Effects of 8 weeks of CPAP on Lipid-based Oxidative Markers in Obstructive Sleep Apnoea: A Randomised Trial

Sheila Sivam[1,5], Paul K Witting[2], Camilla M Hoyos[1,3], Aung M Maw[2], Brendon J Yee[1,3,5], Ronald R Grunstein[1,3,5], Craig L Phillips[1,3,4]

1. Sleep & Circadian Research Group
   Woolcock Institute of Medical Research
   PO Box M77
   Missenden Road
   NSW 2050, Australia

2. Discipline of Pathology
   The University of Sydney
   NSW 2050, Australia

3. Discipline of Sleep Medicine
   Sydney Medical School
   Edward Ford Building, A27
   University of Sydney
   NSW 2006, Australia

4. Department of Respiratory and Sleep Medicine
   Royal North Shore Hospital
   St Leonards

NSW 2065, Australia

5. Department of Respiratory and Sleep Medicine
   Royal Prince Alfred Hospital
   Camperdown
   NSW 2050, Australia

Funding Sources

This work was supported by Australian National Health and Medical Research Council project grant 301936 (to R.R. Grunstein), a Centre for Integrated Research and Understanding of Sleep Clinical Research Fellowship (to S. Sivam), a Centre for Integrated Research and Understanding of Sleep Postdoctoral Research Fellowship (to C.Hoyos), a Health Professional Fellowship (571179; to C.L. Phillips) and a Practitioner Fellowship (202916; to R.R. Grunstein).

Corresponding Author:

Sheila Sivam

Email: sheila.sivam@sydney.edu.au

Contact address

Sleep & Circadian Research Group

Woolcock Institute of Medical Research

PO Box M77

Missenden Road

NSW 2050, Australia

Abstract

Rationale: Altered lipid metabolism has been reported in severe Obstructive Sleep Apnoea (OSA) and may be related to the development of cardiovascular disease. Continuous Positive Airway Pressure (CPAP) treatment for 8 weeks leads to improvements in postprandial triglycerides and total cholesterol. Intermittent episodes of hypoxia and re-oxygenation occur in OSA and have been linked to the formation of reactive oxygen species and increased oxidative stress and inflammation. We investigated the effect of CPAP on lipid markers of oxidative damage and plasma lipophilic anti-oxidant levels before and after sleep.

Methods: We compared the effect of 8 weeks of therapeutic CPAP and sham CPAP, in a randomized cross-over study, on unesterified cholesterol, esterified unsaturated fatty acids (cholesteryl linoleate: C18:2, the major oxidizable lipid in LDL cholesterol, and cholesteryl arachidonate: C20:4), and their corresponding oxidized products (cholesteryl ester derived lipid hydroperoxides and hydroxides (CE-O(O)H) and Vitamin E (as α-tocopherol the most biologically active vitamin E form) in patients with moderate to severe OSA. All analytes were determined by liquid chromatography and concentrations determined by comparison to corresponding standard curves. Measurements were obtained prior to sleep, upon awakening and 2 hours post awakening. Statistical analysis was performed using mixed effects models.

Results: Amongst the 29 patients completing the study, three had incomplete data for part of a visit. The mean AHI, age and BMI were 38, 49 years and 32 kg/m^2

respectively. No differences in lipid based oxidative markers were observed between the CPAP and sham arms at any of the three time points (Unesterified cholesterol 0.01, p=NS; cholesteryl linoleate: C18:2 0.05, p=NS; cholesteryl arachidonate: C20:4 0.02, p=NS; lipid hydroperoxides and hydroxides (CE-O(O)H) -2.5, p=NS) and levels of the lipid-soluble antioxidant Vitamin E (0.03, p=NS).

Conclusion:

In this randomized sham-controlled trial, there was no change in lipid based oxidative markers or lipophilic antioxidant levels after 8 weeks of therapeutic CPAP compared with 8 weeks of sham CPAP.

Keywords

Oxidative stress, Obstructive Sleep Apnoea, Lipid, CPAP

INTRODUCTION

Cardiovascular morbidity and mortality is increased in patients with obstructive sleep apnoea (OSA), an independent risk factor for hypertension and left ventricular diastolic dysfunction [1-4]. Population studies estimate that 2 and 4% of middle age women and men respectively, have OSA [5]. Postulated mechanisms linking chronic intermittent hypoxia and arousals in OSA with cardiovascular disease include transient episodes of hypertension, endothelial dysfunction and increased arterial stiffness, elevated sympathetic activity, intrathoracic pressures changes, inflammation and oxidative stress [6-11].

Oxidative stress is characterized by an imbalance between oxidant-producing systems and antioxidant defence mechanisms, resulting in excessive formation of reactive oxygen species (ROS), chemically reactive by products of oxygen metabolism. At lower levels, ROS participate in physiologic signalling pathways [12]. Detrimental modification of cell structures encompassing membranes, proteins, lipids and DNA, can occur when higher levels of ROS or reactive nitrogen species (RNS) are present. Oxidative modification of low-density lipoproteins (LDL) in the arterial wall is a key feature of atherogenesis [13]. A recently published population based study demonstrated an independent association between 10-year CAD events and plasma, oxidisable LDL level in a general population (Gomez et al, 2013 – not on pubmed yet). In OSA, the presence of increased oxidant molecules is further accompanied by reduced antioxidant capacity in OSA [14, 15].

Several randomized controlled trials in OSA have investigated the impact of continuous positive airway pressure (CPAP) on various oxidative markers. Lipid

peroxidation markers utilized range from 8-isoprostane concentrations in plasma, urine and exhaled breath condensate as a reflection of airway lining fluid composition [16-18], to determination of malondialdehyde (MDA) in the thiobarbituric acid-reactive substance (TBARS) test, as an indirect method of assessment of lipid peroxidation [13, 19, 20]. Other oxidative markers measured include total levels of hydroperoxides [21] and nitrotyrosine, by product of tyrosine nitration mediated by RNS [22]. In addition, antioxidant protective enzyme paraxonase-1 (PON1) [13, 19, 20], total antioxidant, vitamin A, E and gamma-glutamyltransferase levels (GGT) [23] have also been assessed as measures of antioxidant capacity in OSA. Most studies have shown a reduction in oxidative markers with CPAP.

In a previously published study, improvements in postprandial triglycerides and total cholesterol were demonstrated with 8 weeks of CPAP use [24]. Here, we investigate the effect of CPAP on directly measured lipid markers of oxidative damage and plasma lipophilic anti-oxidant levels before and after sleep.

METHODS

Participants

The protocol has previously been reported in a study which assessed the effect of CPAP treatment on postprandial lipidemia [24]. Details of randomization, allocation concealment and blinding and the flow of patients through the trial are included in the original report. Briefly, we recruited patients from sleep apnoea clinics who were aged > 21 years, with OSA severity in the upper moderate or severe range (Apnoea Hypopnoea Index or AHI ≥ 25) with a hypoxic component (Oxygen Desaturation

Index or ODI ≥ 20). We excluded patients with BMI>35kg/m^2, uncontrolled Type 2 diabetes and patients who had previously used CPAP. Other selection criteria are available on the Australian and New Zealand Clinical Trials Registry (ACTRN 12605000066684 available at http://www.anzctr.org.au). The diagnosis of sleep apnoea was based on full overnight polysomnography in a clinical laboratory prior to recruitment. Apnoeas and hypopnoeas were scored using standard scoring techniques [25].

Study design

Patients who met the inclusion/exclusion criteria were randomized to receive CPAP (Remstar Auto, Phillips Respironics, USA) or sham CPAP each for 8 weeks in a crossover design with an intervening 1 month washout period between treatments (Figure 1). Sham CPAP devices are identical in appearance to the therapeutic devices except that the pressure delivered at the mask remains at 0.5 cmH2O, having no effect on sleep disordered breathing. In contrast, therapeutic CPAP was set to a pressure which prevented most sleep disordered breathing. This was determined during an at home pressure determination study as previously described [24]. Compliance data from both devices was obtained from machine downloads at the end of each treatment arm.

Subjects did not change their exercise regime, diet or medications throughout the duration of the study. At predetermined times patients were fed breakfast, lunch and dinner as well as a snack in the mid-morning and mid-afternoon. The meals were

representative of a standard western diet with a total energy of 9433kJ (30.5% fat, 54.6% carbohydrate, 14.9% protein).

Biochemical testing

Biochemical measurements were obtained prior to sleep, upon awakening and 2 h post awakening. All analytes were determined by liquid chromatography and concentrations determined by comparison to corresponding standard curves. Paul to add details.

Statistical Methods

The final analyses included 29 patients who had completed the crossover trial, regardless of compliance with either therapeutic or sham CPAP. Order effect was analysed using unpaired t-tests and main outcomes were analysed using mixed effects models. The treatment, order, time-point and treatment by time-point interaction were included as fixed effects and the patient was a random effect in all models.

Data was analysed with the statistical software package SAS V.9.2 (SAS Institute) and Stata™ version 10 (StataCorp, College Station, TX, USA).

RESULTS

A total of 29 patients completed the trial [24]. Three subjects had incomplete data for part of a visit. Nine participants withdrew or were withdrawn post randomization as previously reported [24].

Patient demographics, anthropometrics, diagnostic polysomnography data and baseline biochemistry data are as shown in table 1 and polysomnographic, CPAP compliance and residual AHI data obtained at the end of CPAP and sham treatment arms are outlined in table 2. There was no significant difference observed in esterified cholesterol, unesterified cholesterol, lipid hydroperoxides and hydroxides or lipid soluble Vitamin E based on treatment modality or measurement time points – Table 3). Log transformation of CEEOH was performed because...The ratio of oxidized substrates (CE-O(O)H) over total substrates (cholesterol esters) in CPAP versus Sham groups, did not demonstrate a significant difference (p=0.71) and neither was there a correlation between this ratio in CPAP users with AHI, minimum oxygen saturation, oxygen desaturation index (ODI) and arousals (r =0.14, r=0.16, r= 0.18 and r = 0.01 respectively).

There was no effect of compliant (>4 hours) versus non-compliant CPAP use on the baseline to CPAP change in oxidative parameters (p= .....for unesterified cholesterol, cholesteryl linoleate: C18:2, cholesteryl arachidonate: C20:4, lipid hydroperoxides and hydroxides (CE-O(O)H) and lipid-soluble antioxidant Vitamin E respectively. In addition, there was no effect of order of CPAP treatment on baseline to CPAP change in oxidative parameters.

DISCUSSION

This is the first sham controlled randomized study to test the effect of CPAP treatment for OSA on directly measured lipid markers of oxidative stress. The study failed to demonstrate any beneficial influence of therapeutic CPAP compared to sham CPAP on unesterified cholesterol, a major potential atherogenic factor, esterified cholesterol, the bulk of oxidisable fat in lipoproteins, hydroperoxides and hydroxides (CE-O(O)H) and lipid soluble vitamin E, in subjects with moderate to severe obstructive sleep apnoea, irrespective of timing of measurements after daytime normoxia or nocturnal recurrent hypoxic episodes.

A previous non-randomized 2 month intervention study with nasal CPAP in subjects with severe OSA demonstrated a significant reduction in overall oxidative stress as measured by morning total hydroperoxides and hydroxides (CE-O(O)H) levels, acutely after 1 night as well as 2 months of CPAP use [21]. Our study did not demonstrate an increase in cholesterol esters or substrate, nor a reduction in the end product of lipid-based oxidant damage. Total oxidative damage reflective of lipid, protein, membrane and deoxyribonucleic acid (DNA) peroxidation was measured in the Christou study compared with the isolated lipid based oxidative damage measured in our study. This suggests that protein and DNA may be more susceptible to the hypoxia related oxidant damage of severe OSA and that CPAP may have more of an influence on non-lipid based oxidative markers. Potential differences in CPAP compliance and measurement techniques may also contribute to the differing results between studies.

Several studies have utilized other common markers of lipid based peroxidation which include 8-isoprostane levels and the determination of malondialdehyde (MDA) in thiobarbituric acid-reactive substance (TBARS).

A non-randomized study investigating the effect of CPAP on 8-isoprostane measured by exhaled breath analysis showed a beneficial effect [17]. A more recent 12 week randomized controlled trial using 8-isoprostane concentrations in plasma, also showed a reduction in 8-isoprostane levels with CPAP use [16]. The accuracy, reliability and validity of 8-isoprostane measurements particularly when using commercial kits however, have been criticized [26-29]. Gas chromatography with mass-spectrometry detection, the gold standard, is laborious and costly, prohibiting its use in large epidemiological studies. As a result, enzyme linked immunosorbent assay (ELISA) is often used but is not felt to be comparable to the gold standard.

Conflicting results have been demonstrated in non-randomized CPAP trials measuring MDA/TBARS levels. While some studies have shown a beneficial response to CPAP in patients with moderate to severe OSA after one night and up to 1 year of CPAP use [13, 19, 20], others have not [30] . Similarly however, measurement of MDA/TBARS has been criticized for its inaccuracy and ambiguity [31].

Reactive oxygen species are increased by several factors ranging from infection and inflammation to hypoxia related to OSA [32, 33]. The importance of further understanding the mechanistic link between various disease entities including atherosclerosis, and the recurrent OSA related hypoxia has lead to an abundance of

basic science and clinical studies. Several antioxidant trials have also been published reflecting the reduced antioxidant capacity in OSA [15, 23, 34] and its partial resolution with CPAP [23]. Improvement in endothelial dysfunction has also been demonstrated in patients with OSA after administration of Vitamin C [35]. The lack of improvement in lipid soluble vitamin E levels in our study is in keeping with previously published data [23].

There are several potential limitations to our study. Firstly, we did not repeat baseline biochemical testing after the washout period and hence assumed that any positive effect of CPAP on oxidative stress would revert to baseline levels after washout. The length of the washout period was long (4 weeks) however and we could not detect any order effect. Some studies showed a difference after 1-2 nights of CPAP suggesting that reversal of any effect from CPAP may be equally prompt [13, 17]. Secondly, our sham CPAP compliance was lower than therapeutic CPAP, although the difference was small (1 hour) and it would have been preferable to have longer nightly CPAP with both devices. In addition, our study may not have been adequately powered to detect a difference in lipid based oxidative markers. Lastly, we did not measure non-lipid based oxidative stress markers including reactive nitrogen species mediated oxidative markers eg. nitrotyrosine. We focused on lipid only markers as altered lipid metabolism has been demonstrated in OSA and CPAP has been shown to decrease total cholesterol and triglycerides [24, 36].

In summary, this is the first randomized sham controlled study investigating the influence CPAP on lipid-based oxidative markers over an 8 week period. No differences in unesterified cholesterol, esterified cholesterol, hydroperoxides and

hydroxides and lipid soluble vitamin E levels were observed between the therapeutic and sham CPAP groups. This suggests that CPAP may have a greater influence on non-lipid based oxidative markers.

REFERENCES

1. Stradling, J.R., J.C. Pepperell, and R.J. Davies, *Sleep apnoea and hypertension: proof at last?* Thorax, 2001. **56 Suppl 2**: p. ii45-9.
2. Arias, M.A., et al., *Obstructive sleep apnea syndrome affects left ventricular diastolic function: effects of nasal continuous positive airway pressure in men.* Circulation, 2005. **112**(3): p. 375-83.
3. Marin, J.M., et al., *Long-term cardiovascular outcomes in men with obstructive sleep apnoea-hypopnoea with or without treatment with continuous positive airway pressure: an observational study.* Lancet, 2005. **365**(9464): p. 1046-53.
4. Shahar, E., et al., *Sleep-disordered breathing and cardiovascular disease: cross-sectional results of the Sleep Heart Health Study.* Am J Respir Crit Care Med, 2001. **163**(1): p. 19-25.
5. Young, T., et al., *The occurrence of sleep-disordered breathing among middle-aged adults.* N Engl J Med, 1993. **328**(17): p. 1230-5.
6. Davies, R.J., et al., *Arterial blood pressure responses to graded transient arousal from sleep in normal humans.* J Appl Physiol (1985), 1993. **74**(3): p. 1123-30.
7. Jelic, S., et al., *Arterial stiffness increases during obstructive sleep apneas.* Sleep, 2002. **25**(8): p. 850-5.
8. Clark, R.W., et al., *Adrenergic hyperactivity and cardiac abnormality in primary disorders of sleep.* Neurology, 1980. **30**(2): p. 113-9.
9. Hedner, J., et al., *Is high and fluctuating muscle nerve sympathetic activity in the sleep apnoea syndrome of pathogenetic importance for the development of hypertension?* J Hypertens Suppl, 1988. **6**(4): p. S529-31.
10. Kent, B.D., S. Ryan, and W.T. McNicholas, *Obstructive sleep apnea and inflammation: relationship to cardiovascular co-morbidity.* Respir Physiol Neurobiol, 2011. **178**(3): p. 475-81.
11. Suzuki, Y.J., et al., *Oxidative stress and oxidant signaling in obstructive sleep apnea and associated cardiovascular diseases.* Free Radic Biol Med, 2006. **40**(10): p. 1683-92.
12. Valko, M., et al., *Free radicals and antioxidants in normal physiological functions and human disease.* Int J Biochem Cell Biol, 2007. **39**(1): p. 44-84.
13. Lavie, L., A. Vishnevsky, and P. Lavie, *Evidence for lipid peroxidation in obstructive sleep apnea.* Sleep, 2004. **27**(1): p. 123-8.
14. Halliwell, B. and J.M. Gutteridge, *Role of free radicals and catalytic metal ions in human disease: an overview.* Methods Enzymol, 1990. **186**: p. 1-85.
15. Christou, K., et al., *Antioxidant capacity in obstructive sleep apnea patients.* Sleep Med, 2003. **4**(3): p. 225-8.
16. Alonso-Fernandez, A., et al., *Effects of CPAP on oxidative stress and nitrate efficiency in sleep apnoea: a randomised trial.* Thorax, 2009. **64**(7): p. 581-6.
17. Carpagnano, G.E., et al., *8-Isoprostane, a marker of oxidative stress, is increased in exhaled breath condensate of patients with obstructive sleep apnea after night and is reduced by continuous positive airway pressure therapy.* Chest, 2003. **124**(4): p. 1386-92.
18. Minoguchi, K., et al., *Association between lipid peroxidation and inflammation in obstructive sleep apnoea.* Eur Respir J, 2006. **28**(2): p. 378-85.
19. Itzhaki, S., et al., *The effects of 1-year treatment with a herbst mandibular advancement splint on obstructive sleep apnea, oxidative stress, and endothelial function.* Chest, 2007. **131**(3): p. 740-9.

20. Barcelo, A., et al., *Abnormal lipid peroxidation in patients with sleep apnoea.* Eur Respir J, 2000. **16**(4): p. 644-7.
21. Christou, K., et al., *Nasal continuous positive airway pressure treatment reduces systemic oxidative stress in patients with severe obstructive sleep apnea syndrome.* Sleep Med, 2009. **10**(1): p. 87-94.
22. Jelic, S., et al., *Inflammation, oxidative stress, and repair capacity of the vascular endothelium in obstructive sleep apnea.* Circulation, 2008. **117**(17): p. 2270-8.
23. Barcelo, A., et al., *Antioxidant status in patients with sleep apnoea and impact of continuous positive airway pressure treatment.* Eur Respir J, 2006. **27**(4): p. 756-60.
24. Phillips, C.L., et al., *Continuous Positive Airway Pressure Reduces Post Prandial Lipidemia in Obstructive Sleep Apnea. A Randomised, Placebo Controlled Crossover Trial.* Am J Respir Crit Care Med, 2011.
25. *Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. The Report of an American Academy of Sleep Medicine Task Force.* Sleep, 1999. **22**(5): p. 667-89.
26. Halliwell, B. and C.Y. Lee, *Using isoprostanes as biomarkers of oxidative stress: some rarely considered issues.* Antioxid Redox Signal, 2010. **13**(2): p. 145-56.
27. Janicka, M., et al., *Isoprostanes-biomarkers of lipid peroxidation: their utility in evaluating oxidative stress and analysis.* Int J Mol Sci, 2010. **11**(11): p. 4631-59.
28. Il'yasova, D., et al., *Epidemiological marker for oxidant status: comparison of the ELISA and the gas chromatography/mass spectrometry assay for urine 2,3-dinor-5,6-dihydro-15-F2t-isoprostane.* Ann Epidemiol, 2004. **14**(10): p. 793-7.
29. Nourooz-Zadeh, J., *Key issues in F2-isoprostane analysis.* Biochem Soc Trans, 2008. **36**(Pt 5): p. 1060-5.
30. Svatikova, A., et al., *Oxidative stress in obstructive sleep apnoea.* Eur Heart J, 2005. **26**(22): p. 2435-9.
31. Moselhy, H.F., et al., *A specific, accurate, and sensitive measure of total plasma malondialdehyde by HPLC.* J Lipid Res, 2013. **54**(3): p. 852-8.
32. Yamauchi, M., et al., *Oxidative stress in obstructive sleep apnea.* Chest, 2005. **127**(5): p. 1674-9.
33. Lavie, L., *Oxidative stress--a unifying paradigm in obstructive sleep apnea and comorbidities.* Prog Cardiovasc Dis, 2009. **51**(4): p. 303-12.
34. Celec, P., et al., *Antioxidant vitamins prevent oxidative and carbonyl stress in an animal model of obstructive sleep apnea.* Sleep Breath, 2013. **17**(2): p. 867-71.
35. Grebe, M., et al., *Antioxidant vitamin C improves endothelial function in obstructive sleep apnea.* Am J Respir Crit Care Med, 2006. **173**(8): p. 897-901.
36. Robinson, G.V., et al., *Circulating cardiovascular risk factors in obstructive sleep apnoea: data from randomised controlled trials.* Thorax, 2004. **59**(9): p. 777-82.

FIGURES

Figure 1

Study Protocol

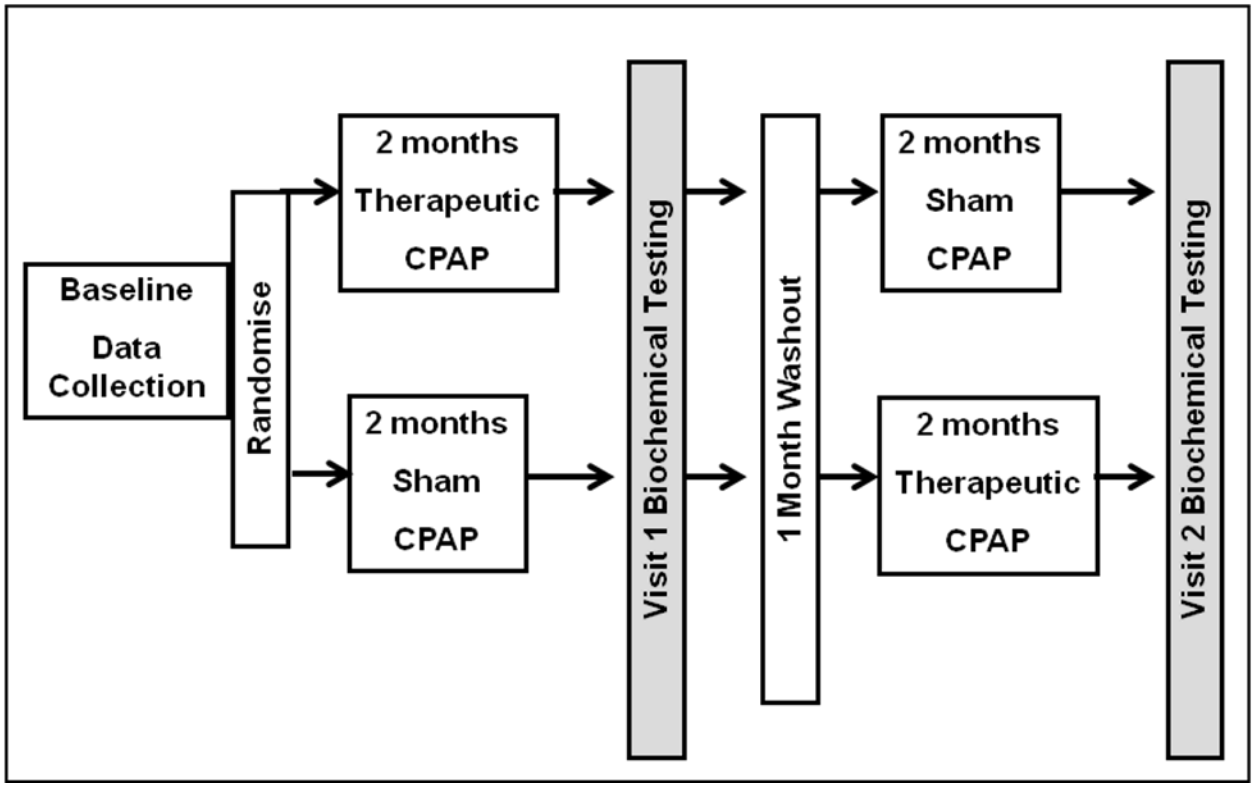


Figure 2

Unesterified cholesterol – need graphpad or other non-excel program to add SD

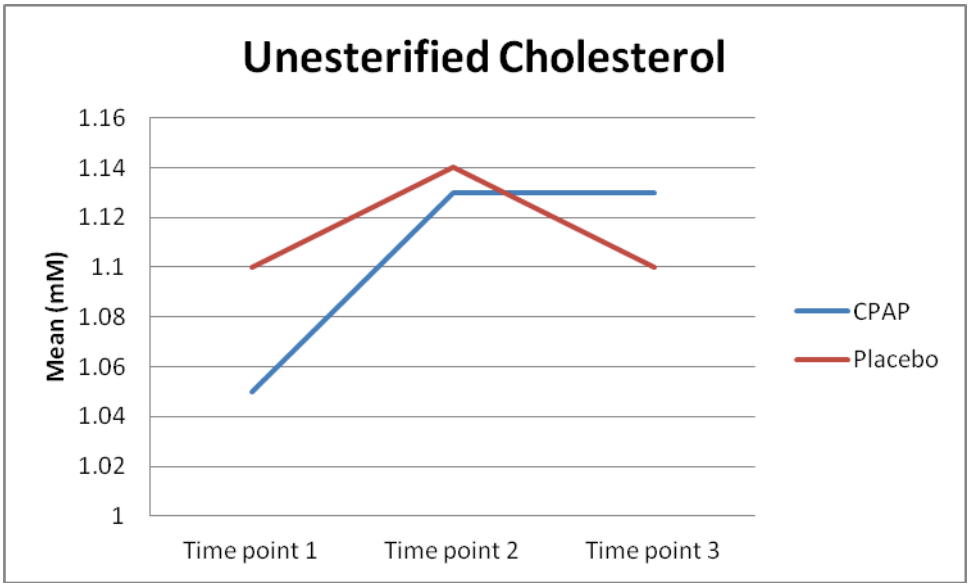


Figure 3

Cholesteryl linoleate (C18:2)

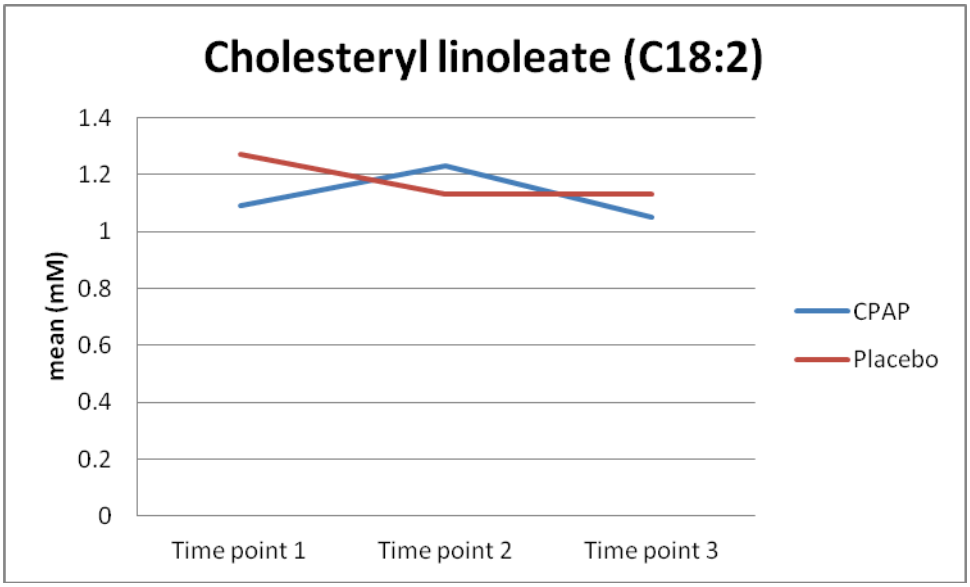


Figure 4

Cholesteryl arachidonate (C20:4)

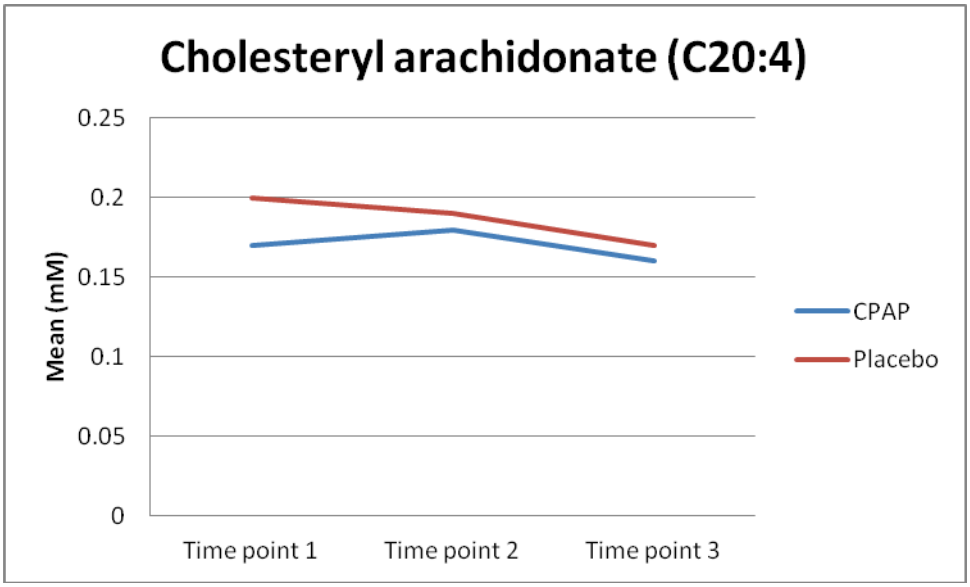


Figure 5

Lipid hydroperoxides and hydroxides

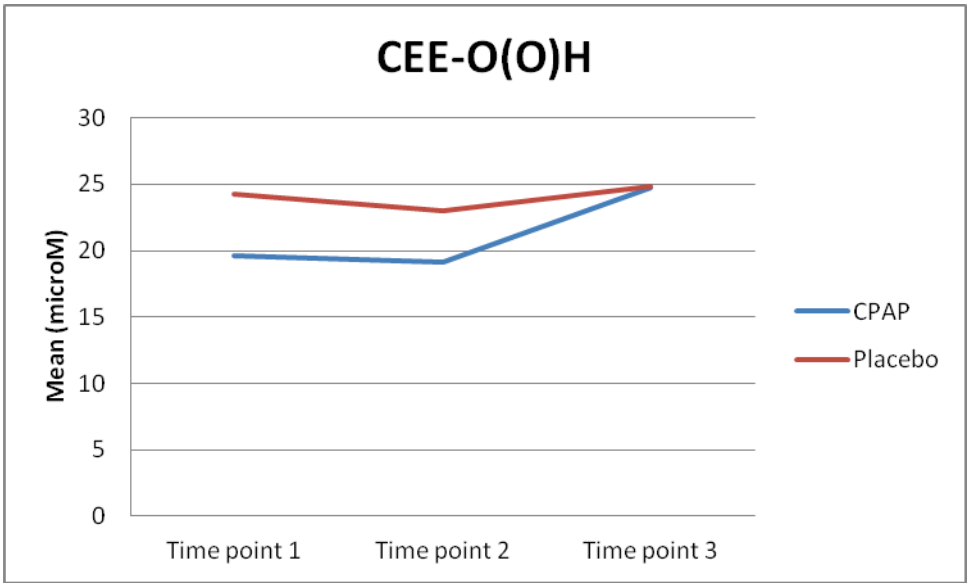
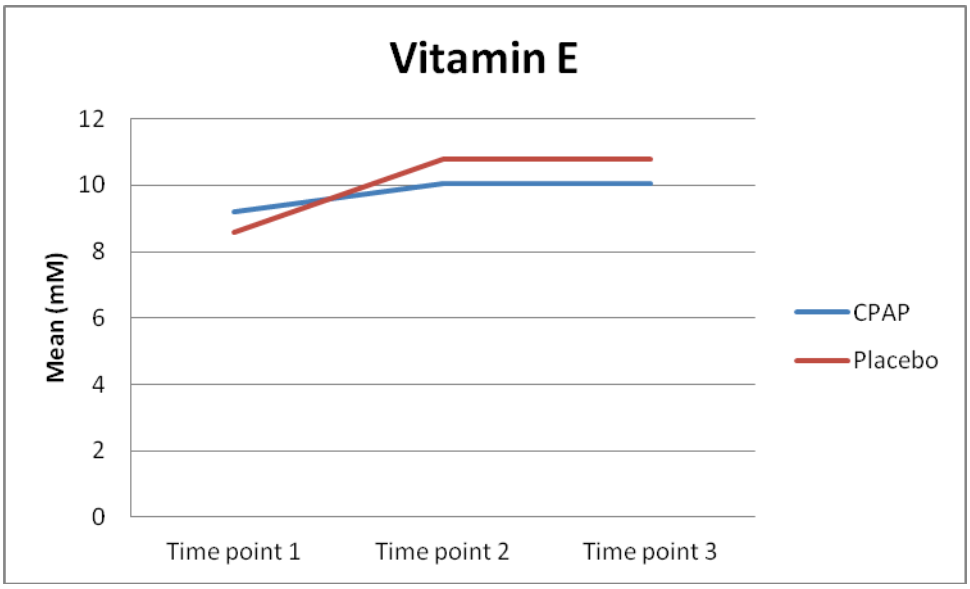


Figure 6

Vitamin E



TABLES

Table 1

Patient demographic, anthropometric and baseline data

Characteristics	Mean ± SD
Demographics	
Age (years)	49 ± 13
Male/Female (n)	28/1
BMI (kg.m-2)	31.6 ± 4.1
Sleep Apnoea	
AHI (events/hr)	38.2 ± 24.3
ODI (events/hr)	31.5 ± 22.8
SaO2-T90 (%TST)	7.04 ± 11.4
Min SaO2 (%)	78.5 ± 11.1
Epworth Sleepiness Score (ESS)	10.1 ± 4.9
Medical History and Medication (n)	
Hypertension	9
Type 2 Diabetes	2
Hypercholesterolemia	10
Antihypertensives	9
Oral hypoglycemics	
Insulin	
Statins	7

AHI: Apnoea Hypopnoea Index; BMI: Body Mass Index; ODI: Oxygen Desaturation Index; SaO2-T90 (TST): Percentage of Total Sleep Time spent with arterial oxygen saturation < 90%; Min SaO2 (%): Minimum arterial oxygen saturation.

Table 2

End of treatment polysomnography data

Characteristics	CPAP	Sham	
	Mean ± SD	Mean ± SD	P-value
AHI (events/hr)	6.9 ± 11.0	40.0 ± 26.9	<0.00001
ODI (events/hr)	5.1 ± 10	38.6 ± 21.7	<0.00001
SaO2-T90 (%TST)	0.98 ± 2.3	9.7 ± 11.7	0.0004
Min SaO2 (%)	89.1 ± 7.2	78.3 ± 8.4	<0.00001
Treatment Compliance (hours/night)	4.6 ± 2.0?	3.4 ± 2.2?	0.

AHI: Apnoea Hypopnoea Index; ODI: Oxygen Desaturation Index; SaO2-T90 (TST): Percentage of Total Sleep Time spent with arterial oxygen saturation < 90%; Min SaO2 (%): Minimum arterial oxygen saturation

Table 3

Effects of CPAP versus sham across all time points – using t-test

	CPAP (mean ± SD)	Sham (mean ± SD)	Mean Difference	P-value
Cholesterol (mM)				
Unesterified	1.10 ± 0.37	1.11 ± 0.34	-0.02	0.86
Esterified (mM)				
Cholesteryl linoleate: C18:2	1.12 ± 0.67	1.18 ± 0.67	-0.05	0.75
Cholesteryl arachidonate: C20:4	0.17± 0.08	0.18 ± 0.09	-0.02	0.40
Oxidized product (μM)				
Lipid hydroperoxides and hydroxides (CE-O(O)H)	20.74 ± 18.62	23.87 ± 19.03	-3.13	0.53
Lipid Soluble Antioxidant (mM)				
Vitamin E (α-tocopherol)	9.72 ± 3.19	9.77 ± 3.72	0.05	0.95

Table 4

Effects of CPAP versus sham – Time point 1 (before sleep) using t-test

	CPAP (mean ± SD)	Sham (mean ± SD)	Mean Difference	P-value
Cholesterol (mM)				
Unesterified	1.05 ± 0.33	1.10 ± 0.37	-0.05	0.61
Esterified (mM)				
Cholesteryl linoleate: C18:2	1.09 ± 0.67	1.27 ± 0.80	-0.19	0.34
Cholesteryl arachidonate: C20:4	0.17± 0.08	0.20 ± 0.01	-0.03	0.25
Oxidized product (μM)				
Lipid hydroperoxides and hydroxides (CE-O(O)H)	19.62 ± 24.18	24.23 ± 24.13	-4.61	0.48
Lipid Soluble Antioxidant (mM)				
Vitamin E (α-tocopherol)	9.21 ± 3.03	8.59 ± 4.05	0.62	0.51

Table 5

Effects of CPAP versus sham – Time point 2 (After sleep) using t-test

	CPAP (mean ± SD)	Sham (mean ± SD)	Mean Difference	P-value
Cholesterol (mM)				
Unesterified	1.13 ± 0.37	1.14 ± 0.37	-0.01	0.89
Esterified (mM)				
Cholesteryl linoleate: C18:2	1.23 ± 0.84	1.13 ± 0.65	0.10	0.64
Cholesteryl arachidonate: C20:4	0.18 ± 0.10	0.19 ± 0.01	-0.01	0.80
Oxidized product (μM)				
Lipid hydroperoxides and hydroxides (CE-O(O)H)	19.13 ± 19.80	22.96 ± 23.36	-3.82	0.52
Lipid Soluble Antioxidant (mM)				
Vitamin E (α-tocopherol)	10.07 ± 3.58	10.79 ± 4.71	-0.72	0.52

Table 6

Effects of CPAP versus sham – Time point 3 (2 hours post sleep) using t-test

	CPAP (mean ± SD)	Sham (mean ± SD)	Mean Difference	P-value
Cholesterol (mM)				
Unesterified	1.13 ± 0.49	1.10 ± 0.07	-0.02	0.83
Esterified (mM)				
Cholesteryl linoleate: C18:2	1.05 ± 0.70	1.13 ± 0.65	-0.10	0.59
Cholesteryl arachidonate: C20:4	0.16 ± 0.07	0.17 ± 0.07	-0.01	0.52
Oxidized product (μM)				
Lipid hydroperoxides and hydroxides (CE-O(O)H)	24.78 ± 23.02	24.87 ± 22.19	-0.10	0.98
Lipid Soluble Antioxidant (mM)				
Vitamin E (α-tocopherol)	10.07 ± 3.58	10.79 ± 4.71	-0.72	0.52