

## **CPAP Treatment rapidly improves Insulin Sensitivity in Patients with OSAS**

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## **Abstract**

The obstructive sleep apnea syndrome is typically associated with conditions known to increase insulin resistance as hypertension, obesity, diabetes. We investigated, whether obstructive sleep apnea itself is an independent risk factor for increased insulin resistance and whether continuous positive airway pressure treatment improves insulin sensitivity. 40 patients (apnea-hypopnea-index > 20) were treated with continuous positive airway pressure. Before, 2 days after and after 3 months of effective continuous positive airway pressure treatment, hyperinsulinemic euglycemic clamp studies were performed. Insulin sensitivity significantly increased after 2 days ( $5.75 \pm 4.20$  baseline vs.  $6.79 \pm 4.91$   $\mu\text{mol/kg} \times \text{min}$ ;  $p = 0.003$ ) and remained stable after 3 months of treatment. The improvement in insulin sensitivity after 2 days was much greater in patients with a body mass index <  $30 \text{ kg/m}^2$  than in more obese patients. The improved insulin sensitivity after two nights of treatment may reflect a decreasing sympathetic activity, indicating, that sleep apnea is an independent risk factor for increased insulin resistance. The effect of continuous positive airway pressure on insulin sensitivity is smaller in obese patients than in non-obese patients, suggesting that in obese individuals insulin sensitivity is mainly determined by obesity and to a smaller extent by sleep apnea.

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## **Key words:**

Obstructive sleep apnea syndrome, continuous positive airway pressure treatment, insulin sensitivity, obesity, leptin

## **Introduction**

Obesity has a high prevalence in many societies and is closely related with the obstructive sleep apnea syndrome (OSAS) (1) as well as with hypertension (2), diabetes mellitus (3,4,5) and other features of the metabolic syndrome (6). Former studies found a high incidence of OSAS within the general population (2-4% of adult men and 1-2% of adult women) (7,8). Patients with OSAS have a higher incidence of morbidity and mortality due to cardiovascular and cerebrovascular disease, even in case of mild obstructive sleep apnea (9). The metabolic syndrome is characterized by increased insulin resistance. Some authors speculate, that in patients with OSAS the increased insulin resistance is partially mediated by an increased sympathetic activity caused by frequent nocturnal microarousals and nocturnal hypoxemia (10-14).

The standard treatment for OSAS is nasal continuous positive airway pressure (CPAP). This treatment rapidly improves the patients' condition by reducing daytime sleepiness and improving vigilance. Recently, a significant reduction of mean arterial ambulatory blood pressure by effective CPAP treatment could be demonstrated (15) and CPAP is known to reduce sympathetic drive in OSAS (10). However, positive metabolic effects of CPAP treatment e.g. improving insulin sensitivity are still under discussion (16-18). Increased insulin resistance seems to play a key role among the mechanisms responsible for metabolic effects in OSAS (19). The presence of increased insulin resistance has repeatedly been reported in patients with OSAS (16-18, 20-24). However, insulin resistance is not uncommon in the general population and is also associated with obesity, immobility, use of different drugs and a variety of other conditions, also frequently present in OSAS (16-25). Investigations addressing the question, whether OSAS itself is an independent risk factor of insulin resistance in these patients have led to conflicting results (16-18, 26,27).

One study showed a tendency to improved insulin sensitivity after 4 months of CPAP treatment measured by hyperinsulinemic euglycemic clamp (16), two other investigations

(measurements after 2 and 3 months) could not confirm this result (17,18). One reason for these conflicting findings could be the lack of statistical power, due to the low patient numbers. Thus, up to now it is not clear, whether the successful treatment of OSAS or the concomitant weight reduction and changes in lifestyle were the key factors in decreasing insulin resistance in non-diabetic patients with OSAS (20). Some authors speculate, that insulin resistance in OSAS is mainly induced by increased sympathetic drive. Since the elevated sympathetic drive is mainly mediated via adrenal hormones with their short half-lives within minutes (28,29), a significant reduction of insulin resistance should be achieved by CPAP therapy within a short period of time without any changes in body weight or lifestyle. To clarify, if such fast effects occur, we investigated non-diabetic patients with moderate to severe OSAS before and 2 days after initiation of CPAP treatment by hyperinsulinemic euglycemic clamp technique (30). Additionally, we investigated the insulin sensitivity 3 months after onset of CPAP treatment to clarify, if the effect of CPAP treatment on insulin sensitivity is maintained for a longer time interval.

Leptin is an adipocyte-derived hormone with important functions in appetite behaviour and energy homeostasis. It decreases during CPAP treatment in patients with OSAS, even without concomitant weight loss (24,31). In animal models, respiratory effects of this hormone have clearly been demonstrated (32). The implications of the decrease of serum leptin in humans treated with CPAP are not that clear. Adipose tissue, the main source of leptin, is an important factor determining insulin resistance. We measured serum leptin levels at the same time points as insulin sensitivity to investigate, whether changes in insulin resistance are paralleled by changes in leptin levels.

Some of the preliminary results of this study have been previously reported as an abstract (33).

## **Methods**

### *Patients*

Forty patients with OSAS (mean age  $53.81 \pm 11.84$  years) participated in the study (n=34: male, n=6 female). The mean body mass index (BMI) was  $32.76 \pm 6.92$  kg/m<sup>2</sup>. The patients were free from severe accompanying diseases. Hyperlipidemia was present in 20 patients, hypertension in 24 patients. Five patients had an impaired fasting glucose. These diagnoses had been made according to standard criteria (34,35,36). No patient had diabetes mellitus. Antihypertensives influencing insulin sensitivity were withdrawn 1 week before the clamp studies (n = 4) (25,37). All patients had OSAS with a mean AHI of  $43.10 \pm 11.38$  and complained about impaired performance and daytime sleepiness (Epworth Sleepiness Scale:  $12.9 \pm 3.6$ ; Range 9-23) (38).

The 40 patients underwent a first night with diagnostic polysomnography as previously described (39,40), a routine blood examination and a measurement of serum leptin. Bioelectrical impedance measurement was performed and insulin responsiveness was measured by a hyperinsulinemic euglycemic clamp at 7.00 a.m.. The following night, CPAP treatment was initiated according to a standard CPAP-titration protocol (41) and in the third night, CPAP was performed with the previously established minimal effective CPAP pressure, followed by a second clamp at the next morning. In the remaining 31 patients, the clamp studies were repeated after about 3 months ( $93.97 \pm 25.22$  days) after a further night with polysomnography with effective CPAP treatment. Nine of the forty patients had removed or discontinued CPAP therapy due to discomfort. The same laboratory parameters as after the first night were repeated, as well as bioelectrical impedance analysis. The medical treatment for all patients remained unchanged throughout the study.

The study protocol was approved by the Ethics Committee of the Friedrich-Alexander-University, Erlangen-Nuremberg. All patients gave written informed consent to participate in this study.

### ***Hyperinsulinemic euglycemic clamp***

Insulin responsiveness was measured by a hyperinsulinemic euglycemic clamp after a 10-h overnight fasting period as described by de Fronzo (30). Insulin (Actrapid U 40 HM; ge, Novo Nordisk, Bagsvaerd, Denmark) was administered at a rate of 1 mU / kg x minute. Serum glucose levels were measured every 10 minutes. The insulin sensitivity index (ISI) was calculated from the insulin measurements and the corresponding glucose infusion rates during that period (given as  $\mu\text{mol} / \text{kg} \times \text{min}$ ).

### ***Sleep studies***

Polysomnography was performed according to the recommendations of the American Thoracic Society (39) and the German Sleep Society (40,41). Sleep staging was performed using the criteria of Rechtschaffen and Kales (42) and microarousals were defined in accordance with the definitions of the American Sleep Disorders Association (ASDA) (43). Variables extracted were the following: Apnea-Hypopnea-Index (AHI), Arousal Index (ARI), Oxygen Desaturation Index (ODI; number of oxygen desaturations  $\geq 4\%$ ), Mean Minimal Arterial Oxygen Saturation (MMAO<sub>2</sub>), as described by Juhasz et al.(44).

### ***Data analysis***

Descriptive analysis was performed using mean values with standard deviation (parametric data). Relationships between two continuous variables were analyzed using scatterplots and Spearman's rho correlation coefficients. Differences between insulin sensitivity (ISI) before and after treatment were evaluated using the Wilcoxon test for paired samples. A logistic regression analysis was performed to evaluate the influence of a baseline BMI  $<30 \text{ kg/m}^2$  on the outcome "change in ISI  $>0.58$  after 2 days" (dichotomization according to sample median) while controlling for age, arterial hypertension, and „baseline apnoea-hypopnea-index (AHI)

<39“ (dichotomization according to sample median). Two-sided p values of  $\leq 0.05$  were considered significant. All computations were performed with SPSS (Version 11.0).

## **Results**

CPAP treatment could initially be effectively established in all 40 patients. AHI and daytime sleepiness were normalized. There were no significant changes of body weight or body fat mass during the three months of CPAP treatment. Further characteristics of the patients during the course of treatment are given in Table 1.

The insulin sensitivity index (ISI) was significantly improved after 2 days of CPAP treatment in the 40 patients ( $p = 0.003$ ). In the 31 patients reinvestigated after 3 months, there was no further significant improvement of ISI, but the difference from baseline remained statistically significant ( $p = 0.001$ ; Figure 1). Similar results could be found in a subgroup with  $\text{BMI} < 30 \text{ kg/m}^2$  (normal weight and overweight grade I according to the WHO criteria,  $n = 16$ ). In patients with  $\text{BMI} \geq 30 \text{ kg/m}^2$  (overweight grade II and III according to the WHO criteria,  $n = 24$ ), no significant improvement of ISI could be demonstrated after 2 days of CPAP (Table 2). The obese patients and the non-obese patients showed no significant differences in any of the other parameters studied despite serum leptin levels (Table given in the web-based repository). The individual changes of ISI are shown in Figure 2 and 3.

Since the body weight seems to influence the effect of CPAP on insulin responsiveness, a logistic regression analysis of the BMI on the changes between ISI before and after 2 days after CPAP treatment was performed and the results are given in Table 3. There was an independent impact of the variable  $\text{BMI} < 30 \text{ kg/m}^2$  on the  $\text{Delta ISI} > 0.58$  after adjustment for the following factors: age, AHI, hypertension. This means, that patients with a  $\text{BMI} < 30 \text{ kg/m}^2$  have a 7 fold higher chance to experience an improvement of insulin sensitivity of

more than 0.58. The data also show, that the change in ISI is not significantly influenced by age, presence or absence of hypertension and the initial AHI in our patient group.

CPAP adherence could be a further factor influencing the changes in ISI. Nine patients discontinued CPAP treatment later on, but 31 patients showed good compliance during the first 3 months of treatment (the built-in data stores of the CPAP devices were read out, the number of days of use within the past 42-day period established, and the mean duration of use per treatment night calculated). The CPAP devices were used on  $38.1 \pm 6.4$  nights (range 23-41). The mean duration of use per night was  $5.2 \pm 0.91$  h (range 2.3-7.8). The possible association between mean duration of CPAP use per night and change in insulin sensitivity between baseline and 3 months was evaluated by scatterplot and by calculation of the Spearman correlation coefficient. No significant association could be demonstrated between the mean duration of CPAP use per night and the change in insulin sensitivity between baseline and 3 months ( $r=0.21$ ,  $p=0.27$ ).

Parameters of the severity of OSAS were studied to investigate a possible influence on ISI and changes in ISI. We found only minor to moderate correlations between the insulin sensitivity before treatment and the parameters AHI ( $r=-0.30$ ,  $p=0.06$ ), ARI ( $r=-0.30$ ,  $p=0.06$ ), ODI ( $r=-0.35$ ,  $p=0.03$ ) and  $MMAO_2$  ( $r=0.12$ ,  $p=0.54$ ) on one hand as well as between the difference in insulin sensitivity at baseline and after 2 days of treatment and the parameters AHI ( $r=-0.32$ ,  $p=0.047$ ), ARI ( $r=-0.11$ ,  $p=0.50$ ), ODI ( $r=-0.31$ ,  $p=0.05$ ) and  $MMAO_2$  ( $r=0.71$ ,  $p<0.001$ ) on the other hand. Furthermore, in an additional exploratory logistic regression analysis, no significant independent influence ( $p=0.08$ ) of the variable “baseline AHI < 39” on the variable “baseline ISI < 3.94” was found when controlling for the interfering variables “percent body fat < 21.5”, “BMI < 30”, and hypertension. Due to the limited case number, inclusion of the variable age was not feasible in this additional analysis.

Serum leptin was studied before and during the course of CPAP treatment. Serum leptin and the ISI were well correlated before, as well as 2 days and 3 months during CPAP treatment ( $r=-0.40$ ,  $r=-0.51$ ,  $r=-0.61$ ;  $p=0.01$  in all cases). We found no significant changes between baseline fasting leptin levels after 2 days of CPAP treatment (Table 1). Serum fasting leptin levels were significantly lower at 3 months of CPAP treatment when compared to baseline levels ( $p < 0.05$ ). Serum fasting leptin levels at baseline showed a strong correlation with the BMI ( $r = 0.71$ ), and the percentage of body fat ( $r = 0.57$ ), the correlation with AHI was low ( $r = 0.34$ ).

## **Discussion**

There is a growing body of literature suggesting, that sleep-disordered breathing may be a causal factor for glucose intolerance and insulin resistance (26,27). Thus, the goal of our study was to investigate, whether OSAS itself is an independent risk factor for increased insulin resistance and whether effective CPAP treatment improves insulin sensitivity. Preceding studies have already investigated the effect of CPAP treatment on insulin sensitivity in patients with OSAS by euglycemic clamp studies, the standard method to measure insulin sensitivity since 1979 (30). Those studies had conflicting results. Whereas the study of Brooks et al. (16) showed a tendency to improved insulin sensitivity after 4 months of CPAP treatment measured by hyperinsulinemic euglycemic clamp in severely obese and diabetic patients, two other investigations (measurements after 2 and 3 months) could not confirm this result in non-diabetic patients with OSAS (17,18). A major problem of those investigations was the small patient number (always less than 10 patients) that may not offer enough statistical power to detect significant associations between insulin sensitivity and indices of sleep-disordered breathing. Furthermore, if the reassessment of insulin sensitivity is done 2-4

months after onset of CPAP treatment, other factors influencing insulin sensitivity (weight, body fat distribution, treatment of concomitant diseases, changes in dietary behaviour, smoking, alcohol consumption, physical activity) may be of considerable or major importance, too. Especially changes in body weight or fat distribution as important factors influencing insulin sensitivity have to be taken into consideration when interpreting these former studies (12). In these studies, the BMI did not change significantly, which is in accordance to our observation. The percentage of body fat had not been measured in those investigations. However, in recent studies, a growing body of data suggest an independent association between OSAS and insulin resistance independent of body weight. Using the homeostasis assessment method (HOMA) to determine insulin sensitivity and applying stepwise multiple linear regression analysis, Ip et al. (26) were able to demonstrate that obesity was the major determinant of insulin resistance in a group of 270 patients with OSAS, but that parameters of sleep-disordered breathing (AHI, minimal oxygen saturation) were also independent determinants of insulin resistance. Punjabi et al. (27) could also demonstrate an association between an increasing AHI and insulin resistance independent of obesity in mildly obese men.

In our study, we wanted to investigate direct effects of OSAS on insulin resistance with hyperinsulinemic euglycemic clamp studies performed soon after the onset of CPAP treatment to remove confounding variables like fluctuations in body fat or body composition. Assuming that increased nocturnal sympathetic drive is one important mechanism leading to increased insulin resistance in OSAS and considering the short half-lives of adrenal hormones mainly mediating the sympathetic drive (28,29), this effect should be quickly reversible after effective treatment of OSAS. It has to be pointed out, that the hypothesis of an elevated sympathetic drive is only one possible mechanism, alterations of the hypothalamic-pituitary-adrenal function due to sleep disruption and/or hypoxemia also have to be taken into consideration, and should also be reversible soon. To summarize our results, we could clearly

demonstrate an improvement in insulin sensitivity as soon as 2 days after onset of effective CPAP therapy in most of our patients.

In our study, we did not perform computed tomographies to differentiate between visceral fat accumulation and subcutaneous fat. Changes in body fat distribution may have an impact on insulin sensitivity, but significant changes of the body fat distribution within 2 days can be excluded. However, after 3 months of CPAP treatment, changes in body fat distribution might have taken place. The different results compared to Saarelainen et al. and Smurra et al. (17,18) can be explained by a lack of statistical power in these investigations. With this in mind, changes in lifestyle hardly to record completely (e.g. physical activity, diet, nicotine and alcohol consumption) will also influence the statistical calculations more in small samples.

In our attempt to identify further factors influencing the change in insulin sensitivity, we investigated the BMI of our patients, as well as the glucose metabolism (normal fasting glucose vs. impaired fasting glucose according to the ADA criteria). Fasting glucose at baseline was investigated and 5 patients had an impaired fasting glucose. Unfortunately, due to this low patient number, there was insufficient statistical power to detect an effect of the elevated fasting glucose on the changes in insulin sensitivity (Figure 2). We also analysed the impact of the severity of OSAS on insulin sensitivity, since there is experimental evidence, that the exposure to hypoxia can induce insulin resistance (45,46), and furthermore, an independent association of an increasing AHI with worsening insulin resistance independent of obesity has recently been reported (26,27). In our study, we were unable to demonstrate a substantial association between parameters indicating the severity of OSAS with the degree of initial insulin resistance or the degree of improvement of insulin sensitivity 2 days after onset of CPAP treatment. It is unlikely, that the different methods used for the measurement of insulin sensitivity (HOMA vs. euglycemic hyperinsulinemic clamp) could explain this discrepancy. Probably our study was underpowered to demonstrate such effects. However, we would like to point out, that the AHI cannot be considered a parameter that well reflects the

severity of all aspects of OSAS as loss of vigilance, daytime activity and cardiovascular effects and may also be a poor predictor of metabolic effects in OSAS.

In our study, we identified obesity as the main predictor for an improvement in insulin sensitivity during CPAP. The leaner patient group had a more rapid improvement of insulin sensitivity, but the effect could be also demonstrated at a later stage of treatment in the more obese group. At all time points, the insulin sensitivity was significantly better in the lean group.

In an attempt to characterize further important factors associated with metabolic effects in OSAS, we have measured serum levels of leptin, the product of the ob-gene. Leptin is produced within the adipose tissue, that is known to be a main determinant of insulin resistance. We could observe close correlations between serum leptin and the insulin sensitivity index before, as well as 2 days and 3 months during CPAP treatment. These data rather suggest a linkage between insulin resistance and leptin, perhaps via the common main determinant, the adipose tissue and do less support concepts of leptin as a respiratory stimulus as demonstrated in animal models (32). The decrease of serum leptin after 3 months of treatment may be related to factors apart from sleep-disordered breathing as another distribution of adipose tissue or changes in dietary habits.

In our study, we were able to demonstrate, that effective CPAP treatment rapidly improves the insulin sensitivity in patients with OSAS. Nevertheless, our study also shows, that obesity is a more important determinant of insulin resistance. The less obese the patients are, the higher is the improvement of insulin sensitivity by CPAP treatment. Our data are in accordance with the recent investigation of Ip et al. (26). A question still controversially discussed is, whether insulin resistance or hyperinsulinemia are independent cardiovascular risk factors. The question is controversially discussed (47-50), since insulin resistance is typically accompanied by clinical conditions such as obesity, diabetes mellitus or hypertension, *per se* putting the patient at an elevated cardiovascular risk. In the insulin

resistance syndrome, the main pathophysiology probably is the dissociation between intermediate metabolic effects of insulin and its growth promoting effects. However, recent studies like the Quebec Cardiovascular Study (47) have provided data, that insulin resistance could be an independent risk factor for cardiovascular disease with plasma insulin levels predicting a 1.6 fold higher odds ratio for coronary heart disease with every increase of one standard deviation in insulin concentration. Data from the UKPDS study (50) found no relation between (exogenous) insulin treatment and cardiovascular disease. In a more recent paper by Resnick et al. (51), insulin resistance was found to predict diabetes mellitus type 2, but not cardiovascular disease in American Indians. Rutter et al. (52) found insulin resistance associated with increased left ventricular mass, a premier risk factor for cardiovascular disease events in women, not in men. Data from the prospective Insulin Resistance Atherosclerosis Study (53) supported the concept of a protective association between greater insulin sensitivity and a reduced risk for the development of hypertension and cardiovascular disease. The authors claim, that these findings support the conclusion, that interventions improving insulin sensitivity may be beneficial in reducing the atherogenic risk. There is evidence, that CPAP treatment is able to lower blood pressure (16). Furthermore, in medically treated patients with heart failure, CPAP treatment reduces systolic blood pressure and improves left ventricular systolic function (54).

Together with an improvement of blood pressure and heart function due to CPAP therapy, the improvement of insulin sensitivity, clearly demonstrated in our investigation, might be an important factor contributing to a reduction of cardiovascular risk in patients with OSAS treated with CPAP.

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## Figure Legends

Figure 1. Improvement in the insulin sensitivity index (ISI) at baseline, 2 days after onset of CPAP treatment and 3 months under CPAP treatment in 31 patients. The insulin sensitivity index is given as  $\mu\text{mol}/\text{kg} \times \text{min}$ .

Figure 2. Changes of the insulin sensitivity index (ISI, given as  $\mu\text{mol}/\text{kg} \times \text{min}$ ) between baseline and 2 days after onset of CPAP treatment. The changes are significantly different for the whole group ( $p = 0.003$ ). The degree of changes are furthermore significantly different between the patients with  $\text{BMI} < 30 \text{ kg}/\text{m}^2$ , not in the patients with  $\text{BMI} \geq 30 \text{ kg}/\text{m}^2$ . The changes of ISI in patients with an impaired fasting glucose are given as white circles.

Figure 3. Changes of the insulin sensitivity index (ISI, given as  $\mu\text{mol}/\text{kg} \times \text{min}$ ) between baseline and 3 months after onset of CPAP treatment in the 31 patients with baseline  $\text{BMI} < 30 \text{ kg}/\text{m}^2$  and the subgroup with  $\text{BMI} \geq 30 \text{ kg}/\text{m}^2$ .

## Tables

Table 1. Characteristics of the patients with OSA at baseline and during the course of their treatment with CPAP, data given as means  $\pm$  standard deviation. 31 of the initial 40 patients were reinvestigated 3 months after onset of CPAP treatment. None of the parameters given in the table are significantly different in this subgroup of 31 patients at the time points “before treatment” and “2 days CPAP treatment” and all 40 patients.

Definition of abbreviations: BMI: Body Mass Index; AHI: Apnea-Hypopnea Index; ARI: Arousal Index; ODI: Oxygen Desaturation Index; MMAO<sub>2</sub>: Mean Minimal Average Oxygen Saturation; ESS: Epworth Sleepiness Scale.

	Before treatment	After 2 days CPAP treatment	After 3 months CPAP treatment
Number of patients	40	40	31
BMI (kg/m <sup>2</sup> )	32.76 $\pm$ 6.92	n.d.	32.33 $\pm$ 6.69
Percentage Body Fat	23.10 $\pm$ 9.89	n.d.	21.29 $\pm$ 8.72
AHI	43.10 $\pm$ 11.38	5.50 $\pm$ 4.39	4.43 $\pm$ 3.40
ARI	37.81 $\pm$ 2.12	5.09 $\pm$ 3.41	5.43 $\pm$ 3.74
ODI	40.12 $\pm$ 16.41	5.12 $\pm$ 4.79	4.82 $\pm$ 4.13
MMAO <sub>2</sub> (%)	88.72 $\pm$ 4.16	92.98 $\pm$ 0.93	91.31 $\pm$ 0.84
ESS	12.90 $\pm$ 3.60	n.d.	6.90 $\pm$ 4.00
Leptin ( $\mu$ g/l)	20.56 $\pm$ 17.04	20.63 $\pm$ 18.67	10.19 $\pm$ 10.90

Table 2: Changes in the insulin sensitivity index (ISI), given as  $\mu\text{mol}/\text{kg} \times \text{min}$ , before and during CPAP treatment in the whole patient group, as well as in two subgroups with BMI  $< 30 \text{ kg}/\text{m}^2$  and BMI  $\geq 30 \text{ kg}/\text{m}^2$ .

	ISI (whole group) n=40  (after 3 months n=31)	ISI (BMI $< 30 \text{ kg}/\text{m}^2$ ) n=16  (after 3 months n=13)	ISI (BMI $> 30 \text{ kg}/\text{m}^2$ ) n=24  (after 3 months n=18)
baseline	$5.75 \pm 4.20$	$8.53 \pm 4.48$	$3.89 \pm 2.80$
after 2 days CPAP therapy	$6.79 \pm 4.91$	$10.47 \pm 5.09$	$4.33 \pm 2.87$
Improvement compared to baseline	p=0.003	p=0.001	p=0.13
after 3 months CPAP therapy <sup>a</sup>	$7.54 \pm 4.84$	$10.71 \pm 4.95$	$5.26 \pm 3.50$
Improvement compared to baseline	p=0.001	p=0.001	p=0.03

<sup>a</sup>: statistically significant differences remain, if tested in the 31 individuals at all three time points

Table 3: Results of logistic regression analysis of the influence of “BMI < 30 kg/m<sup>2</sup>” on “change in insulin sensitivity (ISI) before and after 2 days of CPAP treatment > 0.58” (0.58 was the median change in ISI). BMI has an independent impact on the change of ISI while controlling for age, hypertension, and AHI.

Variable	Regression coefficient	p-value	Odds ratio and confidence interval
BMI < 30 kg/m <sup>2</sup>	2.19	0.02	8.89 (1.51 – 52.22)
Age	0.01	0.77	1.01 (0.95 – 1.08)
Presence of hypertension	0.73	0.39	2.07 (0.39 – 10.91)
Baseline AHI < 39	1.36	0.09	3.91 (0.81 – 18.90)
Constant	-2.36	0.24	0.10

Figure 1

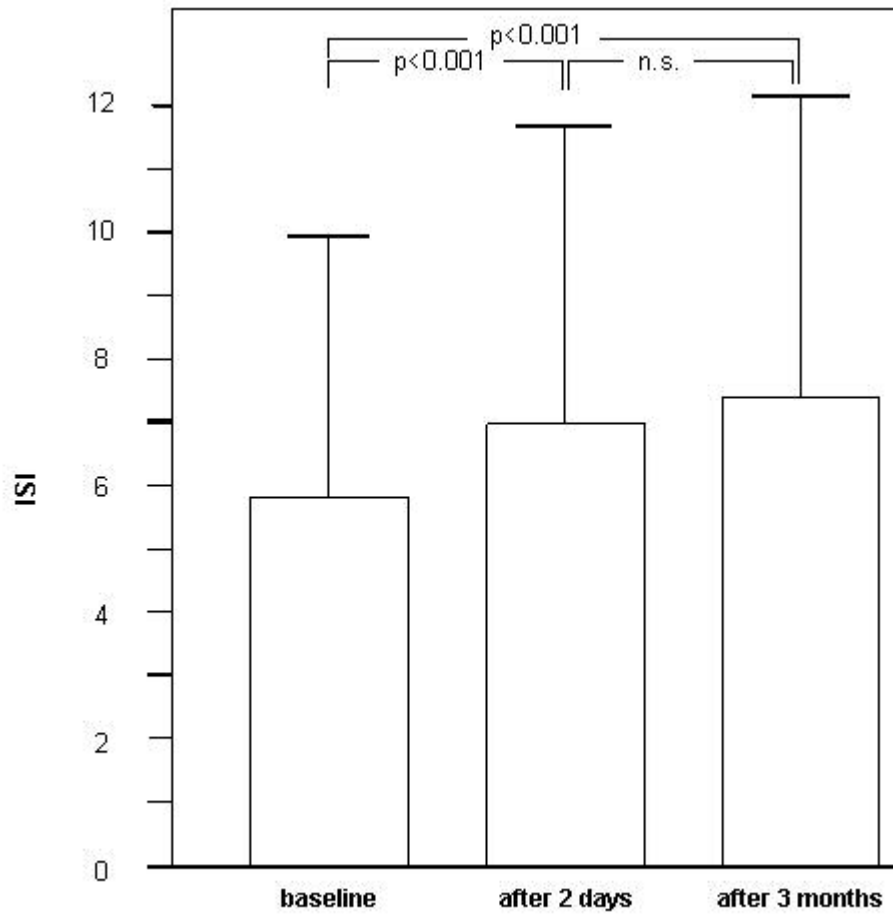


Figure 2

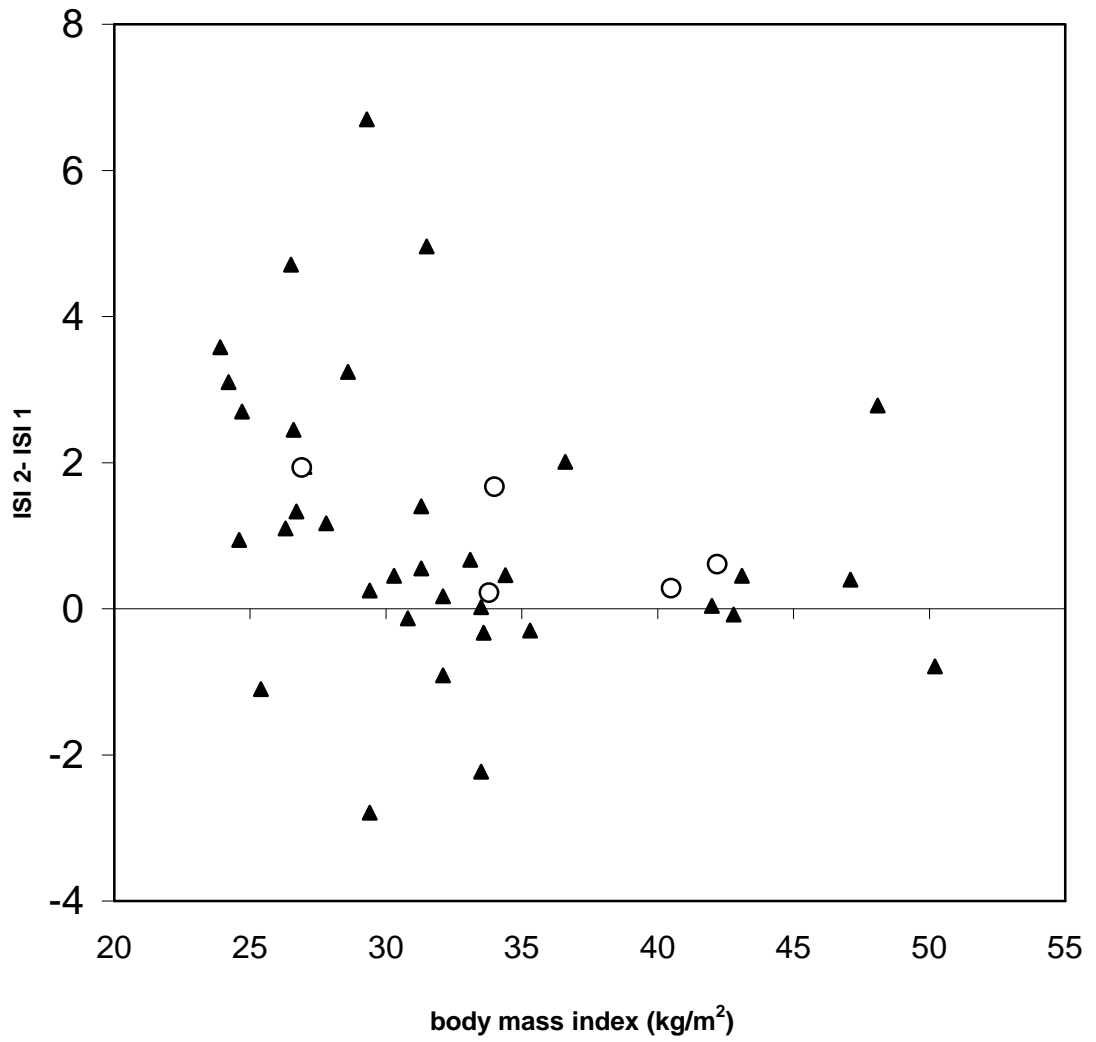
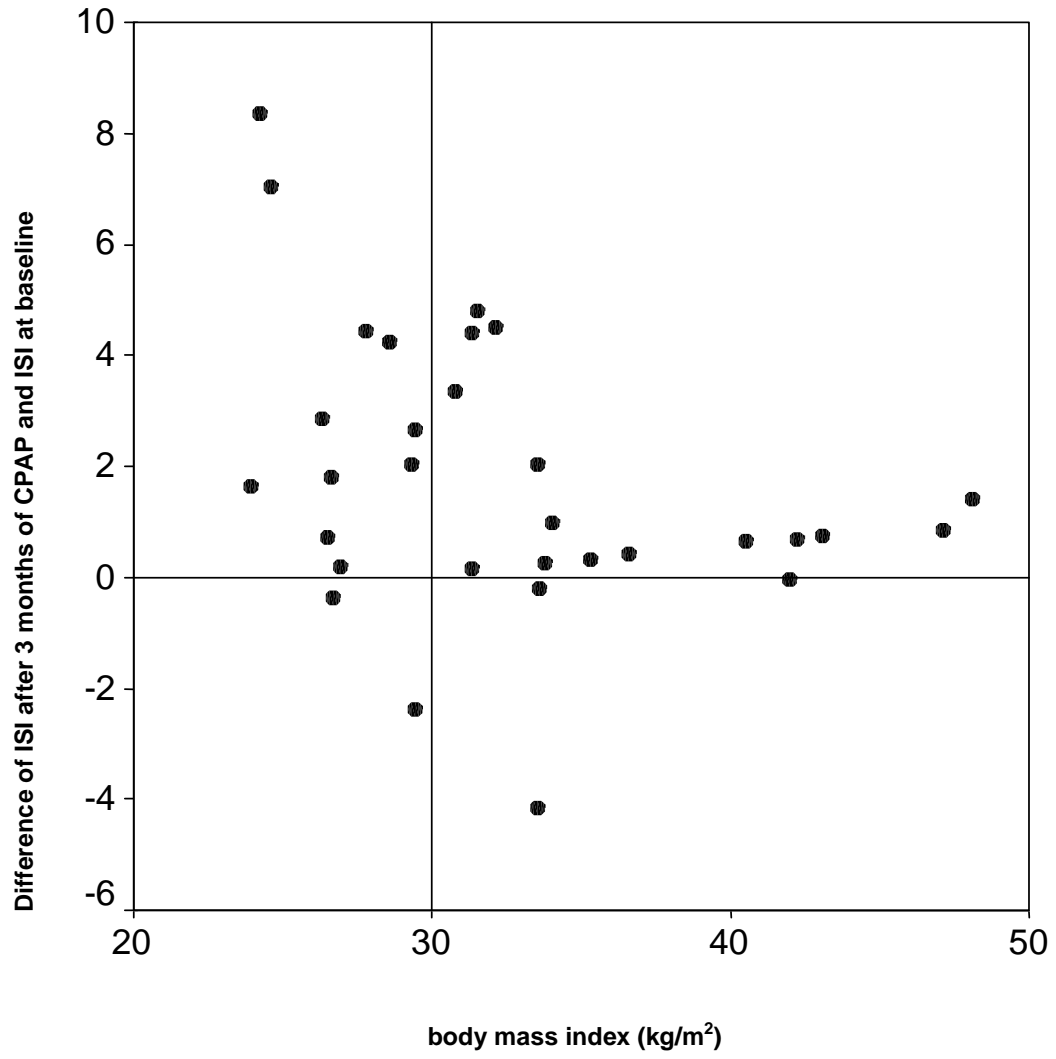


Figure 3



## **Appendix: Online-Only Supplement**

### **CPAP Treatment rapidly improves Insulin Sensitivity in Patients with OSAS**

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This supplementary material contains an extended version of the “Methods” part and one additional table for the “Results” part. The references and their numbers are identical with the numbers of references in the Print Version. The online version of the manuscript should contain the extended Methods part and the one “Results” table as given below.

## **Methods**

### ***Design and setting***

We did a longitudinal study of patients in the Sleep Laboratory of the University of Erlangen-Nuremberg, Germany. The unit takes patients who have been referred with suspicion of obstructive sleep apnea syndrome from the surrounding region.

### ***Patients***

Forty patients with OSA with a mean age of  $53.81 \pm 11.84$  years agreed to participate in the study by written informed consent. 34 patients were male, 6 patients female. The mean BMI was  $32.76 \pm 6.92$  kg/m<sup>2</sup>. The patients had to be free from severe accompanying diseases as malignant diseases, liver diseases and endocrinopathies (TSH and IGF-I were found normal in all patients). Hyperlipidemia was present in 20 patients. Hyperlipidemia was defined according to the NCEP and ATP III classification (National Cholesterol Education Program; Adult Treatment Panel III) as LDL cholesterol > 130 mg/dl and total cholesterol > 200 mg/dl (34). Hypertension was present in 24 patients. Hypertension was defined according to the WHO definition as a blood pressure higher than 140/90 mmHg (35). None of the patients had diabetes mellitus (fructosamine and HbA1c were normal and fasting glucose < 126 mg/dl). 5 patients had an impaired fasting glucose ( $116.2 \pm 4.79$  mg/dl) according to the ADA (American Diabetes Association) criteria (36). Five of the hypertensive patients were treated with calcium antagonists, 6 with ACE inhibitors, 2 with AT-II antagonists and 4 with Beta-blockers, the latter withdrawn 1 week before the clamp studies (37). 3 patients had asthma and were treated with inhaled antiobstructive medication. 6 patients were smokers. Patients with a medication influencing insulin sensitivity (e.g. thiacides or steroids) were excluded from the study (25). All patients had moderate to severe OSA with a mean AHI of  $43.10 \pm 11.38$ . They all complained about impaired psychophysical performance and daytime sleepiness (Epworth Sleepiness Scale:  $12.9 \pm 3.6$ ; Range 9-23) (38).

## ***Procedures***

The 40 patients were admitted to the sleep lab of our clinic at 8 p.m. and underwent a first night with diagnostic polysomnography (PSG) as previously described (39-41). Before admission, all patients had been scored positive for OSA by ambulatory screening (SOMNOcheck; Fa. Weinmann, Hamburg, Germany). If the diagnosis of moderate to severe OSA was confirmed by PSG, the patients underwent a physical examination and a routine blood examination (sodium, potassium, fasting glucose, hepatic and renal parameters), as well as serum leptin, lipoprotein (a), cholesterol and its subfractions. Furthermore, a bioelectrical impedance measurement was performed and the insulin responsiveness measured by a hyperinsulinemic euglycemic clamp at 7.00 a.m.. In the following night, CPAP treatment was initiated according to a standard CPAP-titration protocol (41) and in the consecutive third night, CPAP treatment was performed with the previously established minimal effective CPAP pressure, followed by a second hyperinsulinemic euglycemic clamp at the next morning, and another measurement of fasting leptin.

In the remaining 31 patients, the clamp studies were repeated after about 3 months ( $93.97 \pm 25.22$  days) after a further night with polysomnography in the sleep lab with effective CPAP treatment. Nine of the initial forty patients had removed ( $n = 3$ ) or discontinued CPAP treatment due to discomfort. In addition, the built-in data stores of the CPAP devices were read out, the number of days of use within the past 42-day period established, and the mean duration of use per treatment night calculated. The same laboratory parameters as after the first night were repeated, as well as bioelectrical impedance analysis. The medical treatment for all patients remained unchanged throughout the study, one patient had undergone coronary bypass surgery in the meantime.

The study protocol was examined and approved by the Ethics Committee of the Friedrich-Alexander-University, Erlangen-Nuremberg. All patients gave informed consent to participate in this study.

### ***Methods***

Hyperinsulinemic euglycemic clamp:

Insulin responsiveness was measured by a hyperinsulinemic euglycemic clamp after a 10-h overnight fasting period (30) by a trained team of three medical students. An i.v.-cannula was inserted into one arm for blood sampling, with a warming blanket wrapped around the forearm to arterialize the blood. A second i.v.-cannula was inserted at the other arm for the insulin and glucose infusions. Human insulin (Actrapid U 40 HM; ge, Novo Nordisk, Bagsvaerd, Denmark) was administered continuously at a rate of 1 mU / kg x minute. The glucose infusion, calculated according to a predetermined algorithm, was commenced, when glucose fell under 80 mg/dl. Glucose was administered continuously at a variable rate and serum glucose levels were measured every 10 minutes. The rate of glucose infusion was varied to maintain serum glucose levels at 90 mg/dl according to a given algorithm with variations  $\pm 4$  mg/dl regarded as acceptable. At 90, 100, 110 and 120 minutes, blood samples were taken to measure serum insulin levels and the actual glucose infusion rates were documented. The insulin sensitivity index (ISI) was calculated from these insulin measurements and the corresponding glucose infusion rates during that period and was given as  $\mu\text{mol} / \text{kg} \times \text{min}$ . The glucose values were determined successively by a Beckman Glucose Analyzer 2 (Beckman Coulter Instruments, Inc.; Galway, Ireland) with a glucoseoxidase method. Blood samples for insulin measurements were centrifuged at 3.000 rpm for 20 min at 4°C, and were stored at -80°C. Insulin levels were measured by an immunenzymometric assay (SR 1 Insulin; Biochem ImmunoSystems; Freiburg, Germany). The intraassay precision of this kit is 3.3%, the interassay precision 4.8%.

#### Bioelectric Impedance Analysis:

The Bioelectric Impedance Analysis was done with the following device: Akern-RJL BIA 101/5 Body Composition Analyzer (Data Input Inc., Frankfurt, Germany). The measurements were done before the hyperinsulinemic euglycemic clamp with the patients fasting for at least 10h, the bladder emptied and without having done sports for at least 12h.

#### Serum Leptin Measurements:

The blood samples were collected in ethylenediamine tetraacetate-coated polypropylene tubes kept on ice, were promptly centrifuged at 3.000 rpm for 20 min at 4°C, and the supernatant clear plasma was then stored at -35°C until measurement of plasma leptin levels using an ELISA kit (IBL ELISA kit ; IBL Inc., Hamburg, Germany). The intraassay variation of this kit is 4.13 %, the interassay variation 3.59 %.

#### Sleep studies:

Diagnostic polysomnography was performed in our sleep laboratory by trained sleep laboratory technicians according to the recommendations of the American Thoracic Society (39) and the German Sleep Society (40) as previously described (41). All variables were recorded on a computer (SleepLab, Jaeger and Toennies, Wuerzburg, Germany), and included electroencephalography (C4/A1, C3/A2), bilateral electrooculography (EOG), submental electromyography (EMG), nasal airflow measured by nasal cannulas during diagnostic polysomnographies and by a pneumotachograph during CPAP studies, snoring detected by microphone, electrocardiography, thoracic and abdominal movements measured by uncalibrated inductive plethysmography and oxyhaemoglobin saturation using an finger oxymeter (Microspan 3040G, Jaeger and Toennies; Wuerzburg, Germany).

Obstructive apneas were defined as the absence of oronasal airflow for at least 10 s. Hypopneas were defined as reduction in airflow to  $\leq 60$  % of the preceding stable baseline for 10 s or longer together with a dip in oxygen saturation  $\geq 4\%$ . The mean number of apneas and hypopneas per hour of sleep was calculated as the apnea/hypopnea index (AHI). Oxygen desaturations were defined by a drop in oxygen saturation of more than 4% from a stable baseline and the oxygen desaturation index (ODI) was calculated as the number of oxygen desaturations per hour. Further variables extracted were the following: Arousal Index (ARI) and Mean Minimal Arterial Oxygen Saturation (MMAO<sub>2</sub>), as described by Juhasz et al.(44). Sleep staging was performed using the criteria of Rechtschaffen and Kales (42) and microarousals were defined in accordance with the definitions of the American Sleep Disorders Association (ASDA) (43). The polysomnographies were analyzed manually by experienced sleep lab technicians.

CPAP titration and therapy:

For CPAP treatment we used a standard CPAP device Somnotron 4<sup>®</sup> (Weinmann, Germany). Manual titration of the CPAP pressure was performed in our sleep laboratory under polysomnographic control by a trained sleep laboratory technician. For each patient, the minimum effective pressure at which most of the apnoeas, hypopneas and snoring were abolished in all body postures and all stages of sleep was established. Starting from an initial 4 mbar, the pressure was increased in steps of 1 mbar at intervals of at least 5 minutes when obstructive events (apneas, hypopneas or snoring) occurred. If no further events occurred during the next 30 minutes, the pressure was then reduced every 10 minutes in steps of 1 mbar until such events re-occurred, whereupon the pressure was increased once more in the manner described above. The possibility to reduce the pressure temporarily and then increase it again helps to establish the minimal effective pressure needed more accurately, thus, avoiding the

use of unnecessarily high pressures. On the second night of CPAP the patients were treated with the minimum effective pressure established during the previous night.

#### Data analysis:

Normally distributed variables are described using mean values and standard deviation (mean  $\pm$  SD). Scatterplots are used for graphical analysis of the relationships between two continuous variables. Associations between continuous non-parametric variables were evaluated using Spearman's rho correlation coefficient. Differences between insulin sensitivity (ISI) before and after treatment were evaluated using the Wilcoxon test for paired samples. Differences between frequencies of unpaired samples were evaluated using Fisher's exact test. Differences between unpaired samples of continuous data were evaluated using the Mann-Whitney test. For evaluation of the influence of BMI on the changes of insulin sensitivity during the course of treatment, a logistic regression analysis was performed with the binary variable "ISI 2 d after onset of CPAP treatment – ISI before CPAP treatment > 0.58" as dependent variable and the independent variable "BMI < 30 kg/m<sup>2</sup>" while controlling for the variables age, hypertension, and "baseline AHI < 39". In this analysis, based on the finding that the continuous variables AHI and ISI change after 2 days and were not approximately normally distributed, we chose to dichotomize them according to the sample median prior to regression analysis as described above. BMI was dichotomized according to a clinically useful cutpoint (BMI < 30). An additional exploratory logistic regression was performed with the dependent variable „baseline ISI < 3.94“ and the independent variables "baseline AHI < 39", "percent body fat < 21.5", "BMI < 30", and hypertension. Likewise, in this analysis, the sample median was used for dichotomization of the covariates "baseline ISI" and "percent body fat". The resulting odds ratios can be seen as estimates of the relative risk of a subject with the respective covariate value to experience the outcome of interest compared to subjects with the respective reference covariate value. Two-sided p values  $\leq$  0.05

are considered as significant. All statistical calculations were performed with SPSS (Version 11.0).

## Results:

Additional table: Characteristics of the patient group 1 (BMI < 30 kg/m<sup>2</sup>) and 2 (BMI ≥ 30 kg/m<sup>2</sup>) before onset of CPAP treatment.

Definition of abbreviations: BMI: Body Mass Index; AHI: Apnea-Hypopnea Index; ARI: Arousal Index; ODI: Oxygen Desaturation Index; MMAO<sub>2</sub>: Mean Minimal Average Oxygen Saturation.

	Group 1 (n = 16)	Group 2 (n = 24)	p value
BMI (kg/m <sup>2</sup> )	25.78 ± 4.78	36.80 ± 6.05	< 0.001 <sup>a</sup>
Percentage Body Fat	20.08 + 5.93	25.47 + 8.64	0.04 <sup>a</sup>
Age (years)	61.81 ± 9.30	54.83 ± 12.71	0.07 <sup>a</sup>
Hypertension	8	16	0.32 <sup>b</sup>
Female sex	1	5	0.25 <sup>b</sup>
AHI	37.87 ± 14.05	46.58 ± 20.67	0.21 <sup>a</sup>
ARI	35.50 ± 20.55	41.58 ± 21.62	0.40 <sup>a</sup>
ODI	33.25 ± 14.86	46.04 ± 22.60	0.09 <sup>a</sup>
MMAO <sub>2</sub> (%)	89.91 ± 3.56	87.68 ± 5.00	0.14 <sup>a</sup>
Leptin (µg/l)	10.12 ± 7.72	27.52 ± 18.09	0.001 <sup>a</sup>

<sup>a</sup>: Mann-Whitney-U-Test; <sup>b</sup>: Fisher's exact test.