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Sleeping oxygen saturation, rapid eye movement sleep, and the adaptation of postprandial metabolic function in insulin sensitive and resistant individuals without diabetes

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Abstract

Aims—Sleeping oxygen saturation (SaO₂) and sleep stage duration have been linked with prediabetic alterations but the pathogenic pathways are not well understood. This study of insulin sensitive and resistant adults examined the effect on postprandial metabolic regulation of repeated mixed-meal challenges of different carbohydrate loading. The aim was to examine whether the

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Data availability

The datasets generated during and/or analyzed during the current study are not publicly available because of data protection issues. An anonymized form is available from the corresponding author on reasonable request.

Duality of interest

The authors have no conflict of interest to disclose. The authors have the following competing interests: EF received honoraries for invited talks from AstraZeneca, Takeda, Novo Nordisk, Sanofi, Mitsubishi, Eli Lilly & Co., Boehringer Ingelheim, and Merck Sharp & Dohme, consultancy from Janssen, Sanofi, AstraZeneca, and advisory boards meetings from Boehringer Ingelheim, Eli Lilly & Co., and Merck Sharp & Dohme. In addition, JSS received honoraries for consultancy from Sanofi, AstraZeneca, Intarcia, Merck, Actobiotics, Genentech, Servier, Boehringer Ingelheim, Adocia, Dance Biopharm, vTv Therapeutics, Valeritas, Mesoblast, and Halozyme, and advisory boards meetings from Dexcom Inc.

Author contributions

BEH conceived and executed the study. KG, WKW, EF and BEH performed the study interpretation and write-up. AG, AJM, JSS, and NS participated in the study conception and execution. AJM and EF were responsible for the laboratory analytics. AM and RB performed the pancreatic function data modeling. KG and WKW carried out the statistical analyses. JSS, AM, and NS contributed to discussion. BEH is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

relationship between lower sleeping oxygen saturation (SaO₂) and poorer fasting and postprandial metabolic function may be linked with reduced slow wave sleep (SWS) and rapid eye movement (REM) duration, independent of age, sex and total adiposity.

Methods—The 24 men and women, aged 25–54 years, had no diabetes or other diagnosed conditions, were evaluated with polysomnography to derive indices of SaO₂ and sleep architecture. In addition, an OGTT and two 14-hour serial mixed-meal tests were administered over 3 successive in-patient days. The carbohydrate content of the mixed-meals was manipulated to compare a standard-load day with a double-load day (300 vs. 600 kcal/meal). Quantitative modeling was applied to derive β -cell glucose sensitivity (β -GS), early insulin secretion rate sensitivity (ESRS), and total postprandial insulinemia (AUC_{INS}).

Results—Analyses showed that, for the 14-hour tests, the SaO₂ relationship with metabolic outcomes was associated significantly with percent time spent in REM but not SWS, independent of age, sex and total adiposity. Specifically, indirect pathways indicated that lower SaO₂ was related to shorter REM duration, and shorter REM was respectively associated with higher β -GS, ESRS, and AUC_{INS} for the 300- and 600-load days (300 kcal/meal: β =−8.68, p <.03, β =−8.54, p <.002, and β =−10.06, p <.008; 600 kcal/meal: β =−11.45, p <.003, β =−11.44, p <.001, and β =−11.00, p <.03).

Conclusion—Sleeping oxygen desaturation and diminished REM duration are associated with a metabolic pattern that reflects a compensatory adaptation of postprandial insulin metabolism accompanying preclinical diabetic risk.

Keywords

sleeping oxygen saturation; REM; insulin resistance; postprandial; insulin secretion; glycemia

Introduction

A large number of studies have shown that OSA is associated with insulin resistance and glucose intolerance in prediabetic individuals [1]. In OSA, the repeated apneic (complete) and hypopneic (partial) obstruction of the upper airway induce respiratory pauses during sleep that lead to reductions in blood SaO₂ levels and often to levels below 90% [2]. The Sleep-Ahead study reported OSA prevalence was about 86% in overweight or obese persons with T2DM [3]. Although when controlling for age, gender and body habitus, 23% of persons in the Wisconsin Sleep Cohort had both OSA and T2DM [4]. Thus, there appears a sizeable comorbidity of OSA and metabolic dysfunction. The Sleep Heart Health Study has shown that even mild sleeping oxygen desaturation (2–4%) during apneic and hypopneic episodes is associated with up to 72% greater likelihood of having impaired or diabetic fasting glucose levels [5]. Thus, it has been proposed that chronic depression or intermittent decline of sleeping SaO₂ is causally related to the development of insulin resistance and impaired glucose regulation prior to T2DM onset [6]. These pathogenic pathways are not well understood, but may be operational prior to OSA diagnosis and long before the development of frank diabetes or even prediabetes is detected.

Among a number of other possible sleep-disrupting conditions, altered sleep architecture may negatively influence glucose homeostasis [7–9]. The epidemiological literature has

linked shortened TST, which may occur due to repetitive apneic and hypopneic episodes that lead to arousals, with obesity and metabolic dysfunction [6]. In this context, experimental sleep restriction has been shown to decrease acutely insulin sensitivity in the days following deprivation [10]. PSG studies of obese adolescents have reported that reduced TST was associated with greater postprandial glucose excursions to the OGTT, whereas reduced SWS time was associated with greater postprandial insulin secretion; these findings were independent of AHI severity and body mass [11]. In a small sample of healthy lean men, metabolic function following two nights of baseline sleep was compared with three nights of selective SWS deprivation, without a TST reduction [12]. The study findings indicated that the extent of SWS suppression was associated with poorer insulin sensitivity and glucose tolerance. In contrast, other PSG studies have found that shorter REM was associated with obesity in both adults and children [13–15]. In a study of T2DM patients, compared to persons without OSA, those with severe OSA had 38% higher glucose levels during REM than NREM sleep [16]. Therefore, the literature suggests that sleep stage architecture may be altered in persons experiencing acute and long-term metabolic dysregulation. However, it is not clear whether SWS or REM reduction or some combination is involved.

In the studies of sleep and metabolic function, measures typically have included fasting glucose and insulin, and OGTT-measures of postprandial metabolism [17]. These measures have been used to estimate insulin sensitivity (HOMA-IR), β -cell insulin secretion, and gluco-regulatory function [18]. Although metabolic function studies have used mixed-meal assessments to provide more information about postprandial metabolism [19], accumulating evidence suggests longer-term, serial mixed-meal assessments that include repeated meal ingestion may better approximate free-living conditions [20, 21]. In our previous study of insulin sensitive and insulin resistant men and women, who were otherwise healthy, we have used mathematical modeling to quantitate postprandial β -cell and insulin secretion function using two 14-hour repeated mixed-meal challenges, over two sequential days [22]. In these challenges, the meal carbohydrate content was manipulated so that participants received mixed-meals with 300 kcal/meal of carbohydrates on one day, and mixed-meals with 600 kcal/meal carbohydrates on the other day. Compared with insulin sensitive individuals, those with insulin resistance showed higher postprandial glycemia, but their glycemic levels were constrained by insulin hypersecretion that was due, in part, to greater β -GS and ESRS. It would follow that if there were a robust pathophysiological association between subclinical metabolic adaptations and alterations in sleeping SaO_2 and architecture, evidence of these relationships should be apparent even in persons with mild or no OSA, who have not been diagnosed with cardiovascular conditions or T2DM.

Therefore, the present study examined the interrelationships of sleeping SaO_2 with postprandial metabolic function indicators, and the extent to which SWS and/or REM duration accounts for these associations, independent of age, sex and total adiposity. Therefore, it is hypothesized that:

1. Lower SWS% and REM% would be associated with poorer pancreatic β -cell (β -GS and ESRS) and metabolic function (FPG, FPI, insulin sensitivity, AUC_{GLU} and AUC_{INS}) during OGTT, and 300 and 600 kcal carbohydrate mixed-meal challenges, independent of covariates (age, sex and total adiposity).

2. Lower sleeping SaO₂ will be associated with poorer pancreatic β -cell (β -GS and ESRS) and metabolic function (FPG, FPI, insulin sensitivity, AUC_{GLU} and AUC_{INS}) during OGTT, and 300 and 600 kcal carbohydrate mixed-meal challenges, when controlling for covariates.
3. Lower SWS% and REM% will be associated with poorer pancreatic β -cell (β -GS and ESRS) and metabolic function (FPG, FPI, insulin sensitivity, AUC_{GLU} and AUC_{INS}) during OGTT, and 300 and 600 kcal carbohydrate mixed-meal challenges, when controlling for sleeping SaO₂, and other covariates.
4. The indirect effects of sleeping SaO₂ on pancreatic β -cell (β -GS and ESRS) and metabolic function (FPG, FPI, insulin sensitivity, AUC_{GLU} and AUC_{INS}) during the meal challenges via SWS% and REM% will be significant and independent of covariates.

Methods

Study participants

The parent study examined cardiometabolic function in response to fast-food style meals with low and high carbohydrate content by enrolling 143 adult insulin sensitive ($n = 79$) and insulin resistant ($n = 64$) participants recruited via flyer advertisement and chain-referral from Miami-Dade, Broward, and other South Florida counties [22]. During a 1-year period of study recruitment, participants were offered the opportunity to enroll in an additional protocol to evaluate sleep function. Of the 59 study-eligible persons enrolled during this period, 24 participants provided consent. Study eligibility included participants who: 1) were aged 18–55 years; 2) had no nicotine use in the past year, no history of substance or alcohol dependency in the ten years pre-study entry, and negative urine toxicology screen; 3) were taking no prescribed cardiovascular, carbohydrate, endocrine, or psychiatric medication; 4) had no history of diagnosed cardiovascular, metabolic, or endocrine disorder; and 5) for women, were not pregnant, with regular menstrual cycling (26–35 days) for the 3 months before study entry. The protocol was approved by the Institutional Review Board of the University of Miami in accordance with the principles of the Declaration of Helsinki, and informed consent was obtained prior to inclusion.

Procedures

The parent study protocol consisted of three separate assessment sessions. These procedures will be briefly summarized below [cf. 22 for a more detailed description]. The initial screening session confirmed study eligibility. In the second assessment session, fasting blood sampling for lipid profile, insulin, glucose and C-reactive protein, was followed by a 150-min euglycemic hyperinsulinemic procedure (insulin infusion rate 40 mU.min.m²) to clamp glucose within 5% of fasting levels [22, 23]. The third assessment session was a 3-day, overnight in-patient laboratory stay to evaluate postprandial metabolic function over two meal challenge days (standard vs. double carbohydrate load), wherein four meals per day were provided. The first day included a standard 75-g, 3-hour OGTT [24]. Subsequently, VAT and SAT abdominal fat was measured using multi-slice computed tomography performed by Siemens Somaton-Sensation-16 scanner (Siemens, Malvern, PA) [22]. TAT

volume was the sum of VAT and SAT [25, 26]. On days 2 and 3, subjects were given a meal every 3.5 hours over 14 hours (8:00 am, 11:30 am, 3:00 pm and 6:30 pm), and a snack at 9:00 pm, and then an overnight fast ensued. The meal carbohydrate content was manipulated so that on one day subjects received a ‘standard’ level of carbohydrate intake per meal (as administered in the OGTT e.g., 300 kcal) and on the other day received a ‘double’ carbohydrate content per meal (e.g., 600 kcal). The sequence (SEQ) of standard and double carbohydrate days was randomized. The calories from fats and proteins per meal were kept identical throughout. The macronutrient content per meal was adjusted on the basis of the subjects’ body surface area and sex, approximating the U.S. national criteria prescribed for normal daily consumption (i.e., 50% carbohydrate, 35% fat and 15% protein) [27] and tailored to a sedentary to light daily energy utilization. Thus, the average daily caloric intake for the cohort met the current average U.S. dietary intake of about 3000 kcal/day for men and 2300 kcal/day for women [28]. Carbohydrate calories were provided from OGTT drink and white rice (75% drink/25% rice ratio), whereas fat and protein calories were provided by hamburger and sausage. Total daily sodium intake was 1/3 of the recommended daily allowance [29]. Timed blood samples were taken prior to (–15 and 0 min) and following meal consumption (0, 15, 30, 60, 90, 120 150 and 180 min). Samples assay of fasting/pre-meal and postprandial levels of glucose, insulin, C-peptide, and other constituents were performed [22]. Deconvolution modeling was used to derive indices of β -cell function and glucose and insulin metabolism to the OGTT and the multiple meal test days [cf. 20, 30]. Thus, in addition to fasting measures, the analysis generated the β -GS from the dose-response relationship of the insulin secretion rates to the concomitant plasma glucose concentrations. The modeling analyses also yielded a second insulin secretion component (i.e., ESRS), which reflects the dependence of insulin secretion on the rate of initial glucose concentration change following ingestion. The total glucose and insulin AUC were denoted as AUC_{GLU} and AUC_{INS} , respectively.

Upon completion of the initial 3 sessions, those persons participating in the follow-up study had a PSG examination at the University of Miami Sleep Disorders Center to assess sleep architecture and function. The Medcare Embla system was used to acquire the PSG data and the Medcare Rembrandt system to score them. PSG records were scored on reader stations by experienced sleep technologists using standard scoring criteria [31] to derive sleep staging and durations, and indices of sleep latency and efficiency. In addition, standard methods were employed to identify apneas and hypopneas and derive the AHI [32]. The oximetry data were used to derive measures of SAO_2 while asleep and per sleep stage, and included mean SAO_2 , nadir SAO_2 , and ODI (defined as number of decreases in SAO_2 3% per hour).

Statistical analysis

Preliminary data screening included descriptive statistics, outlier detection, and tests of normality. Regression analyses evaluated whether mean SAO_2 and percent of TST spent in SWS and REM were associated with metabolic parameters, including pancreatic β -cell (β -GS and ESRS) and metabolic function (FPG, FPI, insulin sensitivity, AUC_{GLU} and AUC_{INS}), separately for the OGTT, the standard-carbohydrate mixed-meal (300 kcal) and the double-carbohydrate mixed-meal (600 kcal) tests, while controlling for age, sex, and

TAT. SEQ was also used as a covariate in the standard- and double-carbohydrate meal analyses. The analyses were undertaken to assess the relationship with metabolic outcomes of sleeping oxygen saturation (mean SaO₂, nadir SaO₂, ODI) and sleep architecture measures separately, and then, together in a single model. Thus, the following three regression models were tested consecutively per outcome measure per meal challenge: Model 1 included SWS% and REM%; Model 2 included an SaO₂ index, but not sleep stage durations; and Model 3 included SaO₂ index, SWS% and REM%. If these analyses suggested that a variable might be operating as a mediator [33], then this variable was assessed accordingly, using the Process procedure for SPSS [34]. The significance of the observed indirect effects for mediation analyses was tested by calculating bias-corrected 95% CI using bootstrapping with 10,000 resamples.¹

Results

Demographic characteristics, adiposity, cardiometabolic function, and sleep architecture and function

Of the 143 subjects who completed the full protocol, 24 persons completed the PSG exam. Of these persons, two were excluded from the present data analysis because their AHI exceeded 15, which would possibly indicate moderate or severe OSA. As seen in Table 1, the participants' age ranged from 25 to 54 years, with a mean age of about 42 years. Similar to the overall cohort, these participants included about 64% men, were comprised mostly of minority race/ethnicity (~91%) and, on average, had low total family income but had accrued some college education. As per enrollment criteria, none of the participants were current smokers, although the sample included about 14% who were former smokers. Blood pressure measures indicated that all participants were normotensive. Notably, measures of BMI indicated that, of the sample, 32% were classified as overweight, and 46% as obese. Accordingly, TAT volume in obese participants was, respectively, 1.3-fold and 2.6-fold greater than overweight and normal-weighted participants, independent of sex ($F(2,18)=16.8$, $p<0.001$). Lipid profile measures indicated that about 23% of the cohort had hypercholesterolemia, and 27% had hypertriglyceridemia. Although average FPG was in the normal range, about 27% had IFG, 23% had IGT, and 41% were prediabetes as per ADA definitions [36]. By the euglycemic clamp, 50% of the participants had insulin resistance (i.e., insulin-mediated glucose disposal $4.5 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) [37, 38]. Of the sample, mean sleeping SaO₂ was within normal limits (95% or above) for all participants [39, 40]. Time spent in sleep stages relative to TST was comparable with previous studies of healthy adults [40, 41]. In sum, although the participants were healthy, having no clinical conditions or prescribed cardiometabolic or sleeping medications, a large proportion of subjects were overweight or obese, about one-quarter of them evidenced dyslipidemia, and about half of the persons displayed signs of early glucose dysregulation. Parameters of β -cell function, glucose and insulin response to the meal challenges are shown in Table 2. As can be seen,

¹The term, mediation, in this analysis, does not imply causality, but simply describes the possibility that one variable may partially account for the relationship between two other variables. In a regression analysis, the total effect of the relationship between an independent variable and an outcome, following the introduction of a possible mediating variable, may be decomposed into a direct and indirect effect [33]. If introducing this mediator variable eliminates a significant direct effect, then the relationship of the independent variable to the outcome is said to be fully mediated. Alternatively, if a direct effect remains but is reduced, then the relationship is said to be partially mediated [33–35].

these findings confirmed that the carbohydrate loading manipulation resulted in no response difference between OGTT and 300 kcal tests, but significantly more elevated β -GS and insulin secretion during the 600 kcal test that resulted in comparable glycemic levels.

Relationships among sleeping oxygen saturation, sleep stage duration, and metabolic function

Table 3 displays the regression analyses for the outcome measure AUC_{INS} with independent variables, SWS% and REM% (Model 1), mean SaO_2 (Model 2), and the combination of these sleep measures (Model 3). Model 1 showed that REM% in all 3 meal tests was significantly inversely related to AUC_{INS} , independent of covariates (OGTT: $\beta=-0.61$, $p<0.004$; 300 kcal: $\beta=-0.61$, $p<0.005$; 600 kcal: $\beta=-0.61$, $p<0.005$); in this model, SWS% was not significantly related to AUC_{INS} ($ps>0.477$). The total variance explained in AUC_{INS} (i.e., R^2 change), when REM% and SWS% were added to the model, increased about 35–40%. This finding represents a 5.3-fold to 6.6-fold increase in explained variance relative to the model containing covariates alone. The Model 2 analyses also showed for the 3 meal tests that mean SaO_2 was significantly inversely related to AUC_{INS} , independent of covariates (OGTT: $\beta=-0.62$, $p<0.03$; 300 kcal: $\beta=-0.55$, $p=0.05$; 600 kcal: $\beta=-0.60$, $p<0.04$). The total variance explained in AUC_{INS} , when mean SaO_2 was added to the model, increased about 17%–22% for these meal tests. This finding represents a 2.7-fold to 3.5-fold increase in explained variance relative to the model containing the covariates alone. The nadir SaO_2 (2%–6%) and ODI (0%–0.1%) indices of sleeping oxygen saturation did not account for as much variance in AUC_{INS} as the mean SaO_2 . Thus, mean SaO_2 was used as the predictor in forthcoming analyses. In the Model 3 analyses, REM%, SWS%, and mean SaO_2 were entered with the covariates. Findings indicated that REM%, but not SWS% ($ps>0.56$) or SaO_2 ($ps>0.50$), was significantly related to AUC_{INS} (OGTT: $\beta=-0.55$, $p<0.02$; 300 kcal: $\beta=-0.60$, $p<0.02$; 600 kcal: $\beta=-0.53$, $p<0.03$). REM% did not significantly correlate with either SWS% and mean SaO_2 ($r = -0.36$ and 0.31 , respectively), nor did SWS% with mean SaO_2 ($r = -0.18$), and variance inflation factors were less than 1.24, suggesting that the Model 3 findings were not the result of multicollinearity. The total variance explained in AUC_{INS} , in this model increased about 36–46%. This finding represents a 5.6-fold to 7.6-fold increase in explained variance in the full model relative to the model containing traditional covariates alone. In sum, these findings suggest that REM% may function as a mediator between mean SaO_2 and the AUC_{INS} outcome measure.

As planned, analyses for the insulin sensitivity, FPG, FPI, β -GS, ESRS, and AUC_{GLU} outcome measures evaluated whether REM% may be similarly operating as a mediating influence. In brief, these analyses showed that SWS% did not appear to mediate the relationship of SaO_2 with any of these outcome measures ($ps>0.23$). However, REM% trended toward significance for insulin sensitivity ($\beta=.311$, $p=.09$). Similarly, REM% mediation appeared significant or trended toward significance for two or more of the three meal challenges for FPG (OGTT: $\beta=0.52$, $p<0.04$; 300 kcal: $\beta=0.44$, $p=0.06$; 600 kcal: $\beta=0.50$, $p=0.06$) and also FPI (OGTT: $\beta=-0.36$, $p>0.18$; 300 kcal: $\beta=-0.46$, $p=0.06$; 600 kcal: $\beta=-0.48$, $p=0.08$). In addition, REM% mediation was suggested for β -GS (OGTT: $\beta=-0.59$, $p<0.03$; 300 kcal: $\beta=-0.63$, $p<0.02$; 600 kcal: $\beta=-0.81$, $p<0.01$), and also ESRS (OGTT: $\beta=-0.32$, $p>0.28$; 300 kcal: $\beta=-0.82$, $p<0.001$; 600 kcal: $\beta=-0.87$, $p<0.01$). Of

note, REM% mediation was not significant for AUC_{GLU} for all meal challenges ($p > 0.41$). Therefore, follow-up analyses were conducted to determine whether there was statistical evidence supporting the conclusion that REM% may be operating as a mediator between mean SaO_2 and metabolic outcomes (i.e., insulin sensitivity, FPG, FPI, β -GS, ESRS, AUC_{INS}).

Evidence of partial and full REM% mediation

Table 4 summarizes these follow-up analyses, which indicate statistical evidence of mediation by REM% for FPI, β -GS, ESRS, and AUC_{INS} but not for measures of insulin sensitivity, FPG or AUC_{GLU} . As can be seen in this table, the findings indicate that lower mean SaO_2 was associated with lower REM%, and less REM% was associated with greater FPI, β -GS, ESRS, and AUC_{INS} , independent of age, sex, total adiposity, and sequence of carbohydrate loading. Of note, these results were maintained when analyses controlled for AHI. In addition, as displayed in Table 4, the detection of REM% mediation differed as a function of the meal challenges (OGTT vs. 300 kcal vs. 600 kcal). Specifically, for FPI, β -GS and ESRS, the mediation by REM% was not significant for the OGTT. However, in the analyses of the two 14-hour mixed-meal challenges, REM% partially accounted for the significant relationship between SaO_2 with β -GS and SaO_2 with ESRS. In contrast, full REM% mediation was observed in all three meal challenges for AUC_{INS} . Figure 1 displays the mediation models on the 600 kcal carbohydrate load day, wherein the partial- and full-mediation indirect effects can be observed.

Discussion

To maintain metabolic regulation within normative ranges in persons with prediabetes and those without diabetes but with diminished insulin sensitivity, adaptive changes in β -cell function may occur that act to compensate for the prevailing decrease in insulin-mediated glucose uptake [20, 21, 42]. Our previous study demonstrated that postprandial glycemia was well-controlled even in the insulin resistant individuals due to an upregulation of β -cell function and insulin responsivity [22]. The present study extended these findings, demonstrating that in adult men and women without diabetes with mild to no OSA, both sleeping SaO_2 and percent time spent in REM sleep were associated with measures of β -cell function, and fasting and postprandial glucose metabolism. Although the initial analyses suggested that REM% may be functioning as a mediator in the relationship of SaO_2 with many of these outcome measures, statistical tests of mediation in subsequent analyses showed that this finding was only the case for a subset of metabolic outcomes. Specifically, REM% did not account for the relationships between SaO_2 with insulin sensitivity, fasting glucose, and AUC_{GLU} . In contrast, REM% was found to partially or fully account for the relationships between SaO_2 and FPI, β -GS, ESRS, and AUC_{INS} . These findings were present even when controlling for AHI. Thus, the main study findings indicated that lower sleeping oxygen saturation was associated with a) shorter percentage of TST in REM sleep, b) more elevated FPI, c) higher β -GS, d) higher ESRS, and e) greater total postprandial insulinemia. Of note, these relationships tended not to be significant in response to the OGTT, but were present in the 14-hour serial-meal challenge data. In addition, these findings were independent of age, sex, total adiposity and the carbohydrate loading

sequence. Across the two days of serial-meal challenges, sleeping SaO₂ and percent REM duration accounted for about 5–8-fold more variance in metabolic outcomes than did these control variables. Thus, this study extends our previous findings and suggests that one possible underlying mechanism by which lower SaO₂ affects β -cell and insulin secretory function involves the influence of reduced REM sleep duration.

Mechanisms mediating the association between sleeping oxygen desaturation and metabolic dysfunction are likely to be multifactorial, involving a cascade of pathogenic mechanisms such as central and autonomic dysregulation, development of non-alcoholic fatty liver, changes to growth hormone secretion, inflammation and oxidative stress, abnormalities in orexigenic and anorexigenic signaling, and changes in blood glucose uptake during sleep [17, 43–45]. In addition to epidemiological evidence that links OSA severity and metabolic dysfunction in persons with diabetes, studies of individuals without diabetes have shown that acute intermittent hypoxia exposure induces negative glucoregulatory consequences [5, 46–49]. OSA severity is also associated with lower glucagon-like peptide-1 (GLP-1) intestinal secretion response to a meal challenge (50). GLP-1 is an anorexigenic hormone that inhibits gut motility, and gastric acid and glucagon secretion, but also stimulates pancreatic insulin secretion in a glucose-dependent manner (51). Our studies have shown that GLP-1 secretion to oral glucose in both Type 2 diabetes and non-diabetic persons is inversely related to BMI, independent of hyperglycemia (52). Moreover, we have shown in persons with non-diabetic insulin resistance that fasting GLP-1 is up-regulated, whereas GLP-1 meal-related responses are down-regulated (53). Others have shown that increasing AHI was associated with higher fasting GLP-1 levels, but lower GLP-1 response to a meal tolerance test (54). Thus, defective GLP-1 secretory response may be one mechanism that predisposes persons with diminished sleeping oxygen saturation to impaired postprandial insulin release and glycemic metabolism, weight gain, and increased diabetes risk.

Strengths and limitations

There are several issues of consideration. First, we enrolled only healthy individuals, whereby the impact of diabetic status or OSA severity could not be examined. The current findings, however, do suggest that a very mild lowering of mean sleeping SaO₂, albeit within the normal range may have an important association with β -cell and metabolic functional adaptations, independent of age, sex and total adiposity. The finding that the shorter REM duration may underlie the observed relationship between sleeping SaO₂ and metabolic function is novel. The extent to which shortened REM duration plays a causal role in the sleeping SaO₂-metabolic dysfunction relationship, and whether these metabolic adaptations are preclinical or prognostic indicators of diabetic pathogenesis, would require longitudinal study. Second, the limitations of cross-sectional studies apply to our study, including the difficulty excluding reverse causality. Although we have discussed primarily how sleep disorders are risk factors for the development of preclinical dysglycemia, prediabetes and T2DM, the opposite pathophysiological progression may be operational as well. For example, a 6-year prospective study of 3,565 persons has shown that both elevated FPI and greater HOMA-IR predicted OSA diagnosis, independent of age, sex and adiposity [55]. Therefore, the direction of the relationships may be reversed or bi-directional, and this issue cannot be determined without experimental manipulation. Third, the present study evaluated

mean sleeping SaO₂, which may not reflect the desaturation frequency or the magnitude of the SaO₂ decline reached during the process of cyclical sleeping desaturations that may occur through the sleep period. Justification for the present approach was based on the findings that mean SaO₂ was more strongly associated than the nadir SaO₂ and the ODI were with total postprandial insulinemia. It is possible that the sample size restricted the range for the latter variables, and thus the present findings in this regard should be viewed with caution. Fourth, the PSG study is laboratory-based, and as such it is not possible to be sure that the sleep architecture observed is similar to that occurring when participants sleep at home. Therefore, study findings need replication, wherein home PSG evidence is collected. Fifth, sleep architecture was assessed in this study using one night of PSG examination. It is established that sleep architecture has enhanced night-to-night variability, which will reduce the reliability of these measures. However, the presently observed time spent in sleep stages relative to TST was comparable with reported values from previous studies of healthy adults [40, 41]. It is notable, in this respect, that the present findings have demonstrated significant relationships among objective measures obtained using various methodologies and independent postprandial assessments. Sixth, although the cohort composition was balanced with regard to proportion of insulin resistant persons, the sample size was relatively small. Nevertheless, the study design provided a comprehensive and extensive metabolic evaluation of fasting and postprandial function, employing two days of 14-hour serial meal challenges, in comparison with the 3-hour standard OGTT assessment performed on the preceding day. The use of the three meal challenges provided opportunity to observe the replicability of mediation findings under single vs. multiple meal conditions, and under standard vs. double carbohydrate loading conditions. Indeed, the main findings that the observed relationships were significantly accounted for by percent time spent in REM, but not SWS, became fully consistent when the postprandial outcomes were analyzed during these more prolonged meal challenges. Whether the presence of these findings during the more prolonged postprandial assessments was due to the composition of the mixed-meals, enhanced measurement reliability obtained from the use of multiple meal challenges, or to additional information provided by postprandial responses beyond the initial meal remains to be clarified.

Conclusions

Clinical management of glycemia in T2DM patients consists mainly of lifestyle advice and pharmacotherapy. The emerging literature has indicated that sleep disturbances have potential clinical metabolic implications, even in persons who are not obese and are not diagnosed with diabetes. The present study in adults without diabetes supports this suggestion, showing that alterations in sleep, specifically decreased sleeping oxygen saturation and REM sleep duration, are robustly associated with compensatory adaptation of β -cell glucose sensitivity and other metabolic functions influencing insulin secretion. The issue of whether shortened REM sleep duration is an underlying causal mechanism in this context, resulting in diabetic pathophysiology, is largely unexplored. The present findings suggest this hypothesis warrants further detailed study using longitudinal designs.

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Abbreviations

AHI	apnea-hypopnea index
AUC_{GLU}	total glycemic area under the curve
AUC_{INS}	total insulinemic area under the curve
BMI	body mass index
β-GS	β-cell glucose sensitivity
ESRS	early insulin secretion rate sensitivity
DBP	diastolic blood pressure
FPI	fasting plasma insulin
FPG	fasting plasma glucose
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
ODI	number of desaturation events (decline in SaO ₂ ≥ 3%) per hour
OSA	obstructive sleep apnea
PSG	polysomnography
REM	rapid eye movement
SaO₂	oxygen saturation
SAT	subcutaneous adipose tissue
SBP	systolic blood pressure
SEQ	sequence
SWS	slow wave sleep
T2DM	type 2 diabetes mellitus

TAT	total adipose tissue
TST	total sleep time
VAT	visceral adipose tissue
2-hr PG_{OGTT}	OGTT 2 hour plasma glucose

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Highlights

- Obstructive Sleep Apnea is linked with preclinical dysglycemia and incident diabetes.
- Not known whether altered sleep architecture mediates.
- Decreased sleeping oxygen saturation and REM duration (not SWS) are associated with compensatory metabolic adaptations.
- Pancreatic β -cell and other metabolic factors affecting insulin secretion are involved.

Figure 1. The models of the standardized indirect effects (IE) of SaO₂ on FPI, β-GS, ESRS, and AUC_{INS} during the double carbohydrate (600 kcal) meal challenge

The SaO₂–β-GS relationship and the SaO₂–ESRS relationship are both partially mediated by REM% as SaO₂ is also directly affecting these outcome measures. REM% appears to fully mediate the relationship between SaO₂ with FPI and with AUC_{INS}. Significant paths are indicated with a solid line and nonsignificant paths with a dashed line. ^t $p < .10$; * $p < .05$; ** $p < .01$; *** $p < .001$

Table 1

Demographic characteristics, adiposity, cardiometabolic measures, and sleep architecture and function parameters (n = 22).^{ab}

<u>Demographics</u>			
Age (yr)	42.3	±	1.9
Sex (% men)	64.4		
Ethnicity/Race (%)			
	Hispanic	81.8	
	Black	9.1	
	non-Hispanic White	9.1	
Family annual income (\$k)	16.8	±	3.6
Education (yr)	13.3	±	0.7
<u>Adiposity</u>			
BMI (kg/m ²)	28.7	±	0.9
TAT (L)	11.1	±	0.9
VAT (L)	3.7	±	0.4
SAT (L)	7.4	±	0.7
<u>Cardiometabolic Function</u>			
SBP (mmHg)	118.8	±	3.3
DBP (mmHg)	69.1	±	1.6
LDL-c (mg/dl)	116.3	±	8.0
HDL-c (mg/dl)	42.3	±	2.3
Total cholesterol (mg/dl)	181.2	±	8.1
Triglycerides (mg/dl)	144.4	±	26.3
HbA _{1c} (%)	5.3	±	0.1
HbA _{1c} (mmol/mol)	34.2	±	0.5
FPI (pmol/L)	91.9	±	15.2
FPG (mmol/L)	5.2	±	0.1
2-hr PG _{OCTT} (mmol/L)	5.9	±	0.4
Insulin sensitivity (mg·min ⁻¹ ·kg ⁻¹)	5.5	±	0.9
<u>Sleep Architecture & Function</u>			
Sleep latency (min)	32.4	±	8.6
TST (min)	333.9	±	11.9
%TST in Stage 1	11.9	±	1.3
%TST in Stage 2	49.2	±	2.3
%TST in SWS	15.9	±	2.1
%TST in REM	22.9	±	1.5
Mean SaO ₂ (%)	96.6	±	0.3
Nadir SaO ₂ (%)	91.1	±	0.9

ODI (events/hr)	3.6	±	0.8
AHI (events/hr)	3.8	±	0.7

^aData are mean ± SE unless otherwise indicated.

^bAbbreviations are: BMI, body mass index; TAT, total adipose tissue; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; HbA1c, glycosylated hemoglobin; FPI, fasting plasma insulin; FPG, fasting plasma glucose; 2-hr PGOGTT, 2 hour plasma glucose to oral glucose tolerance test; TST, total sleep time; SWS, slow wave sleep; REM, rapid eye movement; SaO₂, sleeping oxygen saturation; ODI, number of desaturation events (decline in SaO₂ ≥ 3%) per hour; AHI, apnea-hypopnea index.

Table 2

Depicted are the mean $\pm$ SE β -cell and glucose/insulin outcomes observed during the oral glucose tolerance test (OGTT), standard carbohydrate load (300 kcal/meal) and double carbohydrate load (600 kcal/meal) assessments.^a

	OGTT	300 kcal	600 kcal
FPI (pmol/L)	91.9 $\pm$ 15.2	110.5 $\pm$ 14.0	107.7 $\pm$ 13.0
FPG (mmol/L)	5.2 $\pm$ 0.1	5.2 $\pm$ 0.1	5.3 $\pm$ 0.1
β -GS (pmol min ⁻¹ ·m ⁻² ·[mmol/L] ⁻¹) ^{b,c}	90.3 $\pm$ 8.4	93.1 $\pm$ 7.7	118.9 $\pm$ 10.1
ESRS (nmol·m ⁻² ·[mmol/L] ⁻¹)	1.2 $\pm$ 0.1	1.2 $\pm$ 0.1	1.2 $\pm$ 0.2
AUC _{INS} (pmol ⁻¹ L·m·h ⁻¹) ^{b,c}	24,934.1 $\pm$ 3598.1	29,254.8 $\pm$ 4552.2	50,510.3 $\pm$ 10,065.6
AUC _{GLU} (mmol ⁻¹ L·m·h ⁻¹)	390.4 $\pm$ 17.8	372.8 $\pm$ 11.4	397.4 $\pm$ 17.1

^a Abbreviations are: FPI, fasting plasma insulin; FPG, fasting plasma glucose; β -GS, β -cell glucose sensitivity; ESRS, early secretion rate sensitivity; AUC_{INS}, total insulinemia; AUC_{GLU}, total glycemia.

^b Significant mean difference between 300 kcal vs 600 kcal ($p < .01$).

^c Significant mean difference between OGTT vs 600 kcal ($p < .01$).

Table 3

Multiple regressions assessing the relationship of sleep outcomes with postprandial total insulinemia (AUC_{INS}) during OGTT, standard carbohydrate load (300 kcal/meal), and double carbohydrate load (600 kcal/meal) assessments.^a

OGTT			300 kcal			600 kcal		
Model 1	β	t	β	t	p	β	t	p
Age	0.143	0.786	0.443	0.083	0.466	0.648	0.034	0.174
Sex	-0.198	-1.109	0.284	-0.373	-2.102	0.053	-0.327	-1.781
TAT	0.352	2.113	0.051	0.299	1.829	0.087	0.319	1.821
SEQ	-	-	-	0.013	0.075	0.941	-0.038	-0.214
SWS%	0.122	0.676	0.509	0.132	-0.728	0.478	0.055	0.302
REM%	-0.613	-3.477	0.003	-0.612	-3.449	0.004	-0.613	-3.429
Model 2	β	t	β	t	p	β	t	p
Age	-0.225	-1.043	0.311	-0.273	-1.177	0.256	-0.335	-1.484
Sex	0.036	0.171	0.866	-0.152	-0.675	0.509	-0.121	-0.552
TAT	0.208	0.989	0.336	0.178	0.814	0.428	0.178	0.835
SEQ	-	-	-	0.042	0.204	0.841	-0.012	-0.060
SaO ₂	-0.615	-2.504	0.023	-0.551	-2.079	0.054	-0.596	-2.311
Model 3	β	t	β	t	p	β	t	p
Age	0.074	0.338	0.740	0.068	0.302	0.767	-0.070	-0.283
Sex	-0.133	-0.625	0.541	-0.358	-1.613	0.129	-0.240	-1.070
TAT	0.307	1.643	0.121	0.290	1.565	0.140	0.267	1.379
SEQ	-	-	-	0.019	0.107	0.917	0.001	0.004
SaO ₂	-0.166	-0.596	0.560	-0.035	-0.120	0.906	-0.213	-0.694
SWS%	0.083	0.425	0.677	0.122	0.596	0.561	-0.004	-0.021
REM%	-0.549	-2.628	0.019	-0.601	-2.901	0.012	-0.532	-2.461

^a Abbreviations: TAT, total adipose tissue; SEQ, carbohydrate load administration sequence (300 day 1, 600 day 2 vs. 600 day 1, 300 day 2); SWS%, percent time in slow wave sleep; REM%, percent time in rapid eye movement sleep; SaO₂, sleeping oxygen saturation.

Table 4

Mediation analyses assessing the indirect effect (IE) of REM% on the relationship of sleeping oxygen saturation with insulin sensitivity, β -cell glucose sensitivity, and fasting and postprandial glucose and insulin outcomes.^{a,b}

	OGTT				300 kcal				600 kcal			
	IE	SE	BC 95% CI		IE	SE	BC 95% CI		IE	SE	BC 95% CI	
			Lower	Upper			Lower	Upper			Lower	Upper
Insulin sensitivity	0.14	0.13	-0.04	0.47	-	-	-	-	-	-	-	-
FPG	0.24	0.15	-0.01	0.59	0.21	0.17	-0.05	0.63	0.24	0.22	-0.03	0.87
FPI	-0.17	0.15	-0.64	0.01	-0.22 [*]	0.15	-0.55	-0.04	-0.22 [*]	0.15	-0.63	-0.01
β -GS	-0.29	0.21	-0.82	0.01	-0.30 [*]	0.18	-0.77	-0.01	-0.36 [*]	0.20	-0.79	-0.01
ESRS	-0.17	0.22	-0.73	0.18	-0.40 [*]	0.23	-0.72	-0.01	-0.41 [*]	0.23	-0.95	-0.01
AUC _{INS}	-0.26 [*]	0.15	-0.64	-0.02	-0.29 [*]	0.17	-0.67	-0.01	-0.25 [*]	0.16	-0.62	-0.01
AUC _{GLU}	-0.01	0.27	-0.48	0.42	0.08	0.16	-0.11	0.53	0.08	0.17	-0.10	0.59

^a Refer to Table 2 for abbreviations.

^b SE and bias-corrected (BC) 95% CIs were estimated using bootstrapping with n = 10,000 samples [35].

^{*} Standardized IE coefficients were significant when the CI did not contain 0.