METHODS-online extended version

Study design

One hundred and forty four men, between the ages of 30-65 years, presenting abdominal obesity (waist circumference ≥90 cm), triglyceride levels ≥1.69 mmol/L and/or HDL-cholesterol <1.03 mmol/L, were recruited in general community, by solicitation in the media, for a three-year lifestyle modification program. Subjects with type 2 diabetes, body mass index (BMI) values <25 or >40 kg/m^2, or taking medication targeting glucose or lipid metabolism or blood pressure were excluded. From these 144 men, 86 volunteered to participate in an ancillary study addressing nocturnal respiratory disturbances at baseline and after one year. From these 86 men, 77 had a technically acceptable sleep home-recording, and were kept for baseline analyses. After one year of intervention, 11 men had dropped out the program, leaving 66 men for the one-year evaluation. From this 66 men, 11 refused to participate to the one-year sleep evaluation, the sleep recording was not technically satisfactory in 8 men, leaving 47 men for the one-year sleep-related analyses (Figure 1). Informed written consent was obtained from all participants prior to their inclusion in the study which had been approved by the Medical Ethics Committees of Université Laval and Institut universitaire de cardiologie et de pneumologie de Québec.

Lifestyle intervention

Subjects were individually counseled to improve their nutritional and physical activity/exercise habits, once every two weeks during the first four months of management with subsequent monthly visits. Each visit included an interactive session with a registered nutritionist followed by a meeting with a kinesiologist. The nutritional counseling was adapted to elicit a 500 kcal daily energy deficit during the first year, which was the "moderate

weight loss" phase of the study. The daily caloric intake was estimated at baseline and at one year by a three-day dietary record including a nonworking day.

The physical activity program was individualized based on subjects' history and preferences. The goal was to reach 160 min/week of moderate intensity endurance exercise which also included, as additional objective, an increase in occupational activity. In order to help participants to be more active between exercise sessions, they were asked to wear a pedometer and to reach a target of 10 000 daily steps.

Anthropometric measurements and body composition

Height, weight and waist circumference were measured according to standardized procedures. [1] Body composition (fat mass and fat free mass) was assessed by DEXA (Lunar Prodigy, GE, Madison, WI, USA). Three sitting blood pressure and pulse rate measurements were taken 3 minute apart on the non dominant arm with an appropriate cuff size measured after the patient had been resting in the sitting position for 5 minutes.

Computed tomography

Visceral adipose tissue and subcutaneous adipose tissue cross-sectional areas were assessed by computed tomography, using previously described procedures [2-3]. Calculations of the partial volumes of visceral adipose tissue and subcutaneous abdominal adipose tissue between L2-L3 and L4-L5 were performed using the product of the mean of L2-L3 and L4-L5 areas multiplied by the distance separating the two slices, as previously described [4].

Cardiorespiratory fitness

Cardiorespiratory fitness was assessed using a submaximal standardized exercise test on a TMX 425 treadmill (Trackmaster, Newton, KS) linked to a QuarkB2 monitor (Cosmed, Rome, Italy). After 3 minutes of warm-up at 2.5 miles per hour (mph), 0% slope, the exercise

physiologist adapted the speed and the slope in three to four steps of 5 minutes each, including a standardized workload of 3.5 mph at 2% slope, in order to obtain a linear progression to reach between 70% and 80% of the predicted maximal heart rate, which corresponds to approximately 150 beats/min for all subjects. According to the American College of Sports Medicine formulas,[5] the VO_2 was calculated for each step. In the present study, two variables were retained as fitness endpoints to evaluate CRF: (i) the subject's heart rate (mean of the last three minutes) at a standardized treadmill stage (3.5 mph, 2% slope) and (ii) the estimated metabolic equivalent of task (MET) reached by the subject at a heart rate of 150 beats/min.

Oral glucose tolerance test

After a 12-hour overnight fast, participants were subjected to a three hours, 75 g oral glucose load. Blood samples were taken for the measurement of plasma glucose and insulin concentrations. Plasma glucose was measured enzymatically,[6] whereas plasma insulin was determined by radioimmunoassay. The total glucose and insulin areas under the curve (AUC) during the oral glucose tolerance test (OGTT) were determined by the trapezoid method between 0 and 180 minutes.

Plasma lipoprotein-lipid profile

Fasting plasma triglycerides and HDL-cholesterol were determined according to standardized procedures.[7-10]

Adipokine and inflammatory markers

Adipokines and inflammatory markers were measured on frozen plasma samples (-80°C). Briefly, plasma leptin and adiponectin concentrations (B-Bridge, CA, USA) as well as plasma interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha (R&D Systems Inc, Mineapolis,

MN, USA) were determined by ELISA. Highly sensitive C-reactive protein (CRP) levels were measured with by immunoassay (Dade Behring, Germany). Plasma CRP levels >10 mg/L were excluded from the analyses.[11]

Nocturnal respiratory recording

Subjective daytime sleepiness was evaluated with Epworth sleepiness scale (ESS). The presence of nocturnal respiratory disturbances was assessed at home by an ambulatory monitoring device (Remmers Sleep Recorder Model 4.2; Saga Tech Electronic, Calgary, AB, Canada) providing a continuous recording of nasal pressure and a percutaneous oxyhaemoglobin saturation (SpO_2). Oxygen desaturation index (ODI) was automatically determined as the number of SpO_2 fall ≥ 4% followed by a return within 1% of baseline value.[12] Mean SpO_2, minimal SpO_2, time spent under 90% of SpO_2 and total monitoring time were also determined. Respiratory disturbance index (RDI) was manually determined by nasal pressure signal analysis. A minimum of 4 hours of suitable nasal pressure, SpO_2 and heart rate were required to determine RDI. Apnea (absence of airflow) and hypopnea (reduction of nasal pressure magnitude ≥50% from baseline value or a ≥30% reduction of airflow associated with a fall of SaO_2 ≥4%, or an heart rate acceleration ≥8 beats/min) lasting ≥10 s were scored to determine RDI. Since RDI validity criteria ended with significant missing data, the ODI was chosen to establish sleep apnea phenotype. ODI and apnea+hypopnea index determined by polysomnography are highly correlated [12] and a threshold of 10 events/hour in ODI has been shown to predict an apnea+hypopnea index above 15 events/hour with a 85% sensitivity and a 93% specificity.[13] Therefore, an ODI threshold of 10/hour was chosen to separate men with or without moderate to severe OSAS.

Statistical analysis

Results were expressed as means ± SD. Changes after one year were assessed by one-way ANOVA repeated measure on the 77 patients who completed the sleep recording at baseline. Men were classified according to baseline ODI value. Comparisons between the two groups were made by one-way ANOVA, with and without adjustments for baseline VAT volume and baseline daily step count. Pearson correlation coefficients were calculated between sleep variables and cardiometabolic risk markers. ODI, RDI, triglycerides, HOMA-IR, CRP, TNF-alpha and IL-6 values were log transformed. Significance was set at p-values ≤0.05. SAS, 9.2 (SAS Institute Inc, Cary, NC, U.S.A.).

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