SUPPLEMENTAL MATERIAL

Detailed Summary of Exclusion Criteria

The exclusion criteria included: current use of oral hypoglycemic medications or insulin; overt diabetes defined by a mean fasting plasma glucose (FPG) level ≥ 126; a mean 2-h OGTT glucose ≥ 250 mg/dL; a single abnormal FPG or 2-h OGTT glucose with diabetes symptoms or marked metabolic derangement (e.g., acidosis); use of supplemental oxygen; a primary sleep disorder other than SDB; severe chronic insomnia or circadian rhythm disorder with < 4 h of sleep per night; unstable medical conditions (e.g., new onset or changing angina, myocardial infarction, or congestive heart failure exacerbation documented within the previous 3 months, uncontrolled hypertension, etc.); daytime sleepiness with reports of sleepiness while driving or otherwise in situations which would present a risk for the subject or public (e.g., operating heavy equipment); alcohol abuse; or pregnancy.

Detailed Description of Study Design

This is a randomized, double-blind, 2×2 crossover trial in patients with sleep apnea and IGT. A schema of the study design is depicted in Figure 1. In brief, if subjects met initial study eligibility criteria, they underwent a polysomnography titration study. Each subject was then enrolled in a 2-week run-in period where they were asked to use CPAP at home for 2 weeks at the pressure settings identified by titration to resolve sleep apnea events. Those subjects meeting minimal CPAP adherence (5 h of use or 70% of sleep time) during the 2-week run-in period received a one-month washout period and were then randomized to receive 8 weeks of CPAP or 8 weeks of sham CPAP (Period 1). Following Period 1, patients again received a one-month washout period and then crossed over to the alternate 8 weeks of therapy (Period 2). Throughout the study, each patient attended a series of research visits at the Dahms Clinical Research Unit of University Hospitals Case Medical Center. These research visits included 4 overnight examinations: polysomnography with CPAP titration prior to the run-in period; polysomnography without CPAP therapy before the start of Period 1 (baseline visit); and polysomnography at the end of Period 1 and 2 (follow-up visits). The details of the measurements, which included anthropometry, venipuncture, actigraphy and abdominal CT imaging made in conjunction with each visit are summarized below. Sequence order (Sequence 1: CPAP/sham CPAP; Sequence 2: sham CPAP/CPAP) was determined by a computerized program that generated random numbers.

Detailed Description of Diet, Nutrition and CPAP Education and CPAP Interventions

Sleep hygiene, dietary counseling, and CPAP support: All subjects met with a sleep technician and research nutritionist prior to CPAP titration and received approximately 30 min of instruction on sleep hygiene (including written materials) and diet. The nutritionist used a standardized instrument to guide a problem-specific approach to dietary counseling. A designated, unblinded research assistant oversaw the research titration studies and provided participant support for CPAP use across the duration of each intervention. The assistant was responsible for fitting masks, educating participants on CPAP use and for providing ongoing troubleshooting of problems with mask fit, pressure sensations, leak, nasal congestion, and behavioral resistance to use of CPAP. The assistant met with each participant at the time of titration and then periodically in person or by phone throughout the 8-month study period.

Active CPAP: Prior to the run-in period, a trained research technician titrated the pressure during an overnight sleep study with the goals of eliminating obstructive apneas and reducing hypopneas to an AHI < 5, reducing respiratory-related arousals, maintaining oxygen saturation > 90%, and eliminating snoring. Each participant was provided a Philips-Respironics RemStar Pro CPAP machine, equipped with humidification and expiratory relief as needed.

Sham-CPAP: Sham therapy was delivered using a customized Philips-Respironics device and masks with increased expiratory ports, configured to deliver a marginal pressure (0.4 ± 0.1 SD cm H_2O). CPAP use was objectively monitored using data exported from the CPAP units, quantifying effective duration of treatment (time spent at prescribed pressure). Data were exported at the end of the run-in period and Period 1 and 2.

Detailed description of sleep measurements, biochemical outcomes, anthropometry and blood pressure measurements.

Biochemical outcomes: Following each polysomnography study and a 12-h fast, venipuncture was performed and again after ingestion of 75 grams of anhydrase glucose (OGTT). To identify potential early changes in glucose metabolism, fasting blood and 2-h OGTT were also obtained 5-7 days after the start of Period 1 and 2. Fasting blood and 2-h OGTT were also measured within 3 days of the baseline visit and at each of the 2 follow-up visits so that at each time point, duplicate measurements were available to enhance the reliability. Whole blood was assayed for hemoglobin A1C using a BioRad VARIANT instrument based on ion-exchange high performance liquid chromatography. Insulin was determined using Coat-A-Count radioimmunoassay kits (Siemens Healthcare Diagnostics, Deerfield, IL). Glucose was determined using a Beckman Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA) or a YSI 2300 StatPlus analyzer (YSI Life Sciences, Yellow Springs, OH). Lipids were measured by enzymatic methods.[33]

The insulin sensitivity index was calculated using the Gutt Index (ISI (0,120) ([75 000 mg + (fasting glucose – 2 h glucose) × 0.19 × body weight] / 120 min[35]). Insulin resistance was also estimated using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR: Ins0 (μU/mL) × Glc0 (mmol/L)) / 22.5; "0" represents the fasting value).HOMA-B (β-cell function), a measurement used to assess pancreatic β-cell function (which we postulated would not vary with CPAP treatment) was calculated as: (fasting insulin in μU/mL) × 3.33 / (fasting glucose in mg/dL - 3.5). The ISI (0,120), HOMA-IR, and HOMA-B were log transformed to achieve approximate normality.

Anthropometry: The following were measured in duplicate at the baseline visit and at the 2 follow-up visits by staff blinded to the interventions: height; weight; neck, waist, and hip circumferences; and skin-fold thickness. At the 2 follow-up visits, abdominal computed tomography (CT) was obtained to quan-

tify abdominal total, visceral, and subcutaneous fat with 2 slices taken at lumbar 4-5 level (Siemens Sensation 16 scanner).

Blood pressure: At each of the overnight examinations, blood pressure was measured supine at approximately 22:00 and then at 07:00 in triplicate.

Detailed Description of Statistical Analysis

Preliminary analyses: Baseline characteristics were summarized by randomized sequence group (Sequence 1: CPAP then sham CPAP; Sequence 2: sham CPAP then CPAP) using the mean and standard deviation (SD) for quantitative variables and a frequency and percentage for categorical variables. A Wilcoxon-Mann-Whitney test, 2-sample *t*-test, or Fisher exact test was used as appropriate to compare baseline characteristics between the 2 sequence groups.

Indices of sleep, anthropometry, and CPAP adherence at each follow-up visit were summarized for each therapy in each of the 2 sequences. A paired 2-sample *t*-test was used to compare these indices between CPAP and sham CPAP. Indices of sleep included AHI, arousal index, oxygen saturation, %N3 (slow wave) sleep, and sleep duration. Indices of anthropometry included BMI and CT visceral abdominal tissue fat. Indices of adherence included average usage and percentage of days when therapy was used for > 4 hours.

Primary analyses: Based on an intent-to-treat approach, a generalized estimating equation (GEE) approach was used to estimate the effect of therapy (CPAP or sham-CPAP) on the odds of normalization of IGT. The model treated subjects as clusters and included fixed effects for period, therapy, and baseline 2-h OGTT. Such a model provides an estimate of the odds ratio of normalizing the 2-h OGTT with CPAP compared with sham-CPAP, while controlling for period and baseline 2-h OGTT glucose level. Models were also extended to include additional adjustment for baseline BMI, baseline AHI, gender, and race.

The effect of carryover on normalization of IGT was also estimated. In this context of this 2×2 crossover study, carryover effects refer to when the effect of therapy in Period 1 persists into Period 2 and distorts the effect of the therapy in Period 2 on normalization of IGT. To assess this, we tested the interaction between therapy and period. Separate analyses were also conducted for each period to estimate the effect of therapy on normalization of IGT via a parallel group design. In this case, we adjusted for the baseline 2-h OGTT glucose, baseline BMI, baseline AHI, gender, and race.

Secondary analyses: Secondary outcomes included continuously measured indices of glucose and insulin resistance that were obtained after fasting and 2 h after the OGTT, as well as the ISI (0,120), HOMA-IR, and HOMA-B. Based on an intent-to-treat approach, a linear mixed effect model was used to estimate the association between therapy and each secondary outcome. Each model included a random intercept to account for within subject correlation as well as fixed effects for period, therapy, and the baseline measure of each outcome. For normally distributed outcomes, these models estimated the adjusted mean difference in the outcome between CPAP and sham CPAP, while for log-normally distributed outcomes (such as ISI), these models estimated the adjusted geometric mean ratio of the outcome between CPAP and sham CPAP. Models were also extended to include baseline BMI, AHI, gender and race as additional covariates. As with the primary analyses, the carryover effect was estimated for each secondary outcome by testing an interaction between therapy and period although separate analyses were conducted for each period as well.

Post hoc analyses: Post hoc stratified analyses were also performed to assess whether differences in the effect of CPAP on outcomes were observed according to baseline sleep apnea severity (defined by an AHI > 30), gender, race, and baseline weight (dichotomized using the sample median BMI 32). In this case, an interaction between treatment and each baseline factor was incorporated into each model and the effect of therapy on each outcome was estimated at each level of the baseline factor. Using only the data obtained when subjects received CPAP, we also estimated the association between the average hours of use of CPAP and changes in outcomes.

A priori, for 80% power and an α level of 0.05, assuming a within-subject correlation of 0.5, we estimated that a final sample of 37 subjects would be needed to detect a treatment response of 25% if the CPAP group improved while 5% of the sham group improved. For analyses of group differences in levels of metabolic or vascular parameters, we estimated that an n = 50 provides > 90% power to detect effect sizes of > 0.50.