ERJ Express. Published on July 16, 2009 as doi: 10.1183/09031936.00047709

A Randomized Controlled Trial of nCPAP on Insulin Sensitivity in Obstructive Sleep Apnea

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Short title: Effects of nCPAP on insulin sensitivity in OSA

This study was supported by Lee Wing Tat Cardiorespiratory Research Fund, The University of Hong Kong, Hong Kong, SAR.

Abstract

The effects of treatment of obstructive sleep apnea (OSA) on glucose metabolism have been investigated with conflicting results. This study evaluated the impact of nasal continuous positive airway pressure (nCPAP) treatment of OSA on insulin sensitivity.

Men with moderate/severe OSA and no significant comorbidity were randomized to therapeutic or sham nCPAP group for 1-week treatment, and then reassessed. Those who received therapeutic nCPAP were further evaluated at 12 weeks. Insulin sensitivity (K_{itt}) was estimated by the short insulin tolerance test. Other evaluations included blood pressure, metabolic profile, urinary catecholamines and intra-abdominal fat.

61 Chinese subjects were randomized. Subjects receiving therapeutic nCPAP (n=31) showed an increase in K_{itt} (6.6+/-2.9 to 7.6+/-3.2, %/min; p=0.017), while those on sham CPAP (n=30) had no significant change, and the changes in K_{itt} were different between two groups (p=0.022). At 12 weeks, improvement in K_{itt} was seen in subjects with BMI ≥25 (n=20, median BMI 28.3 (26.6,31.5), p=0.044), but not in those with BMI <25 (n=9) nor the entire group.

The findings indicate that therapeutic nCPAP treatment of OSA for one week improved insulin sensitivity in non-diabetic men, and the improvement appeared to be maintained after 12 weeks of treatment in those with moderate obesity.

Word count: 199

Keywords

Insulin sensitivity

nasal Continuous Positive Airway Pressure

Obstructive sleep apnea

Randomized controlled trial

Introduction

Obstructive sleep apnea (OSA) is associated with increased cardiovascular risk [1-3], and its prevalence is closely linked to the epidemic of obesity worldwide [4]. Obesity is not only a major risk factor for sleep apnea but also for cardiometabolic diseases. Insulin resistance / impaired glucose tolerance is strongly associated with visceral adiposity, which may play a role in the underlying pathophysiologic pathways to altered glucose metabolism [5].

A number of studies have reported impaired glucose metabolism in OSA patients, independent of obesity [6]. However, a four-year follow up of a population cohort failed to find increased incidence of diabetes in those with baseline OSA [7]. Studies on the effects of nasal continuous positive airway pressure (nCPAP) treatment on insulin resistance / diabetes in subjects with OSA have reported conflicting results [6, 8-17]. Many of these studies were limited by their variable use of control groups, small sample sizes, and inadequate control of confounding factors.

We hypothesize that OSA may impair insulin sensitivity, which can be reverted by effective treatment of OSA, when diabetes has not set in. This study was thus designed to investigate the effects of nCPAP treatment of OSA on insulin sensitivity in men without overt diabetes mellitus.

Methods and materials

Patient recruitment

Chinese men, aged between 21 and 65, who underwent overnight diagnostic polysomnography (PSG) at the Sleep Laboratory, Queen Mary Hospital, Hong Kong, were recruited from October 2002 to June 2007 (except March to September 2003 when sleep

services were suspended). Some of these subjects participated in the cohort study of OSA and cardiometabolic risk [18]. Inclusion criteria were: OSA with an apnea-hyponea index (AHI) ≥15, CPAP naïve, no significant medical history, not taking any medications, and able to give written informed consent. Exclusion criteria were: fasting glucose ≥ 6.1 mmol/L, sleep disorders other than OSA, excessive sleepiness causing potential harm (e.g. in drivers) necessitating immediate institution of nCPAP therapy, habitual drinker of more than three times per week, morbid obesity with a body mass index (BMI) of ≥35.

Polysomnography: Subjects underwent an overnight 16-channel PSG (Alice 3 / 5 Diagnostics System; Respironics, Pennsylvania, USA) as previously described [13]. PSG recordings were manually scored according to standard criteria [18].

CPAP machines

Subjects receiving therapeutic nCPAP were given autotitrating machines for 1 week (Remstar Pro, Respironics Inc., USA), and those receiving sham (Placebo) CPAP had the same machines, but which have been modified to deliver a pressure between 0 – 1 cm H_2O according to previously used methodology [19,20], with a flow restricting connector and extra holes created at the exhalation port of the mask to allow air escape and to prevent rebreathing of carbon dioxide. The subtherapeutic pressure was confirmed with a manometer before and after the 1-week study period. Compliance data were downloaded from built-in memory cards in the nCPAP machines.

Protocol and Randomization

Clinical assessments included anthropometric and clinical evaluation, and sleep questionnaires. Full in-laboratory PSG was performed, and overnight urine was collected for

catecholamines. Fasting blood was taken in the morning for glucose, insulin and lipids, and short insulin tolerance test was performed. Magnetic Resonance Imaging (MRI) of abdomen was performed within 2 weeks of PSG, and before CPAP or sham CPAP treatment.

The 1-week study followed a double-blinded randomised controlled design. Following acquisition of baseline data according to protocol, subjects were randomized to either nCPAP or sham CPAP group for 1 week of treatment. Randomization schedule was generated by a research assistant who was independent of subject enrolment. Each randomized patient was coded numerically in a consecutive manner for analyses by the investigators at a later stage. A block randomization was used with a block size of 4, and stratified with severity. The subjects were stratified to 2 severity groups: AHI > 15 to 30 and AHI >30, to ensure that the proportions of severity groups were similar in the two treatment groups. Consecutive subjects were allocated to either of the two arms according to the randomization schedule. Subjects were strongly advised to keep their diet and exercise habits for the duration of the study. All baseline studies (except MRI abdominal fat) and PSG were repeated in both nCPAP and sham CPAP groups after 1 week, after which the treatment code was made known. Subjects in the sham CPAP group were then prescribed therapeutic nCPAP as clinically indicated, while those in nCPAP group were advised to continue treatment with a fixed pressure machine for another 11 weeks, at the optimal pressure (95^{th} percentile) as recorded on the autotitrating machine. Those in the nCPAP group were contacted by phone at 6 weeks to enhance CPAP compliance, and reassessed at 12 weeks with all tests including PSG and MRI abdominal fat. After completion of the study, all study subjects were seen at the outpatient clinic for regular clinical management.

This study was approved by the Institutional Research Board Ethics Committee of the University of Hong Kong /Hong Kong Hospital Authority, Hong Kong West Cluster, and written informed consent was obtained from all study subjects.

Measurements:

Anthropometric and blood pressure (BP) measurements

Body mass index (BMI) was calculated from body weight and height in kg/m^2. Waist circumference was measured at a level half-way between the lower rib margin and the iliac crest.

Blood pressure was measured with appropriate cuff size in supine position, using Dinamap (Critikon Inc, Florida), in the evening between 9 – 10 pm, and in the morning on waking, between 7 - 8 am. The average of three readings taken at one-minute interval on each occasion was documented as evening and morning blood pressure respectively. Morning blood pressure was used for all analyses in this study.

Short Insulin Tolerance Test (SITT)

This was conducted according to an established protocol [21,22]. The test was carried out between 9:00 to 9:30 am after a 12-hour overnight fast. An indwelling catheter was inserted into an antecubital vein, with blood collection for glucose and insulin starting at -10 minutes. A bolus of insulin (Human Actrapid) at 0.1unit/kg body weight was then given through the catheter at time zero. Plasma glucose levels were measured at 1,3,5,7,9,11,13, and 15 minutes on arterialized venous samples after insulin administration. To arterialize the venous blood, the hand was placed on a bag of gel beads which was held at a constant temperature of 43

degrees Celsius for 5 minutes prior to the start of the test and kept until the end of the study. The test was terminated by intravenous glucose bolus at 15 minutes.

Insulin sensitivity was estimated by measuring the glucose disappearance rate, represented by the K constant (K_{itt}). Serum glucose levels were logarithmically transformed and were modeled by linear regression to estimate the slope of decline in plasma glucose concentration after insulin administration. The slope was multiplied by –100 to derive the rate constant (K_{itt}) which was equivalent to the percentage decline in blood glucose per minute calculated by the formula 69.3/t ½, where t ½ was the half life of the fall in plasma glucose. The coefficient of variation of K_{itt} was 9 % as reported in Chinese patients from our institution (23).

Homeostasis model assessment method for estimating insulin resistance (HOMA-IR) (see on-line supplement)

The average insulin and glucose values of three blood samples taken over ten minutes (-10, -5 and 0 minutes of SITT) were used for calculation of HOMA-IR : fasting plasma glucose (mmol/litre) x fasting serum insulin (mIU/litre)/22.5 [24].

Lipid Profile (see on-line supplement)

Plasma total cholesterol and triglyceride were determined enzymatically on a Hitachi 912 analyzer (Roche Diagnostics, GmbH, Mannheim, Germany). Apolipoprotein (apo) B was measured by rate nephelometry using the Beckman Array System (Beckman Instruments).

Urinary catecholamines (see on-line supplement)

Urine between 10 p.m. to 8 a.m. was collected on the night of sleep study for assays of catecholamines and their metabolites (epinephrine, E; norepinephrine, NE; metanephrine, ME; and normetanephrine, NME).

Magnetic resonance imaging for intra-abdominal fat (see on-line supplement)

Subjects underwent abdominal MRI using a 1.5 T magnet (Signa Horizon LX, General Electric Medical Systems, Milwaukee, Wisc., USA) in the morning, having fasted for at least four hours.

Statistical Analysis

The primary endpoint was the difference in the change in K_{itt} after 1 week of therapeutic or sham nCPAP treatment. The intention–to-treat (ITT) principle was applied in the analyses between treatment groups, but missing data were not imputed to avoid dilution effect. Within-group comparisons were examined by paired t test for normally distributed data or Wilcoxon Signed rank test for non-normally distributed data; and between-group comparisons were examined by independent sample t test for normally distributed data or Mann-Whitney U test for non-normally distributed data. The changes over 12 weeks in the nCPAP group were all analyzed by Wilcoxon Signed rank tests due to small sample size. Stepwise forward regression analyses were performed to identify predictors for the changes in K_{itt} at 1 week using patients in both groups, and at 12 weeks using patients in nCPAP group. The selected models were re-fitted to all patients with available data in the selected predictors to obtain final estimates of the regression coefficients. Additional subset analysis was performed for the changes in K_{itt} or other variables by BMI groups with Wilcoxon signed ranks. All statistical tests were two-sided and the p-values for the secondary analyses were

not adjusted for multiple tests due to the exploratory nature. Analysis was performed with SPSS version 15.0.

Sample size calculation

Our pilot work on changes of K_{itt} in 5 subjects who received nCPAP treatment for 5-7 days estimated the mean change of K_{itt} as 1.7 mmol/l/min and the standard deviation (SD) of the change as 1.5 mmol/l/min. Assuming a common SD of 1.5 mmol/l/min for both groups and a difference in change of 1.5 mmol/l/min between the two groups, at a significance level of 5% and with a power of 90%, we required a minimum of 21 subjects in each group. Allowing for spontaneous dropouts, defaults and poor nCPAP adherence, 30 subjects per group was targeted.

Results

Figure 1 shows the flowchart of the study. 964 diagnostic PSGs were performed during the study period, only 70 (7.3%) subjects were eligible for the study, but one was newly diagnosed of familial hypertriglyceridemia and three of diabetes mellitus after having baseline blood tests, and 5 refused to participate. Thus 61 subjects who fulfilled the criteria were recruited, with a mean age of 46.3±10.2 years, mean BMI of 27.5±3.7 kg/m^2, and mean AHI of 39.7±22.1 events/hour.

They were randomized to either nCPAP (n=31) or sham CPAP (n=30) group for 1 week of treatment. In the nCPAP group, one subject did not return for reassessment at one week but at 2 weeks, 1 SITT failed technically, and several samples of urinary catecholamines had assay interference (Table 1). In the sham CPAP group, one subject did not use the treatment device at all during the study period, one subject modified the CPAP machine to block the extra

holes in the exhalation port of the mask, 1 SITT failed technically, and several samples of urinary catecholamines had assay interference (Table 1). All subjects were included and analysed in the groups that they were first being assigned to, with the exception of failed SITT and urinary assay interference where no data were available for analyses.

At baseline, the two groups had no significant difference in Kitt and other parameters (Table 1). 1 week CPAP use was higher in the nCPAP group than the sham CPAP group (6.2±1.5 hours/night versus 4.5±2.0 hours/night, p=0.001).

After 1 week of therapeutic nCPAP treatment, the group had a significant increase in K_{itt} (p=0.017) and decrease in systolic and diastolic BP (p=0.008, p=0.004), while the sham CPAP group, which also had a significant but much smaller reduction in AHI, showed no significant change in K_{itt} or systolic BP, but a decrease in diastolic blood pressure (p=0.021) (Table 1, Figure 2a). Between group comparisons showed that the changes in K_{itt} with treatment were significantly different (p=0.022) (Table 1). All subjects reported no changes in diet and physical activity. Further subgroup analysis within the nCPAP group according to BMI <25 and ≥25, the Asian criteria for obesity (25), was conducted, and improvement was only seen in those with BMI ≥25 (n=22, median BMI 28.3 (26.6,31.7)) : K_{itt} (p=0.002), systolic BP (p=0.007) and diastolic BP (p=0.011), but not in those with BMI <25 (n=9, median BMI 24.5 (24.1, 25), with a significant difference for between-group change in K_{itt} (p=0.022). In the stepwise forward regression analysis, the only significant predictor for the changes in K_{itt} after 1 week of treatment was urinary normetanephrine (Table 2a).

After 12 weeks of therapeutic nCPAP treatment, 29 subjects were reassessed as 2 refused to return. Mean nCPAP use during 12 weeks of treatment was 4.9±1.4 hours/night. No

significant changes occurred in BMI, waist circumference and total abdominal fat on MRI over the study period (Table 3). As a group, K_{itt} showed no significant change compared to baseline, while there were significant reductions in systolic and diastolic BP, total cholesterol, triglycerides, apolipoprotein B, and urinary norepinephrine and epinephrine (Table 3). On further subgroup analysis for obesity (BMI<25, n=9; BMI ≥25, n=20), K_{itt} was significantly improved in obese subjects (p=0.044) (Figure 2b), but not in non-obese subjects, and the between group comparison of changes was also significantly different (p=0.009). In the multiple regression analysis (Table 2b), BMI <25/ ≥25 was the only independent predictor for the changes in K_{itt} at 12 weeks. Other parameters including systolic and diastolic BP, total cholesterol, triglycerides, apolipoprotein B, urinary norepinephrine and epinephrine also showed significant changes in the obese but not the non-obese group.

Discussion

In this short term randomized controlled study in otherwise healthy men with moderate to severe OSA, we were able to demonstrate that one week of effective nCPAP treatment significantly increased insulin sensitivity. This improvement was only maintained in those who were obese in the open treatment group over 12 weeks. These findings suggest that OSA is an independent risk factor for adverse glucose metabolism, but the detrimental effects may be less prominent in non-obese subjects compared to moderately obese subjects. The pathogenetic pathway of impaired insulin sensitivity may involve sympathetic activation and other adiposity-related mechanisms.

A number of cross-sectional studies have reported that OSA is independently associated with insulin resistance / glucose intolerance, suggesting that OSA may play a causative role in the development of type 2 diabetes and the metabolic syndrome, while some other studies did not

identify any independent association after adjusting for obesity [6]. Despite more prevalent diabetes in OSA subjects at baseline, no independent relationship could be found between OSA and incident diabetes at 4-year follow-up [7].

An effect of intervention of one condition on another is regarded as strong evidence for a causal link. Previous studies of the effects of nCPAP treatment of OSA on insulin resistance or glycemic control have reported variable results. Many failed to show any improvement in glucose metabolism [6]. Using the hyperinsulinemic euglycemic clamp, two nights of nCPAP treatment was reported to improve insulin sensitivity in a group of non-diabetic Caucasian men with moderate OSA [10], and the improvement was maintained at 3 years [26]. However, in a randomized controlled study with a cross-over design, no change in HOMA-IR was found in Caucasian men with severe OSA after 6 weeks of CPAP treatment compared to sham-CPAP [13]. Recently, a case-control study showed that 3 months of nCPAP treatment reduced HOMA-IR in sleepy OSA subjects but not in non-sleepy subjects [16]. Observational studies further reported that CPAP compliance determined a positive outcome in HOMA-IR [15], or a decrease in glycosylated haemoglobin but not HOMA-IR (17). Data on changes in fasting insulin or HOMA-IR in children before and after adenotonsillectomy were similarly variable [27,28]. In diabetic subjects with OSA, despite several positive observational studies [6,8,11,12], a randomized controlled study reported that neither HbA_{1C} nor insulin sensitivity measured by the euglycemic clamp changed [14].

It is obvious that different methods have been used in the evaluation of *in vivo* glucose metabolism [6], ranging from simple measurements of fasting blood glucose and insulin to the sophisticated hyperinsulinemic euglycemic clamp study which is considered as the "gold standard" for measuring insulin sensitivity [29]. The limitations and strengths of each of

these methods cast significant impact on the interpretation of their results [21,24, 29]. In our study, the primary outcome measure was obtained by the short insulin tolerance test, a test for insulin sensitivity which has been validated against clamp studies [21] and shown to be reasonably reproducible [22]. It is a relatively rapid and simple test in which an estimate of insulin sensitivity is obtained from the rate of decline in glucose levels following an intravenous bolus of insulin. The test reflects the combination of suppression of hepatic glucose output and stimulation of peripheral glucose uptake by insulin, representing peripheral insulin resistance [29]. The short duration of the test avoids the problem of interference from the release of counter-regulatory hormones [29]. Compared to K_{itt}, HOMA-IR is a mathematical modelling of basal plasma insulin and glucose which expresses predominantly the ability of basal insulin to suppress hepatic glucose production in a fasting state. The test represents hepatic insulin resistance and is subject to variability in beta-cell function in a feed-back loop [24]. Catecholamines can inhibit insulin secretion by activating alpha2-adrenoreceptors in beta-cells [30]. Exposure to intermittent hypoxia in young healthy adults was shown to impair insulin sensitivity with a lack of compensatory hyperinsulinemia, suggesting a concomitant suppression of beta-cell function [31]. Similarly using the intravenous glucose tolerance test, OSA subjects were found to have impairment in insulin sensitivity, beta-cell function and glucose effectiveness as well [32]. It is logical to surmise that effective treatment of OSA may also improve beta-cell function with increased insulin secretion. With these intrinsic limitations, the HOMA-IR may under-estimate any improvement in insulin sensitivity, and remains unchanged when Kitt improves.

The exact mechanisms by which OSA leads to insulin resistance are not fully understood. One potential mediating mechanism is the elevation of sympathetic activity [33]. Our regression analysis for predictors of changes in insulin sensitivity at one week supported this

postulated mechanism, albeit the regression model indicated that it only accounted for a small degree of the variance.

The effect of co-existent obesity on glucose metabolism in OSA has been a contentious issue. Obesity, especially visceral obesity, is an important determinant of insulin sensitivity, and an inevitable confounder in studies of adverse metabolic effects of OSA. Previous studies suggested that both obese and non-obese subjects had adverse glucose metabolism related to OSA [34,35]. However, insulin sensitivity in response to nCPAP treatment was found to be better in non-obese Caucasian men (BMI <30 kg/m^2) compared to those with BMI >=30 kg/m^2 [10]. In contrast, a paediatric study suggested that OSA played a more significant role in insulin sensitivity in obese compared to non-obese children [27]. We have excluded by design those who were morbidly obese, with the speculation that severe obesity may have an overwhelming effect on insulin resistance masking any contribution by OSA. Our data showed no changes of BMI, waist circumference and the volume of visceral fat after 12 weeks of nCPAP treatment, and moderately obese subjects had a better metabolic response compared to the non-obese. MRI abdomen might not be the best tool to estimate the body fat content as it does not reflect total body fat mass. Nevertheless, we have used various indicators of body habitus and body fat, and showed no significant change in adiposity. Although the physical mass is static, the composites of adiposity might have changed at the molecular levels after treatment. A number of inflammatory and neurohumoral mediators are produced from the adipose tissues, especially from the visceral adipose tissues, which have been demonstrated to be involved in the pathogenesis of insulin resistance [33,36]. With the application of nCPAP treatment in the obese apneic group, the changes of these mediators and hence insulin sensitivity might have been more pronounced than the non-obese apneic group.

Apart from the randomized controlled design, and the use of a more precise measurement of insulin sensitivity, other strengths of our study include the satisfactory CPAP compliance, and the inclusion of only subjects with no significant comorbidity to allow clear delineation of the impact of OSA *per se* on glucose metabolism. Our findings strongly support that OSA has a causal role on insulin resistance, and the improvement with CPAP treatment carries clinical relevance, as similar improvement in Kitt has been shown after weight reduction by different methods [37,38]. In a prospective study of 31 morbidly obese women with a reduction of mean BMI from 54 to 35 kg/m^2 one year post bariatric surgery, K_{itt} was improved by 1 to 3 % in groups of different glucose profiles [37]. In a randomized placebo-controlled study of sibutramine-assisted weight reduction of 5.6 kg in non-diabetic subjects , mean K_{itt} was enhanced significantly from 4 to 5 %/min in the treatment group as compared to no changes in the control group which had no weight reduction [38].

However, the sample characteristics by design also mean that our results may not be extrapolated to women, those with mild to moderate OSA, and those with comorbidities including diabetes who form a large proportion of sleep clinic patients. A previous study has suggested that nCPAP treatment only improved insulin resistance, as reflected by HOMA-IR, in sleepy but not non-sleepy OSA subjects (16). Our study subjects on average had only mild sleepiness, and the sample size was not adequate for further sub-group analysis for any influence of sleepiness on the response in insulin sensitivity. For further delineation of the impact of OSA on glucose metabolism, it is necessary to evaluate the effect of nCPAP on beta-cell function [39] and non-insulin dependent mechanisms, and the interaction of OSA and adiposity. Finally, we only studied the pathogenetic role of sympathetic activation in insulin resistance, while many other potential mechanisms, such as inflammation, have not been evaluated.

In conclusion, the findings of this study demonstrate that treatment of OSA improved insulin sensitivity in the short term, suggesting a causal relationship between OSA and insulin resistance. The effect appeared to be maintained in the moderately obese subjects over 12 weeks. Early effective nCPAP treatment of OSA may alter the natural course of glucose metabolism favourably in otherwise healthy subjects.

Word count: 3708

Acknowledgments: The authors are thankful to Ms Ku Pui Pui and Mr Jack Lam, for their technical support in the manual scoring of all polysomnography done for this study.

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Figure legends

Figure 1: Study profile

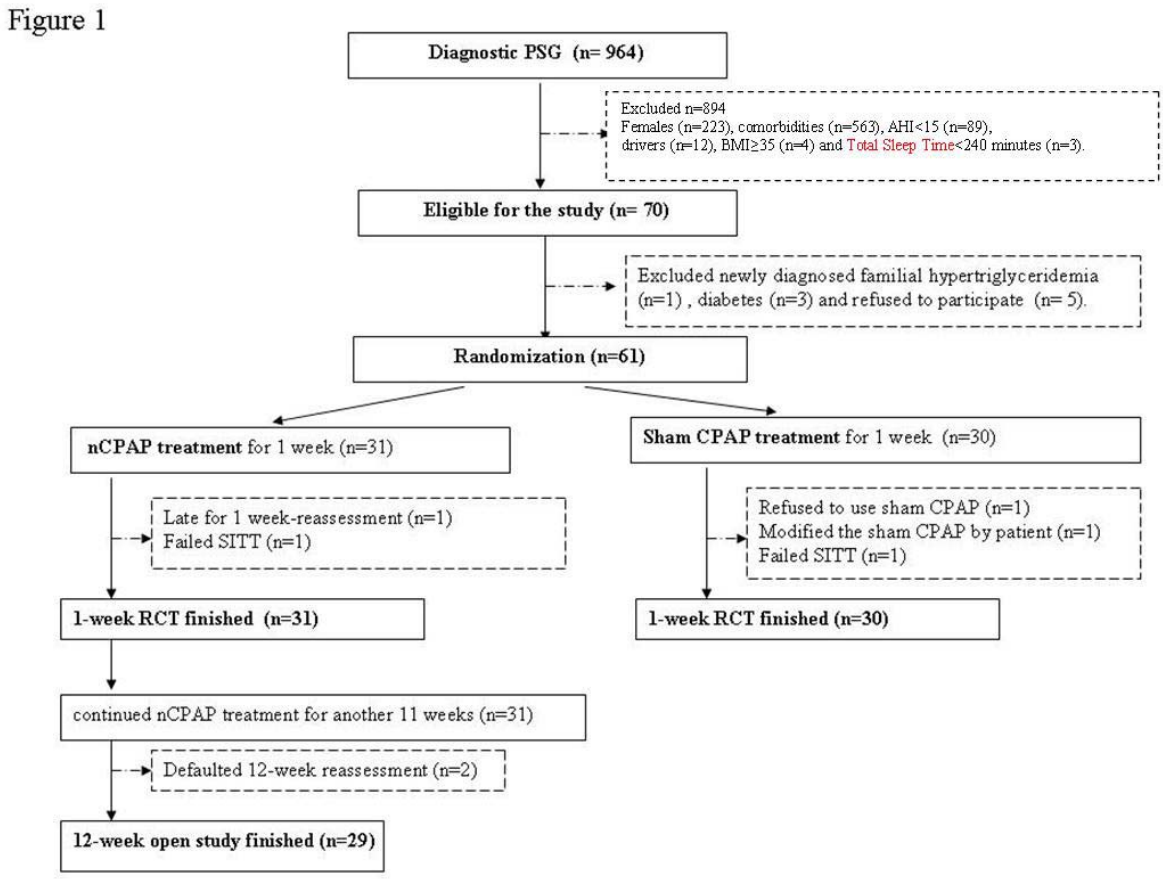


Figure 2a: Changes of K_{itt} after 1 week of CPAP and Sham CPAP treatments.

Figure 2a

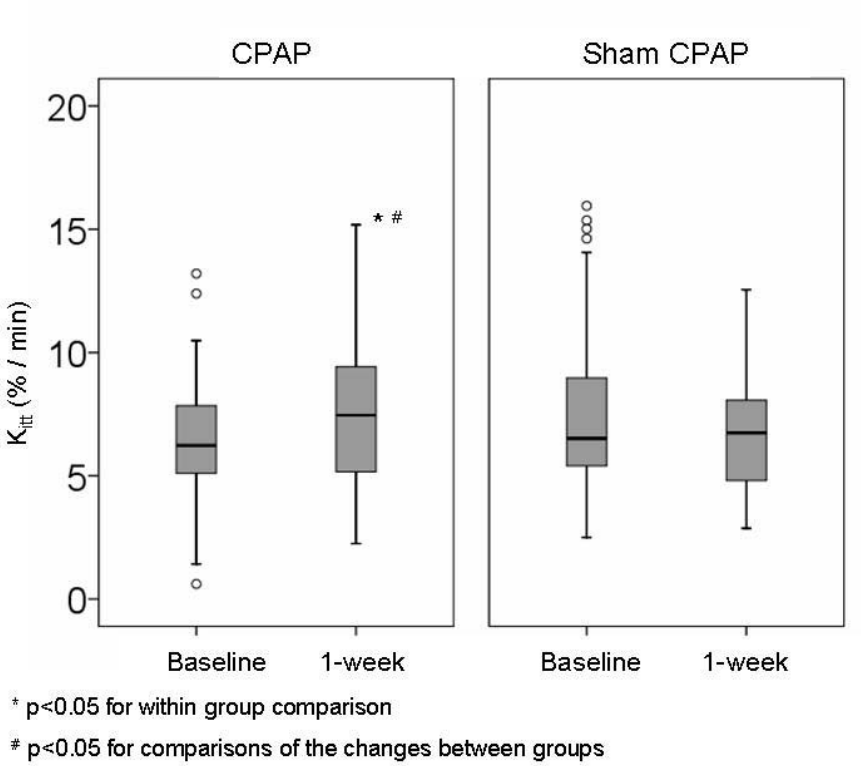
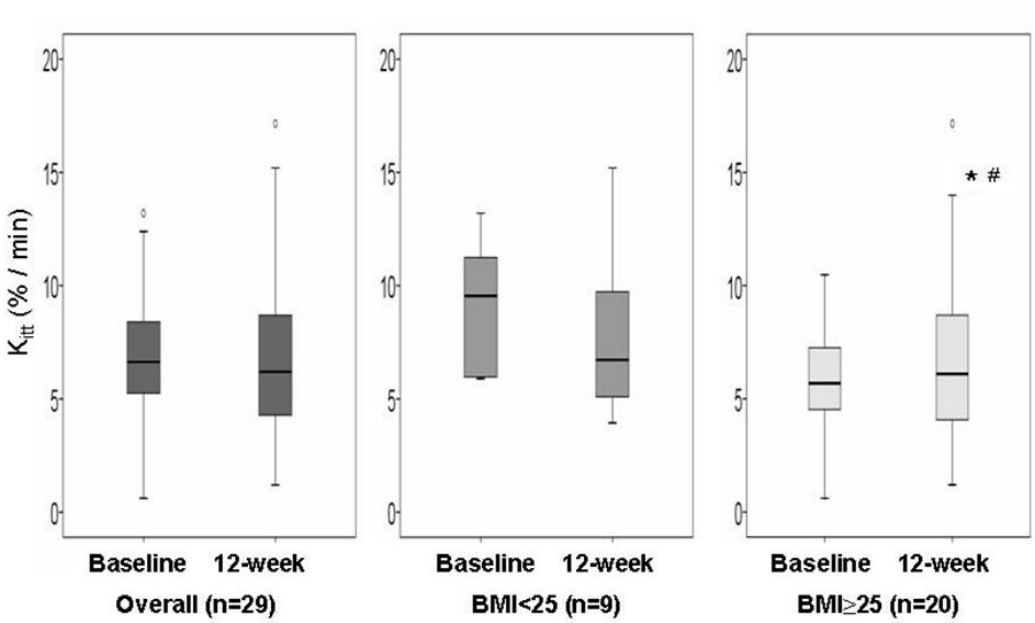


Figure 2b: Changes of K_{itt} after 12 weeks of CPAP treatment, with BMI < 25 and BMI ≥ 25.

Figure 2b



*** p<0.05 for within group comparisons**
 # p<0.05 for comparisons of changes between BMI<25 and BMI≥25

Table 1. Comparisons between before and after 1 week of nCPAP and sham CPAP treatments

	nCPAP n=31			Sham CPAP n=30			p[a] values for the difference in changes between nCPAP and sham CPAP groups
	Baseline	After 1week treatment	Baseline & 1 week difference	Baseline	After 1week treatment	Baseline & 1 week difference	
Age, years	46.5 ±10.8			46.1 ± 9.8			
ESS	10.3 ± 4.9	10.5 ± 5.2	0.3 ± 3.6	10.8 ± 5.5	10.5 ± 5.6	-0.35 ± 1.60	0.370
Waist circumference, cm	95.1 ± 9.3	95.0 ± 9.3	-0.1 ± 2.3	92.7 ± 8.1	92.2 ± 8.4	-0.57 ± 1.55	0.350
BMI, kg/m^2	27.8 ± 3.7	27.8 ± 3.8	0.05 ± 0.39	27.2 ± 3.7	27.1 ± 3.7	-0.12 ± 0.33	0.074
AHI, events/hour	33.4 (20.7, 58.8)	0.6 (0.1, 1.4)**	-33.3 (-58.5,-17.0)	31.3 (19.0, 56.6)	19.7 (8.7, 54.4)**	-8.7 ± (-16.7,-4)	<0.001
Sleep duration, minutes	405 ± 45	394 ± 65	-11 ± 53	403 ± 49	387 ± 48	-15 ± 54	0.759
Kitt, %/min	6.6 ± 2.9 [30][b]	7.6 ± 3.2*	0.98 ± 2.13	7.6 ± 3.5[29] [b]	7.0 ± 2.9	-0.70 ± 3.24	0.022
HOMA	2.7 ± 1.4	2.7 ± 1.2	-0.04 ± 0.75	2.9 ± 2.1	2.8 ± 1.9	-0.19 ± 1.23	0.577
Serum insulin, mIU/L	12.1 ± 6.0	11.8 ± 4.8	-0.28 ± 3.03	12.2 ± 7.6	11.6 ± 7.1	-0.63± 3.89	0.696
Plasma glucose, mmol/L	5.1 ±0.6	5.1 ± 0.4	-0.01 ± 0.41	5.2 ± 0.6	5.2 ± 0.6	0.002 ±0.39	0.934
Systolic blood pressure, mmHg	130.8 ± 14.7	127.8 ± 13.9*	-3.01 ± 8.37	129.5 ± 16.5	127.4 ± 15.9	-2.06 ± 8.47	0.662
Diastolic blood pressure, mmHg	80.1 ± 10.8	76.4 ± 8.2*	-3.77 ± 6.72	82.0 ± 11.6	78.9 ± 11.8*	-3.16 ± 7.09	0.728
Urinary norepinephrine, n/m	18.2 ± 4.7 [28][b]	17.6 ± 5.2	-0.6 ± 3.9	17.0 ± 7.2 [27] [b]	16.6 ± 7.3	-0.4 ± 5.0	0.905
Urinary normetanephrine, n/m	12.3 ± 4.0 [30][b]	11.7 ± 3.8	-0.5 ± 3.5	12.0 ± 5.4 [29] [b]	11.9 ± 4.8	-0.1 ± 5.7	0.695
Urinary epinephrine, n/m	4.8 ± 2.3 [27][b]	4.9 ± 3.6	0.1 ± 3.4	4.6 ± 2.5 [28] [b]	4.7 ± 2.5	0.2 ± 2.2	0.948
Urinary metanephrine, n/m	10.2 ± 4.2 [27] [b]	10.0 ± 3.4	-0.2 ± 2.6	8.6 ± 3.4 [27] [b]	8.5 ± 3.2	-0.1 ±2.8	0.844

Definitions of abbreviations: ESS=Epworth sleepiness score; BMI=body mass index; AHI = apnea-hypopnea index; HOMA= homeostasis model assessment for estimating insulin resistance; Kitt = glucose disappearance rate for estimating insulin sensitivity; n/m = nmol/mmol creatinine.
Data are expressed as mean ± SD for normally distributed data and as median (inter-quartile ranges) for non-normally distributed data.
*p<0.05, **p<0.001 for within group comparisons by paired t-test or Wilcoxon Signed Ranks test.
[a]Comparisons of the changes between groups by independent t-test or Mann-Whitney U test.
[ ][b] represents the number of patients with specimens available both at baseline and 1 week reassessment.

Table 2 Forward stepwise regression models for the changes in insulin sensitivity

a. After 1 week of nCPAP treatment

n=57	Adjusted R^2	Estimate (SE)	p value
Changes in urinary normetanephrine, nmol/mmol creatinine	7.4%	-0.184 (0.078)	0.023

Independent variables considered:
Age, waist circumference, BMI<25/BMI ≥ 25, sleep parameters at baseline (AHI, arousal index, duration with O_2 saturation <90% and minimum O_2 saturation), changes in urinary norepinephrine, normetanephrine, epinephrine and metanephrine.

2b. After 12 weeks of nCPAP treatment

n=29	Adjusted R^2	Estimate (SE)	p value
BMI<25/BMI ≥ 25	22.8 %	2.928(0.962)	0.005

Independent variables considered:
Age, waist circumference, BMI<25/BMI ≥ 25, sleep parameters at baseline (AHI, arousal index, duration with O_2 saturation <90% and minimum O_2 saturation), changes in urinary norepinephrine, normetanephrine, epinephrine and metanephrine.

Table 3 Before and After 12 weeks of nCPAP treatment

n=29	Baseline	12 weeks	[a]p value
AHI, events/hour	36.8 (19.0, 59.3)	0.6 (0, 1.1)	<0.001
ESS	10 (7, 15)	9 (5, 14.)	0.253
Waist circumference, cm	94 (89, 100)	95 (88.3, 100.0)	0.966
BMI, kg/m^2	26.7 (25.0, 30.3)	26.8 (25.4, 29.8)	0.421
MRI Visceral fat, cm^3	1113.9 (730.1, 1431.3)	1002.4 (870.2, 1270.7)	0.407
MRI Subcutaneous fat, cm^3	2211.4 (1510.5, 2945.6)	2102.9 (1465.1, 2731.9)	0.527
K_{itt} , %/min	6.6 (5.2, 8.7)	6.3 (4.3, 9.1)	0.690
HOMA	2.5 (1.5, 3.4)	2.1 (1.5, 3.4)	0.511
Serum insulin, mIU/L	10.9 (7.3, 14.0)	10.7 (7.1, 15.4)	0.199
Plasma glucose, mmol/L	4.9 (4.7, 5.4)	5.1 (4.7, 5.3)	0.954
Systolic blood pressure, mmHg	132 (122, 141)	123 (117, 134)	0.021
Diastolic blood pressure, mmHg	81 (73, 88)	76 (71, 82)	0.042
Total Cholesterol, mmol/L	5.1 (4.8, 5.7)	4.8 (4.2, 5.6)	0.016
Triglycerides, mmol/L	1.8 (1.4, 2.3)	1.5 (1.2, 2.1)	0.028
HDL-Cholesterol, mmol/L	1.1 (0.9, 1.3)	1.1 (1.0, 1.3)	0.974
LDL-Cholesterol, mmol/L	3.2 (2.8, 3.6)	3.1 (2.5, 3.7)	0.189
Apolipoprotein B, mmol/L	1.1 (0.9, 1.2)	1.0 (0.8, 1.1)	0.003
Urinary norepinephrine, nmol/mmol creatinine	19.0 (15.3, 22.0) [22][b]	15.5 (11.0, 17.8)	0.022
Urinary normetanephrine, nmol/mmol creatinine	12.0 (9.4, 15.0) [27][b]	11 (7.5, 13.0)	0.063
Urinary epinephrine, nmol/mmol creatinine	4.1 (3.4, 6.1) [22][b]	3.6 (1.9, 5.2)	0.027
Urinary metanephrine, nmol/mmol creatinine	9.5 (7.0, 12.0) [26][b]	9.3 (6.8, 12.3)	0.399

Definitions of abbreviations: ESS=Epworth sleepiness score; BMI=body mass index; MRI=magnetic resonance imaging; AHI = apnea-hypopnea index; HOMA= homeostasis model assessment for estimating insulin resistance; Kitt = glucose disappearance rate for estimating insulin sensitivity; LDL-cholesterol=low density lipoprotein-cholesterol; HDL-cholesterol=high density lipoprotein-cholesterol.

Data are presented as median (inter-quartile ranges).

[a]Within group comparisons by Wilcoxon Signed Rank tests.

[ ][b] represents the number of patients with specimens available both at baseline and 12-week reassessment.