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# Treatment of obstructive sleep apnoea leads to improved microvascular endothelial function in the systemic circulation.

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## ABSTRACT

*Background.* Obstructive sleep apnoea (OSA) is a common and potentially reversible cause of systemic hypertension. The mechanisms whereby OSA leads to hypertension and the effects of treatment on arterial function, however, are not well established. We therefore assessed microvascular arterial endothelial and smooth muscle function in subjects with OSA, before and after treatment with continuous positive airways pressure (CPAP).

*Methods and Results.* Ten subjects aged 49 ±8 years with at least moderately severe OSA had detailed forearm vascular reactivity studies, before and after 3 months of CPAP. The systemic circulation was assessed by measuring brachial artery pressure, flow and resistance responses to intra-arterial infusions of acetylcholine (ACh - an endothelium dependent vasodilator), sodium nitroprusside (SNP - an endothelium independent vasodilator), L-NMMA (a nitric oxide –NO-antagonist) and L-arginine (the substrate for NO).

Before CPAP, ACh and SNP infusions increased forearm blood flow in a dose- dependent manner ($p<0.01$). After CPAP, endothelium-dependent dilation to ACh was significantly increased (434±23% of baseline post-CPAP vs 278±20% pre-CPAP, $p<0.001$), whereas SNP-induced dilation was unchanged. Resting NO production was higher after CPAP, evidenced by a significantly greater reduction in basal flow by L-NMMA ($p=0.05$). L-arginine reversed the L-NMMA effect in all cases.

*Conclusion.* In patients with OSA, CPAP therapy improves baseline endothelial NO release and stimulates endothelium-dependent vasorelaxation in the systemic circulation. This is a potential mechanism for improvement in systemic and vascular function in patients with OSA treated with CPAP.

## INTRODUCTION

Obstructive sleep apnoea (OSA) is a common disease, associated with a significantly increased risk of systemic hypertension[1 2]. Moderate to severe OSA, with an apnoea hypopnoea index (AHI) > 15/minute, affects 4% of middle-aged women and 9% of middle-aged men [3]. Fifty percent of individuals with OSA have systemic hypertension [4]. OSA is characterized by repetitive airway occlusion, resulting in cyclical surges of hypoxia, which may occur hundreds of times a night. In addition, OSA is associated with heart failure[5], stroke[6 7] and coronary artery disease[8 9]. The mechanisms that explain the relationship between OSA and daytime systemic hypertension, and the ways to reduce OSA-related vascular risk, are unknown[10].

Endothelial dysfunction, a key early event in hypertension and atherosclerosis, has been implicated as a possible mechanism linking the acute cyclical vascular stresses during sleep in OSA and the increased prevalence of chronic vascular diseases. We therefore hypothesized that nocturnal continuous positive airway pressure (CPAP), which alleviates airway obstruction, might improve systemic endothelial function.

## METHODS

### Subjects

Consecutive consenting patients with newly diagnosed untreated moderate to severe OSA (AHI >15/minute) were enrolled from the Royal Prince Alfred Sleep Disorders Clinic, Sydney, Australia. Exclusion criteria included cigarette smoking, systemic hypertension, bleeding disorders, needle phobias, any regular vasoactive or antioxidant medications and previous failed attempts at CPAP therapy.

Twelve subjects met these inclusion criteria and underwent baseline (pre-CPAP) forearm vascular reactivity studies. Of these, 2 were unable to tolerate CPAP therapy and therefore 10 were invited to complete the second study. In all cases, the study procedures were uncomplicated.

Institutional ethics committee approval was obtained for this study and written informed consent obtained from all subjects.

### CPAP Treatment

The subjects were auto-titrated and treated with an automated CPAP device (Autoset T ResMed, Sydney, Australia). CPAP compliance was monitored nightly through out the study, with adequate compliance prospectively defined as being a mean of 5 hours usage per night, with a reduction of obstructive episodes to AHI<5, for three months.

### Measurements

During the vascular studies, ECG, heart rate and arterial blood pressure were measured continuously (Hewlett Packard, USA). Forearm blood flow was measured by strain-gauge plethysmography (EC, Hokanson), in which stretch of a mercury-in-silastic strain gauge correlates to increase in volume of the forearm, which is proportional to forearm blood flow [11]. Cholesterol was measured by an automated enzymatic analyzer (Hitachi 917, Japan; Roche Diagnostics, USA).

### Study Protocol

All subjects fasted from midnight before the experimental procedure and were studied in the morning, in the supine position. The room was kept quiet and at a constant temperature (22-24$^0$C). A 20 G arterial line (Arrow RA-04120, Germany) was inserted, by the Seldinger technique, into the brachial artery of the non-dominant arm of each subject, under sterile conditions with local anaesthetic. Blood was drawn for analysis and the cannula was connected to heparinised saline 1000 units in 500ml (Baxter), at an infusion rate of 60ml/hr. The subjects were then rested for 30 minutes before initial baseline measurements.

Subjects were randomly assigned to either ACh or SNP as the first drug infusion. The vasoactive drugs infused were acetylcholine (ACh; a vasodilator that stimulates the release of NO and other dilator substances from the endothelium), sodium nitroprusside (SNP; an endothelium-independent vasodilator), L-monomethylarginine (L-NMMA; a competitive antagonist of nitric oxide-NO-synthesis) and L-arginine (L-Arg; the physiologic substrate for NO synthesis).

Serial infusions were made into the brachial circulation via the arterial line, in the following sequence, using a syringe pump (Terumo STC-521, Tokyo, Japan): (1) a 4 minute control infusion (0.9% saline), (2) two consecutive 4 minutes of either 2 and 20mcg/min ACh or SNP; 1 and 2.5mcg/min, (3) a repeat control infusion, (4) two 4 minute infusions of the drug *not* infused in step 2, *either* ACh or SNP, (5) a repeat

control infusion, (6) a 4 minute infusion of L-NMMA (4mg/min), (7) L-NMMA 4mg/min with ACh 20 mcg/min and (8) a 4 minute infusion of L-arginine (10mg/min) (Figure 1). All measurements were made with the subject awake. Forearm blood flow is expressed as a percentage of the control forearm blood flow, during the baseline infusion of saline. Forearm blood flow in the contralateral arm was also measured throughout, to exclude effects from external stimuli.

**Analysis**

All analyses were conducted by observers blinded to the subject identity and whether the study was conducted before or after CPAP treatment. FBF was expressed as mL/min per 100ml forearm volume. Changes in blood flow were reported as percentage change from baseline.

**Statistical Methods**

Results are expressed as mean±SEM. Differences in resistance vessel dilation to drug infusions were determined by 2-way ANOVA with repeated measures (SPSS version 9). Differences between subject characteristics before and after CPAP were determined by paired students *t* tests. Statistical significance was inferred with a two-sided p-value $\leq 0.05$.

## RESULTS

All ten subjects were healthy non-smokers, with an average age of 49 ±8 years. Nine of the subjects were male. The female subject was post-menopausal. All subjects had at least moderate OSA with an AHI of 39/min (range, 15-104). The subjects were not on any regular medications and did not start any medications during the study.

**Table 1. Patient Characteristics.**

| **Patient Characteristic** | | **Pre-CPAP** | | **Post-CPAP** | |
|---|---|---|---|---|---|
| Age | (Years ) | 49 | (±8) | 49 | (±8) |
| AHI | (/hour) | 39 | (15-104) | 4.4 | (2.1-6.1) (p<0.01) |
| BMI | ($kg/m^2$) | 31 | (24-41) | 31 | (25-41) |
| BP Systolic | (mmHg) | 122 | (±11) | 125 | (±13) |
| Diastolic | (mmHg) | 80 | (±6) | 76 | (±12) |
| Total Cholesterol | (mmol/L) | 4.9 | (±0.9) | 4.6 | (±0.9) |

**AHI= apnea hypopnea index, BMI=body mass index, BP= blood pressure**

**Baseline Study** (Pre-CPAP)

Prior to CPAP, systemic blood pressure was 122/80mmHg (±11/6). The body mass index (BMI) was in the obese range, 31 $kg/m^2$ (range, 24-41), as is frequently seen in the obstructive sleep apnoea population. Total cholesterol, LDL and HDL were all within normal limits. Between study visits, there were no significant changes in resting blood pressure or heart rate, BMI or the lipid profile of the subjects (Table 1).

In response to ACh, there was a dose-dependent increase in brachial artery flows, compared to the baseline value (p<0.01). There was also a dose-dependent increase in flow to SNP (p<0.01). By contrast, there was no significant change in flow to L-NMMA. Co-infusion of L-NMMA partially inhibited the forearm blood flow increase with ACh
(p<0.01) (fig 1).

**Post – CPAP Study**

AHI was reduced to 4.4/minute (range, 2.1-6.1) on successful CPAP (p<0.01). Baseline forearm blood flow was not significantly changed, nor was the arterial BP (125/76mmHg ±13/12).

In response to ACh, there was a significantly *greater* increase in flow compared to pre-CPAP (434±23% vs 278±20%, p<0.001) (Table 2). This CPAP-related increase was observed at both low and high dose ACh. By contrast, SNP responses were similar to pre-CPAP values (fig 1). After CPAP, L-NMMA induced vasoconstriction and a greater reduction in the brachial artery flow, compared to pre-CPAP values (p=0.05) (fig 2). Co-infusion of L-NMMA reduced the high dose ACh-related increase in forearm blood flow, however the flow remained greater than the pre-CPAP co-infusion flow values (p<0.001). L-arginine reversed the L-NMMA related constriction, consistent with an inhibitory effect of L-NMMA on the NO pathway (Figure 2).

There was no correlation between severity of noctural hypoxaemia (r=0.18, p=0.62), AHI (r=0.61, p=0.062) or CPAP compliance (r=0.71, p=0.85) and the magnitude of change of vascular function with treatment. This finding is, however, consistent with the small size of the study.

**Table 2. Forearm Vascular Reactivity**

**A) Before CPAP (expressed as a % baseline flow).**

| Infusion | Ach low | Ach high | SNP Low | SNP high | LNMMA high | Ach/ LNMMA | LArg |
|---|---|---|---|---|---|---|---|
| Subject 1 | 167 | 209 | 624 | 1250 | 164 | 149 | 80 |
| Subject 2 | 132 | 296 | 281 | 357 | 67 | 60 | 57 |
| Subject 3 | 362 | 474 | 372 | 650 | 95 | 431 | 159 |
| Subject 4 | 141 | 156 | 149 | 222 | 113 | 124 | 138 |
| Subject 5 | 167 | 572 | 376 | 476 | 94 | 420 | 128 |
| Subject 6 | 135 | 168 | 151 | 214 | 114 | 137 | 135 |
| Subject 7 | 122 | 183 | 224 | 285 | 63 | 174 | 135 |
| Subject 8 | 300 | 442 | 264 | 354 | 62 | 372 | 161 |
| Subject 9 | 87 | 163 | 134 | 194 | 67 | 106 | 125 |
| Subject 10 | 106 | 163 | 88 | 101 | 78 | 104 | 111 |

**B) After CPAP (expressed as a % baseline flow).**

| Infusion | Ach low | Ach high | SNP Low | SNP high | LNMMA high | Ach/ LNMMA | LArg |
|---|---|---|---|---|---|---|---|
| Subject 1 | 122 | 289 | 188 | 368 | 62 | 159 | 176 |
| Subject 2 | 228 | 369 | 249 | 340 | 94 | 227 | 146 |
| Subject 3 | 508 | 764 | 403 | 538 | 82 | 470 | 167 |
| Subject 4 | 495 | 504 | 336 | 561 | 103 | 138 | 130 |
| Subject 5 | 190 | 661 | 472 | 571 | 103 | 520 | 147 |
| Subject 6 | 130 | 267 | 291 | 393 | 66 | 59 | 61 |
| Subject 7 | 131 | 253 | 382 | 523 | 80 | 183 | 122 |
| Subject 8 | 248 | 411 | 153 | 337 | 70 | 278 | 136 |
| Subject 9 | 206 | 412 | 153 | 293 | 70 | 273 | 137 |
| Subject 10 | 105 | 410 | 207 | 260 | 67 | 459 | 131 |

## DISCUSSION

OSA is a major and under-diagnosed risk factor for hypertension and its complications [1 2] In this study, we have found that CPAP, an effective therapy for OSA, significantly improves microvascular function in the systemic circulation of OSA subjects.

Specifically, the current data demonstrate that CPAP treatment of OSA enhances endothelial nitric oxide release in the forearm microcirculation (as evidenced by an enhanced L-NMMA-related vasoconstriction), as well as improving stimulated endothelium-dependent vasodilatation (as shown by greater ACh vasodilator responses). This improvement in vasodilatation after ACh was only partially inhibited by co-infusion of L-NMMA, indicating that not only nitric oxide endothelium-dependent pathways, but also non-nitric oxide pathways were enhanced. These significant vascular reactivity changes were observed after only three months of effective CPAP treatment. By contrast, the responses to the endothelium-independent dilator SNP were not different after compared to before CPAP treatment, consistent with improved endothelial but not smooth muscle vasodilator function.

It has previously been shown that subjects with OSA have impaired systemic endothelial function, compared with control subjects. Carlson et al [12] found impairment of the ACh response in normotensive and hypertensive OSA subjects compared to those without OSA, loosely matched for age, sex and weight. In this study, impairment in the SNP response was also reported in the hypertensive OSA subjects. Recently Kato et al [13] compared the vascular responses in 8 subjects with OSA, however, to a tightly matched control group on no medications; in this study, ACh responses were impaired and there was no difference in the SNP response.

Several mechanisms have been proposed to explain the reduction in endothelial responsiveness in patients with OSA. Animal studies have shown that intermittent hypoxia plays a key role in the development of persistent daytime endothelial dysfunction. Sprague-Dawley rats exposed to intermittent hypoxia have less *in vivo* responsiveness to increasing doses of ACh than control rats. There is also significantly greater vasoconstriction to L-NAME (a NO antagonist) in intermittently hypoxic rats, suggesting impairment of basal NO production [14]. Human studies have also pointed to hypoxia as a possible cause of endothelial impairment; abnormal vascular responses have been observed in OSA patients exposed to acute isocapnic hypoxia [15].

Possible mechanisms of hypoxia-induced impairment of endothelial function might include direct damage to the endothelium or altered biosynthesis of NO from L-arginine, which is an oxygen dependent process [16]. Such hypoxia-induced endothelial impairment may occur in isolation or it may be additive to other acute effects of apnea, such as cyclical surges of blood pressure.

Our data have shown (for the first time to our knowledge) that microvascular endothelial vasodilator dysfunction in subjects with OSA is reversible with appropriate long term therapy; indeed basal and stimulated endothelial vasodilator release are significantly enhanced. Previous studies have shown that CPAP might reduce the acute physiologic effects of apnoeas in OSA, including cyclical hypoxia, surges in blood pressure and sympathetic nerve activity [17-19], but have not addressed longer term effects on vascular physiology. Imadojemu et al[20] found an improvement in forearm vasoreactivity in response to a limited noninvasive reactivity study after two weeks of CPAP therapy.

Consistent with our findings are the results from two studies on the effect of CPAP, in subjects with OSA, on plasma levels of nitric oxide derivatives. Ip et al [21] reported reduced serum nitrites and nitrates in subjects with moderate to severe OSA compared to matched controls, which returned to normal levels after one night of CPAP therapy. Similarly, Schultz et al[22] also found increased serum NO derivatives in subjects with OSA after short-term CPAP therapy, and this increase in NO derivatives was maintained at long-term follow up. CPAP treatment also improves hypertension control, both in the night-time and during the day[23] , by an average of 10 mmHg (systolic, mean and diastolic), which may in part be explained by the improvement in microvascular endothelial vascular function [24].

Although most studies of endothelial function in OSA have concentrated on the microvasculature, the level of the systemic circulation considered most relevant in the pathogenesis of hypertension [25], other groups have recently reported studies of endothelial function in peripheral conduit vessels, such as the brachial artery. Both Kraiczi et al [26]and Ip et al [27] have found impaired flow mediated dilation (FMD) in OSA subjects, consistent with a predisposition to atherosclerosis, with Ip et al also showing a beneficial effect of CPAP on FMD (although no mechanistic data regarding the role of NO were available).

The limitations of our study include its relatively small size, due to the invasive nature of the protocol and the restrictions of our enrolment criteria. The study therefore was not able to find a correlation with the severity of OSA and the degree of endothelial impairment. Furthermore we elected not to perform these invasive studies on a control group of normal subjects without OSA, in whom forearm vascular responses to ACh, SNP and L-NMMA have been extensively published previously, with consistent results [13 25 28] .

. Kato et al found a similar dose-dependent increase in forearm blood flow in untreated OSA patients in response to brachial artery infusions of ACh and SNP, with the increase in endothelium dependent vasodilatation to ACh blunted in comparison to normal controls, indicating impaired microvascular endothelial function[13]. For our study, each patient was their own control for the vascular responses after CPAP and no difference was found in the subjects' characteristics before and after CPAP.

Finally, we elected to study normotensive rather than hypertensive subjects with OSA. This was to allow study of the underlying vascular mechanisms in the absence of any confounding effects of anti-hypertensive medications many of which are known to influence endothelial function *per se*[29] even several half-lives after discontinuation.[30] Furthermore, as CPAP is known to reduce blood pressure in hypertensive OSA subjects [24], we also wished to avoid the possible confounding effects of blood pressure lowering itself on systemic endothelial and smooth muscle dependent vascular reactivity.

## CONCLUSION

Treatment of obstructive sleep apnoea with CPAP improves systemic vascular endothelial function. OSA has been implicated in the pathogenesis of hypertension, cardiovascular disease, heart failure and stroke; all of these are associated with impaired endothelial responses. CPAP treatment may therefore provide an opportunity to reduce the vascular risk attributable to obstructive sleep apnoea; long-term endpoint studies will be needed to address this important possibility.

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**FIGURE LEGENDS.**

**Figure 1.** Infusion protocol for all studies. The order of ACh and SNP infusions were randomised. Infusion rates for all substances were 1 ml/min. BL, baseline blood flow measurements with infusion of 1 mL/min 0.9% saline; S, 0.9 saline infusion; ACh, acetylcholine; SNP, sodium mitroprusside; L-NMMA, $N^G$-monomethyl-L-arginine.

**Figure 2.** Forearm blood flow responses before and after CPAP, showing significantly enhanced responses to ACh alone or ACh with L-NMMA, after compared with before CPAP (**p<0.001). SNP responses were unchanged with CPAP treatment. The vasoconstrictor responses to L-NMMA was significantly greater after compared to before CPAP (*p=0.05), consistent with an enhanced release of nitric oxide from the vascular endothelium.