  $<15$  per hour. The ODI was defined as the number of oxygen desaturations  $\geq 4\%$  per hour of sleep.<sup>29</sup> An ODI of  $\geq 5$  per hour was considered to be abnormal. In addition to REI and ODI, mean night-time SpO<sub>2</sub> and the per cent of total sleep time with SpO<sub>2</sub><90% were calculated.

### BP assessment

The 24 hours BP measurements were obtained using a validated device (BR-102 plus, Schiller AG, Switzerland). Trained study nurses handled the devices which were programmed to obtain BP measurements every 15 min during daytime and every 30 min during night-time. If participants had  $<80\%$  of valid measurements, the BP study was repeated whenever possible. BP measurements were only included in the analysis if  $\geq 25$  daytime and  $\geq 8$  night-time measurements were available. Daytime and night-time periods were individually defined through a 24-hour diary completed by each participant. Daytime hypertension was defined as mean systolic BP $\geq 135$  mm Hg and/or mean diastolic BP $\geq 85$  mm Hg,<sup>30</sup> respectively. Night-time hypertension was defined as mean systolic BP $\geq 120$  mm Hg and/or diastolic BP $\geq 70$  mm Hg, respectively.<sup>30</sup> Individuals currently taking antihypertensive medication were considered to be hypertensive.

### Blood sampling

Fasting venous blood samples were obtained from each participant and immediately centrifuged. Blood aliquots were immediately stored at  $-80^{\circ}\text{C}$ . A research-use high-sensitive, single-molecule counting assay (Erenna Immunoassay System, Singulex, Alameda, California, USA) was used to measure ET-1 from frozen EDTA plasma samples.<sup>22</sup> The ET-1 assay's limits of blank and quantification were 0.07 and 0.33 pg/mL, respectively. Interassay coefficients of variation were 7% at an ET-1 concentration of 1.2 pg/mL and 6% at an ET-1 concentration of 1.8 pg/mL.

Plasma levels of glucose, creatinine, high-sensitive C reactive protein (hs-CRP), insulin, triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol were analysed on a Roche Cobas 6000 analyser (F. Hoffmann La Roche, Switzerland) using fresh blood samples.<sup>23</sup> Glycated haemoglobin (HbA1c) was analysed using high-performance liquid chromatography (Bio-Rad D-10, Bio-Rad Laboratories AG, Switzerland).<sup>23</sup> Pre-diabetes was defined as HbA1c between 5.7% and 6.4%. For the estimation of the glomerular filtration rate (eGFR), we used the creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.<sup>23</sup>

### Assessment of other study variables

Questionnaires were used to obtain information about personal, medical, lifestyle and nutritional factors. Smoking status was self-assessed using three categories: current, past and never. BMI was calculated as body weight in kilogram divided by height in metres squared.

### Statistical analysis

Distributions of continuous variables were evaluated using skewness, kurtosis and inspection of the histogram. Continuous variables were presented as medians (IQRs) and compared with the Wilcoxon rank sum test. Categorical variables were compared using  $\chi^2$  tests. Owing to its distribution, ET-1 was used as a log-transformed variable in all analyses. Multivariable linear regression analyses were constructed using ET-1 as the outcome variable. Separate models were used to evaluate different sleep apnoea parameters, including REI, ODI, mean night-time SpO<sub>2</sub> and per cent of sleeping time with a SpO<sub>2</sub><90%. Age, sex and BMI adjusted models were further adjusted for a predefined set of covariates, including smoking status, HDL cholesterol, LDL cholesterol, TG, eGFR, HbA1c, mean systolic and diastolic ambulatory BP and hs-CRP.

Subgroup analyses were performed for age, sex, BMI, eGFR, hs-CRP, pre-diabetes, TG, smoking status, and daytime and/or night-time hypertension. Differences across subgroups were assessed by including multiplicative interaction terms in the non-stratified models. All analyses were performed using SAS V.9.4 (SAS Institute,

**Table 1** Baseline characteristics according to subclinical sleep apnoea

Characteristic n=1255	REI<5 n=1150 (92%)	REI 5–14 n=105 (8%)	p Value*
Age	35 (30–39)	37 (33–39)	0.02
Males (%)	505 (44%)	85 (81%)	<0.0001
Body mass index (kg/m <sup>2</sup> )	23.9 (21.7–26.5)	27.0 (23.5–30.1)	<0.0001
Endothelin (pg/mL)	2.5 (2.1–3.0)	2.9 (2.4–3.6)	<0.0001
eGFR, CKD-EPI (mL/min/1.73 m <sup>2</sup> )	113 (105–119)	110 (101–118)	0.02
Creatinine ( $\mu$ mol/L)	67 (57–76)	74 (67–83)	<0.0001
High-sensitive CRP (mg/L)	0.9 (0.5–1.9)	1.4 (0.6–3.2)	0.007
HbA1c (%)	5.4 (5.1–5.6)	5.4 (5.2–5.7)	0.17
24-hour blood pressure (mm Hg)			
Systolic	122 (114–130)	128 (119–136)	<0.0001
Diastolic	77 (72–82)	81 (76–86)	<0.0001
Daytime blood pressure (mm Hg)			
Systolic	126 (118–133)	133 (123–142)	<0.0001
Diastolic	80 (75–86)	85 (80–89)	<0.0001
Night-time blood pressure (mm Hg)			
Systolic	107 (101–115)	112 (107–120)	<0.0001
Diastolic	65 (60–70)	68 (63–74)	<0.0001
Office blood pressure (mm Hg)			
Systolic	120 (111–127)	128 (120–133)	<0.0001
Diastolic	78 (73–84)	84 (78–86)	<0.0001
Low-density lipoprotein (mmol/L)	2.8 (2.3–3.5)	3.2 (2.6–3.9)	<0.0001
High-density lipoprotein (mmol/L)	1.5 (1.3–1.8)	1.3 (1.1–1.5)	<0.0001
Triglycerides (mmol/L)	0.8 (0.6–1.1)	1.1 (0.8–1.6)	<0.0001
Smoking			
Current	244 (21%)	32 (30%)	0.01
Past	262 (23%)	22 (21%)	0.09
Never	644 (56%)	51 (49%)	0.03

Values are median (IQRs) or counts (percentages).

\*Based on Kruskal-Wallis tests for continuous variables and  $\chi^2$  tests for categorical variables.

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CRP, C reactive protein; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; REI, respiratory event index.

Cary, North Carolina, USA). A *p* value <0.05 was used to indicate statistical significance.

## RESULTS

### Baseline characteristics

Baseline characteristics stratified by the presence or absence of subclinical sleep apnoea are presented in [table 1](#). Of 1255 participants, 590 (47%) were male. Subclinical sleep apnoea was found among 105 (8%) participants according to a REI score between 5 and 14, 85 (81%) men and 20 (19%) women (*p*<0.0001). Median ET-1 levels were 2.9 pg/mL (IQR 2.4–3.6) and 2.5 pg/mL (IQR 2.1–3.0) among patients with and without subclinical sleep apnoea (*p*<0.0001), respectively. Patients with subclinical sleep apnoea were significantly older (37 (IQR 33–39) vs 35 years (IQR 30–39)

*p*=0.02), and had a higher BMI (27 vs 24 kg/m<sup>2</sup>, *p*<0.0001) and higher hs-CRP levels (1.4 (IQR 0.6–3.2) vs 0.9 mg/L (IQR 0.5–1.9) *p*=0.0007). They also had higher 24-hour ambulatory systolic (128 (IQR 119–136) vs 122 mm Hg (IQR 114–130) *p*<0.0001) and diastolic (81 (IQR 76–86) vs 77 mm Hg (IQR 72–82) *p*<0.0001) BP levels, a higher prevalence of current smokers (30% vs 21%, *p*=0.01), and lower HDL (1.3 (IQR 1.1–1.5) vs 1.5 mmol/L (IQR 1.3–1.8) *p*<0.0001) and higher LDL levels (3.2 (IQR 2.6–3.9) vs 2.8 mmol/L (IQR 2.3–3.5) *p*<0.0001), as shown in [table 1](#).

### Results of sleep study

The results of the sleep study according to subclinical sleep apnoea are presented in [table 2](#). All sleep apnoea indices were significantly different between both groups.

**Table 2** Results of sleep study according to subclinical sleep apnoea\*

Characteristic n=1255	REI<5 n=1150 (92%)	REI 5–14 n=105 (8%)	<i>p</i> Value†
REI	1 (0–2)	8 (6–10)	
Oxygen desaturation index	1 (0–2)	8 (6–11)	<0.0001
Oxygen desaturation index ≥5 (%)	36 (2%)	88 (71%)	<0.0001
Mean night-time SpO <sub>2</sub> (%)	95 (94–96)	93 (92–95)	<0.0001
Per cent of time with SpO <sub>2</sub> <90% (%)	0.2 (0–1.2)	2.8 (0.6–10.5)	<0.0001
Apnoeas per hour	0 (0–1)	2 (1–4)	<0.0001
Hypopnoeas per hour	0 (0–1)	5 (3–6)	<0.0001
Sleep duration (hours)	7.3 (6.5–8.0)	7.3 (6.3–8.0)	0.8
Mean heart rate (bpm)	62 (57–68)	62 (58–71)	0.13
Mean breathing rate (breaths/min)	13 (10–15)	12 (9–14)	0.01

Values are median (IQRs), counts or percentages.

\*Subclinical sleep apnoea was defined as REI≥5, but <15.

†Based on Kruskal-Wallis tests for continuous variables.

REI, respiratory event index; SpO<sub>2</sub>, blood oxygen saturation.

**Table 3** Sleep apnoea indices and plasma levels of endothelin-1

n=1255	β (95% CI)	<i>p</i> Value
	Respiratory event index 5–14	
Crude model	0.17 (0.10 to 0.24)	<0.0001
Age, sex and BMI adjusted model	0.14 (0.07 to 0.21)	<0.0001
Multivariable model*	0.13 (0.06 to 0.20)	0.0002
	Oxygen-desaturation index ≥5	
Crude model	0.14 (0.08 to 0.20)	<0.0001
Age, sex and BMI adjusted model	0.11 (0.04 to 0.17)	0.0012
Multivariable model*	0.10 (0.03 to 0.16)	0.003
	Mean night-time SpO <sub>2</sub> (%)	
Crude model	−0.034 (−0.046 to −0.021)	<0.0001
Age, sex and BMI adjusted model	−0.022 (−0.036 to −0.007)	0.003
Multivariable model*	−0.018 (−0.033 to −0.003)	0.02
	Per cent of time with SpO <sub>2</sub> <90%	
Crude model	0.021 (0.011 to 0.032)	<0.0001
Age, sex and BMI adjusted model	0.015 (0.004 to 0.025)	0.008
Multivariable model*	0.012 (0.002 to 0.023)	0.02

Multivariable linear regression models. Endothelin-1 was log-transformed. Per cent of sleeping time with SpO<sub>2</sub><90% was log-transformed.

\*Adjustment for age, sex, BMI, high-sensitivity C reactive protein, glomerular filtration rate, HbA1c, systolic and diastolic ambulatory blood pressure, low-density lipoprotein, high-density lipoprotein, triglycerides and smoking status.

β, β coefficient; BMI, body mass index; HbA1c, glycated haemoglobin; SpO<sub>2</sub>, blood oxygen saturation.

Patients with subclinical sleep apnoea had a lower breathing rate, whereas sleep duration and mean heart rate were not different.

### Subclinical sleep apnoea and plasma ET-1 levels

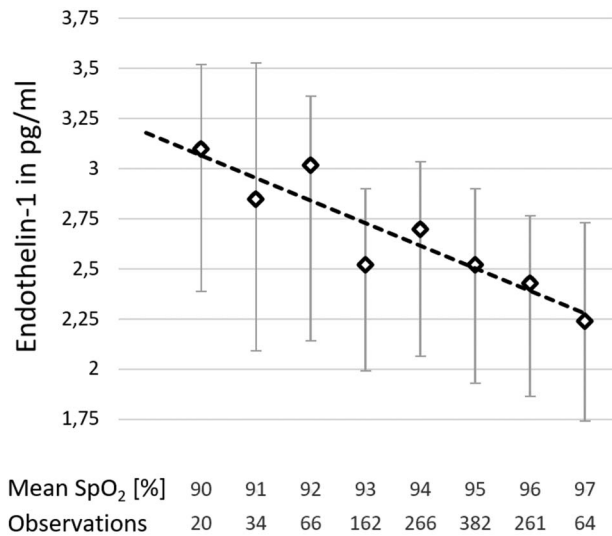
Linear regression models on the relationship between OSA indices and ET-1 levels are shown in table 3. In unadjusted models, subclinical sleep apnoea was significantly associated with log-transformed ET-1 ( $\beta=0.17$  (95% CI 0.10 to 0.24)  $p<0.0001$  for a REI 5–14). Multivariable adjustment slightly attenuated this relationship, but subclinical sleep apnoea remained strongly associated with ET-1 levels ( $\beta=0.13$  (95% CI 0.06 to 0.20)  $p=0.0002$  for a REI 5–14). A similar robust association was found between ET-1 levels and an elevated ODI, present in 124 participants (multivariable-adjusted  $\beta=0.10$  (95% CI 0.03 to 0.16)  $p=0.003$ ). Every 1% decrease in mean night-time SpO<sub>2</sub> resulted in an increase in the plasma concentration of ET-1 of 0.1 pg/mL, as shown in figure 1. This association remained significant after multivariable adjustment ( $\beta=0.02$  (95% CI 0.003 to 0.033)  $p=0.02$ ) (table 3). In addition, the per cent of sleeping time with SpO<sub>2</sub><90% was also associated with plasma levels of ET-1 (multivariable-adjusted  $\beta=0.012$  (95% CI 0.002 to 0.023)  $p=0.02$ ). Figure 2 summarises the effect sizes of the different sleep apnoea indices on log-transformed plasma levels of ET-1.

### Subgroup analyses

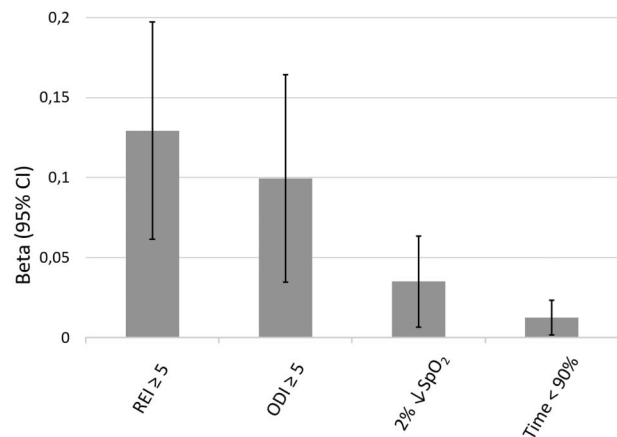
Subgroup analyses are presented in table 4. We found a borderline significant sex by REI interaction ( $p$  value 0.049), suggesting that the association between subclinical sleep apnoea and ET-1 may be somewhat stronger among women ( $\beta=0.27$  (95% CI 0.12 to 0.43)  $p=0.0005$  for a REI 5–14). No evidence for effect modification was found for the other parameters investigated, including age, BMI, pre-diabetes, smoking status, hypertension, eGFR, hs-CRP and TG.

## DISCUSSION

To the best of our knowledge, this is the first large population-based study investigating the relationship between subclinical sleep apnoea and plasma levels of ET-1 among young and healthy adults. In our analysis, we found a strong and independent relationship between several OSA indices and plasma levels of ET-1 that was mainly mediated by hypoxaemia subsequent to apnoeas and hypopnoeas. All parameters related to intermittent or continuous hypoxaemia were significantly associated with ET-1 levels, including mean night-time SpO<sub>2</sub>, per cent of sleeping time with SpO<sub>2</sub><90%, ODI $\geq$ 5 and REI 5–14. According to these findings, our data suggest that ED might already be present in subclinical sleep apnoea and that it may be directly caused by hypoxaemia. It is important to re-emphasize that confounding effects of undiagnosed OSA were minimised by excluding all participants with REI $\geq$ 15.



**Figure 1** Median levels of plasma ET-1 according to mean night-time SpO<sub>2</sub>. Dashed line=regression line adjusted for age, sex, body mass index, high-sensitivity C reactive protein, glomerular filtration rate, HbA1c, systolic and diastolic ambulatory blood pressure, low-density lipoprotein, high-density lipoprotein, triglycerides and smoking status. ET-1, endothelin-1; HbA1c, glycated haemoglobin; Mean SpO<sub>2</sub>, mean night-time blood oxygen saturation.



**Figure 2** Sleep apnoea indices and plasma levels of endothelin-1. Multivariable linear regression models. Endothelin-1 was log-transformed. Adjustment for age, sex, body mass index, high-sensitivity C reactive protein, glomerular filtration rate, HbA1c, systolic and diastolic ambulatory blood pressure, low-density lipoprotein, high-density lipoprotein, triglycerides and smoking status. SpO<sub>2</sub>, mean night-time blood oxygen saturation. The 2% ↓ SpO<sub>2</sub> defined as the reduction of the mean night-time SpO<sub>2</sub> by 2%, for example, from 97% to 95%.  $\beta$ ,  $\beta$  coefficient; HbA1c, glycated haemoglobin; ODI, oxygen desaturation index; REI, respiratory event index; Time <90%, per cent of sleeping time with SpO<sub>2</sub><90%.

If we assume that ET-1 is a direct marker of ED,<sup>17 31 32</sup> then taking into account competing risk factors for ED such as hypertension, diabetes and obesity is of utmost

**Table 4** Subclinical sleep apnoea and plasma levels of ET-1, stratified by selected baseline characteristics

n=1255	$\beta$ (95% CI)	p Value	p Value for interaction
Age			
<35 years (n=599)			0.26
Multivariable model*	0.17 (0.05 to 0.28)	0.004	
$\geq$ 35 years (n=656)			
Multivariable model*	0.10 (0.01 to 0.18)	0.03	
Sex			
Men (n=590)			0.049
Multivariable model*	0.10 (0.03 to 0.17)	0.008	
Women (n=665)			
Multivariable model*	0.27 (0.12 to 0.43)	0.0005	
BMI			
$\geq$ 25 kg/m <sup>2</sup> (n=507)			0.16
Multivariable model*	0.15 (0.06 to 0.24)	0.001	
<25 kg/m <sup>2</sup> (n=748)			
Multivariable model*	0.08 (-0.03 to 0.19)	0.17	
Pre-diabetes			
Yes (n=278)			0.28
Multivariable model*	0.11 (-0.01 to 0.23)	0.06	
No (n=977)			
Multivariable model*	0.14 (0.05 to 0.22)	0.001	
Smoking status			
Current Smoking* (n=276)	0.17 (0.03 to 0.31)	0.015	0.09
Past Smoking* (n=284)	0.02 (-0.13 to 0.16)	0.83	
Never Smoking* (n=695)	0.18 (0.08 to 0.28)	0.0003	
Hypertension			
Yes (n=433)			0.22
Multivariable model*	0.10 (0.01 to 0.19)	0.03	
No (n=822)			
Multivariable model*	0.16 (0.06 to 0.27)	0.002	

ET-1 was log-transformed. Hypertension was defined as mean ambulatory daytime blood pressure  $\geq$ 135/85 mm Hg. Pre-diabetes was defined as HbA1c $\geq$ 5.7%.

\*Adjustment for age, sex, BMI, high-sensitivity C reactive protein, glomerular filtration rate, mean systolic and diastolic ambulatory blood pressure, low-density lipoprotein, high-density lipoprotein, triglycerides, HbA1c and smoking status as appropriate.

$\beta$ ,  $\beta$  coefficient; BMI, body mass index; ET-1, endothelin-1; HbA1c, glycated haemoglobin.

importance, because of the multifactorial and complex genesis of vascular dysfunction. It is therefore important to emphasise that our results remained highly significant after comprehensive multivariable adjustment. It is also noteworthy that our results were obtained in a cohort of young and healthy adults with very low plasma ET-1 levels and a low burden of comorbidities. Our findings suggest that even subclinical forms of OSA may impair endothelial function. In this context, our findings are in accordance with prior basic experimental studies showing that intermittent hypoxaemia impairs endothelial function<sup>33–35</sup> and increases the production of ET-1 in cultured human endothelial cells<sup>14</sup> as well as in vivo among untreated patients with severe OSA.<sup>36</sup> Kato *et al*<sup>37</sup> showed an impairment of resistance-vessel endothelium-dependent vasodilation among eight middle-aged patients with OSA compared with nine control patients underscoring the relationship between OSA and hypertension. Finally, the magnitude of the association between subclinical sleep apnoea and ET-1 should also be stressed. We previously showed that in this cohort, elevated ET-1 levels were strongly related to currently

smoking cigarettes.<sup>38</sup> The  $\beta$  coefficients for subclinical sleep apnoea exceeded those for smoking, underscoring the potential importance of subclinical sleep apnoea in the pathogenesis of cardiovascular disease.

Sex-specific analyses suggested that the relationship between subclinical sleep apnoea and ET-1 levels seems to be stronger among women. While the p value of interaction was of borderline significance, there are recent studies also reporting sex-specific differences in the relationship between OSA and plasma levels of cardiac troponin T.<sup>39</sup> This study showed a significant association between a diagnosis of OSA and plasma levels of troponin T among middle-aged women but not men.<sup>39</sup> These findings may suggest that the effect of OSA may be particularly deleterious among women, but further studies are needed to confirm this hypothesis.

The major strengths of this study include its population-based design and the large number of well-characterised young and healthy participants undergoing night-time pulse oximetry with nasal airflow measurement. In this population, exposure to environmental confounders is relatively short and individuals with pre-

existing cardiovascular comorbidities were excluded. There are also some potential limitations that should be taken into consideration for the interpretation of our results. First, the cross-sectional design does not allow us to draw causal inferences or to assess the directionality of the observed effects. However, it is very unlikely that increased ET-1 levels induce night-time hypoxaemia, such that the latter seems to be the driving force for elevated ET-1 levels.<sup>40</sup> Second, the generalisability of our results to other populations is uncertain. Third, we did not use polysomnography as the gold standard diagnostic tool for sleep apnoea. However, it has been shown that combining pulse oximetry with nasal flow measurement has a high sensitivity and specificity for detecting patients with sleep apnoea.<sup>24–25</sup> Finally, we did not directly measure endothelial function in our population. However, previous studies showed a good correlation between plasma ET-1 levels and endothelial function.<sup>18</sup>

In conclusion, this study of young and healthy adults provides strong evidence of an independent relationship between subclinical sleep apnoea and plasma levels of ET-1. Our results further suggest that the increased ET-1 levels might be directly related to apnoea-induced hypoxaemia and that this phenomenon is already present in young and healthy individuals at a subclinical stage. These findings are independent of other important cardiovascular risk factors (eg, smoking, BMI, BP, renal function) and may, at least in part, explain the tight relationship of clinical sleep apnoea syndrome with hypertension and other adverse cardiovascular events.

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**Competing interests** JT and JE are employees of Singulex and hold stock options in Singulex. They are also co-inventors on patents filed in regard to assay methods for measuring endothelin-1 levels.

**Patient consent** Obtained.

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