METABOLIC HEALTH IN NORMAL AND ABNORMAL SLEEP

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METABOLIC HEALTH IN NORMAL AND ABNORMAL SLEEP

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EDITORIAL
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Editorial: Metabolic Health in Normal and Abnormal Sleep

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Editorial on the Research Topic

Metabolic Health in Normal and Abnormal Sleep

Sleep comprises one third of the human lifespan and therefore has a significant impact on metabolism. There is a growing recognition that sleep disorders are associated with obesity, metabolic syndrome, and cardiovascular disease. This Research Topic, "Metabolic Health in Normal and Abnormal Sleep" contains a collection of studies that address complex interactions between sleep and metabolic health.

Obstructive sleep apnea (OSA) is a common disorder closely associated with cardiometabolic disease. Framnes and Arble write a timely review of the bidirectional relationship between OSA and metabolic disease (Framnes and Arble). First, they cover established impacts of obesity on the pathogenesis of OSA. Then, they review evidence for the reverse, whereby changes in metabolic/endocrine function can affect OSA in a weight-independent manner. In keeping with this theme, several studies in this collection examine associations between OSA, and glucose metabolism. Temple et al. examined whether associations between OSA and glucose metabolism are modified by sex. They recruited non-diabetic men and women from the community (n = 145) and performed polysomnography followed by a morning oral glucose tolerance test (OGTT) and derived measurements of insulin resistance and insulin response. In this cohort, 75% of men and 43% of the women had OSA. In those without OSA, they observed no sex differences in glucose metabolism. Among those with OSA, men exhibited higher glucose and insulin levels than women. The Authors ascribed their findings to OSA conferring a higher risk of diabetes in men (Temple et al.).

Fewer studies have examined the association of OSA and glucose metabolism in adolescents. Hannon et al. enrolled obese patients referred to a weight management clinic (age 12–18, BMI 38.9, n = 57) and performed polysomnography as well as OGTT. Thirty-six subjects had normal OGTT results while 21 exhibited impaired glucose tolerance or fasting hyperglycemia. Those with dysglycemia had higher body fat, but were not different in terms of OSA metrics. The Authors concluded that larger studies will be needed to clarify metabolic risks of OSA in obese youth.

One of the frequently cited mechanisms linking OSA to diabetes risk is exposure to intermittent hypoxia. Pham et al. examined this phenomenon by analyzing the pattern of oxygen desaturations in relation to frequently sampled overnight glucose levels in subjects during CPAP withdrawal (n = 30). They found that the depth of desaturation did not predict glucose elevation, whereas oxyhemoglobin "overshoots" mitigated hyperglycemia (Pham et al.). This suggests that the *pattern* of hypoxemia—not simply the average oxygen level—is a novel predictor of the glycemic response to OSA.

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The liver is a target organ of metabolic syndrome, manifesting as non-alcoholic fatty liver disease (NAFLD). Scartabelli et al. examined associations between OSA and NAFLD, assessed using ultrasound in a cohort of obese women referred to an obesity center in Italy ($n = 97$, BMI = 50). Seventy patients had OSA, of which 53, 26, and 21% were classified as mild, moderate, and severe, respectively. Those with OSA were older, had greater liver size, intra-abdominal fat, and modestly elevated liver function tests levels compared to those without. Regression analysis showed expected associations between neck circumference, abdominal fat, and OSA severity. Liver size was also positively associated with AHI. These findings confirm liver enlargement as a marker of visceral adiposity but do not necessarily imply causation from OSA (Scartabelli et al.).

OSA is much more common in men than women, related to neuro-anatomical and hormonal differences. Morselli et al. examined whether this sex difference might also be related to slow-wave activity (SWA) during sleep, as (a) SWA is higher in women than age-matched men and (b) SWA transiently improves OSA, possibly due to favorable arousal threshold and airway collapsibility physiology. They performed polysomnography in 101 subjects (44 men, 57 women) and used spectral analysis to quantify SWA. As expected, OSA was much more common among the men with an adjusted OR = 3.17. Interestingly, AHI was inversely related to SWA in men, but not in women. Higher testosterone levels were independently associated with lower SWA after controlling for age, race, and AHI. The authors speculated that higher testosterone predisposes to lower SWA/arousal threshold, leading to greater ventilatory instability and greater risk for OSA in men (Morselli et al.).

OSA is also associated with gestational diabetes (GDM), although it is not clear whether OSA is causally implicated. Pamidi et al. plan to address this question by planning a randomized clinical trial to determine the feasibility and efficacy of CPAP (vs. nasal dilator placebo) to improve 24-hr glucose profiles in pregnant women (week 24–28 gestation) with OSA and GDM. The women will be followed until delivery and undergo OGTT at 12 weeks postpartum (Pamidi et al.).

Besides OSA, altered sleep duration and circadian rhythm are associated with obesity. This Research Topic also contains studies that examine this phenomenon. Anothisintawee enrolled 2133 Thai patients with prediabetes and used questionnaires to obtain Composite Scale of Morningness (CSM) score and self-reported sleep duration/timing. They found that evening preference (low CSM score) was associated with increased BMI and this relationship was mediated via reduced sleep duration. The novelty of their analysis is the implication that evening chronotype might be a modifiable risk factor for obesity, if insufficient sleep drives the association (Anothaisintawee et al.).

Titova et al. also examined the association between short sleep duration and adverse metabolic outcomes by examining the prevalence of metabolic syndrome in the Swedish EpiHealth cohort study ($n = 19{,}691$). They found increased prevalence of metabolic syndrome in those who reported either short sleep duration (< 6 h/day) or long sleep duration (≥ 9 h/day). The authors then stratified the analysis into middle-aged (45–65) and older (>65) subjects. Middle-aged subjects showed the same "U-shaped" curve of metabolic risk whereas in older subjects, only long sleep was linked to a higher prevalence of metabolic syndrome. This study reveals novel interactions between sleep duration and age on metabolic health (Titova et al.).

Gohil and Hannon contributed a related article reviewing the association between poor sleep and obesity in adolescents. They cite several cross-sectional studies showing that reduced sleep time, worse sleep quality, altered sleep architecture (reduced REM), delayed sleep phase, and social jetlag are each associated with increased obesity. They also discuss putative mechanisms including obesogenic food choices and changes in leptin and ghrelin signaling (Gohil and Hannon). Building upon the theme of sleep and its influence over appetite control, McHill et al. studied the impact of chronic insufficient sleep on appetite and related hormones. They enrolled 17 participants in a 32-days inpatient protocol that involved sleep restriction (4.6 vs. 6.67 h sleep) superimposed upon a 20-h day (forced desynchrony) to decouple timing of blood samples from the circadian phase. They observed a strong circadian pattern to hunger, leptin, ghrelin, insulin, and glucose, but did not find a differential impact of sleep restriction on this pattern. Although this was a "negative" study, it is positive in the sense that it highlights the powerful influence of circadian rhythm on metabolism (McHill et al.).

Pizinger et al. pose an important corollary question to several of the above studies: Does the literature support the concept that sleep *extension* improves cardiometabolic outcomes? They summarize studies that examined both the acute effects of sleep restriction and subsequent recovery on metabolic outcomes including leptin, glucose, insulin, and inflammatory profiles. In addition, they review a small number of interventional studies examining lifestyle changes, with or without a sleep extension component, and their impact on metabolic outcomes. Some studies found modest improvements in appetite, food intake, blood pressure, and insulin resistance. They conclude with a call to improve methodologies in this area, including a focus on short sleepers (Pizinger et al.).

Metabolism can also be viewed through the lens of energy expenditure, and sleep has a significant impact on metabolic rate. Chapman et al. provide a systematic review on the effect of insomnia on metabolic rate. Their literature search was confined to original studies of healthy adults with a comparator group, yielding only four articles (three from the same lab) evaluating 75 participants. In two studies, both day and night oxygen consumption was modestly elevated in those with insomnia compared to age/gender matched controls. One study showed no difference but only measured metabolic rate at one time point after awakening and included only females. The fourth study showed a minor reduction of metabolic rate during treatment of insomnia with lorazepam (Chapman et al.).

Lipid metabolism has been shown to affect sleep, and vice-versa. In particular, some studies show that fatty acids promote sleep, while sleep restriction increases fatty acids. Acyl-CoA synthetases are enzymes that bind intracellular fatty acids to CoA to form acyl-coA. The activated fatty acid is then able to undergo several metabolic or signaling fates. The Acyl-CoA synthetase pudgy (pdgy) is upregulated in starvation. Thimgan et al. evaluated the role of pdgy in Drosophila sleep. They determined that decreased expression of pdgy (transcriptional mutants) decreased baseline sleep, sleep rebound after sleep deprivation, survival during 48-h starvation in association with higher baseline heart rate and lower triglyceride levels. Similar results were observed with reduced pdgy transcription in fat bodies. The mechanism by which altered lipid metabolism curtails sleep is not known but could be related to impaired energy storage or utilization, signaling, or compensatory changes in endocrine axes (Thimgan et al.).

The articles in this e-book illustrate the importance of sleep toward healthy metabolic function, and the reciprocal effects of metabolic function on healthy sleep.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Associations Between the Prevalence of Metabolic Syndrome and Sleep Parameters Vary by Age

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Objective: To examine whether the relationship between the metabolic syndrome (MetS) and various sleep parameters [sleep duration, symptoms of sleep-disordered breathing (SDB), and sleep disturbances] varies by age.

Methods: Waist circumference, blood pressure, triglycerides, high-density lipoprotein cholesterol, and fasting glucose were used to determine MetS status in a cohort (N = 19,691) of middle-aged (aged 45–64 years) and older (aged ≥65 years) subjects. Habitual sleep duration (short, ≤6 h/day; normal, 7–8 h/day; and long ≥9 h/day), sleep disturbances (such as problems with falling and staying asleep), and symptoms of sleep-disordered breathing (SDB, such as snoring and sleep apneas) were measured by questionnaires.

Results: Among the participants, 4,941 subjects (25.1%) fulfilled the criteria for MetS. In the entire sample, both short and long sleep durations were associated with higher prevalence of MetS as compared to normal sleep duration. When stratified by age, a similar pattern was observed for middle-aged subjects (<65 years old; prevalence ratio (PR) [95% CI], 1.13 [1.06–1.22] for short sleep and 1.26 [1.06–1.50] for long sleep duration). In contrast, in older individuals (≥65 years old), only long sleep duration was linked to a higher prevalence of MetS (1.26 [1.12–1.42]; $P < 0.01$ for sleep duration × age). In the entire cohort, having at least one SDB symptom ≥4 times per week was linked to an increased prevalence of MetS; however, the PR was higher in middle-aged subjects compared with older subjects (1.50 [1.38–1.63] vs. 1.36 [1.26–1.47], respectively; $P < 0.001$ for SDB × age). Finally, independent of subjects' age, reports of sleep disturbances (i.e., at least one symptom ≥4 times per week) were associated with a higher likelihood of having MetS (1.12 [1.06–1.18]; $P > 0.05$ for sleep disturbance × age).

Conclusion: Our results suggest that age may modify the associations between some sleep parameters and the prevalence of MetS.

Keywords: sleep duration, sleep disturbance, sleep-disordered breathing, metabolic syndrome, age

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INTRODUCTION

The metabolic syndrome (MetS) is defined as a cluster of several cardio-metabolic risk factors. This includes hypertension, hyperlipidemia, hyperglycemia, reduced blood concentrations of high-density lipoprotein (HDL) cholesterol, and abdominal obesity (1). Accumulating evidence suggests that chronic poor sleep patterns can increase the risk of having MetS, or some of its components

(2–4). For instance, in a recent meta-analysis involving 76,027 participants, short (defined as ≤6 h/day) and long sleep durations (defined as >8 h/day) were associated with increased risk of MetS (+27 and +23%, respectively), as compared with normal sleep duration (5). Additionally, suffering from sleep disturbances or sleep-disordered breathing (SDB) might also increase the risk of having MetS, or some of its components (6–10). This is for instance supported by findings of a recent meta-analysis demonstrating that subjects with obstructive sleep apnea (OSA; hallmarked by recurrent episodes of either partial or full cessation of breathing while asleep) were at 1.72 times higher risk for MetS (11), than those without OSA. Collectively, existing evidence indicates worrisome connections between poor sleep patterns and MetS.

Noteworthy, associations between poor sleep patterns and parameters of the MetS appear to vary by age. For instance, an association between short sleep duration (≤5 and 6 h) and prediabetes—defined by the authors as blood levels of glycated hemoglobin ≥5.7% (HbA1c; a proxy of the 3-month average plasma glucose concentration)—was found in Japanese subjects aged <40 years (n = 32,929). In contrast, long sleep duration (≥ 8 h) was associated with lower odds of having prediabetes in adults aged <40 years (12). In the same study, in older subjects (≥40 years; n = 42,543), short sleep duration (≤5 h) was associated with higher odds for prediabetes. Long sleep duration was, however, unrelated to prediabetes in older subjects (12). In a separate Korean study involving 5,393 subjects, it was found that young and middle-aged adults (19–64 years) who slept <6 h a day, compared to those who slept 7 h a day, had increased odds of hypertension. This association was not found among those aged ≥65 years (13). This is in line with a Spanish study (N = 3,686) in which self-reported sleep duration was not associated with hypertension among those aged 60 years and above (14). Overall, these results suggest that associations between poor sleep and components of the MetS may vary by age. However, the effects of age on the association between sleep parameters and the prevalence of MetS are not fully disentangled.

With this in mind, this study sought to examine whether relationships of sleep duration outside the recommended range (7–8 h per day), sleep disturbances (such as problems with falling and staying asleep), and SDB symptoms (such as snoring and sleep apneas) with MetS vary by age (45–64 vs. ≥65 years, common retirement age in Sweden). We hypothesized that poor sleep patterns increase the prevalence ratio (PR) of MetS.

MATERIALS AND METHODS

Study Population

Our analysis was based on data from the EpiHealth cohort study (www.epihealth.se). At the time of retrieving data for the present analysis, the EpiHealth study was still recruiting subjects. This explains the difference in initial sample sizes between previous publications (15) and the present analysis. A detailed description of the study has been reported previously (16).

From the initial sample size (n = 20,534), two participants were excluded because their age was more than two SD apart from the population mean; 360 individuals were then excluded because of missing data on biochemical parameters or waist circumference. Finally, 437 participants had missing data on covariates, and 44 subjects provided no reports on sleep duration. After exclusions, data from 19,691 subjects (96% of the initial sample size) were available to investigate the association between sleep duration and MetS. From them, 19,142 (93% of the initial sample size) participants had complete data on sleep disturbance and 16,467 (80% of the initial sample size) on SDB symptoms.

The Ethics Committee at Uppsala University approved the general procedures of the EpiHealth study. All subjects gave written informed consent in accordance with the Declaration of Helsinki. An additional ethical approval for the current data analyses was obtained from the Ethics Committee at Uppsala University. A short description of the research proposal was displayed on EpiHealth's homepage for 1 month which allowed participants in EpiHealth study to withdraw their consent.

Metabolic Syndrome

Participants visited a test center for collection of physical measurements and blood samples (located in Malmö or Uppsala, Sweden). Blood pressure was recorded twice in the sitting position by trained personnel with automatic device (Omron, Kyoto, Japan). Waist circumference was measured at the umbilical level. Blood was collected for determination of fasting glucose, LDL- and HDL-cholesterol, and serum triglycerides at the hospital laboratory using an Architect Ci8200 analyzer (Abbott Laboratories, Abbott Park, IL, USA) (16). The same equipment as well as the same biochemical laboratory for analysis of glucose and lipids was used in both test centers (16).

Metabolic syndrome was defined as the presence of at least three of the following conditions: elevated waist circumference (≥102 cm for men; ≥88 cm for women); hypertriglyceridemia, defined as a serum triglyceride concentration ≥150 mg/dL [≥1.7 mmol/L]; low HDL cholesterol (<40 mg/dL [<1.0 mmol/L] for men and <50 mg/dL [<1.3 mmol/L] for women); elevated blood pressure (systolic ≥130 and/or ≥85 diastolic mmHg) or antihypertensive drug treatment (1); and elevated fasting glucose (≥110 mg/dL [≥6.1 mmol/L]) or drug treatment for diabetes (17).

Sleep Variables

Participants were asked to indicate how many hour per day they usually sleep ("4 h or less," "5h," "6h," "7h," "8h," "9h," "10 h or more," and "don't know/don't want to answer"). The answer "don't know/don't want to answer" was treated as missing value. Short sleep duration was defined as sleep ≤6 h per day, normal sleep duration corresponded to 7–8 h sleep per day, and sleep ≥9 h per day was defined as long sleep duration.

Sleep disturbances were determined based on the following symptoms: difficulties in falling asleep, early awakenings, difficulties getting back to sleep after nighttime awakenings, and disturbed sleep. Symptoms of SDB included witnessed sleep apnea and heavy snoring (witnessed or according to participate him/herself). Participants were required to indicate the frequency of each symptom by the following options: "never/seldom," "1 to 3

times a month," "1 to 3 times a week," "4 or more times a week," and " don't know/don't want to answer." Participants who reported that they suffered from at least one of the above-mentioned sleep disturbance symptoms for "4 or more times a week" were defined to have a sleep disturbance. Participants who indicated to experience either witnessed sleep apnea or heavy snoring for "4 or more times a week" (or both) was defined to have SDB symptoms. The option "don't know/don't want to answer" was treated as missing value.

Covariates

Age and gender were recorded in the test center. Participants' educational attainment, physical activity (PA) during leisure time, alcohol consumption frequency, and current smoking were assessed by the Internet-based questionnaire. Participants' educational attainment was defined as primary and elementary school (up to 9 years of formal schooling), upper secondary school (up to 12 years of formal schooling), university, or other (e.g., further training). PA during leisure time was measured on an eight-point scale. A low level of PA was defined as spending most leisure time mostly sedentary or having light PA about 2–4 h per week, such as walking, gardening, and light housework, etc. A medium level of PA was defined as moderate PA at least 1–2 times a week, such as jogging, swimming, heavy gardening, etc., or light PA for more than 4 h per week or taking care of all the housework, both light and heavier. A high PA level was defined as more strenuous PA at least three times a week, such as playing tennis, swimming, and running, etc. Based on responses to six questions about smoking habits, the participants were assigned to non-smokers or smokers. Alcohol consumption frequency during the last 12 months was categorized as "never," "≤1 time/week," "2–3 times/week," and "≥4 times/week," yielding a four-level ordinal variable.

Statistical Analysis

All statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Descriptive data are presented as mean (SD) for continuous variables and as percentages for categorical variables. The Pearson chi-square test was used to analyze group differences for categorical variables. Numerical data were analyzed with Mann–Whitney *U* test.

Binomial regression with log link function was performed to examine associations between the prevalence of MetS and sleep parameters. This statistical approach was used because the outcome (MetS) was common (25%) in the present cohort. Under such circumstances, log-binomial regression analysis is generally considered to be more conservative than logistic regression (18). PRs derived from log-binomial regression were adjusted for participants' age (expressed in years), gender, educational attainment, leisure PA level, current smoking status, and alcohol consumption frequency.

Note that job-related questions (including those on shift work history) were presented only to participants who were still working at the time of the online survey (15). Hence, the shift work variable was not included in the main analysis due to a large proportion of missing values (n = 9,061, 44%) and risk of potential misclassification. Additionally, shift work was the only variable that was not significantly associated with MetS in fully adjusted models. Finally, comparison of regression models using Akaike's Information Criterion (AIC) revealed that models not adjusted for shift work had lower AIC, indicating a better model fit.

Potential confounders were selected based on existing information on risk factors for impaired sleep and MetS using the method of directed acyclic graphs (DAGs) (19). DAGs is a widely used method to depict graphically assumed causal relationships between predictor, outcome, and confounder variables. Overall, no interactions between gender and sleep parameters were found ($P > 0.05$). Possible multiplicative interaction effects of sleep parameters with age (in y) on MetS risk were investigated in fully adjusted models (i.e., sleep parameter × age). We considered interaction present at $P < 0.05$.

The proportion of missing data on main exposure variables was less than 1% for sleep duration, 3% for sleep disturbance, 17% for SDB symptoms, and ≤1% for covariates. To assess if exclusions of subjects because of missing values could have affected the observed associations between sleep variables and the prevalence of MetS, we performed multiple imputation with the assumption that data were missing at random. The imputation procedure resulted in five imputation data sets.

RESULTS

Study Population

Of the total 19,691 participants with complete data on sleep duration, 21% of middle-aged subjects (age <65 years) and 31.2% of older individuals (≥65 years) met criteria for MetS, respectively. Compared with middle-aged subjects, older individuals had more often an elevated waist circumference (≥102 cm for men; ≥88 cm for women), hypertension (systolic ≥130 and/or ≥85 diastolic mmHg or antihypertensive drug treatment), elevated serum triglycerides (≥150 mg/dL [≥1.7 mmol/L]), and elevated fasting glucose (≥110 mg/dL [≥6.1 mmol/L] or drug treatment for diabetes). In contrast, low HDL cholesterol level (<40 mg/dL [<1.0 mmol/L] for men and <50 mg/dL [<1.3 mmol/L] for women) did not differ between age groups. For more details, see **Table 1**.

Sleep Duration and MetS

Results are summarized in **Table 2**. A significant interaction between sleep duration and age (in years) was observed ($P = 0.009$). Similar results were obtained for the interaction between sleep duration and age when the latter was treated as binary variable ($P = 0.028$). Compared to normal sleep duration, long sleep duration increased the prevalence of MetS in both middle-aged and old participants ($P = 0.008$ and $P < 0.001$, respectively). In contrast, short sleep duration was linked to a higher prevalence of MetS in middle-aged but not older subjects ($P < 0.001$ for middle-aged subjects; $P = 0.716$ for older subjects).

A secondary analysis was performed dividing the sleep duration variable into four instead of three categories (i.e., ≤5; 6; 7–8; and ≥9 h per day). In the entire sample, for those who reported to sleep ≤5 or ≥9 h per day a higher prevalence of MetS was found (PR [95% CI]; ≤5 h: 1.16 [1.07–1.25]; and ≥9 h: 1.26 [1.14–1.39]), compared with those who slept between 7 and 8 h per day. No such

TABLE 1 | Participants' characteristics, stratified by age.

Parameter	Total	Age	
		<65 years	≥65 years
Total participants, *n* (%)	19,691	11,804 (74.9)	7,887 (25.1)
Age, years, mean (SD)*	60.8 (8.5)	55.1 (5.7)	69.3 (3.1)
Metabolic syndrome, *n* (%)*	4,941 (25.1)	2,479 (21.0)	2,462 (31.2)
Females, *n* (%)*	11,139 (56.6)	7,078 (60.0)	4,061 (51.5)
Educational status, n (%)*			
Primary/elementary school	3,105 (15.8)	1,066 (9.0)	2,039 (25.9)
Upper secondary school	5,005 (25.4)	3,645 (30.9)	1,360 (17.2)
University	9,351 (47.5)	6,067 (51.4)	3,284 (41.6)
Other	2,230 (11.3)	1,026 (8.7)	1,204 (15.3)
Leisure PA level, n (%)*			
Low	7,834 (39.8)	4,520 (38.3)	3,314 (42.0)
Medium	8,258 (41.9)	4,675 (39.6)	3,583 (45.4)
High	3,599 (18.3)	2,609 (22.1)	990 (12.6)
Current smoking, n (%)*	1,493 (7.6)	989 (8.4)	504 (6.4)
Alcohol consumption frequency, n (%)*			
Never	1,110 (5.6)	646 (5.5)	464 (5.9)
≤1 time/week	10,484 (53.2)	6,597 (55.9)	3,887 (49.3)
2–3 times/week	6,316 (32.1)	3,810 (32.3)	2,506 (31.8)
≥4 times/week	1,781 (9.0)	751 (6.4)	1,030 (13.1)
Elevated waist circumference*			
(≥102 cm for men; ≥88 cm for women)	7,977 (40.5)	4,407 (37.3)	3,570 (45.3)
Elevated serum triglycerides*			
(≥150 mg/dL [≥1.7 mmol/L])	3,177 (16.1)	1,847 (15.6)	1,330 (16.9)
Low high-density lipoprotein cholesterol level			
(<40 mg/dL [<1.0 mmol/L] for men and <50 mg/dL [<1.3 mmol/L] for women)	2,012 (10.2)	1,245 (10.5)	767 (9.7)
Elevated blood pressure*			
(systolic ≥130 and/or ≥85 diastolic mmHg or antihypertensive drug treatment)	14,228 (72.3)	7,450 (63.1)	6,778 (85.9)
Elevated fasting glucose*			
(≥110 mg/dL [≥6.1 mmol/L] or drug treatment for diabetes)	6,761 (34.3)	3,371 (28.6)	3,390 (43.0)

*Analysis was based on Mann–Whitney U test; Pearson Chi-square test. *P-values <0.05.*
PA, physical activity.

TABLE 2 | Associations between sleep variables and metabolic syndrome (MetS) in the Swedish EpiHealth cohort study.

	MetS			MetS			MetS		
	Absent, *n*(%)	Present, *n*(%)	PR (95% CI)	Absent, *n*(%)	Present, *n*(%)	PR (95% CI)	Absent, *n*(%)	Present, *n*(%)	PR (95% CI)
Sleep duration (h/day)	**Total (*n* = 19,691)**			**Age <65 years (*n* = 11,804)**			**Age ≥65 years (*n* = 7,887)**		
≤6	4,674 (31.7)	1,712 (34.6)	**1.08 (1.03–1.13)**	3,067 (32.9)	965 (38.9)	**1.13 (1.06–1.22)**	1,607 (29.6)	747 (30.3)	1.01 (0.94–1.09)
7–8	9,576 (64.9)	2,962 (59.9)	Ref	6,040 (64.8)	1,426 (57.5)	Ref	3,536 (65.2)	1,536 (62.4)	Ref
≥9	500 (3.4)	267 (5.4)	**1.26 (1.14–1.39)**	218 (2.3)	88 (3.5)	**1.26 (1.06–1.50)**	282 (5.2)	179 (7.3)	**1.26 (1.12–1.42)**
Sleep disturbance[a]	**Total (*n* = 19,142)**			**Age <65 years (*n* = 11,553)**			**Age ≥65 years (*n* = 7,589)**		
No	11,182 (77.7)	3,518 (74.0)	Ref	7,076 (77.4)	1,757 (72.8)	Ref	4,106 (78.3)	1,761 (75.2)	Ref
Yes	3,205 (22.3)	1,237 (26.0)	**1.12 (1.06–1.18)**	2,065 (22.6)	655 (27.2)	**1.14 (1.05–1.23)**	1,140 (21.7)	582 (24.8)	**1.11 (1.03–1.20)**
Sleep-disordered breathing symptoms[a]	**Total (*n* = 16,467)**			**Age <65 years (*n* = 10,059)**			**Age ≥65 years (*n* = 6,408)**		
No	10,583 (84.6)	2,870 (72.6)	Ref	6,866 (85.2)	1,419 (70.8)	Ref	3,717 (83.3)	1,451 (74.5)	Ref
Yes	1,933 (15.4)	1,081 (27.4)	**1.44 (1.36–1.53)**	1,189 (14.8)	585 (29.2)	**1.50 (1.38–1.63)**	744 (16.7)	496 (25.5)	**1.36 (1.26–1.47)**

The results derived from log-binomial regression analysis. This analysis was controlled for participants' exact age (in years), gender, educational level, physical activity during leisure time, smoking status, and alcohol consumption.
[a]*Participants reported that at least one symptom (see methods for description) occurred ≥4 times per week. Bold values = P-values <0.05.*
PR, prevalence ratio; Ref, reference group for the analysis.

difference in the prevalence of MetS was observed between the 6 h-sleep duration and reference groups (1.05 [0.99–1.10]). When stratified by age, a similar pattern was observed among middle-aged subjects (≤5 h: 1.29 [1.17–1.43]; 6 h: 1.08 [0.99–1.16]; and ≥9h: 1.27 [95% CI 1.07–1.50]). In contrast, in the ≥65 years age group only long sleep duration was linked to a higher prevalence of MetS (1.26 [CI 1.12–1.42]).

Sleep Disturbance and MetS

Results are summarized in **Tables 2** and **3**. No significant interaction between sleep disturbance and age was observed [$P = 0.189$ for sleep disturbance × age (in years); $P = 0.521$ for sleep disturbance × age group]. Participants with sleep disturbance were more likely to have MetS, than those without sleep disturbance ($P < 0.001$; **Table 2**). A separate analysis in the entire cohort (i.e., including both age groups) demonstrated that the number of sleep disturbance symptoms showed a positive association with the prevalence of MetS (**Table 3**).

TABLE 3 | Association between the number of self-reported sleep disturbance symptoms and prevalence of MetS.

	MetS		
Number of sleep disturbance symptoms	**Absent, *n*(%)**	**Present, *n*(%)**	**PR (95% CI)**
0	11,182 (77.7)	3,518 (74.0)	Ref
1–2	2,530 (17.6)	919 (19.3)	**1.08 (1.02–1.15)**
3–4	675 (4.7)	318 (6.7)	**1.25 (1.14–1.37)**
1–2	2,530 (78.9)	919 (74.3)	Ref
3–4	675 (21.1)	318 (25.7)	**1.15 (1.04–1.28)**

The results derived from log-binomial regression analysis. This analysis was controlled for participants' exact age (in years), gender, educational level, physical activity during leisure time, smoking status, and alcohol consumption. Note that the analysis was performed in the entire group, as the interaction between age and sleep disturbance did not reach significance (P > 0.05). Bold values = P-values <0.05.
PR, prevalence ratio; Ref, reference group for the analysis; MetS, metabolic syndrome.

SDB Symptoms and MetS

Sleep-disordered breathing symptoms were associated with a higher prevalence of MetS in both age groups ($P < 0.05$; **Table 2**). However, the prevalence of MetS was higher among middle-aged subjects with SDB, as compared to older subjects with SDB ($P < 0.001$ for SDB × age). A separate analysis in the entire cohort, as well as within the age groups, revealed that the higher the number of SDB symptoms, the higher the prevalence of MetS (**Table 4**).

Sensitivity Analysis

In a sensitivity analysis, a multiple imputation approach was used to investigate whether exclusions because of missing values may have influenced our results. The associations between sleep parameters and the prevalence of MetS (revealed by our main analyses, see **Table 2**) remained significant, both in the entire sample and when stratified by age group (data not shown).

As mentioned above, significant interactions between age and sleep duration, as well as between age and SDB symptoms were found. To further examine the influence of age on observed associations between these sleep parameters and the prevalence of MetS, an additional sensitivity analysis dividing subjects into three instead of two age categories (early midlife: 45–54 years; older midlife: 55–64 years; and older ≥65 years old) was performed. This analysis demonstrated that early and older midlife age groups had a higher prevalence of MetS when reporting short sleep duration (≤6 h per day). In contrast, long sleep duration (≥9 h per day) was linked to a higher prevalence of MetS only in the older midlife age group and among individuals ≥65 years old (see Table S1 in Supplementary Material). Finally, in all age groups an association between SDB symptoms and increased prevalence of MetS was noticed, with highest PR for subjects of the early midlife age group (see Table S1 in Supplementary Material).

TABLE 4 | Association between the number of self-reported SDB symptoms and prevalence of MetS.

	MetS			MetS			MetS		
Number of SDB symptoms	**Absent, *n*(%)**	**Present, *n*(%)**	**PR (95% CI)**	**Absent, *n*(%)**	**Present, *n*(%)**	**PR (95% CI)**	**Absent, *n*(%)**	**Present, *n*(%)**	**PR (95% CI)**
	Total (*n* = 16,467)			**Age <65 years (*n* = 10,059)**			**Age ≥65 years (*n* = 6,408)**		
0	10,583 (84.6)	2,870 (72.6)	Ref	6,866 (85.2)	1,419 (70.8)	Ref	3,717 (83.3)	1,451 (74.5)	Ref
1	1,489 (11.9)	687 (17.4)	**1.30 (1.22–1.40)**	922 (11.4)	389 (19.4)	**1.39 (1.26–1.53)**	567 (12.7)	298 (15.3)	**1.19 (1.08–1.31)**
2	444 (3.5)	394 (10.0)	**1.76 (1.64–1.90)**	267 (3.3)	196 (9.8)	**1.78 (1.59–1.99)**	177 (4.0)	198 (10.2)	**1.74 (1.57–1.92)**
	Total (*n* = 3,014)			**Age <65 years (*n* = 1,774)**			**Age ≥65 years (*n* = 1,240)**		
1	1,489 (77.0)	687 (63.6)	Ref	922 (77.5)	389 (66.5)	Ref	567 (76.2)	298 (60.1)	Ref
2	444 (23.0)	394 (36.4)	**1.41 (1.28–1.54)**	267 (22.5)	196 (33.5)	**1.33 (1.17–1.52)**	177 (23.8)	198 (39.9)	**1.48 (1.30–1.69)**

The results derived from log-binomial regression analysis. This analysis was controlled for participants' exact age (in years), gender, educational level, physical activity during leisure time, smoking status, and alcohol consumption. Note that the analysis was performed in the entire group, as well as for both age groups separately (P < 0.001 for SDB symptoms × age). Bold values = P-values <0.05.
PR, prevalence ratio; Ref, reference group for the analysis; SDB, sleep-disordered breathing; MetS, metabolic syndrome.

DISCUSSION

Our study demonstrates that the association between sleep duration and MetS varies by age. In middle-aged participants (45–65 years), both short (defined as ≤6 h sleep per day) and long (defined as ≥9 h sleep per day) duration sleepers exhibited an increased prevalence of MetS, compared with normal-duration sleepers (defined as 7–8 h sleep per day). In contrast, in older individuals (aged ≥65 years), long but not short sleep duration was linked to a higher likelihood of having MetS. Collectively, our study provides an important piece of evidence that the relation between sleep duration and metabolic health may change during adulthood. Age-specific associations between sleep duration and MetS components have also been observed by others. For instance, a study using the NHANES I follow-up data showed that daily sleep durations of ≤5 h compared to 7–8 h were associated with a significantly increased risk of incident hypertension in participants aged 32–59 years. This association was, however, not found among people aged ≥60 years (20). In a cross-sectional study of 29,333 individuals at age ≥50 years, it has further been shown that long sleep (≥9 h/days) but not short sleep was associated with an increased risk of MetS (21).

Sleep-disordered breathing comprises alterations in respiratory rate, rhythm, and depth present during sleep (22). Obstruction of the upper airway during sleep has been attributed to hypoxia, pulmonary hypertension, and light sleep (8, 23), all of which may cause metabolic perturbations (3, 8, 24–27). In this study, we show that both middle-aged and older subjects were at higher risk of MetS, when suffering from at least one SDB symptom ≥4 times per week. This association became stronger the higher the number of SDB symptoms. Additionally, our analysis revealed that middle-aged individuals with SDB exhibited a higher risk of MetS, than older subjects with SDB. One possible explanation for the latter finding could be that older humans, due to sleep hallmarked by lighter sleep stages and reduced time in rapid eye movement (REM) sleep (28), may run a lower risk to suffer from apneas during REM, than middle-aged subjects. Apneas during REM sleep have been proposed to be particularly detrimental to metabolic health (29–31). Overall, our findings could suggest that screening for SDB symptoms, e.g., by means of questionnaires or anamnestic interviews, may be particularly relevant for metabolic risk assessment in middle-aged people.

Another finding of our study was that reports of sleep disturbances, including difficulties in initiating and maintaining sleep, increased the prevalence of MetS. In contrast to sleep duration and SDB symptoms, associations between sleep disturbances and MetS did not differ between age groups. Relationships between measures of sleep disturbance and MetS have also been described by others (6, 9). For instance, data from a nationwide epidemiological survey conducted on middle-aged residents (mean age <60 years; $n = 4{,}197$) showed that problems with falling and staying asleep were associated with an increased prevalence of MetS (24 and 28%, respectively) (9). In a separate study involving 210 volunteers with a mean age of 46 years, it was shown that self-reported global sleep quality, measured with the Pittsburgh Sleep Quality Index, was related to MetS (6). Specifically, an increase of the global sleep score of 2.6 points was associated with an odds of 1.44 of having MetS (6). However, it must be noted that there are also negative results. In a study of 796 Taiwanese male police officers (mean age 37.4 years), no association between sleep quality and MetS was observed (32).

Strengths and Limitations

A major strength of this study is that the analysis was based on a relatively large sample. Moreover, results were robust to adjustments for multiple potential confounders, such as lifestyle factors. To our best knowledge, our study is also among the first to investigate systematically how various characteristics of poor sleep link to the risk of MetS in middle-aged and older subjects. Several limitations, however, apply to our cross-sectional study. It cannot prove cause and effect. Another limitation is that sleep variables were based on self-reports. Thus, our observations should be confirmed by further studies utilizing objectively measured sleep parameters. An additional limitation of our study is that no measures of circadian misalignment have been collected. Sleeping 7–8 h during circadian improper time windows (e.g., during daytime because of night shift work) has been shown to adversely affect metabolic health (33, 34). Moreover, other potential confounders, such as pathological conditions (e.g., chronic or acute pain) and sleep-related medication, have not been included in the present analysis. It must also be noted that criteria underlying the definition of components of MetS (e.g., hypertension) can vary between studies. Finally, most of participants were of northern European origin which may reduce the generalizability of our results to other ethnic groups.

Conclusion

In our study, we demonstrate that both sleep duration outside 7-8 hours per day and having at least one SDB symptom ≥4 times per week increase the risk of MetS in an age-specific manner. Sleep disturbances (i.e., at least one symptom ≥4 times per week) were also associated with an increased prevalence of MetS. The latter relationship was not modified by subject's age. Given the high prevalence of sleep problems and metabolic perturbations in modern society, educational programs aiming to optimize sleep could, therefore, represent promising interventions to improve metabolic health in middle-aged and older subjects.

ETHICS STATEMENT

The Ethics Committee at Uppsala University approved the general procedures of the EpiHealth study. All subjects gave written informed consent in accordance with the Declaration of Helsinki. An additional ethical approval for the current data analyses was obtained from the Ethics Committee at Uppsala University. A short description of the research proposal was displayed on EpiHealth's homepage for 1 month which allowed participants in EpiHealth study to withdraw their consent.

AUTHOR CONTRIBUTIONS

OT: wrote the manuscript, performed the literature search, data and statistical analysis, and data interpretation. EL: reviewed and

revised the manuscript, and supported data interpretation. SE: contributed to the study design, acquired data, and gave scientific advice. LL: contributed to the study design, acquired data, and reviewed the manuscript. HS: reviewed and revised the manuscript. CB: reviewed and revised the manuscript, supported data interpretation, and gave scientific advice. All authors approved the final version of the manuscript. OT and CB had full access to all of the data and take responsibility for the integrity and accuracy of the data analysis.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fendo.2018.00234/full#supplementary-material.

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Characteristics of Obstructive Sleep Apnea Across the Spectrum of Glucose Tolerance in Obese Adolescents

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Background: It is not known if dysglycemia and sleep-disordered breathing are linked in adolescents, as in adults.

Objective: To perform a pilot study evaluating measures of sleep-disordered breathing across the spectrum of glucose tolerance in obese adolescents. We hypothesized that dysglycemia would be associated with sleep-disordered breathing.

Participants/methods: This was a prospective, cross-sectional clinical pilot study that included 57 adolescents [body mass index (BMI) 38.9 ± 8.4 kg/m^2] aged 12–18 years (14.5 ± 1.6) with normal glucose tolerance (NGT), or dysglycemia [impaired glucose tolerance (IGT) or type 2 diabetes (T2D)].

Measures: Anthropometrics, overnight polysomnogram, and oral glucose tolerance tests were performed. Participant characteristics and outcome measures were compared by glucose tolerance status. Correlational analyses were conducted to assess the associations between variables of interest.

Results: Participants with dysglycemia (n = 21) were not different from those with NGT (n = 36) for BMI, waist circumference, body fat, or sleep characteristics. Nocturnal oxygen desaturation was associated with higher BMI (r = –0.334, p = 0.012). The apnea–hypopnea index (AHI) was not associated with physical and metabolic parameters. Although participants with dysglycemia tended to have higher AHIs (median 3.2, 2.2, and 1.6 events/h for T2D, IGT, and NGT, respectively), there was not a linear relationship between measures of glycemia and AHI.

Conclusion: Further study with a larger proportion of youth with prediabetes and T2D is necessary to determine whether evaluation for sleep-disordered breathing is uniformly warranted.

Keywords: obesity, prediabetes, pediatric, sleep-disordered breathing, apnea, insulin sensitivity

INTRODUCTION

Prediabetes and type 2 diabetes (T2D) are strongly associated with obstructive sleep apnea (OSA) in adults (1). In the Look AHEAD study, >80% of overweight adult participants with T2D were found to have OSA on screening polysomnogram (PSG) (2). In adults, OSA is linked with higher hemoglobin A1C values across the range of glucose tolerance (3), and treatment of OSA has been associated with improvement in blood glucose control (4, 5). In youth, it is unknown if prediabetes and/or T2D and OSA or sleep-disordered breathing are linked, as few similar studies have been performed (6). In this pilot study, we sought to evaluate if sleep-disordered breathing is prevalent in obese adolescents with dysglycemia, and whether sleep-disordered breathing is associated with blood glucose measures. This is important to study in adolescents because: (1) adolescents have physiologic insulin resistance which increases risk for dysglycemia and development of prediabetes and T2D (7); (2) adolescents with obesity and T2D have particularly poor health outcomes (8, 9); and (3) longer exposure to T2D and sleep-disordered breathing may further exacerbate these poor health outcomes.

The primary objective of this pilot study was (a) to evaluate PSG-derived measures of sleep-disordered breathing across the spectrum of glucose tolerance in obese adolescents. We utilized oral glucose tolerance tests (OGTT) to categorize youth as having normal glucose tolerance (NGT), or dysglycemia [OGTT values consistent with prediabetes defined as impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), and T2D], and (b) to examine the associations between OGTT-derived measures of glucose tolerance, insulin sensitivity, and insulin secretion and PSG-derived measures of OSA. We hypothesized that obese youth with dysglycemia would have more characteristics of OSA than obese youth with NGT.

MATERIALS AND METHODS

This cross-sectional study was approved by the University of Pittsburgh and Indiana University Institutional Review Boards and performed in the Pediatric Clinical and Translational Research Center (Children's Hospital of Pittsburgh) and the Indiana Clinical Research Center with collaboration from the clinical sleep centers at these institutions. Informed and written consent was obtained from a parent/guardian, and assent was obtained from participants. A convenience sample of eligible subjects referred to outpatient weight management or endocrinology clinics for obesity, prediabetes, or T2D, and who also reported snoring were approached for participation. Eligibility criteria were 12- to 18-year-old male or female adolescents (Tanner stage >1) of any race with body mass index (BMI) ≥95th percentile. Exclusion criteria included diagnosis of type 1 diabetes, current tonsillar hypertrophy, chronic disease, or medications that may interfere with sleep, endocrine function or glucose regulation, syndromic obesity, chronic upper or lower airway disease, smoking, or current upper respiratory tract infection. Participants treated with either metformin and/or lifestyle recommendations were eligible, after discontinuing metformin/exercise treatment for 48 h before study. If there was a previous diagnosis of T2D, duration was less than 2 years for all participants. Ninety-six patients were screened for the study, and 57 participants enrolled and completed study visits after informed consent/assent was obtained. Data for part of this study cohort have been previously published (10–12).

Study Procedures and Assays

All participants had an examination by a pediatric endocrinologist that included assessment of pubertal development, anthropometric measurements, and 53/57 had body composition by dual-energy X-ray absorptiometry (4 had body weights greater than machine limits). A fasting laboratory evaluation including HbA1C, glucose, and insulin, and 2-h OGTT (1.75 g/kg, maximum 75 g; glucose and insulin at −15, 0, +15, +30, +60, +90, and +120 min time points) was performed. Fifty-three of fifty-seven participants completed the OGTT without complications. The remaining participants had minor complications, usually related to loss of intravenous access, which prohibited frequent blood sampling or reduced sample volume. Plasma glucose was measured by the glucose oxidase method (Pittsburg) and the glucose hexokinase method (Indianapolis). Samples were re-measured in Indianapolis using the glucose hexokinase method when available. Plasma insulin was determined by Beckman Coulter DXI 800 using a chemiluminescent sandwich assay (CV 6%).

Polysomnograms

Overnight PSGs were performed in the sleep lab either the night immediately preceding the OGTT or the night immediately following the OGTT. Due to scheduling issues, it was not possible to conduct all studies in the same order. There were no differences in outcomes based on timing of the OGTT. Data were recorded using either Sensormedics Somnostar Pro version 7.2 or the Sandman Elite 9.1 sleep diagnostic software, and applying the following EEG montage: F3M2, F4M1, C3M2, C4M1, O2M1, O1M2, L-EOG, R-EOG, chin EMG, limb EMG, and the following cardiorespiratory parameters: SpO_2 and pulse (Masimo), ETCO2 (Microstream NPB 70 and Capnograph Sleep by BCI), nasal pressure, airflow (nasal or oral thermistor), thoracic and abdominal excursion (uncalibrated respirator inductance plethysmography), pulse, and ECG. The PSG data were interpreted by one of two sleep Board-certified co-investigators specific to the site where the PSG was performed, who had no knowledge of the participants' metabolic status. Sleep architecture and respiratory disturbances including the minimum percent oxyhemoglobin saturation (SpO_2) by pulse oximetry, arousal events per hour (sleep fragmentation), and the apnea–hypopnea index (AHI, total number of central and obstructive apnea and hypopnea events per 1 h of sleep), were hand-scored utilizing pediatric criteria and calculated following the AASM manual for scoring guidelines (13). Obstructive apneas were scored for a drop in the peak flow signal by ≥90% of the pre-event baseline using an oral thermal sensor lasting ≥90% for the duration of at least two breaths during baseline breathing and associated with the presence of respiratory efforts throughout the entire period of the absent flow. Hypopneas were scored for a drop by ≥30% of the pre-event baseline using nasal pressure lasting ≥two breaths duration in association with either ≥3% oxygen desaturation

from the pre-event baseline or the event is associated with an arousal. Nocturnal hypoxia is defined as the time spent with SpO_2 less than 90% for more than 5 min.

Calculations

Insulin sensitivity was estimated using inverse fasting insulin (14). Insulin secretion was expressed as the ratio of the incremental response of insulin to glucose at 30 min during the OGTT [insulinogenic index (IGI), $\Delta I_{0-30}/\Delta G_{0-30}$], i.e., the IGI (15). Insulin secretion in relation to insulin sensitivity (oral disposition index) was calculated as ($\Delta I_{0-30}/\Delta G_{0-30} \times 1$/fasting insulin) (15). OGTT glucose and insulin areas under the curve (AUC) were calculated using the trapezoidal method.

Statistical Analysis

Demographic and clinical characteristics of the cohort were summarized, and participants were compared by glucose tolerance status [NGT or having dysglycemia (IFG, IGT, or T2D)]. Dysglycemia was defined as having IFG or IGT using American Diabetes Association (ADA) criteria (16). T2D was defined using ADA criteria for OGTT results (16). Data were log-transformed when not normally distributed. Scatter plots were used to examine the relationships between variables of interest for the entire study cohort, and for NGT and dysglycemia groups. Non-parametric tests for independent samples were performed using Kruskal–Wallis one-way ANOVA. Spearman's correlation coefficients were used to quantify linear correlations, as data were not uniformly distributed for the cohort. Given the pilot nature of the study, we did not perform a pre-study sample-size calculation to address whether or not OSA was related to prediabetes or T2D in adolescent youth. *Post hoc* power analysis showed that with the current sample size, we had 80% power to detect correlation coefficients greater than 0.37 (17). Considering the preliminary nature of the investigation, we did not perform multiplicity adjustment. p Values < 0.05 were considered statistically significant. All analyses were implemented using SPSS software (Version 24).

RESULTS

Characteristics of the participants are reported in **Table 1**. Thirty-six (63.2%) had OGTT results consistent with NGT; 21 had dysglycemia on OGTT [IFG ($n = 5$), IGT ($n = 11$), both

TABLE 1 | Characteristics of the study participants.

	Total study population	Normoglycemic	Dysglycemic	*p* Value
Total (%)	57 (100)	36 (63.2)	21 (36.8)	
Age, years (SD)	14.5 ± 1.6	14.5 ± 1.6	14.2 ± 1.7	0.50
Sex				
Female	30 (52.6%)	15 (41.7%)	15 (71.4%)	0.03
Male	27 (47.4%)	21 (53.3%)	6 (28.6%)	
Race				
White	29 (50.9%)	19 (52.8%)	10 (47.6%)	0.66
Black	27 (47.4%)	16 (44.4%)	11 (52.4%)	
Mixed race	1 (1.8%)	1 (2.8%)	0 (0%)	
Body mass index (BMI) (kg/m^2)	38.9 ± 8.4 (35.0, 28.4–61.6)	38.3 ± 8.4 (34.8, 28.4–61.6)	39.8 ± 8.5 (35.5, 28.7–57.1)	0.53
BMI SDS	3.88 ± 1.50 (3.43, 1.80–8.31)	3.83 ± 1.57 (3.35, 1.80–8.31)	3.95 ± 1.39 (3.80, 2.04–6.43)	0.77
Waist circumference (cm)	113.8 ± 17.8 (109.0, 78.1–155.0)	115.3 ± 16.6 (111.6, 91.4–155.0)	111.1 ± 19.8 (106.0, 78.1–149.0)	0.39
Body fat (%)	46.4 ± 6.1 ($n = 53$) (46.3, 36.7–58.5)	45.3 ± 5.7 ($n = 32$) (44.7, 36.8–58.5)	48.9 ± 6.1 (48.9, 36.7–57.2)	0.035
Laboratory characteristics				
HbA1c (%)	5.8 ± 1.3 (5.4, 4.5–12.0)	5.5 ± 0.8 (5.4, 4.5–9.6)	6.3 ± 1.8 (5.9, 5.0–12.0)	0.014
Fasting glucose (mg/dL)	98.3 ± 7.4 (91.5, 74–231)	88.6 ± 6.5 (89.3, 74–99)	114.9 ± 37.4 (101.0, 79–231)	<0.001
OGTT 2-h glucose (mg/dL)	143 ± 52 ($n = 56$) (128, 89–332)	118.8 ± 13.7 (122, 89–138)	187.2 ± 66.5 (157.5, 98–332)	<0.001
1/Fasting insulin (μU/mL)	0.0421 ± 0.0210 ($n = 52$) (0.0385, 0.0100–0.0900)	0.0481 ± 0.0210 (n = 34) (0.0446, 0.0200–0.0900)	0.0307 ± 0.0159 ($n = 18$) (0.0321, 0.0100–0.0700)	0.003
Insulinogenic index	3.79 ± 2.64 ($n = 51$) (3.01, 0.30–12.3)	3.60 ± 2.0 ($n = 33$) (3.27, 0.36–7.83)	4.14 ± 3.57 ($n = 18$) (2.63, 0.30–12.26)	0.95
Oral disposition index	13.6 ± 10.0 ($n = 51$) (11.8, 0.81–56.6)	15.4 ± 10.3 ($n = 33$) (13.9, 2.5–56.6)	10.2 ± 8.6 ($n = 18$) (7.0, 0.8–34.5)	0.022
Sleep characteristics				
Polysomnogram sleep time, min	411 ± 53 (415, 233–494)	406 ± 53 (415, 233–478)	419 ± 54 (414, 314–494)	0.61
Sleep latency, min	15.5 ± 14.4 (12.0, <1.0–67.0)	14.8 ± 13.6 (12.3, 1.0–67.0)	16.6 ± 16.0 (12.0, <1.0–54.0)	0.93
REM sleep, % of total	18.9 ± 5.2	18.9 ± 5.5	18.9 ± 4.6	0.99
Slow wave sleep, % of total	19.4 ± 7.1	20.0 ± 6.9	18.3 ± 7.6	0.42
Apnea–hypopnea Index (AHI), events/h	4.7 ± 8.1 (2.2, 0.0–50.4)	3.3 ± 4.1 (1.6, 0.0–20.0)	7.0 ± 11.0 (2.9, 0.0–50.4)	0.19
AHI <1	17 (29.8)	12 (33.3)	5 (23.8)	
Mild obstructive sleep apnea (OSA); AHI ≥1 to <5	24 (42.1)	17 (47.2)	7 (33.3)	
Moderate OSA; AHI ≥5 to <10	8 (14.0)	4 (11.1)	4 (19.0)	
Severe OSA; AHI ≥10	8 (14.0)	3 (8.3)	5 (23.8)	
Adult OSA cutoff met, AHI >5, *n* (%)	16 (28.1)	7 (19.4)	9 (42.9)	0.07[a]
Arousal index, events/h	9.7 ± 6.3 (8.4, 2.0–39.2)	9.4 ± 4.7 (8.7, 3.5–25.3)	10.3 ± 8.8 (7.9, 2.0–39.2)	0.67
Nadir SpO_2, %	88.6 ± 6.5 (91, 58–96)	90.3 ± 4.7 (92, 77–96)	86.0 ± 8.4 (87.0, 58–94)	0.06

Data are means ± SD unless otherwise indicated. Median, range are given in parentheses for variables not normally distributed.
[a]*Fisher's Exact Test for the proportion with AHI >5 in each group (normoglycemic and dysglycemic).*

IFG and IGT ($n = 3$), FPG ≥126 mg/dL ($n = 3$), OGTT 2-h glucose ≥200 mg/dL ($n = 5$)]. Participants with dysglycemia were not significantly different from those with NGT for BMI, BMI SDS, or waist circumference. Percent body fat was higher in the group with dysglycemia (**Table 1**). Sleep characteristics among the groups divided by glycemia categories are shown in **Table 1**. There were no sex- or race-related differences in sleep characteristics. Seventeen (29.8%) had normal sleep studies with AHI < 1; 24 (42.1%) had AHI ≥1 to <5; 8 (14.0%) had AHI ≥5 to <10; 6 (10.5%) had AHI ≥10 to <15; 2 (3.5%) had AHI ≥15.

The numbers and percentages of participants meeting criteria for OSA based on the AHI (mild, 1 to <5 events/h; moderate, 5 to <10 events/h; severe, ≥10 events/h) are shown in **Table 1**. The majority of participants had an AHI <5 events/h. The percentage of participants meeting criteria for any degree of OSA was 66.7% in those with normal glycemia and 76.2% in those with dysglycemia. The between-group difference was not statistically significant ($p = 0.07$). Spearman's correlation coefficients for the associations between laboratory markers of glycemia and β-cell function, and indices of sleep fragmentation and sleep-disordered breathing (arousal events per hour, AHI, and nadir SpO_2 during PSG) are shown in **Table 2**. BMI, waist circumference, percent body fat, and laboratory measures were not associated with the arousal index or the AHI. Higher BMI, higher waist circumference, and higher fasting and 2-h glucose concentrations were associated with lower nadir nocturnal SpO_2. However, there was an outlier for nadir nocturnal SpO_2 (58%, >2 SD from the mean). When the outlier was removed, the association between nadir nocturnal SpO_2 and BMI remained ($r = -0.334$, $p = 0.012$), but the associations with waist circumference ($r = -0.0258$, $p = 0.055$), and with fasting ($r = -0.229$, $p = 0.09$) and 2-h glucose ($r = -0.244$, $p = 0.07$) were no longer significant (**Figure 1**).

When only participants with NGT were evaluated, lower nadir SpO_2 was associated with higher BMI ($r = -0.34$, $p = 0.04$), higher waist circumference ($r = -0.37$, $p = 0.03$), lower inverse fasting insulin ($r = 0.41$, $p = 0.02$), and IGI ($r = -0.40$, $p = 0.02$) (**Table 3**). There were no significant relationships between laboratory and sleep measures in the participants with dysglycemia (**Table 4**). However, among those with T2D, nadir SpO_2 was significantly associated with waist circumference ($r = -0.86$, $p = 0.007$).

TABLE 2 | Correlation coefficients for the associations of sleep measures with physical and metabolic parameters.

	Body mass index	Waist circ.	Body fat (%)	HbA1c	Fasting glucose	2-h Glucose	1/Fasting insulin	Insulinogenic index	Oral disposition index
Arousal index, events/h	−0.074 (0.59)	−0.031 (0.82)	−0.164 (0.26)	0.151 (0.28)	−0.086 (0.54)	−0.074 (0.60)	0.044 (0.76)	−0.064 (0.66)	−0.094 (0.52)
Apnea–hypopnea index, events/h	0.175 (0.19)	0.169 (0.21)	−0.037 (0.79)	0.044 (0.74)	0.184 (0.17)	0.156 (0.25)	−0.089 (0.53)	−0.087 (0.55)	−0.177 (0.21)
Nadir SpO_2, %	−0.318 (0.02)	−0.263 (0.048)	0.081 (0.56)	−0.087 (0.52)	−0.266 (0.046)	−0.284 (0.03)	0.239 (0.09)	−0.056 (0.69)	0.068 (0.64)

Data are Spearman's coefficients (p values).

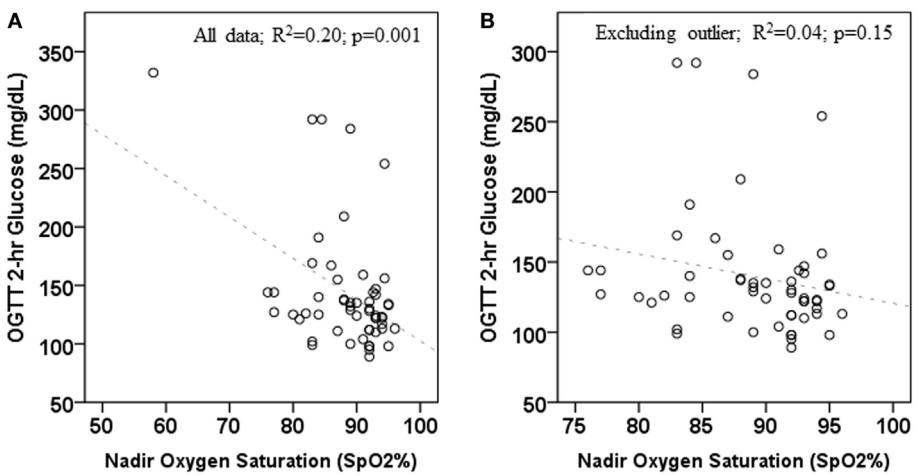

FIGURE 1 | Association between 2-h oral glucose tolerance tests (OGTT) glucose concentration and nadir oxygen saturation during polysomnogram. **(A)** includes all data; **(B)** excludes one outlier.

TABLE 3 | Correlation coefficients for the associations of sleep measures with physical and metabolic parameters, normal glucose tolerance group only.

	Body mass index	Waist circ.	Body fat (%)	HbA1c	Fasting glucose	2-h Glucose	Glucose areas under the curve	1/Fasting insulin	Insulinogenic index	Oral disposition index
Arousal index, events/h	−0.086 (0.74)	−0.104 (0.55)	0.062 (0.74)	0.303 (0.07)	0.034 (0.84)	−0.037 (0.83)	0.234 (0.18)	0.047 (0.79)	−0.236 (0.19)	−0.319 (0.07)
Apnea–hypopnea index, events/h	0.143 (0.41)	0.247 (0.15)	−0.064 (0.73)	−0.029 (0.87)	0.260 (0.13)	0.012 (0.95)	0.149 (0.40)	−0.198 (0.26)	−0.087 (0.55)	−0.177 (0.21)
Nadir SpO_2, %	−0.342 (0.04)	−0.372 (0.03)	0.151 (0.40)	−0.010 (0.95)	−0.283 (0.10)	−0.127 (0.46)	−0.064 (0.72)	0.405 (0.02)	−0.402 (0.02)	−0.143 (0.43)

Data are Spearman's coefficients (p values).

TABLE 4 | Correlation coefficients for the associations of sleep measures with physical and metabolic parameters, dysglycemic group.

	Body mass index	Waist circ.	Body fat (%)	HbA1c	Fasting glucose	2-h Glucose	Glucose areas under the curve	1/Fasting insulin	Insulinogenic index	Oral disposition index
Arousal index, events/h	−0.038 (0.88)	0.009 (0.97)	−0.399 (0.10)	0.046 (0.86)	−0.104 (0.68)	−0.242 (0.35)	−0.164 (0.53)	−0.076 (0.77)	0.147 (0.57)	0.201 (0.44)
Apnea–hypopnea index, events/h	0.172 (0.46)	0.172 (0.46)	−0.088 (0.70)	−0.020 (0.93)	−0.118 (0.61)	0.083 (0.73)	0.170 (0.47)	0.340 (0.17)	−0.171 (0.50)	0.059 (0.82)
Nadir SpO_2, %	−0.225 (0.33)	−0.331 (0.14)	0.047 (0.84)	−0.006 (0.98)	0.031 (0.89)	−0.169 (0.48)	−0.268 (0.25)	−0.369 (0.13)	0.380 (0.12)	0.157 (0.53)

Data are Spearman's coefficients (p values).

DISCUSSION

In this pilot study, we evaluated PSG-measured sleep-disordered breathing in obese adolescents with a range of glucose tolerance. The median AHI in this study was 2.2 events/h, and the majority of participants had an AHI less than 5 events/h, which is the threshold for OSA in adults (18). Although participants with dysglycemia tended to have higher AHIs (median 3.2, 2.2, and 1.6 events/h for T2D, prediabetes, and NGT, respectively), there was not a linear relationship between measures of glycemia and AHI. The percentage of participants meeting criteria for any degree of OSA (AHI greater than or equal to 1 events/h) was 66.7% in those with normal glycemia versus 76.2% in those with dysglycemia, although this did not meet criteria for statistical significance ($p = 0.07$). Individuals in each category of glucose tolerance had PSG evaluations consistent with OSA, with wide variability. Higher BMI, higher waist circumference, and greater insulin resistance (lower inverse fasting insulin) were associated with lower nadir SpO_2 among participants with NGT. Although our hypothesis that youth with dysglycemia would have higher AHI than obese youth with NGT was not verified in this study, our results suggest that a study with a greater proportion of youth with dysglycemia and T2D is warranted and should be pursued through a larger network of clinical sites.

An association with nocturnal hypoxia and hyperglycemia has been shown in adult T2D studies, where greater levels of oxygen desaturation or even relatively mild intermittent hypoxemia were associated with poorer glycemic control (19–23). Also, improving glycemic control in adults with T2D *via* a pharmacological intervention has been associated with a decrease in nocturnal oxygen desaturation (24). The mechanisms of this complex relationship are not yet completely understood, but central, autonomic, and inflammatory mechanisms have been implicated (25–27). Rodent studies have shown that circulating catecholamines act upon α-adrenoreceptors, leading to hyperglycemia and glucose intolerance when intermittent hypoxia is imposed (28).

Pediatric studies have shown that OSA measured by PSG is associated with markers of insulin resistance as measured by fasting insulin and glucose (HOMA-IR) (29, 30), OGTT (10), and intravenous glucose tolerance testing (31). However, pediatric studies examining the relationship between OSA and dysglycemia have had mixed results. Verhulst et al. showed the mean nocturnal SpO_2 was independently correlated with OGTT glucose area under the curve in lean and obese youth aged 6–16 years (32). However, in the Cleveland Children's Sleep and Health Study, oxygen desaturation was not associated with OGTT-stimulated glucose concentrations in a community-based cohort of lean and obese adolescents ($n = 270$; mean age 13.7 years; 41% with BMI ≥85th percentile) (30). The fact that most of the participants were not obese in this previous study may contribute to the different outcomes (30). de Sousa et al. compared PSG variables and OGTT-derived measures of glucose tolerance in obese white adolescents with polycystic ovary syndrome (PCOS, $n = 31$) to those of healthy control females ($n = 19$) (33). This study found a positive correlation between AHI and HOMA-IR ($r = 0.21$, $p = 0.01$), but no association between PSG parameters and OGTT glucose measures. The same group studied changes in PSG variables and OGTT glucose measures in obese adolescents

treated for PCOS longitudinally ($n = 15$, mean age 15.3 ± 1.2 y, 28 ± 6 months between evaluations) (34). Body composition, PSG variables, and OGTT-derived measures of glucose metabolism were unchanged from baseline. Koren et al. also found no association between measures of sleep-disordered breathing and OGTT measures (35). Similar to our study, participants were all obese ($n = 62$, mean age 14.4 years, range 8–17.5; mean BMI 37 kg/m^2) with a range of glucose tolerance. The age range was broader in this previous study, and the range of minimum SaO_2 values was greater in our study.

Shalitin et al. evaluated obese children and adolescents with and without T2D ($n = 11$, $n = 30$, respectively) with PSG and fasting laboratory tests (6). There were no between-group differences in AHI >5/h. The percentage of participants with AHI >5/h was 45% in participants with T2D, 25% in obese participants with IGT, and 18% in obese participants with NGT. Similarly, our study found: AHI >5/h was 43% in participants with dysglycemia and 19% in obese participants without dysglycemia. In both studies, participants with dysglycemia were more likely to have AHI meeting the criteria for OSA; however, between-group differences were not statistically significant in either study. Both studies lacked sufficient participants to adequately test the hypothesis that OSA occurs more frequently in obese adolescents with dysglycemia. It may be that obese youth are at risk for both sleep-disordered breathing and T2D, but the associations are weaker in youth than in adults. It could also be that the pathophysiology linking T2D and hypoxia is much more prevalent in older adults as compared with adolescents. A larger study in adolescents will be required to determine whether or not these speculations are accurate.

This study has limitations that warrant consideration. Participants who reported snoring were recruited from obesity referral clinics, limiting the generalizability to the broader adolescent population. Participants treated with either metformin and/or lifestyle recommendations were eligible, after discontinuing treatment for a short period of time. Previous treatment may have influenced findings in this study. The study was performed in two locations due to the principal investigator relocating, and PSGs were read and evaluated by two sleep investigators without cross-calibrations. However, the same OGTT and PSG protocol was utilized in both locations. To minimize participant burden and enhance enrollment and participation, we opted for OGTT evaluations in lieu of more invasive glucose clamp studies. Measures often performed in adult populations, including neck circumference and level of crowding of oropharynx, were not performed. Sleep data including the percentage of time spent in REM and slow wave sleep and the percent of sleep time below 90% oxygen saturation were not uniformly available. Also, children with a history of tonsillectomy, which is a treatment for OSA in children, were not excluded. The data were not normally distributed, which was expected for glucose measures given the objective of the study. However, considering the preliminary nature of this investigation, we included all of the available data from all participants. There was a predominance of females in the dysglycemia group, as dysglycemia is more commonly occurs in female versus male adolescents. As males are more likely to have sleep-disordered breathing, evidence of sleep-disordered breathing in obese adolescents with dysglycemia could be underestimated in this setting. It must be noted that a larger sample size is required to provide a definitive answer to the question of whether or not OSA is related to dysglycemia in adolescents. Given the nature of this pilot study, an adequate sample-size calculation was not performed at the onset. Perhaps with a larger study, including more participants with T2D, sleep-disordered breathing would be seen more predominantly.

Strengths of the study include a strong rationale, given the epidemiological prevalence of OSA in obese adults and the high percentage of adults with both T2D and OSA, and the lack of previous studies in obese adolescents. The inclusion of only obese pubertal patients aged 12 years and up is of particular value given the differences in glucose metabolism in pre-pubertal and adolescent patients (7). The mean age of diagnosis of T2D in youth is 14 years (36); thus, our cohort is representative of an appropriate population for evaluation of diabetes risk markers.

In conclusion, although we did not confirm the hypothesis that obese youth with dysglycemia have more characteristics of OSA than obese youth with NGT in this pilot study, we have further added to the literature on adolescent obesity, dysglycemia, and sleep characteristics. A larger study involving a network of clinical research sites is warranted to further understand the relationship between sleep-disordered breathing and dysglycemia in obese adolescents, especially since two pediatric studies have now shown a greater proportion of OSA in obese youth with dysglycemia versus NGT. Further study with a larger proportion of youth with prediabetes and T2D will also be necessary to determine whether evaluation for sleep-disordered breathing is uniformly warranted.

ETHICS STATEMENT

This study was approved by the University of Pittsburgh and Indiana University Institutional Review Boards and performed in the Pediatric Clinical and Translational Research Center (Children's Hospital of Pittsburgh) and the Indiana Clinical Research Center with collaboration from the clinical sleep centers at these institutions. All participants and their parent (guardian) gave informed consent before participating.

AUTHOR CONTRIBUTIONS

TH wrote the draft of the manuscript. SW collected data and performed research procedures. HJ interpreted data. SC interpreted data. KM provided key manuscript edits and mentoring. SA provided key manuscript edits and mentoring. No honorarium, grant, or other form of payment was given to anyone to produce the manuscript. There are no prior publications or current submissions to other journals with overlapping information.

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Liver Enlargement Predicts Obstructive Sleep Apnea–Hypopnea Syndrome in Morbidly Obese Women

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Obstructive sleep apnea–hypopnea syndrome (OSAHS) is frequently present in patients with severe obesity, but its prevalence especially in women is not well defined. OSAHS and non-alcoholic fatty liver disease are common conditions, frequently associated in patients with central obesity and metabolic syndrome and are both the result of the accumulation of ectopic fat mass. Identifying predictors of risk of OSAHS may be useful to select the subjects requiring instrumental sleep evaluation. In this cross-sectional study, we have investigated the potential role of hepatic left lobe volume (HLLV) in predicting the presence of OSAHS. OSAHS was quantified by the apnea/hypopnea index (AHI) and oxygen desaturation index in a cardiorespiratory inpatient sleep study of 97 obese women [age: 47 ± 11 years body mass index (BMI): 50 ± 8 kg/m^2]. OSAHS was diagnosed when AHI was ≥5. HLLV, subcutaneous and intra-abdominal fat were measured by ultrasound. After adjustment for age and BMI, both HLLV and neck circumference (NC) were independent predictors of AHI. OSAHS was found in 72% of patients; HLLV ≥ 370 cm^3 was a predictor of OSAHS with a sensitivity of 66%, a specificity of 70%, a positive and negative predictive values of 85 and 44%, respectively (AUC = 0.67, $p < 0.005$). A multivariate logistic model was used including age, BMI, NC, and HLLV (the only independent predictors of AHI in a multiple linear regression analyses), and a cut off value for the predicted probability of OSAHS equal to 0.7 provided the best diagnostic results (AUC = 0.79, $p < 0.005$) in terms of sensitivity (76%), specificity (89%), negative and positive predictive values (59 and 95%, respectively). All patients with severe OSAHS were identified by this prediction model. In conclusion, HLLV, an established index of visceral adiposity, represents an anthropometric parameter closely associated with OSAHS in severely obese women.

Keywords: non-alcoholic fatty liver disease, obstructive sleep apnea–hypopnea syndrome, hepatic left volume, metabolic syndrome, insulin resistance, morbid obesity

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Abbreviations: AHI, apnea/hypopnea index; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AT, abdominal thickness; ATP-III, adult treatment panel; AUC, area under the curve; BMI, body mass index; CT, computed tomography; γ-GT, γ-glutamyltransferase; HDL, high-density lipoprotein; HLLV, hepatic left lobe volume; HOMA, homeostatic model assessment—insulin resistance; IAF, intra-abdominal fat; IDF, International Diabetes Federation; IQR, interquartile range; LDL, low-density lipoprotein; MS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NC, neck circumference; NPV, negative predictive value; ODI, oxygen desaturation index; OGTT, oral glucose tolerance test; OSAHS, obstructive sleep apnea–hypopnea syndrome; PPV, positive predictive value; R, Pearson correlation coefficient; Rho, Spearman correlation coefficient; ROC, receiver operating characteristic curve; SCF, subcutaneous fat; SpO_2, nocturnal O_2 saturation; TG, triglycerides.

INTRODUCTION

Obstructive sleep apnea–hypopnea syndrome (OSAHS), an emerging public health issue, is characterized by recurrent episodes of upper airway occlusion during sleep, which results in reduction or cessation of the airflow, and lead to chronic intermittent hypoxia and sleep fragmentation (1). The pathogenetic factors of OSAHS are manifold and yet not completely understood. The main cause seems to be an anatomical upper airway narrowing, where the increased negative intrathoracic pressure during inspiration exceeds the counteracting forces of the dilating muscles (2–4). The cyclical recurrence of obstructions and arousals may cause instability of central respiratory motor output, thus contributing to the genesis of apneic episodes (5).

Obstructive sleep apnea–hypopnea syndrome affects a significant proportion of the adult population, mainly males, and its prevalence increases with increasing body mass index (BMI) and advancing age (1, 6, 7). Currently, in the United States, among adults, approximately 13% of men and 6% of women have moderate-to-severe OSAHS [apnea/hypopnea index (AHI) $\geq$ 15] while 14% of men and 5% of women have an AHI $\geq$ 5 together with symptoms of daytime sleepiness (1, 8). The mechanisms linking obesity to OSAHS include pharyngeal narrowing due to fatty tissue in the lateral airway walls, muscle functional impairment due to fatty deposits, enlargement of the abdomen resulting in reduced lung volumes, decreased longitudinal tracheal traction forces and increased tendency of pharyngeal collapse during inspiration, the low-grade systemic inflammation associated with obesity (3, 9).

On the basis of animal models (10), it has been postulated that leptin resistance developed by obese patients may impair the neuroanatomic interaction necessary for stable breathing, thereby contributing to the genesis of OSAHS (11). The disruption of normal sleep and chronic intermittent hypoxia starts a vicious circle by worsening obesity and may explain the close association between OSAHS and some of the features of the metabolic syndrome (MS), including hypertension, insulin resistance, and type 2 diabetes, ultimately leading to cardiovascular and cerebro-vascular illness (12–15). The name "syndrome Z" has been proposed for the association between MS and OSAHS (16) and the inclusion of this sleep and breathing disorder among the manifestations of MS has been also suggested (17).

The Cardiometabolic Think Tank convened on June 20, 2014 in Washington, DC, has tried to categorize subtypes and stages of MS in order to find an optimal care model for patients at increased cardiometabolic risk. The subtype with the excess visceral adipose tissue as main pathophysiologic mechanism is characterized by the presence of sleep disordered breathing and non-alcoholic fatty liver disease (NAFLD) (18).

Patients with MS frequently have an increased fat (triglyceride) accumulation in the liver, called NAFLD, and hepatic insulin resistance. With the increasing worldwide prevalence of obesity, NAFLD has become the most common cause of abnormal liver function in the general adult population (19–23). Studies conducted in humans and mice have suggested that OSAHS could be a detrimental factor potentially responsible for the exacerbation of liver injury in obesity (3).

The diagnosis of NAFLD is usually established by ultrasound and can be confirmed by liver biopsy (24).

We have shown that the ultrasound measurement of the hepatic left lobe volume (HLLV) correlated with the total volume of liver measured with MR and is an excellent indicator of visceral adiposity clustering with parameters defining MS (25, 26).

Little is known about the prevalence of OSAHS and the impact of this medical disorder on metabolic risk factors in morbidly obese patients; especially in women in whom it frequently remains underdiagnosed (27).

The aim of this cross-sectional study was to evaluate in a group of morbidly obese women with symptoms and signs associated with OSAHS the prevalence of OSAHS and the relationships between the severity of OSAHS and several anthropometric measurements with particular interest to HLLV.

MATERIALS AND METHODS

This is a retrospective study that included 97 morbidly obese women (BMI > 35 kg/m^2) referred to our Obesity Center for evaluation of obesity and its comorbidities and had, therefore, been submitted to cardio-respiratory sleep study for the clinical suspicion of OSAHS (excessive daytime sleepiness, choking or gasping during sleep, recurrent awakenings from sleep, impaired concentration). All patients signed a written consent for the treatment of their clinical data for purpose of research. The study did not require additional testing beside the protocol for evaluation of patients candidate to bariatric surgery. Exclusion criteria for this study were: history of hypothalamic–pituitary diseases, hypercortisolism, goiter, self-reported daily alcohol consumption > 20 g, current or past use of illicit drugs or hepatotoxic medications, viral hepatitis as assessed by conventional serum markers, a previous diagnosis of a disease potentially causing liver enlargement (e.g., storage diseases, autoimmune hepatitis), pregnancy or breast feeding within the 12-month period before enrollment. A careful endocrinological evaluation was also performed to reveal undiagnosed dysfunctions that required specific therapy and to evaluate the presence of hormonal abnormalities associated with OSAHS. 33 subjects were taking hypoglycemic agents, 7 hypolipidemic agents, and 4 hypouricemic agents.

Anthropometric, Clinical, and Laboratory Measures

Clinical, hematological, and instrumental examinations were performed according to the Italian guidelines for obesity (28). Anthropometric measures were determined in the morning after an overnight fast. Body weight was measured using a stand-on-scale in a hospital gown to the nearest 1/10th of a kilogram (SECA gmbh & co. —Germany) and height was measured to the nearest centimeter using a wall-mounted stadiometer (Health o meter. inc., Bridgeview, IL, USA). Neck circumference (NC) was determined at the level of the cricothyroid membrane. Blood pressure on admission was recorded with a large cuff while the patient was recumbent. Venous blood was obtained after an overnight fasting for measurement of glucose, triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and

total cholesterol, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (γ-GT), and alkaline phosphatase (ALP). The homeostasis model of insulin resistance (HOMA) was calculated based on fasting serum glucose and insulin concentrations (29). In 64 patients who were not taking hypoglycemic agents, an oral glucose tolerance test was performed in the morning with measurement of serum insulin and glucose when fasting, and every 30 min for 3 h after the ingestion of the glucose load (75 g).

HLLV and Abdominal Fat Measurements

Ultrasound examination for measurement of HLLV, subcutaneous fat (SCF) and intra-abdominal fat (IAF) was performed, as previously described (25, 26). Briefly, the ellipsoid formula (width × height × length × 0.52) was employed to calculate the HLLV. Thickness of the SCF was taken 1 cm over the transversal umbilical vein, by measuring the distance between the skin and the external face of the muscular fascia, while IAF thickness was defined as the distance between the internal face of the same muscle and the anterior wall of the aorta. Abdominal thickness (AT) was defined as the distance between the skin and the anterior wall of the aorta.

Sleep Apnea Assessment

An inpatient cardiorespiratory overnight sleep study was performed by means of a polygraph (Somno Check; Vivisol). To consider a study valid, a minimum sleep duration of 5 h was required. Parameters measured included oronasal flow by nasal cannula, thoraco-abdominal movements, pulsoxymetry, snoring, and body position. The results recorded by the instrument were scored by the attending physician expert in sleep studies who was maintained blinded to the characteristics of the patient. Nocturnal O_2 saturation (SpO_2) was recorded during the entire length of the night. Apnea was defined by the absence of airflow for > 10 s while hypopnea was defined as any airflow reduction of >50% that lasted for >10 s and resulted in oxyhemoglobin desaturation (3% dip rate). AHI was defined as the sum of the numbers of apnea and hypopneas per hour of sleep and OSAHS was diagnosed when AHI was ≥ 5. Oxygen desaturation index (ODI) was defined as the number of desaturations per hour of sleep and OSAHS was diagnosed when ODI was ≥5.

According to the American Sleep Disorders Association Task Force criteria (30), sleep-related obstructive breathing events were scored as mild when between 5 and 15 events/h of sleep, as moderate when between 15 and 30, and as severe when they were >30 events/h of sleep. Patients with a negative cardiorespiratory sleep study showing diurnal sleepiness underwent a complete polysomnography to avoid false-negative diagnosis in accordance with the American Sleep Disorders Association report (31).

Statistical Analysis

The sample size calculation was based on the number of patients needed to demonstrate an association between HLLV and AHI. A target sample size of 84 patients provided a power of 0.80 with an alpha of 0.05 to detect a modest correlation equal to 0.30. With 97 patients recruited in this study, the statistical power was equal to 0.85. Descriptive statistics were calculated for the cohort as a whole and then separately by OSAHS diagnosis. Statistical tests used to compare groups included Student's *t* test and ANOVA for difference in mean values, Mann–Whitney *U* and Kruskal–Wallis tests for skewed variables, Pearson Chi-square test for difference in counts and frequency. The Levene's test was used to assess the equality of variances between OSAHS groups. The Kolmogorov–Smirnov test was used to assess normality of data; logarithmic transformations were applied to skewed variables (AHI, insulin concentrations and HOMA index) to approximate a Gaussian distribution. Pearson (R) and Spearman (Rho) correlation coefficients were employed to quantify associations for Gaussian and skewed variables, respectively. Multiple linear regression analyses using the forward selection algorithm were carried out to identify the most significant anthropometric predictors of AHI, SpO_2, and ODI.

The receiver operating characteristic (ROC) curve was calculated to identify the cutoff value of both HLLV and NC, which better discriminated between subjects with and without OSAHS in terms of highest combined sensitivity and specificity (i.e., highest Youden index). The positive group included subjects with OSAHS (AHI ≥ 5) while the negative group those without OSAHS (AHI < 5). Sensitivity was defined as the percentage of subjects having OSAHS who were correctly classified as having this disease, while specificity was the percentage of subjects without OSAHS who were correctly classified as not having this disease.

A multivariate logistic analysis including all the determinants of OSAHS as determined through univariate analysis was carried out to calculate the predicted probability of OSAHS diagnosis (AHI ≥ 5) based on the logistic formula: $1/[1 + \exp(-\text{SCORE})]$, where SCORE was the linear combination of logistic model coefficients multiplied for the values of the respective determinant of OSAHS. A ROC curve analysis was then performed to determine the best cutoff value of the predicted probability of OSAHS diagnosis according to the highest Youden index, as above.

A p-value < 0.05 was considered statistically significant. Data are presented as a mean ± SD or median with inter-quartile range (IQR), as indicated. Statistical analyses were performed in SPPS (version 25; Armonk, NY, USA: IBM Corp.Armonk, NY, USA: IBM Corp.).

RESULTS

Average age of the study cohort (±SD) was 47 ± 11 years (range: 24–67) and BMI was 50 ± 8 kg/m^2 (range: 36–81). The physical, hematological, and clinical characteristics are reported in **Table 1**. OSAHS was found in 70 patients (median AHI = 14, IQR: 8–25). Of all subjects with OSAHS, 53% had a mild OSAHS, 26% moderate OSAHS, and 21% severe OSAHS. Average SpO_2 was lower in patients with OSAHS compared to those without ($p < 0.005$). Median ODI was higher in patients with OSAHS compared to those without ($p < 0.001$). On average, patients with OSAHS were 8-year older ($p < 0.005$) and had greater IAF and HLLV compared to patients without OSAHS (both $p < 0.05$) despite similar body weight, BMI, and SCF. In addition, higher levels of γ-GT were observed in patients with OSAHS ($p < 0.05$), and there was a positive association between AHI and γ-GT (Rho = 0.21; $p < 0.05$). No significant associations were observed between AHI and ALT ($p = 0.21$), AST ($p = 0.22$) or ALP ($p = 0.49$). Out of 70 patients with

TABLE 1 | Demographic and anthropometric (A), clinical (B) and laboratory characteristics (C) of the study population.

	All subjects (*N* = 97)	Without obstructive sleep apnea–hypopnea syndrome (OSAHS) (*N* = 27)	With OSAHS (*N* = 70)
A			
Age (years)	46.6 ± 10.7	40.8 ± 9.5	48.8 ± 10.3*
Body weight (kg)	126.9 ± 20.9	125.1 ± 16.8	127.6 ± 22.3
Body mass index (kg/m^2)	49.6 ± 7.5	48.5 ± 6.0	50.0 ± 8.1
NC (cm)	38.4 ± 3.2	37.5 ± 2.7	38.7 ± 3.3
Subcutaneous fat (mm)	41.6 ± 14.2	41.6 ± 12.9	41.6 ± 14.8
Intra-abdominal fat (mm)	101.1 ± 32.6	90.3 ± 20.0	105.7 ± 35.8*
Abdominal thickness (mm)	142.1 ± 34.6	130.0 ± 21.1	147.3 ± 37.9*
Hepatic left lobe volume (cm^3)	448.0 ± 242.7	344.6 ± 144.5	487.9 ± 261.3*
B			
Metabolic syndrome (%)			
ATP III criteria	62.9%	48.1%	68.6%
IDF criteria	64.9%	48.1%	71.4%*
Apnea/hypopnea index (AHI) (events/h)	18.5 ± 25.4	2.5 ± 1.6	24.6 ± 27.6*
Median [interquartile range (IQR)]	8.3 (4.7–18.4)	2.2 (1–4)	14 (8–25)*
AHI < 5 (no OSAHS)	27 (28%)	27 (100%)	0 (0%)
AHI ≥ 5 and <15 (mild)	37 (38%)	0 (0%)	37 (53%)
AHI 15–30 (moderate)	18 (19%)	0 (0%)	18 (26%)
AHI > 30 (severe)	15 (15%)	0 (0%)	15 (21%)
Mean nocturnal O_2 saturation	92.7 ± 4.5	94.6 ± 2.9	92.0 ± 4.7*
Total sleep time (h)	8 ± 0.7	8.2 ± 0.7	8 ± 0.6
Oxygen desaturation index (events/h)	20.9 ± 24.1	7.0 ± 8.9	26.5 ± 26*
Median (IQR)	13.0 (5.4–23.0)	3.8 (1.3–7.8)	16 (10.2–29.6)*
C			
Fasting glucose (mg/dL)[a]	108.3 ± 45.3	102.2 ± 30.8	111.2 ± 51.0
2-h glucose (mg/dL)[a]	150.4 ± 74.9	145.2 ± 78.1	153.0 ± 74.2
Fasting insulin (μU/mL)[a]	13.3 ± 14.0	11.9 ± 11.2	13.9 ± 15.2
Median (IQR)	9 (6–14.5)	7 (5–13)	10 (6–16)
2-h insulin (μU/mL)[a]	73.0 ± 57.5	64.5 ± 48.2	77.4 ± 62.0
Median (IQR)	63 (34–88)	62 (32–88)	69 (41–89)
Insulin peak (μU/mL)[a]	98.3 ± 67.2	87.5 ± 71.2	103.9 ± 65.2
Median (IQR)	83 (59–113)	72 (45–107)	91 (64–116)
HOMA index[a]	3.7 ± 4.7	3.5 ± 4.9	3.9 ± 4.6
Median (IQR)	2.2 (1.4–3.7)	1.6 (1.3–2.8)	2.3 (1.6–3.9)*
Total cholesterol (mg/dL)[b]	195.5 ± 34.3	192.8 ± 40.1	196.6 ± 31.9
High-density lipoprotein (mg/dL)[b]	50.7 ± 15.9	51.4 ± 14.3	50.4 ± 16.7
Low-density lipoprotein (mg/dL)[b]	123.3 ± 29.9	116.3 ± 31.1	126.3 ± 29.2
Triglycerides (mg/dL)[b]	150.9 ± 67.8	146.1 ± 61.6	152.8 ± 70.5
Uric acid (mg/dL)[c]	5.6 ± 1.2	5.4 ± 1.1	5.7 ± 1.3
Aspartate aminotransferase (U/L)	23.8 ± 12.9	22.1 ± 13.4	24.4 ± 12.7
Alanine aminotransferase (U/L)	29.8 ± 16.8	27.0 ± 14.1	30.9 ± 17.8*
γ-glutamyltransferase (U/L)	35.8 ± 39.6	27.4 ± 23.4	39.0 ± 44.1*
Alkaline phosphatase (U/L)	125.7 ± 73.4	116.0 ± 74.8	129.4 ± 73.1

Data are reported as mean ± SD, or frequency (%). For skewed variables, median values with IQR are also reported.

**p < 0.05 vs. without OSAHS.*

[a]64 patients not taking hypoglycemic agents.

[b]90 patients not taking hypolipidemic agents.

[c]93 patients not taking hypouricemic agents.

OSAHS, 48 (69%) and 50 (71%, $p < 0.05$) patients were classified as with the MS according to ATP-III and IDF criteria, respectively.

Anthropometric Determinants of AHI and SpO_2

Apnea/hypopnea index (Rho = 0.36, $p < 0.005$) and SpO_2 ($R = -0.30$, $p = 0.003$) correlated with HLLV in a positive and negative fashion, respectively (**Figures 1A,C**). Similar associations with AHI (Rho = 0.24, $p < 0.05$) and SpO_2 (Rho = −0.21, $p < 0.05$) were observed for NC (**Figures 1B,D**). ODI correlated with HLLV (Rho = 0.36, $p < 0.001$) and with NC (Rho = 0.27, $p = 0.009$) in a positive fashion (**Figures 1E,F**). Accordingly, both HLLV and NC increased with severity of OSAHS (trend $p < 0.05$, **Figure 2**). Further, IAF (AHI: Rho = 0.33, $p < 0.005$; SpO_2: Rho = −0.36, $p < 0.005$) and AT (AHI: Rho = 0.37, $p < 0.005$; SpO_2: Rho = −0.35, $p < 0.005$) were associated with AHI and SpO_2 while there was no correlation with SCF (AHI: Rho = 0.08, $p = 0.43$; SpO_2: Rho = −0.01, $p = 0.99$). In separate multivariate models each including age and BMI, HLLV ($p < 0.005$) and

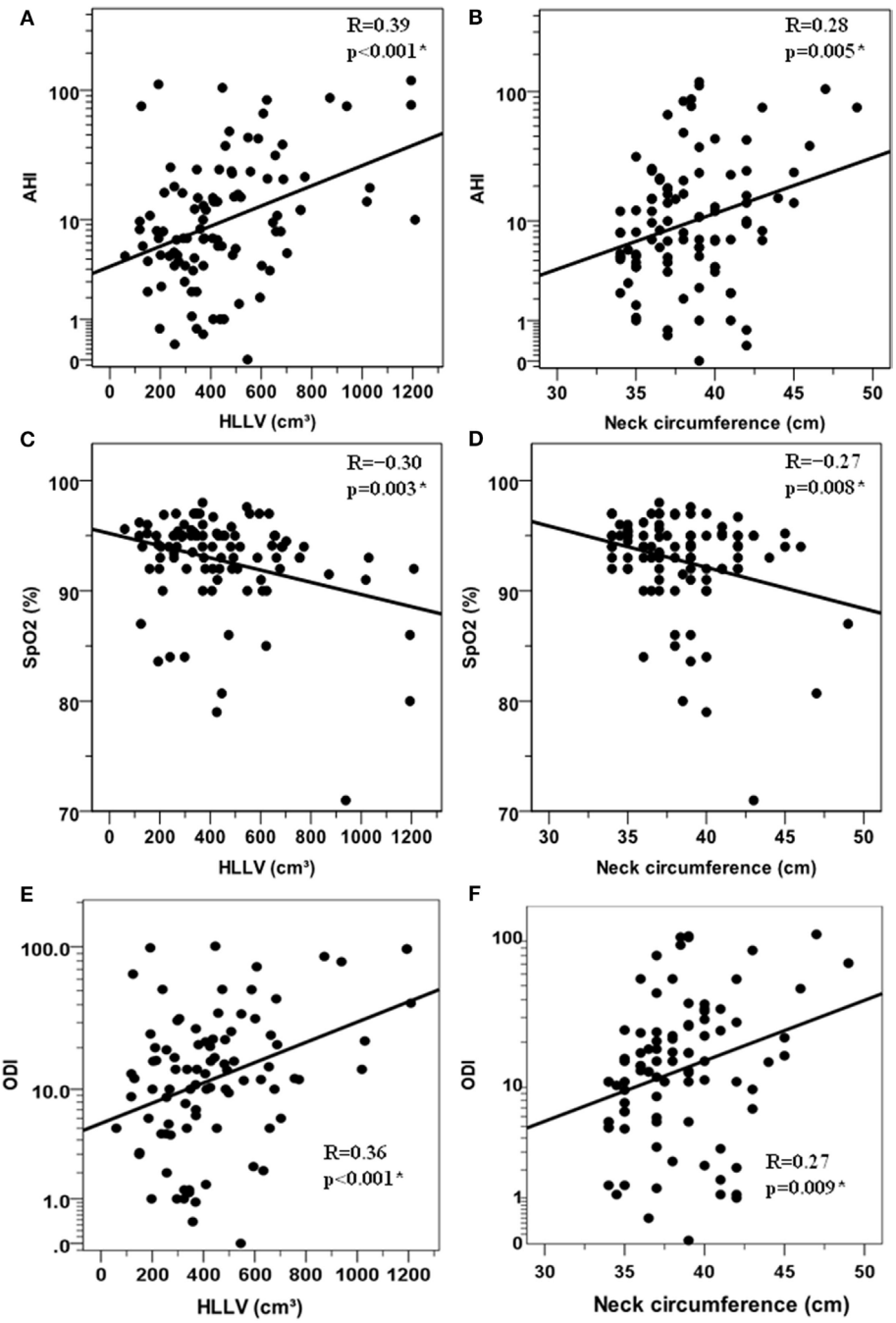

FIGURE 1 | Correlations between anthropometric variables and respiratory paramenters in the cohort of 97 obese women. Relationships between the hepatic left lobe volume (HLLV) and the apnea/hypopnea index (AHI) **(A)** the mean percent oxygen saturation [SpO_2, **(C)**] and the oxygen desaturation index (ODI) **(E)**. Relationship between neck circumference and AHI **(B)** mean percent oxygen saturation [SpO_2, **(D)**] and (ODI), **(F)**. AHI and ODI values are reported on a logarithmic scale, i.e., $LOG_{10}(1 + AHI)$ and $LOG_{10}(1 + ODI)$ which can handle zero values. R: Pearson's correlation coefficient (*$p < 0.05$).

NC ($p < 0.05$), but not IAF ($p = 0.47$), AT ($p = 0.48$) nor SCF ($p = 0.84$), were associated with AHI.

In a full model including age, BMI, HLLV and NC, both HLLV (partial $R^2 = 9\%$, $p = 0.001$) and NC (partial $R^2 = 7\%$, $p = 0.004$) were independent predictors of AHI, such that a 100-cm^3 increase in HLLV and 1-cm increase in NC were independently associated with mean increases in AHI of 15 and 9%, respectively (**Table 2**).

In a full model including age, BMI, HLLV, and NC, HLLV (partial $R^2 = 9\%$, $p = 0.005$) was an independent predictor of ODI, such that a 100-cm^3 increase in HLLV was independently associated with an average 13% increase in ODI (**Table 2**).

To further illustrate the independent effects of HLLV and NC on AHI, we categorized subjects in four subgroups according to the median values of HLLV and NC (**Figure 3**) and women with

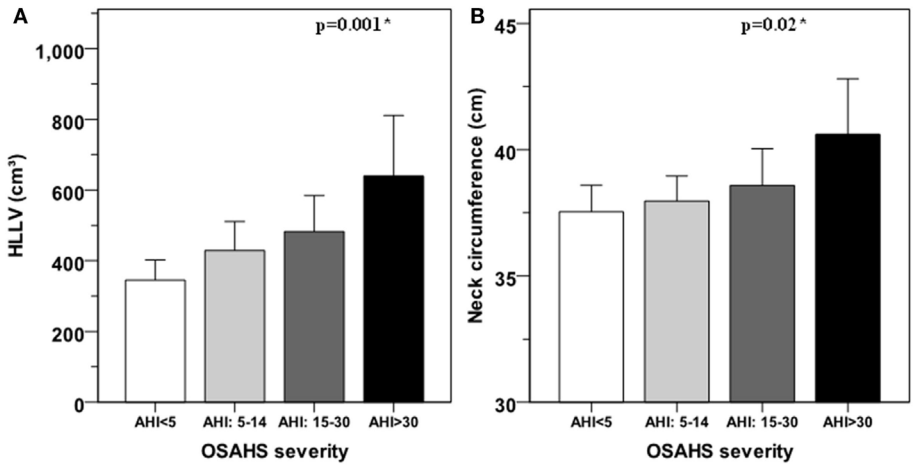

FIGURE 2 | Hepatic left lobe volume (HLLV) **(A)** and neck circumference **(B)** average values for each class of obstructive sleep apnea–hypopnea syndrome (OSAHS). OSAHS severity is defined as mild for apnea/hypopnea index (AHI) ≥ 5 and < 15; moderate for AHI ≥ 15 and ≤ 30; and severe for AHI > 30. Error bars represent the 95% confidence interval of the mean. The variances of HLLV ($p = 0.08$) and neck circumference ($p = 0.38$) were not significantly different among OSAHS categories by the Levene's test. *: $p < 0.05$ for linear trend by ANOVA.

TABLE 2 | Most significant anthropometric predictors of AHI, SpO_2, and ODI according to multiple linear regression analyses.

Predictors	AHI (log. values)	SpO_2 (%)	ODI (log. values)
Age (years)	0.010 (0.004) $p = 0.012$*	−0.123 (0.037) $p = 0.002$*	0.007 (0.004) $p = 0.073$
BMI (kg/m^2)	0.012 (0.006) $p = 0.043$*	−0.161 (0.056) $p = 0.005$*	0.015 (0.006) $p = 0.013$*
NC (cm)	0.039 (0.013) $p = 0.004$*	−0.321 (0.126) $p = 0.012$*	0.033 (0.014) $p = 0.019$*
HLLV (100 cm^3)	0.060 (0.018) $p = 0.001$*	−0.347 (0.17) $p = 0.044$*	0.052 (0.018) $p = 0.005$*
Intercept	−1.775 (0.561)	120.312 (5.405)	−1.473 (0.578)
Explained variance	$R^2 = 0.310$*	$R^2 = 0.297$*	$R^2 = 0.282$*

Beta coefficients in each cell are reported as mean values with SE and significance ($p < 0.05$).*
Beta coefficient of VLES is expressed by increase of 100 cm^3 of VLES.
Prior to regression analysis, AHI and ODI values were expressed in a logarithmic scale, e.g., $LOG_{10}(1 + AHI)/LOG_{10}(1 + ODI)$, to approximate a Gaussian distribution.
AHI, apnea/hypopnea index; BMI, body mass index; HLLV, hepatic left liver volume; NC, neck circumference; SpO_2, mean nocturnal O_2 saturation; ODI, oxygen desaturation index.

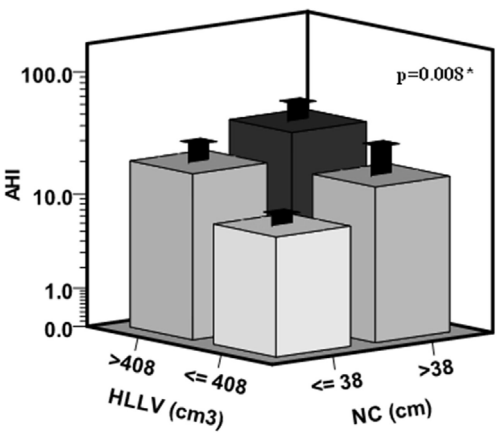

FIGURE 3 | Cumulative effects of hepatic left lobe volume (HLLV) and neck circumference (NC) on the apnea/hypopnea index (AHI). Subjects were categorized in four subgroups according to the median values of HLLV (=408 cm^3) and NC (=38 cm) in the whole cohort. The variances of AHI values were not significantly different among the four subgroups by the Levene's test ($p = 0.07$). *$p < 0.05$ by ANOVA.

a HLLV > 408 cm^3 and NC > 38 ($n = 14$, median AHI = 15) had higher AHI compared to those with an HLLV < 408 cm^3 and NC < 38 cm ($n = 25$, median AHI = 5, $p < 0.005$). Similarly, NC (partial $R^2 = 5\%$, $p < 0.05$) and HLLV (partial $R^2 = 3\%$, $p < 0.05$) were independent predictors of SpO_2 after adjustment for age and BMI (**Table 2**).

Predictors of OSAHS Diagnosis

In the whole cohort of 97 women, HLLV was a parameter significantly associated with OSAHS by ROC curve analysis (AUC = 0.67, 95% CI: 0.57–0.76, $p < 0.005$) (**Figure 4A**). A cutoff value of 370 cm^3 had a sensitivity of 66% (95% CI: 53–77%), a specificity of 70% (95% CI: 50–86%), and positive and negative predictive values (NPVs) of 85 and 44%, respectively, in identifying subjects with OSAHS. Conversely, NC did not discriminate between subjects with or without OSAHS (AUC = 0.59, $p = 0.19$) (**Figure 4B**). Considering the full logistic model, HLLV ($p < 0.05$, OR = 1.003, 95% CI: 1.001–1.006), but not NC, ($p = 0.09$) was the only independent predictor of OSAHS diagnosis after adjustment for age ($p < 0.005$) and BMI ($p = 0.86$) in the multivariate analysis. According to the results of the logistic analysis, a predicted probability of OSAHS (AHI ≥ 5) was calculated on the basis of age, BMI, NC, and HLLV applying the following formula: $1/[1 + \exp(-\text{SCORE})]$, where SCORE = −9.806 + age (years) × 0.075 + BMI (kg/m^2) × 0.007 + NC (cm) × 0.155 + HLLV

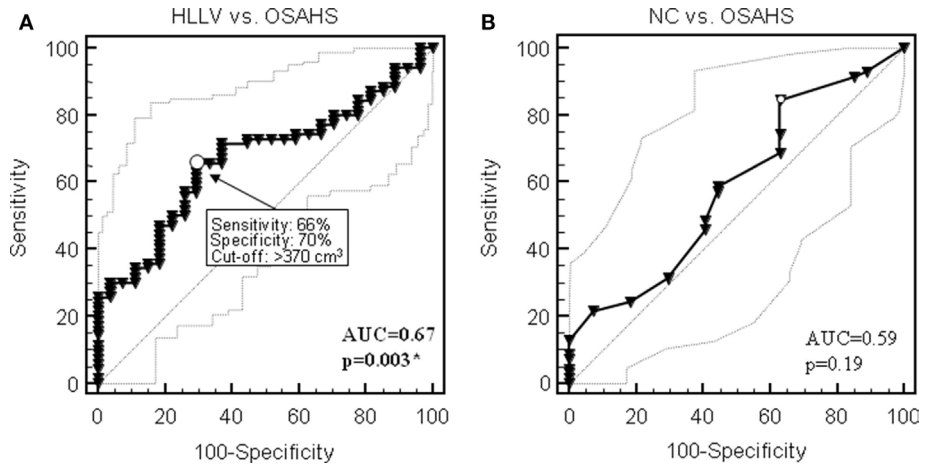

FIGURE 4 | Receiver operating characteristic (ROC) curves for the diagnostic performance of hepatic left lobe volume (HLLV) **(A)** and neck circumference (NC) **(B)** to identify obstructive sleep apnea syndrome (OSAHS) in the cohort of 97 obese patients. Thin lines show 95% confidence intervals; arrows point at the optimal cutoff as defined by the Younden index for diagnostic sensitivity and specificity. AUC, area under the curve. *$p < 0.05$ by ROC curve analysis.

(cm^3) × 0.003. The predicted probability of OSAHS achieved the highest performance in the classification of OSAHS (AUC = 0.79, 95% CI: 0.70–0.87, $p < 0.005$) and a cut-off value equal to 0.70 yielded to the best diagnostic results in terms of combined sensitivity (76%), specificity (89%), NPV (59%), positive predictive value (95%), and accuracy (79%). Among subjects with a predicted probability of OSAHS ≥ 0.70, only 3 (5%) did not actually present OSAHS (false positive patients) while among the subjects with a predicted probability of OSAHS < 70%, 17 (41%) presented OSAHS (false negative patients). Results for each class of OSAHS severity are reported in **Table 3**. Of note, all patients with severe OSAHS were identified by this prediction model.

TABLE 3 | Comparison between the predicted probability of obstructive sleep apnea–hypopnea syndrome (OSAHS) (calculated by multivariate logistic model which includes age, BMI, HLLV, NC) and the actual prevalence of OSAHS.

Predicted probability of OSAHS	No OSAHS [apnea/hypopnea index (AHI) < 5]	Mild OSAHS (AHI ≥ 5 and <15)	Moderate OSAHS (AHI: 15–30)	Severe OSAHS (AHI > 30)
<0.70	*N* = 24 (89%)	*N* = 13 (35%)	*N* = 4 (22%)	*N* = 0 (0%)
≥0.70	*N* = 3 (11%)	*N* = 24 (65%)	*N* = 14 (78%)	*N* = 15 (100%)

DISCUSSION

Obstructive sleep apnea–hypopnea syndrome and NAFLD are very common in patients with central obesity and MS (18). Regional fat distribution is different in each sex, with fat tending to accumulate primarily around the waist in men and around the hips in women; the predominance of central obesity in men partly explains the difference in the prevalence of OSAHS between the sexes. In the Wisconsin Sleep Study Cohort, women had a higher BMI than men at each level of respiratory disturbance index and less severe apnea at an equivalent degree of obesity (8, 32). Male patients with OSAHS have a greater amount of visceral adipose tissue by computed tomography as compared to BMI-matched men without sleep-disordered breathing, and visceral but not subcutaneous fat appears significantly correlated with indices of sleep apnea (33).

Age is another risk factor for OSAHS especially in women (8), probably due to the protective effect of female sex hormones before menopause. Bixler et al. in a epidemiologic study of OSAHS in women found that the prevalence was higher in postmenopausal women that in premenopausal women (3.9 vs 0.6%) (34).

Female subjects are referred less frequently to sleep clinics probably also because of gender-related symptom differences; women with OSAHS may refer atypical symptoms such as fatigue, headaches, mood disorders (32, 35).

In studies conducted in numerically limited populations with predominance of male subjects affected by overweight or mild and moderate obesity, higher values of BMI, waist circumference (or waist–hip ratio), neck circumference were almost always associated with the presence and severity of OSAHS (9, 36–39). However, anthropometric parameters appear inadequate in predicting the risk of OSAHS and the systematic use of polysomnography is recommended (40, 41). Waist circumference may not be reliable in severe obesity due to its imprecision and inability to palpate the iliac crest besides the confounding effect of cutaneous plications; in addition, it cannot distinguish between subcutaneous and intra-abdominal fat (IAF). In severe obesity, it was also found a weak association between AHI and excessive daytime sleepiness or other symptoms and signs of OSAHS especially in females. Obesity itself is a cause of poor subjective assessment of sleep quality and sleepiness (15, 42, 43).

Recognizing the risk factors and predictors of OSAHS may be of particular relevance in women. Indeed, women are more frequently addressed to bariatric surgery than men and patients with OSAHS are particularly vulnerable during anesthesia and sedation, and display an increased risk of developing respiratory and cardiopulmonary postoperative complications (44). Identifying anthropometric, clinical, and laboratory predictors of risk of OSAHS would be useful in clinical practice in order to

avoid expensive investigations such as polysomnography and to select patients that should be submitted to specific diagnostic test.

Few studies have analyzed the prevalence of OSAHS in morbidly obese women; the most important risk factors identified were BMI, age, and menopausal status (42, 43, 45).

In our sample, OSAHS was found to be present in 72% of obese women with a BMI greater than 35. Approximately half of the patients had mild OSAHS, one quarter had moderate OSAHS and one quarter of patients manifested a severe form of obstructive sleep apnea. Patients with OSAHS were older and had a greater visceral fat estimated by ultrasound compared to patients without OSAHS, despite similar body weight, BMI, NC, and SCF. γ-GT, but not transaminase, levels positively correlated with OSAHS quantified by AHI index, suggesting that OSAHS could be an independent risk factor for NAFLD. Lack of association between AHI index and serum transaminases may depend on the limited statistical power of our study sample. However, liver enzymes alone are not sensitive enough to characterize NAFLD. In this regard, it should be noted that non-invasive liver biomarkers-based scores have been developed to evaluate the extent of liver injury, which are independently associated with the severity of nocturnal hypoxia (46, 47).

As expected, neck circumference (NC) and IAF (measured by ultrasound) correlated with AHI and ODI and with the severity of OSAHS. We also observed a positive relationship between HLLV and AHI. ODI correlated with HLLV in a positive fashion as well. Interestingly, HLLV showed a stricter correlation with parameters describing OSAHS than NC.

According to the ROC curve analysis, only HLLV but not NC did discriminate between subjects with or without OSAHS. Similarly, according to the logistic regression, when dichotomizing a continuous variable (AHI) in two groups, the statistic contribution of NC was lost.

These results confirm the role of liver enlargement as a marker of visceral adiposity, which may facilitate the identification of individuals who are at increased risk of OSAHS in addition to the other known risk factors of OSAHS such as age, BMI, and NC. Indeed, prediction logistic model, including age, BMI, NC, and HLLV, displayed a discriminative ability (evidenced by AUC value of 0.79). However, the NPV was too low (59%) to propose this model in a clinical setting. While it is useful to reduce the number of false positives to avoid unnecessary instrumental sleep studies, a relevant number of false negative results may lead to a failure to treat OSAHS with potential adverse consequences. Nevertheless, among the false negative, there were no patients with severe OSAHS (AHI > 30) indicating that the model can identify all patients affected by OSAHS requiring urgent and active treatment.

At present, we cannot establish whether OSHAS and NAFLD can influence each other in terms of occurrence and progression or if they are merely unrelated expressions of ectopic fat deposition. Future studies including HLLV and other non-invasive scores (46, 47) may be helpful in this regard.

In conclusion, the results of this study indicate that in severe obese women HLLV is a powerful and better anthropometric predictor of OSAHS than neck circumference, and we have identified a prediction logistic model based only on four parameters (age, BMI, NC, and HHLV), which is capable of predicting all severe forms of OSAHS requiring active treatment.

ETHICS STATEMENT

Our study is a pure retrospective analysis of data collected from a cohort of patients who gave written consent to the treatment of their clinical data for purpose of research. No additional testing was performed beside those required for the evaluation of patients' candidate to bariatric surgery. There are no ethical issues that may require approval by our EC.

AUTHOR CONTRIBUTIONS

GS and GQ conceived the study design, carried out data collection, and wrote the manuscript. LM performed cardiorespiratory overnight sleep study. PP performed the statistical analysis and drew the figures. SM performed ultrasound examination for measurement of hepatic left volume, subcutaneous and intra-abdominal fat. GC, PF, GS, JV, SB, and GC were involved in data interpretation and writing of the paper. AP and FS conceived the study design, contributed to data interpretation, and writing of the manuscript. All authors approved the final version.

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Chronic Insufficient Sleep Has a Limited Impact on Circadian Rhythmicity of Subjective Hunger and Awakening Fasted Metabolic Hormones

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Weight gain and obesity have reached epidemic proportions in modern society. Insufficient sleep—which is also prevalent in modern society—and eating at inappropriate circadian times have been identified as risk factors for weight gain, yet the impact of chronic insufficient sleep on the circadian timing of subjective hunger and physiologic metabolic outcomes are not well understood. We investigated how chronic insufficient sleep impacts the circadian timing of subjective hunger and fasting metabolic hormones in a 32-day in-laboratory randomized single-blind control study, with healthy younger participants (range, 20–34 years) randomized to either Control (1:2 sleep:wake ratio, 6.67 h sleep:13.33 h wake, $n = 7$, equivalent to 8 h of sleep per 24 h) or chronic sleep restriction (CSR, 1:3.3 sleep:wake ratio, 4.67 h sleep:15.33 h wake, $n = 8$, equivalent to 5.6 h of sleep per 24 h) conditions. Participants lived on a "20 h day" designed to distribute all behaviors and food intake equally across all phases of the circadian cycle over every six consecutive 20 h protocol days. During each 20 h day, participants were provided a nutritionist-designed, isocaloric diet consisting of 45–50% carbohydrate, 30–35% fat, and 15–20% protein adjusted for sex, weight, and age. Subjective non-numeric ratings of hunger were recorded before and after meals and fasting blood samples were taken within 5 min of awakening. Subjective levels of hunger and fasting concentrations of leptin, ghrelin, insulin, glucose, adiponectin, and cortisol all demonstrated circadian patterns; there were no differences, however, between CSR and Control conditions in subjective hunger ratings or any fasting hormone concentrations. These findings suggest that chronic insufficient sleep may have a limited role in altering the robust circadian profile of subjective hunger and fasted metabolic hormones.

Clinical Trial Registration: The study was registered as clinical trial #NCT01581125.

Keywords: sleep restriction, appetite, sleep loss, endocrinology, forced desynchrony, circadian rhythms, fasting hormones, hunger

INTRODUCTION

Over 30% of Americans are obese (1) and 1.4 billion adults worldwide are overweight (2, 3). High body weight is associated with an increased risk for heart disease, stroke, osteoarthritis, diabetes, and cancer (4) and accounts for ~147 billion dollars in healthcare costs in the United States per year (5). Thus, identifying risk factors, behaviors, and mechanisms that promote weight gain is vital to help combat disease and associated decreases in quality of life for patients and their families.

Sleep is a vital component for the optimal functioning of many physiological processes such as metabolism (6), immune function (7), and cognition (8). A recent health survey reported that ~30% of Americans typically sleep less than 6 h per night (9), an amount far below what is needed for optimal health. Epidemiological evidence suggests that shorter sleeping durations are associated with higher body weight (10–12) and poorer glucose tolerance (13). Controlled in-laboratory studies have found that when sleep is acutely restricted and food is provided *ad libitum*, participants consume more nighttime calories than when they are attaining non-restricted sleep (14–17). It is not mechanistically clear, however, why sleep restriction increases caloric intake. Several studies have found that acute sleep restriction can lead to changes in appetitive hormones that would stimulate hunger: specifically, a decrease in the satiety hormone leptin (10, 18–20) and an increase in the appetitive hormone ghrelin (10, 19, 21, 22). If food is provided *ad libitum*, however, participants during acute sleep restriction eat far more calories than needed to meet energy balance, despite increased leptin and decreased ghrelin concentrations (15, 16, 23), thus bringing the hormonal mechanism for increased food intake into question (24). It is not well understood how the levels of these hormones will react to chronic exposure to sleep restriction, which is a common cause of sleep loss and may be more important for metabolic health, as appreciable weight gain occurs chronically (25).

Circadian timing also plays a role in weight gain, subjective hunger, and diabetes risk. Individuals who work during the night, thereby shifting the timing of the majority of their caloric consumption to the nighttime hours when the internal biological clock is promoting sleep and fasting, have increased risk for obesity (26–28) and diabetes (29). Individuals who do not work during the night but eat closer to and during their biological nights (i.e., when the hormone melatonin is high) have higher percentages of body fat (30), potentially due to decreased metabolic rate during that time (31–33). The increased food consumption during the evening is driven by the circadian clock: in controlled in-laboratory settings, hunger ratings on a visual analog scale show robust circadian rhythmicity, peaking at a circadian phase equivalent to ~2000 hours and a trough at ~0750 hours (34). The appetitive hormones leptin and ghrelin have also been found to follow diurnal rhythms (35–38), with large influence from caloric intake (39) and sleep (36, 37, 40). Furthermore, under tightly controlled constant routine conditions when snacks are provided evenly every hour and constant wakefulness and posture are maintained (41), insulin concentrations follow a circadian rhythm, with a peak in the early biological morning (42, 43).

In a protocol combining sleep restriction with circadian disruption, Buxton and colleagues found that during sleep restriction and circadian disruption within a forced desynchrony (FD) protocol—a protocol designed to distribute all behaviors evenly across all phases of the circadian cycle—there were significant circadian rhythms in fasted glucose, insulin, and cortisol, with fasted insulin displaying lower levels after weeks of sleep restriction and no significant difference in fasted glucose, cortisol, or leptin concentrations (31). However, in that study, there was no control group that did not receive sleep restriction independent of circadian disruption, and thus it is difficult to conclude whether the changes in these hormones were actually due to the sleep restriction or the combination of sleep restriction and circadian disruption.

Whether there are hormonal changes during chronic sleep restriction (CSR) that may promote increased hunger and diabetes risk, and how the circadian timing of these hormones interact with CSR, remains unknown. Thus, the aim of the current study was to determine the impact of CSR and circadian timing on subjective hunger and fasted concentrations of hormones that may influence subjective hunger as compared to a Control condition. Furthermore, we wanted to examine the influence of CSR on fasted glucose and insulin concentrations along with the hormones adiponectin and cortisol, which influence glucose tolerance.

MATERIALS AND METHODS

Participants

Seventeen healthy participants completed a 32-day inpatient protocol, but due to blood sample collection issues in 2 participants (one in the Control and one in the CSR condition), data from 15 participants (7 males) are presented in the current study [BMI, 23.6 ± 3.7 kg/m^2, 18.2–28.4 kg/m^2; weight, 64.9 ± 12.0 kg, 47.2–88.9 kg; age 26.7 ± 4.4 years, 20.0–34.0 years; (mean ± SD, range)]. Participants were deemed medically and psychologically healthy based on self-reported health, psychological screening questionnaires, physical examination by a physician, laboratory testing of hematological or metabolic measures, and psychological evaluation from a clinical interview with a psychologist. Participants were also free of any sleep disorders, as determined by questionnaires and an overnight clinical sleep screening. Exclusion criteria consisted of a self-reported habitual sleep duration <7 or >9 h averaged across the entire week, history of night-shift work or transmeridian travel <3 months prior to study, BMI <18 or >29.9, age <18 or >35 years, pregnancy, and use of any prescription medication. For at least 3 weeks prior to the inpatient protocol, participants maintained an approximate 10-h per night sleep schedule at their self-reported habitual timing that was verified by sleep logs, wrist actigraphy (Actiwatch-L Mini Mitter/Respironics), and call-ins to a time-stamped voicemail recording system immediately upon awakening and prior to going to bed. This ~3-week sleep-wake schedule was implemented to ensure participants were not sleep restricted prior to admission to the Brigham and Women's Hospital Center for Clinical Investigation research facility. In addition, participants abstained from any over-the-counter medication or drug use, alcohol, caffeine, nicotine, or other foreign substances during the ~3 weeks of at-home

monitoring and for the duration of the protocol; this was verified *via* urine toxicology upon admission to laboratory. All participants provided written informed consent and all study procedures were approved by the Partners Healthcare Institutional Review Board. The study was registered as on clinicaltrials.gov as NCT01581125.

Inpatient Protocol

Participants were admitted to a sound-attenuated, temperature-controlled suite that was free from time cues. All events were scheduled related to the participant's habitual sleep-wake timing as determined from the ~3 weeks of at-home monitoring. Lighting was maintained at dim (<4 lux) levels during scheduled wakefulness and 0 lux during scheduled sleep. The first 3 days of the protocol were "sleep-satiation" days consisting of 12-h overnight sleep opportunities and 4-h daytime sleep opportunities to minimize any potential residual sleep loss upon entering the study. The next two "baseline" days consisted of 10-h overnight sleep opportunities at habitual timing. Participants were then scheduled to 24 cycles of a 20-h FD protocol and randomized to one of two sleep:wake FD conditions: Control (1:2, 6.67 h sleep opportunity, 13.33 h wake, equivalent to 8 h of sleep per 24 h day; seven participants) or CSR (1:3.3, 4.67 h sleep, 15.33 h wake, equivalent to 5.6 h of sleep per 24 h day; eight participants) (**Figure 1**). Use of the 20-h FD design allowed for the endogenous circadian pacemaker to run at each participant's endogenous circadian period and uncouple the timing of each fasted blood draw from circadian phase. The participants were blinded to which condition they were randomized and the specifics of the FD protocol (e.g., time of day, day length, and date). Following the 24 cycles of FD, participants were provided five recovery days of 10 h sleep opportunities at the same circadian phase as the baseline sleep opportunities. During scheduled wakefulness, participants were allowed to engage in sedentary activities (e.g., read, talk or play board games with a researcher, and watch movies) and wakefulness was verified by continuous monitoring by research staff and continuous polysomnographic recordings.

During each 20 h day, participants were provided a nutritionist-designed, isocaloric diet consisting of 45–50% carbohydrate, 30–35% fat, and 15–20% protein adjusted for sex, weight, and age using the Harris–Benedict equation with an activity factor of 1.3 (44). During each wake episode, participants were provided a breakfast, lunch, and dinner and were instructed to consume all food provided. Subjective hunger was assessed using a visual analog scale that was given to each participant prior to and after each meal. The visual analog scale prompted the participant to identify on a 100 mm horizontal line how they felt at that moment, with each end of the line labeled with the extremes of the subjective continuum of "not at all hungry" to "extremely hungry." Participants were not given any feedback on the "value" that they identified as using this scale.

Blood for fasted hormone concentrations was drawn *via* an indwelling venous catheter approximately 5 min after scheduled awakening (**Figure 1**). Prior to the blood draw, participants remained seated in bed, but were elevated to a semi-recumbent

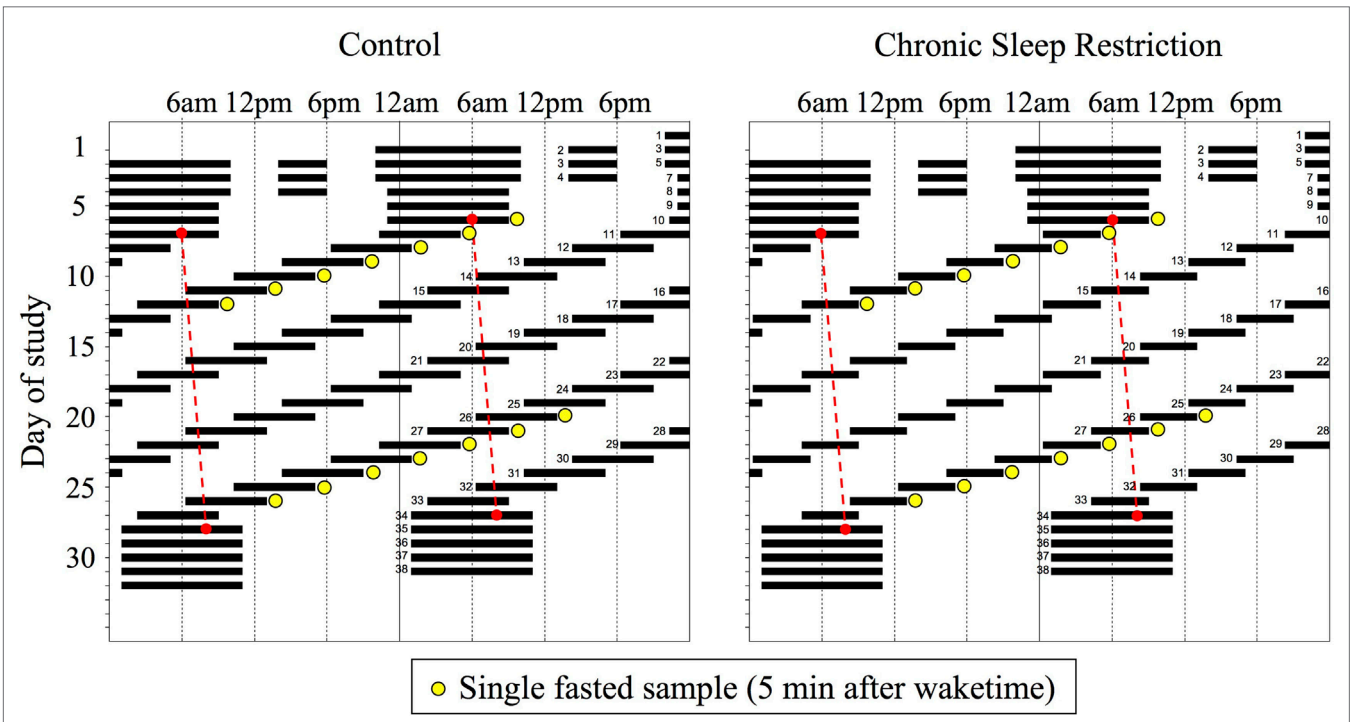

FIGURE 1 | Raster plot of the Control (n = 7, 1:2 sleep:wake ratio, equivalent to 8 h sleep opportunity per 24 h day) and chronic sleep restriction (n = 8, 1:3.3 sleep:wake ratio, equivalent to 5.6 per 24 h day) forced desynchrony protocols. Clock hour is plotted on the horizontal axis and day of study on the vertical axis. Solid black bars represent sleep opportunities, yellow circles represent fasted blood draws, and the red dashed line represents the daily marker of a participant's circadian period. Days are double plotted such that each consecutive study day is plotted next to and below the previous day.

posture for cognitive computer testing. For the current analysis, data are from the first (study days 10–16) and last (study days 27–33) weeks of the FD protocol. Blood for melatonin concentrations was drawn hourly to enable assessment of circadian phase.

Assay Information

All assays were performed by the Brigham and Women's Hospital Research Assay Core; the Core was blinded to study condition. Leptin and active ghrelin were assayed using serum radioimmunoassay techniques (Millipore Research, St. Charles, MS, USA). The leptin assay had a sensitivity of 0.1 ng/mL, within assay coefficient of variation (CV) 5.2–5.7%, and between assay CV of 3.2–8.9%; ghrelin had a sensitivity of 7.8 pg/mL, within CV of 6.5–9.5%, and between CV of 9.6–16.2%. Insulin and cortisol were assayed using Access Chemiluminescent Immunoassay (Beckman Coulter, Fullerton, CA, USA) techniques with the insulin assay having a sensitivity of 0.03 μIU/mL, within assay CV of 2.0–4.2%, and between CV of 3.1–5.6%. The cortisol assay had a sensitivity of 0.4 μg/dL, a within CV of 4.4–6.7%, and a between CV of 6.4–7.9%. Finally, adiponectin was assayed using enzyme-linked immunosorbent assay techniques (ALPCO Diagnostics, Salem, NH, USA) with a sensitivity of 0.10 μg/mL, within assay CV of 5.0–5.4%, and between assay CV of ~6%.

Statistical Analysis

To determine circadian phase for each individual, non-orthogonal spectral analysis of hourly serum melatonin was used to estimate intrinsic circadian period and subsequent circadian phase (45), with melatonin maximum set to 0 circadian degrees, which is ~0300 hours for individuals in entrained conditions. Subjective hunger and fasted hormone concentrations were analyzed as both raw values and normalized within each participant (*z*-scored) and binned into 60° (~4 h) circadian bins. One participant in the CSR condition had raw insulin concentrations much higher than other participants and was identified as an extreme (interquartile range × 3) outlier. Therefore, the data from this participant were omitted from the analysis of raw insulin concentrations. Subjective hunger and hormone concentrations were analyzed using mixed-effects models with circadian phases and condition as fixed factors and participant as a random factor to account for inter-participant differences. All statistical analyses were performed using SAS 9.4.

RESULTS

Impact of Sleep Restriction and Circadian Timing on Subjective Hunger and Appetitive Hormones

Contrary to expectations, there were no significant condition or condition-by-circadian phase interaction effects between CSR and Control conditions for subjective hunger, leptin, or ghrelin (**Figure 2**, all $p > 0.16$). There was, however, a robust circadian rhythm in subjective hunger (**Figures 2A,B**, both $p < 0.001$), such that subjective hunger levels peaked at 240° and reached a nadir at 60° for both Control and CSR conditions. Leptin, a satiety hormone, exhibited significant circadian rhythmicity (**Figures 2C,D**, both $p < 0.05$), with a peak at 0° and a nadir at 120° for both the Control and CSR conditions. Finally, ghrelin, an appetitive hormone, also displayed a significant circadian rhythm (**Figures 2E,F**, both $p < 0.01$) with a peak at 300° and nadir at 120° for both Control and CSR conditions. All findings were similar for raw and *z*-scored variables.

Impact of Sleep Restriction and Circadian Timing on Insulin, Glucose, Adiponectin, and Cortisol

Somewhat surprisingly, fasted insulin and glucose levels did not have significant condition or condition-by-circadian phase interaction effects between CSR and Control conditions (**Figure 3**, all $p > 0.12$). Fasted insulin concentrations exhibited a significant circadian rhythm (**Figures 3A,B**, both $p < 0.05$) with a peak in concentrations between 0° and 60° and a nadir between 180° and 240° for both the Control and CSR conditions, while fasted glucose concentrations also displayed significant circadian variation (**Figures 3C,D**, both $p < 0.001$) with a peak at 0° for the Control condition and 120° for the CSR condition and a nadir between 240° and 300° for both the Control and CSR conditions. All findings were similar for raw and *z*-scored variables.

Adiponectin concentrations did not have significant condition or condition-by-circadian phase interaction effects between CSR and Control conditions (**Figures 4A,B**, both $p > 0.37$). Cortisol concentrations also did not display significant condition effects (**Figures 4C,D**, both $p > 0.18$), but did display a non-significant trend for condition-by-circadian phase interaction effects for higher raw cortisol concentrations in the CSR condition (**Figure 4C**, $p = 0.05$). Fasted adiponectin concentrations did display a significant circadian rhythm (**Figures 4A,B**, both $p < 0.05$) with a peak at 120° and 240°, for the Control and CSR conditions, respectively and a nadir at 0°–60° for both conditions. Finally, fasted cortisol also exhibited an expected robust circadian rhythm (**Figures 4C,D**, both $p < 0.001$) with a peak in the early morning hours at 60° and a nadir during the evening at 300° for both conditions. All findings were similar for raw and *z*-scored variables.

DISCUSSION

Sleep restriction, experienced by millions of individuals on a daily basis, has been associated with weight gain and poor glucose tolerance. In-laboratory studies of *acute* sleep restriction have shown that weight gain occurs due to consumption of excess nighttime calories (14–17); however, the physiological drive for the excess of consumption of calories, particularly in response to chronic insufficient sleep, is unclear. In the current study, we show that there is limited evidence for a role of CSR in levels of subjective hunger, fasted appetitive hormones, and measures of fasted glucose metabolism, however, there is strong circadian phase modulation influencing these outcomes. These findings provide valuable insight into the potentially limited role of CSR, the robust impact of circadian timing, and the potential for circadian timing of therapy (chronotherapy), in influencing fasted physiology.

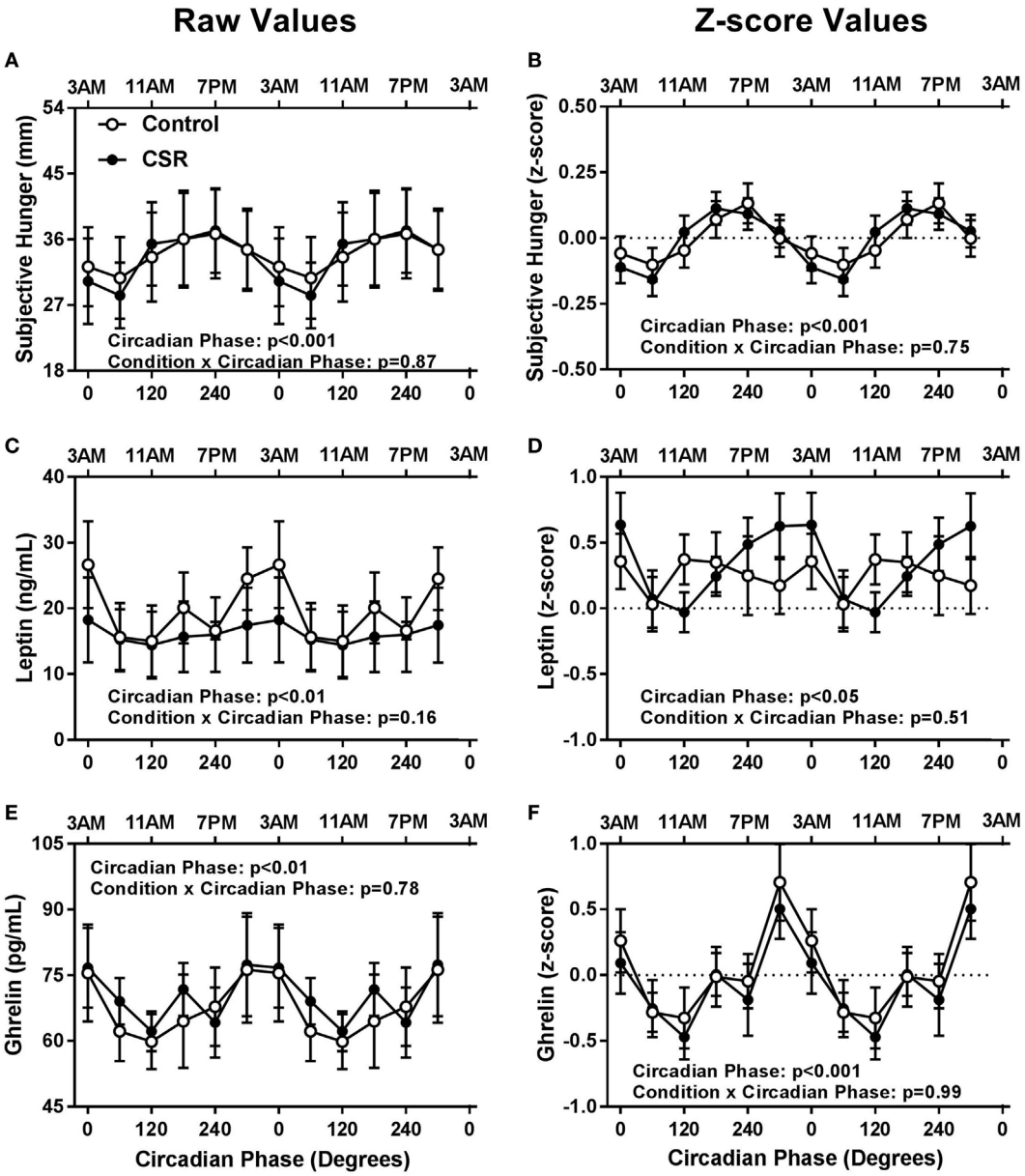

FIGURE 2 | Influence of circadian phase and sleep restriction on **(A,B)** subjective hunger, **(C,D)** leptin, and **(E,F)** ghrelin. The Control (n = 7, 1:2 sleep:wake ratio, equivalent to 8 h sleep opportunity per 24 h day) condition is denoted by open circles and the chronic sleep restriction (CSR, n = 8, 1:3.3 sleep:wake ratio, equivalent to 5.6 h sleep opportunity per 24 h day) condition by closed circles. Data are represented as raw scores (left) and z-scores (right) and double plotted across circadian phase and relative clock hour for an individual with a habitual sleep time of 2300–0700 hours. Error bars represent SEM.

One of the aims of the current study was to determine whether CSR had any impact on subjective hunger ratings, as previous findings have shown sleep restricted individuals eat more calories than non-sleep restricted individuals (14–17). While we did not find a difference between groups in subjective hunger, we did find a robust circadian rhythm in hunger levels, which agrees with another study using validated circadian protocols (34). In that study, Scheer and colleagues found that levels of subjective hunger, independent of the wakefulness/sleep cycle, and thus the feeding/fasting cycle, follow a circadian rhythm such that individuals are most hungry in the evening hours and least hungry in the early morning hours (34). Carnell and colleagues have also shown a diurnal rhythm to hunger levels in obese individuals, with subjective hunger being higher in the evening as compared to the morning hours, coinciding with lower levels of peptide YY (satiety hormone) and higher levels of ghrelin (46). Our subjective hunger results display a similar peak (~1900 hours) and trough (~0700 hours), but we now add the additional findings that CSR does not impact this rhythm. The lack of a significant difference in the hunger rhythm during CSR is of note as it may add insight into

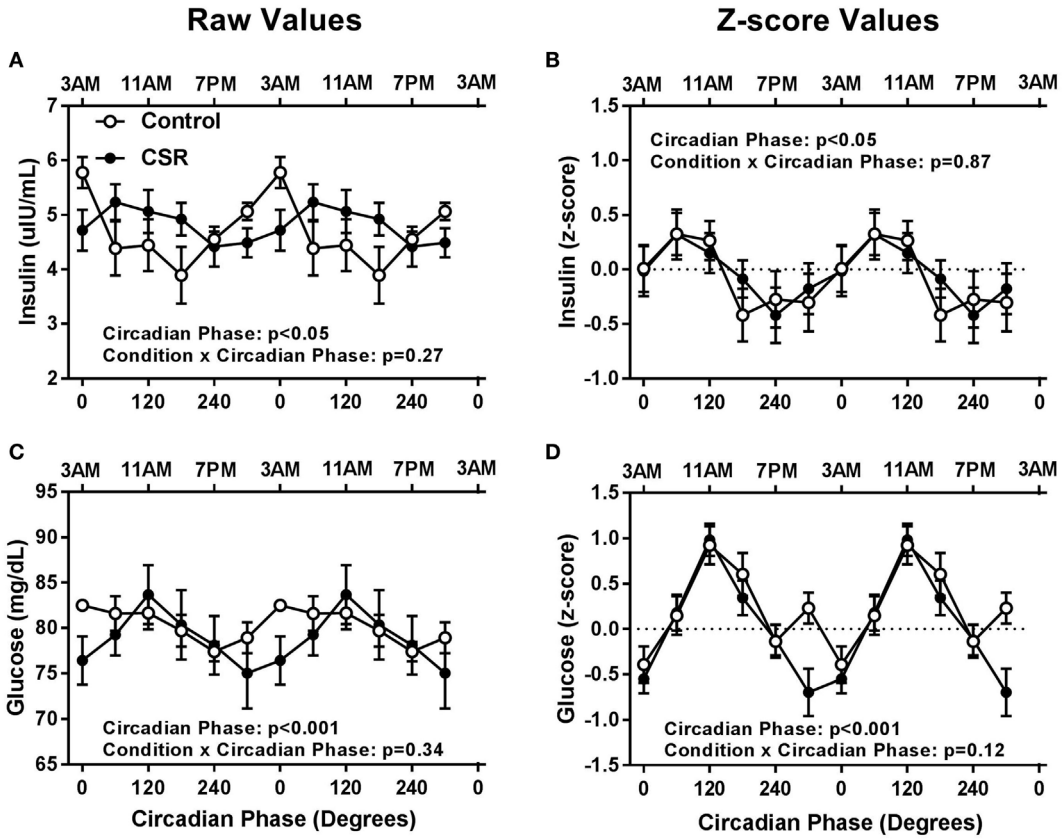

FIGURE 3 | Influence of circadian phase and sleep restriction on fasted **(A,B)** insulin and **(C,D)** glucose concentrations. The Control (n = 7, 1:2 sleep:wake ratio, equivalent to 8 h sleep opportunity per 24 h day) condition is denoted by open circles and the chronic sleep restriction (CSR, n = 7, 1:3.3 sleep:wake ratio, equivalent to 5.6 h sleep opportunity per 24 h day) condition by closed circles. Data are represented as raw scores (left) and z-scores (right) and double plotted across circadian phase and relative clock hour for an individual with a habitual sleep time of 2300–0700 hours. Error bars represent SEM.

why sleep restriction leads to excess caloric intake, particularly at night; sleep restricted individuals may have a higher likelihood of being awake across a longer duration of time when their internal physiology is promoting hunger if sleep is initiated at a later time. Subjective hunger does not begin to decline until between 1900 and 2300 hours, but still remains relatively high during this time. Thus, if one individual were to go to sleep at 2100 hours and another at 2300 hours, the drive for food consumption would still be reasonably high in that second individual during those two additional hours of wakefulness, and this may be driving the excess food consumption and weight gain observed during sleep restriction (14–17). It is unknown, however, if these hunger rhythms can be shifted by the light or other exogenous factors; this should be explored in future research.

In addition to not finding a difference between groups and a circadian rhythm in subjective hunger levels, we also did not find any significant differences between groups or significant rhythms in the fasted concentrations of circulating leptin and ghrelin. Leptin has been observed to follow a diurnal rhythm under fed conditions with concentrations lowest in the mid-afternoon, beginning to rise at ~2200 hours, and highest during the nighttime hours (~0300 hours) (35); however, these concentrations are greatly influenced by caloric intake (39) and sleep (40) and have not demonstrated circadian rhythmicity in a FD circadian protocol (47). Ghrelin has been reported to follow a diurnal rhythm with increases in the early portion of the night and decreases in the second half of the night with a trough in the morning at ~0800 hours (36–38), though these rhythms are blunted with sleep restriction suggesting that ghrelin is influenced by both the circadian and the sleep and wakefulness systems (36, 37). Our findings in leptin and ghrelin may differ from previous FD studies because we only measured fasted concentrations and there is a non-linear interaction between circadian and behavioral effects in leptin concentrations (47). Future work is needed to examine CSR in both the fed and fasted states to fully tease apart the influence of sleep restriction and circadian timing, particularly since levels of ghrelin in sleep restricted individuals have been found to predict subsequent caloric consumption (22).

Our findings of no difference in fasted insulin and glucose concentrations between the two conditions were somewhat surprising. Decreases in glucose tolerance and insulin sensitivity during acute insufficient and fragmented sleep protocols have been well established (48–52), and fasted insulin has been found

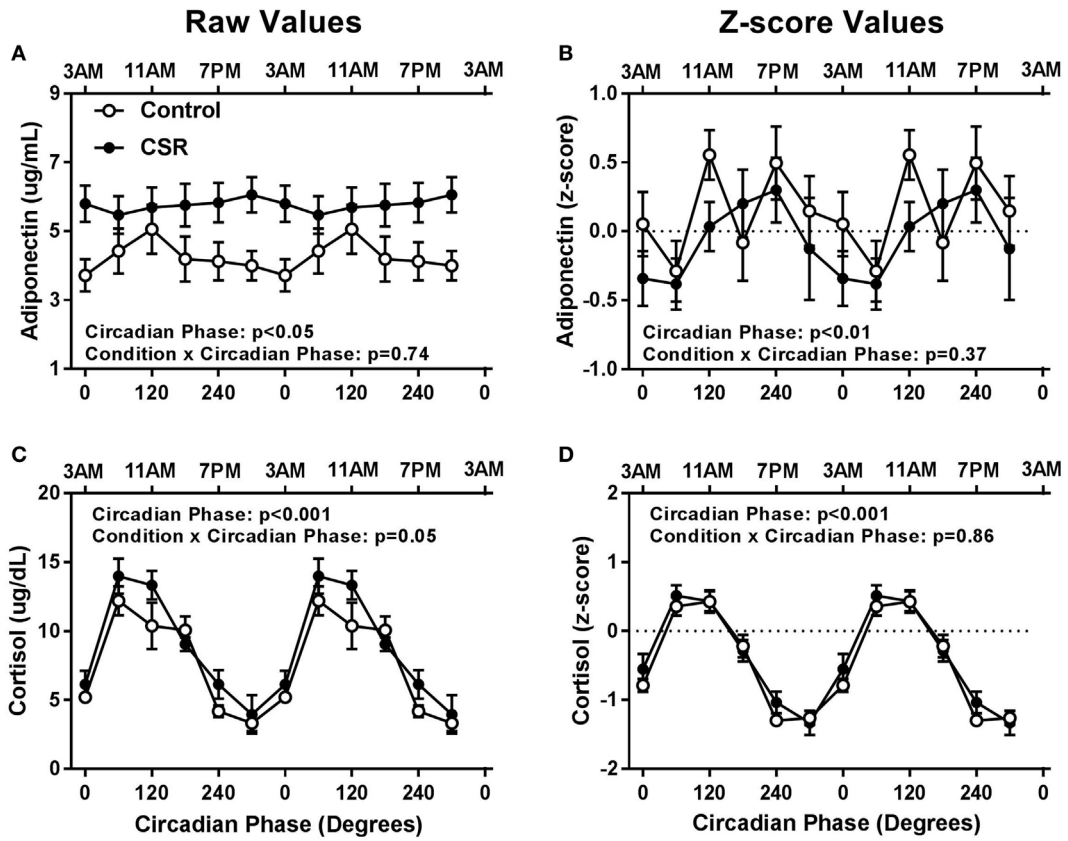

FIGURE 4 | Influence of circadian phase and sleep restriction on fasted **(A,B)** adiponectin and **(C,D)** cortisol concentrations. The Control (n = 7, 1:2 sleep:wake ratio, equivalent to 8 h sleep opportunity per 24 h day) condition is denoted by open circles and the chronic sleep restriction (CSR, n = 8, 1:3.3 sleep:wake ratio, equivalent to 5.6 h sleep opportunity per 24 h day) condition by closed circles. Data are represented as raw scores (left) and z-scores (right) and double plotted across circadian phase and relative clock hour for an individual with a habitual sleep time of 2300–0700 hours. Error bars represent SEM.

to be lower than baseline levels after weeks of sleep restriction and circadian disruption (31). However, by using fasted levels and not oral or intravenous glucose tolerance tests as has been done previously in acute sleep restriction, we may not have challenged the system enough to see appreciable differences. Another hypothesis for why we did not observe differences between our two groups is that in previous sleep restriction studies, researchers may have been studying sleep restricted individuals at a different circadian phase than when the individuals were not sleep restricted (for example, if awakening were earlier in the sleep restriction condition), as circadian timing can independently play a role in impaired glucose metabolism (53, 54). Further supporting the premise that sleep restriction protocols may lead to studying individuals at a different circadian phase, in a previous protocol of 5 days of 5 h sleep restriction, impairments in early morning insulin sensitivity were found to be highly correlated with the circadian timing of high melatonin concentrations (55). In addition, protocols providing exogenous melatonin have found decreases in insulin secretion and sensitivity (56, 57), and individuals carrying a common variant in the melatonin receptor 1b gene have increased risk for impaired glucose regulation (58, 59), further supporting the hypothesis relating the circadian timing of melatonin to impaired glucose regulation. Our findings of no significant difference between conditions in adiponectin concentrations is in agreement with the literature that sleep restriction has limited impact on adiponectin (60, 61), though sleep restriction has been found to influence adiponectin differently depending on sex and ethnicity (61). Our limited sample size did not allow for in-depth examination of the impact of sex and ethnicity during CSR, and should be examined in future research. Finally, our findings of a non-significant trend for higher fasted cortisol in the CSR condition adds to the somewhat inconclusive literature on the impact of sleep restriction on cortisol, as acute sleep restriction has been reported to either elevate (49, 62, 63) or not change (64–66) cortisol concentrations. However, our data are in agreement with previous findings of no change in cortisol across weeks of sleep restriction and circadian disruption (31), but we now include a Control condition to add additional evidence to the limited impact of sleep restriction to the robust circadian rhythmicity of cortisol.

Our study did have several limitations and the conclusions should be considered with caution. First, we only measured the fasted hormone concentrations of the observed measures. It is unknown how these hormones would change after food consumption

during CSR, which may be of importance as (i) individuals tend to ignore satiety signals during acute sleep restriction and continue to eat (15) and (ii) circadian timing without sleep restriction has been shown to dampen leptin levels when measured in the fasted and post-prandial state (47). Furthermore, we only had one fasted draw per wake episode. Additional draws may have added a temporal resolution to help detect more sensitive differences. Second, our population consisted of healthy young participants whose physiology may be able to respond quickly to the physiological challenge of sleep restriction. Though our protocol included a chronic exposure to insufficient sleep spanning across 32-days, it may not have been long enough or a sufficient amount of sleep restriction to see a metabolic inability to respond. Finally, our somewhat small sample size may have limited our ability to observe subtle differences between the two groups and as mentioned previously, reduced our capability to draw conclusions in regard to sex or ethnic differences in response to sleep restriction.

In summary, our findings demonstrate that sleep restriction may have a limited impact on hunger and fasted concentrations of hormones and that circadian timing needs to be considered when examining fasted levels of these outcomes. This information is important for clinicians who may need to make recommendations or prescribe therapies during the early morning hours and our findings could be used to help guide future chronotherapies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, upon request, to any qualified researcher.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of, and with the approval of, the Partners Healthcare Institutional Review Board (IRB #2011P001094). All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

EK obtained funding. EK and JH conducted design, data collection, data analysis, data interpretation, and writing. AM contributed to data collection, data analysis, data interpretation, and writing. CM contributed to data analysis, data interpretation, and writing.

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Sex Differences in the Impact of Obstructive Sleep Apnea on Glucose Metabolism

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Objectives: Obstructive sleep apnea (OSA) is more prevalent in men and is an independent risk factor for type 2 diabetes. We aimed to determine if there are sex differences in the impact of OSA on glucose metabolism in nondiabetic overweight and obese adults.

Methods: One hundred and forty-five men and women (age 33.4 ± 0.6, BMI 37.2 ± 0.7, 70.3% blacks) from the community underwent in-laboratory polysomnography. Severity of OSA was assessed by the apnea-hypopnea index (AHI). Glucose tolerance was assessed using fasting glucose, 1-h glucose, 2-h glucose and the area under the curve (AUC) during the 2-h oral glucose tolerance test (OGTT). Fasting insulin resistance was assessed by HOMA-IR, and insulin sensitivity during the OGTT was assessed by the Matsuda Index. Pancreatic beta-cell function was assessed by fasting HOMA-%B and by $AUC_{insulin/glucose}$, insulinogenic index, and oral disposition index (DI_{oral}) during the OGTT. All comparisons were adjusted for age, BMI, race and severity of OSA.

Results: There were no significant demographic differences between men and women without OSA. Men and women with OSA were similar in age, BMI, and severity of OSA, but there were more black women with OSA. Compared to women with OSA, men with OSA had significantly higher fasting glucose, 1-h glucose levels, $AUC_{glucose}$, and AUC for insulin secretion rate (AUC_{ISR}) but similar 2-h glucose levels. These differences persisted in adjusted analyses. Men with OSA secreted significantly more insulin than women with OSA in order to achieve similar glucose levels. Men with OSA had significantly worse beta cell function as measured by the DI_{oral} than women with OSA. In contrast, there were no significant sex differences in measures of glucose tolerance and beta-cell function in participants without OSA.

Conclusion: Men with OSA secreted more insulin compared to women with OSA in order to maintain glucose homeostasis.The adverse impact of OSA on beta-cell responsiveness was larger in men, which may result in an overall greater risk of type 2 diabetes compared to women.

Keywords: obstructive sleep apnea, sleep-disordered breathing, diabetes, glucose tolerance, insulin resistance, beta-cell, gender, sex

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INTRODUCTION

Type 2 diabetes affects nearly 30 million individuals or 9.4% of the US population with an estimated 1.5 million new cases per year. Even more alarming is the significant increase in the prevalence of prediabetes (impaired fasting glucose and/or impaired glucose tolerance), an intermediate state between normal glucose tolerance and overt diabetes. According to the Centers for Disease Control and Prevention, the number of American adults with prediabetes increased from 57 million in 2008 to 84 million in 2015 (1). Undoubtedly the high prevalence of obesity has played a pivotal role in this epidemic. In parallel, the obesity epidemic has also resulted in an increased prevalence of obstructive sleep apnea (OSA) in the general population (2, 3).

Several clinic-based and community-based cross-sectional studies have found a robust association between the presence and severity of OSA—as measured by the apnea-hypopnea index or AHI—and insulin resistance in both men and women, independent of age and various measures of adiposity (4–10). A recent meta-analysis of 9 longitudinal studies that included 64,101 participants, with follow up ranging from 2.7 to 12.8 years, revealed that OSA is associated with incident type 2 diabetes with an adjusted pooled relative risk of 1.35 (95% CI, 1.24–1.47) (11). However, the sex-specific difference of the impact of OSA on glucose metabolism remains mostly unexplored. To that end, we aimed to quantify the impact of OSA on glucose tolerance, insulin sensitivity, beta-cell responsiveness and diabetes risk in a community-based cohort of overweight and obese men and women without diabetes.

METHODS

Participants

Subjects were recruited using flyers and public advertisement. The flyer requested healthy obese men and women between ages 18–50 to volunteer for a research study related to sleep and metabolism. Interested participants who called in to the recruitment phone line underwent a brief phone screen to assess eligibility for the study. Eligible participants were admitted to the University of Chicago General Clinical Resource Center. All subjects were between 18 and 50 years of age, with a body mass index (BMI) >25 kg/m^2, and free of psychiatric, endocrine and cardiovascular disorders except for well-controlled hypothyroidism and hypertension. Sleep complaints or symptoms of OSA were not used as selection criteria for the study. Shift workers, subjects with chronic insomnia, and subjects with self-reported habitual sleep duration of <6.5 h per night or more than 9 h per night as well as any subjects with diagnosis of a sleep disorder other than OSA were excluded. Other exclusion criteria included any prior or current treatment for OSA (upper airway surgery, CPAP therapy, oral appliances or supplemental oxygen), active cigarette smoking, habitual alcohol intake above 2 drinks per day, previous diagnosis of type 2 diabetes, use of antihypertensives that impact sleep or glucose metabolism (e.g., thiazide diuretics and beta blockers), caffeine intake above 300 mg per day, pregnancy, and women taking hormonal therapy. We also excluded post-menopausal women and women with established diagnosis of polycystic ovary syndrome (PCOS) or suspicion of PCOS based on hyperandrogenemia. All study participants gave written informed consent prior to participating in this study. This study was approved by the University of Chicago Institutional Review Board, and was conducted in accordance with the Declaration of Helsinki.

Consented subjects had a physical examination, and a complete medical history was obtained. Height and weight were measured in all participants on the night of the polysomnography (PSG). Race was self-reported and categorized as non-Hispanic white or black. Subjects had an overnight in-laboratory PSG to assess the presence and severity of OSA. The following morning, a standard 75-g oral glucose tolerance test (OGTT) was performed to measure glucose tolerance and insulin sensitivity.

Polysomnography

Subjects were admitted to the University of Chicago Clinical Resource Center (CRC) and underwent an overnight in-laboratory PSG. Lights were turned off at 11 pm and turned on at 7 am. The PSG (Neurofax EEG 1100 system; Nihon Kohden, Foothill Ranch, CA) included recordings of six electroencephalogram channels, bilateral electro-oculograms, chin and tibialis electromyogram, electrocardiogram, airflow by nasal pressure transducer and oronasal thermocouples, chest and abdominal wall motion by piezo electrodes, and oxygen saturation by pulse oximeter. All PSGs were staged and scored according to the 2007 American Academy of Sleep Medicine Manual for the Scoring of Sleep and Related Events (12). Apneas were defined as total cessation of airflow for at least 10 s (obstructive if respiratory effort was present and central if respiratory effort was absent). Hypopneas were scored if the magnitude of ventilation signal decreased by at least 50% of the baseline amplitude of the nasal pressure transducer for at least 10 s and were associated with either a 3% or greater drop in oxygen saturation as measured by finger pulse oximetry, or an electroencephalographic microarousal (12). AHI was defined as the total number of obstructive apneas and obstructive hypopneas per hour of sleep. Severity of OSA was measured by the AHI. A subject was considered not to have OSA if the AHI was <5, to have mild OSA if the AHI was 5–14, moderate OSA if the AHI was 15–29, and severe OSA if the AHI was ≥ 30. The oxygen desaturation index (ODI) was defined as the total number of oxygen desaturations of at least 3% per total sleep time (TST) in hours. The microarousal index (MAI) was calculated as the total number of microarousals per hour of sleep.

Oral Glucose Tolerance Test (OGTT)

After a 12-h overnight fast, an intravenous catheter was placed into an antecubital vein for blood drawing. Baseline blood samples were drawn at -15 and 0 min for measurement of glucose, insulin, and C-peptide concentrations. At time 0 min, subjects consumed a 75-g glucose beverage over a period not to exceed 5 min. Subsequent blood samples were drawn at 30, 60, 90, and 120 min for measurement of glucose, insulin, and C-peptide concentrations. If the fasting glucose concentration was

≥100 mg/dl but <126 mg/dl, a diagnosis of impaired fasting glucose (IFG) was assigned; a fasting glucose concentration >126 mg/dl was diagnostic of type 2 diabetes. The glucose concentration post-2h glucose challenge was used to diagnose normal glucose tolerance (<140 mg/dL), impaired glucose tolerance (IGT; 140–199 mg/dl) and type 2 diabetes ≥200 mg/dL) (13). Area under the curve (AUC) for glucose and insulin response was calculated for the first 2-h interval after glucose load using the trapezoidal rule (14, 15).

The degree of insulin resistance was quantified using the homeostasis model assessment index of insulin resistance (HOMA-IR) [(glucose (mmol/L) • insulin (mIU/L))/22.5]. (16) Fasting HOMA-IR and area under the curve of glucose (AUC glucose) were used as measures of insulin resistance and glucose tolerance, respectively. The Matsuda Index was used as a measure of insulin sensitivity (17). Beta-cell responsiveness was assessed using the fasting HOMA-%B [(20 • insulin (mIU/L))/(glucose (mmol/L) – 3.5)], $AUC_{insulin}/AUC_{glucose}$ [$AUC_{insulin/glucose}$] (16), AUC_{ISR}, and insulinogenic index (IGI) (18). Oral disposition index (DI_{Oral}) was used as a measure of beta-cell function adjusted for insulin sensitivity (18).

Assays

Plasma glucose was assayed by the glucose oxidase method (YSI Life Sciences). Serum insulin and C-Peptide were measured by chemiluminescence assays using the Immulite immunochemistry system (Diagnostic Products Corp., Los Angeles, CA). As a result of hemolysis, <1.5% of insulin values were adjusted by linear interpolation or extrapolation after examination of corresponding C-Peptide values. Hemoglobin A1c was measured using turbidimetric inhibition immunoassay (Roche Diagnostics).

Determination of Insulin Secretory Rates

In each blood sampling interval during the OGTT, the insulin secretion rate (ISR) (19) was mathematically derived from plasma C-Peptide levels using a two-compartment model for C-Peptide disappearance kinetics (20, 21). The kinetic parameters were obtained from published demographic data taking into account sex, age, and body surface area (22). The mean (±SEM) parameter values were 4.55 ± 0.0 min for the short half-life, 33.8 ± 0.08 min for the long half-life, and 0.78 ± 0.0 for the fraction of decay associated with the short half-life. The volume of distribution averaged 4.69 ± 0.05 L.

Statistical Analyses

Continuous variables are expressed as mean ± SEM for normally distributed data, or median with 25–75% interquartile range (IQR) when the assumption of normality was not met; categorical variables are summarized as percentages (%). The main objective of this study was to determine if sex differences exist in the impact of OSA on insulin resistance and glucose tolerance (fasting HOMA-IR, AUC glucose, and Matsuda Index) and beta-cell function (HOMA-%B, $AUC_{insulin/glucose}$, insulinogenic index and oral disposition index). We therefore created four groups based on sex (men, women) and OSA (presence, absence). Comparisons were made separately between men and women with OSA and men and women without OSA. Unadjusted group comparisons were performed using a *t*-test for normally distributed continuous variables and the Wilcoxon/Mann-Whitney test for non-normally distributed continuous variables. Pearson Chi Square test was used to compare categorical variables. Multivariate linear regression models were constructed to examine whether sex was independently associated with measures of glucose metabolism after adjusting for age and BMI as continuous variables, race, and severity of OSA based on the natural log of AHI (*Ln*AHI). Dependent variables that were not normally distributed were log transformed. Given that the calculation for insulin secretion rate (ISR) takes into account body surface area, the multivariate regression models examining AUC_{ISR} did not adjust for BMI. For AHI values equal to zero, we used the formula Ln (AHI + 0.1) to log transform. *P*-values <0.05 were considered statistically significant. All statistical calculations were performed using JMP 9.0 statistical software for Macintosh (SAS Institute).

RESULTS

A total of 157 subjects participated in the study. An OGTT was not performed in 6 subjects who had a fasting plasma glucose ≥126 mg/dl, consistent with the presence of undiagnosed type 2 diabetes. Four additional subjects were excluded because their HbA1c level was ≥6.5%. Two subjects were excluded because of uncontrolled hypothyroidism. Thus the final analytic cohort included 145 subjects (90 women and 55 men). OSA, defined as AHI ≥5 events per hour, was present in 72.7% of men and 43.3% of women ($p < 0.001$). We categorized the subjects by sex and presence of OSA. **Table 1** summarizes the demographics of the four groups of subjects. There were no significant differences between men and women without OSA. In those with OSA, men and women had similar age and BMI, but there were more black women than black men with OSA ($p = 0.0016$).

Table 2 summarizes polysomnographic differences between men and women with and without OSA. There was a statistically significant, but likely clinically less relevant, difference in AHI between men and women without OSA (1.9 vs. 1.1; $p = 0.0265$). In participants with OSA, the only polysomnographic difference between men and women was a lower percentage of slow wave sleep in men ($p = 0.0017$). Importantly, the severity of OSA was not significantly different between men and women with OSA.

The proportion of individuals with IFG varied between the 4 groups ($p = 0.0004$). A higher prevalence of IFG was observed in men with and without OSA (35.0 and 26.7%, respectively) compared to women with and without OSA (10.3 and 3.9%, respectively). After adjusting for age, BMI, race, and severity of OSA (*Ln*AHI), men were more likely to have IFG (odds ratio 10.2, CI 2.9–42.7; $p = 0.0001$). The prevalence of impaired glucose tolerance (IGT) was higher in men and women with OSA (27.5 and 20.5%, respectively) compared to men and women without OSA (6.7 and 9.8%, respectively). After adjusting for confounders, there was no significant group differences for IGT ($p = 0.094$).

TABLE 1 | Subject demographics.

Variable	Women without OSA ($n = 51$)	Men without OSA ($n = 15$)	p* value ($n = 66$)	Women with OSA ($n = 39$)	Men with OSA ($n = 40$)	p* value ($n = 79$)
Age (years)	30.2 ± 0.7	27.6 ± 1.6	0.091	36.0 ± 1.3	37.1 ± 1.0	0.485
BMI (kg/m^2)	35.6 ± 0.9	35.0 ± 1.6	0.757	40.3 ± 1.5	37.3 ± 1.4	0.152
Blacks, *n* (%)	37 (72.5)	9 (60.0)	0.353	34 (87.2)	22 (55.0)	**0.0016**
Non-hispanic White, *n* (%)	14 (27.5)	6 (40.0)		5 (12.8)	18 (45.0)	

Data are given as mean ± SEM or n (%)
**p-values reflect student's t-test for continuous variables and Pearson coefficient for categorical variables. P-values in bold are statistically significant.*

TABLE 2 | Polysomnographic variables.

Variable	Women without OSA ($n = 51$)	Men without OSA ($n = 15$)	p-value* ($n = 66$)	Women with OSA ($n = 39$)	Men with OSA ($n = 40$)	p-value* ($n = 79$)
Total sleep time (minutes)	447 (422–460)	454 (433-491)	0.239	434 (393-450)	422 (377-457)	0.791
Sleep efficiency (%)	91.4 (85.9–94.5)	94.3 (85.7–96.4)	0.449	85.6 (82.7–92.4)	86.7 (81.0–92.7)	0.961
Slow wave sleep (%)	11.6 (5.7-16.3)	5.6 (2.0–13.9)	0.076	9.1 (3.6–14.2)	1.9 (0.1–9.9)	**0.0017**
REM sleep (%)	23.5 (20.6–26.2)	22.5 (17.8–26.7)	0.245	19.9 (16.6–23.5)	22.2 (17.8–26.3)	0.109
AHI	1.1 (0.5–2.3)	1.9 (1.7–2.6)	**0.0265**	15.2 (7.3–21.7)	18.4 (11.8–28.5)	0.096
REM AHI	1.9 (0.6–4.1)	2.8 (1.3–5.3)	0.229	22.3 (5.8–37.2)	23.8 (14.2–51.4)	0.281
NREM AHI	0.7 (0.2–2.2)	1.4 (0.8–3.0)	**0.0187**	12.8 (7.4–21.6)	16.6 (8.6–25.1)	0.184
MAI	10.6 (7.4–14.5)	10.7 (7.3–14.1)	0.748	19.2 (17.0–26.7)	23.5 (13.4–29.3)	0.764

Data are given as median (interquartile range).
**p-values are from Wilcoxon test. P-values in bold are statistically significant.*
REM, Rapid Eye Movement; AHI, Apnea-Hypopnea Index; NREM, non-Rapid Eye Movement; MAI, Microarousal Index.

Figure 1 illustrates profiles of plasma glucose, serum insulin, and ISR from the OGTT. In unadjusted analyses, men with OSA had a significantly higher fasting, 30-min and 1-h glucose levels compared to women with OSA. Men with OSA had significantly higher $AUC_{Glucose}$ than women with OSA ($p = 0.005$). Although men without OSA had a higher fasting and 30-min glucose levels compared to women without OSA, the $AUC_{Glucose}$ was similar between men and women without OSA ($p = 0.204$). In unadjusted analysis, the AUC_{ISR} was significantly greater in men with OSA compared to women with OSA ($p = 0.0069$), as well as in men without OSA compared to women without OSA ($p = 0.0030$).

Tables 3, **4** summarize metabolic differences between men and women. In subjects without OSA, men had a higher fasting glucose in both unadjusted and adjusted comparisons. There were no significant differences between men and women without OSA in any of the measures of beta-cell function, measures of insulin sensitivity, and glucose tolerance. In contrast, in subjects with OSA, men had significantly higher fasting glucose, 1-hr glucose, and $AUC_{Glucose}$. Men with OSA secreted more insulin compared to women with OSA, as evidenced by a significantly higher AUC_{ISR}. These differences remained significant after adjusting for covariates. Men with OSA had significantly lower beta-cell function as assessed by the DI_{Oral} even after adjusting for covariates.

DISCUSSION

Our study rigorously examines sex-specific differences in the impact of OSA on glucose metabolism in relatively young overweight and obese subjects without diabetes. There were no significant differences in glucose tolerance and beta-cell function between men and women without OSA. In contrast, men with OSA had worse glucose tolerance than women with OSA despite secreting more insulin. As such, the adverse impact of OSA on beta-cell responsiveness is of greater magnitude in men, which may result in an overall greater risk of developing type 2 diabetes. Higher glucose levels and insulin responses during the OGTT (i.e., 1-h glucose and the shape of the plasma glucose curve) are indeed related to the risk of developing type 2 diabetes, particularly if the 2-h plasma glucose does not return to or below the fasting plasma glucose levels (23–25). A slower rate of decrease in plasma glucose concentration during an OGTT is

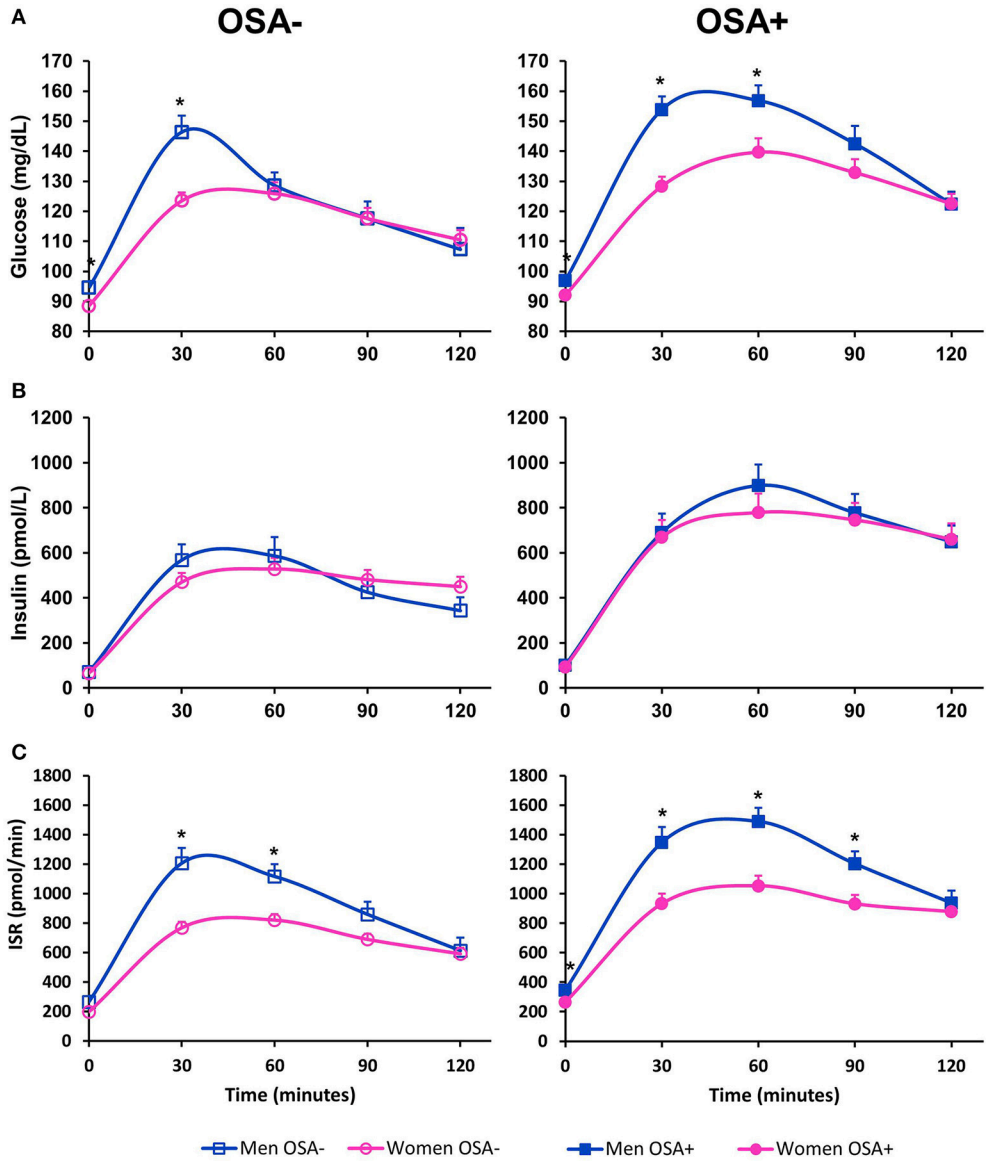

FIGURE 1 | Oral glucose tolerance test in men and women with and without OSA. **(A)** Illustrates plasma glucose, **(B)** illustrates serum insulin and **(C)** illustrates insulin secretion rate (ISR) as measured during a 2-h 75-g oral glucose challenge. Data are represented as mean + SEM. *denotes an unadjusted p-value < 0.05 from a student's t-test for variables with normal distribution, and Wilcoxon test for variables that are not normally distributed.

indicative of increased insulin resistance and/or impaired beta-cell responsiveness, both of these being important risk factors for future type 2 diabetes.

The underlying pathogenesis of impaired glucose metabolism due to OSA is not fully understood but factors such as activation of the sympathetic nervous system, intermittent hypoxemia, oxidative stress, and low-grade systemic inflammation have been implicated. In a study of 118 nondiabetics recruited from the community who underwent a frequently sampled intravenous glucose tolerance test, the severity of OSA as measured by the AHI was independently associated with insulin sensitivity after controlling for age, sex, race and percent body fat. Moreover, the acute insulin response to glucose, a measure of beta-cell function, did not increase across OSA severity categories. Together these findings suggest that the increased diabetes risk in OSA is associated with increased insulin resistance without adequate compensation by the beta-cell (26). The sex-specific differences of the impact of OSA on glucose metabolism, however, remains mostly unexplored as most studies have predominantly included men or only women or did not take sex differences into account (4–10, 27, 28). A few cross-sectional epidemiologic studies have suggested that markers of OSA, namely observed apneas and habitual snoring, are independently associated with type 2 diabetes or prediabetes in women only (29–32). These studies

TABLE 3 | Fasting metabolic measures.

Variable	Women OSA- ($n = 51$)	Men OSA- ($n = 15$)	Unadjusted p ($n = 66$)	Adjusted p* ($n = 66$)	Women OSA+ ($n = 39$)	Men OSA+ ($n = 40$)	Unadjusted p ($n = 79$)	Adjusted p* ($n = 79$)
Fasting glucose (mg/dL)	88.5 ± 0.9	94.5 ± 2.3	**0.0039**	**0.0028**	92.1 ± 1.0	97.0 ± 1.0	**0.0007**	**0.0010**
Fasting insulin (pmol/L)	49 (31–90)	52 (21–97)	0.963	0.952	83 (56–118)	89 (48–149)	0.814	0.416
Fasting ISR[a] (pmol/min/m^2)	192 (144–240)	228 (169–340)	0.102	0.224	212 (166–334)	292 (207–453)	**0.0354**	0.115
HOMA–IR (mIU/mmol)	1.61 (0.98–2.89)	1.78 (0.64–3.34)	0.842	0.736	2.77 (1.92–3.70)	2.98 (1.54–5.21)	0.580	**0.255**
HOMA–%B (mIU/mmol)	120 (71–190)	82 (48–151)	0.335	0.318	161 (88–246)	126 (85–215)	0.430	0.800
HbA1c (%)	5.44 ± 0.04	5.43 ± 0.07	0.875	0.826	5.63 ± 0.04	5.59 ± 0.06	0.619	0.582

Data are given as mean ± SEM or median (interquartile range) for variables that are not normally distributed.
Unadjusted p-values are from a student's t-test for variables with normal distribution, and Wilcoxon test for variables that are not normally distributed.
**p-values are from a multiple regression model adjusting for age, BMI, race, and natural log (Ln)AHI. P-values in bold are statistically significant.*
[a]Multiple regression models for ISR did not adjust for BMI because the calculation for ISR includes an adjustment for body surface area.
ISR, Insulin Secretion Rate; HOMA-IR, Homeostatic Model Assessment Insulin Resistance; HOMA-%B, Homeostatic Model Assessment Beta Cell Function.

TABLE 4 | Metabolic measures derived from response to oral glucose.

Variable	Women OSA- ($n = 51$)	Men OSA- ($n = 15$)	Unadjusted p ($n = 66$)	Adjusted p* ($n = 66$)	Women OSA+ ($n = 39$)	Men OSA+ ($n = 40$)	Unadjusted p ($n = 79$)	Adjusted p* ($n = 79$)
2-h Glucose (mg/dL)	110.5 ± 3.3	107.3 ± 7.2	0.660	0.904	122.5 ± 3.3	122.4 ± 4.2	0.978	0.512
1-h Glucose (mg/dL)	125.9 ± 3.7	128.6 ± 4.3	0.709	0.718	139.7 ± 4.6	156.9 ± 5.0	**0.0135**	**0.0225**
$AUC_{Glucose}$	13,995 ± 312	14,805 ± 472	0.204	0.212	15,247 ± 375	16,893 ± 427	**0.0050**	**0.0066**
$AUC_{Insulin}$	42,920 (28,179–66,307)	54,429 (35,477–70,750)	0.520	0.786	56,8312 (35,367–119,603)	70,813 (42,415–106,936)	0.691	0.539
AUC^{a}_{ISR}	2,867 (2,213–3,630)	4,094 (3,384–5,111)	**0.0030**	0.055	3,993 (2,833–5,197)	4,973 (4,087–6,409)	**0.0069**	**0.0319**
$AUC_{Insulin/Glucose}$	57.9 (40.4–85.8)	68.4 (42.1–76.7)	0.807	0.918	71.6 (46.1–129.6)	77.0 (50.7–117.8)	0.841	0.970
DI_{Oral}	3.60 (2.20–6.74)	2.94 (2.11–7.67)	0.481	0.529	3.15 (2.24–4.72)	2.16 (1.09–3.27)	**0.0090**	**0.0116**
IGI	187 (122–290)	179 (132–205)	0.391	0.493	258 (127–425)	149 (81–299)	**0.0314**	0.123
Matsuda index	5.10 (3.21–8.08)	4.17 (2.61–7.45)	0.592	0.643	3.21 (1.86–4.54)	2.44 (1.58–5.16)	0.424	0.226

Data are given as mean ± SEM or median (interquartile range) for variables that are not normally distributed.
Unadjusted p values are from a student's t-test for variables with normal distribution, and Wilcoxon test for variables that are not normally distributed.
**p-values are from a multiple regression model adjusting for age, BMI, race, and LnAHI. P-values in bold are statistically significant.*
[a]Multiple regression models for AUC_{ISR} did not adjust for BMI because the calculation for ISR includes an adjustment for body surface area.
AUC, Area under the curve; ISR, Insulin Secretion Rate; DI_{Oral}, Oral disposition index; IGI, Insulinogenic index.

were questionnaire based and lacked objective polysomnographic evaluation. In contrast, we quantified the presence and severity of OSA using the gold standard in-laboratory polysomnography. Our findings of increased risk of type 2 diabetes with OSA in men are consistent with findings from the largest nationwide health claims database analysis which revealed a higher prevalence of type 2 diabetes in men with OSA compared to women with OSA (33). In a Swedish clinic-based longitudinal study with 16 years of follow up, OSA was independently associated with incident type 2 diabetes in women only (34). However, this study was limited due to lack of full in-lab polysomnography, it included only 10 women with OSA and the presence of incident type 2 diabetes was obtained by questionnaires mailed to the patients. The questionnaire based nature of the study and lack of OGTT precludes any inferences regarding sex-specific mechanistic differences by which OSA may impact glucose metabolism.

A number of population-based and clinic-based studies have reported a stronger association between OSA and hypertension in men than in women (35–37). There is a paucity of studies examining sex differences of the adverse metabolic impact of OSA. Our cross-sectional analysis suggests that the adverse impact of OSA on beta-cell function is more prominent in men. Harsch and colleagues performed hyperinsulinemic euglycemic clamps after 2 days of all-night CPAP therapy in the sleep laboratory in 40 nondiabetic subjects (36 men and 4 women)

with severe OSA. Although they did not measure beta-cell function, they demonstrated significant improvement in insulin sensitivity after only 2 nights of effective all-night CPAP therapy (38). Clinical trials assessing the impact of CPAP therapy on glucose metabolism in patients with OSA and type 2 diabetes, on the other hand, have yielded mixed results, in part due limited adherence to CPAP therapy (11). However, clinical trials with higher CPAP adherence in patients with either type 2 diabetes (39, 40) or prediabetes (41, 42) have found that therapeutic CPAP improves glycemic control or insulin sensitivity compared to the control group. These findings suggest that in order to derive metabolic benefits from CPAP therapy, treatment should include the great majority of the sleep period (43).

Our study has several limitations. First and foremost, its cross-sectional design does not establish the direction of causality. Second, we used derived measures of insulin resistance, glucose tolerance and beta-cell function from an OGTT alone. Although some of the derived measures of beta-cell function have been previously validated (18, 44), there is no clear consensus on how best to measure beta-cell function based on an OGTT. We therefore explored several derived measures of beta-cell function. Undoubtedly, additional studies with more detailed and intensive assessments of beta-cell function and insulin sensitivity are needed to better elucidate mechanistic pathways. Third, our study includes one night of sleep in the laboratory and therefore does not take into account variability in sleep patterns as well as participants' habitual sleep duration which can influence glucose metabolism. Fourth, although we excluded post-menopausal women, metabolic testing was not standardized to a particular phase of the menstrual cycle. Lastly, we did not assess measures of body fat distribution. This may be relevant given the established sex differences in fat distribution.

In summary, the adverse impact of OSA on beta-cell responsiveness is greater in men, which may result in an overall higher risk of future development of type 2 diabetes compared to women.

AUTHOR CONTRIBUTIONS

BM and EV designed the protocol. LM, KT, and RL recruited subjects and collected data. KT, DE, EV, and BM analyzed the data. BM drafted the manuscript. KT, EV, DE, and BM reviewed and edited the manuscript.

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REVIEW
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Poor Sleep and Obesity: Concurrent Epidemics in Adolescent Youth

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Poor sleep and obesity are both extraordinarily common in the US adolescent population and often occur simultaneously. This review explores the links between obesity and sleep, outlining what is known about the relationships between sleep characteristics, obesity, and cardiometabolic risk factors in youth. Sleep duration is less than optimal in teens, and decreases as age increases. This is detrimental to overall well-being and is associated with obesity in children, adolescents, and young adults. Accordingly, inadequate sleep duration is associated with poor diet quality, decreased insulin sensitivity, hyperglycemia, and prevalent cardiometabolic risk factors. Evidence suggests that poor sleep quality and altered circadian timing characterized by a preferred later sleep onset, known as "adolescent chronotype," contributes to shortened sleep duration. Obstructive sleep apnea (OSA) occurs more frequently among youth with obesity, and is associated with autonomic nervous system activity promoting higher blood pressure, increased markers of cardiovascular disease risk, and insulin resistance. While there is a clear association between OSA and type 2 diabetes in adults, whether or not this association is prevalent in youth is unclear at this time. Interventions to improve both sleep duration and quality, and obesity in adolescents are scarce and more evidence is needed to determine if such interventions can improve obesity-related health outcomes.

Keywords: obesity, poor sleep, obstructive sleep apnea, sleep duration, sleep quality, diet quality, cardiometabolic risk, insulin sensitivity

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INTRODUCTION

Data from the National Health and Nutrition Examination Survey (NHANES) shows the prevalence of obesity, defined as having a body mass index (BMI) at or above the 95th percentile for age and sex, in 2–19 year old youth was 16.8% in 2007–2008 and increased to 18.5% (nearly 1 in 5) in 2015–2016 (1). In addition, since 2013–2014, there has been a significant increase in obesity among youth ages 2–5 years, with an increase in prevalence from 9.3 to 13.7% in 2015–2016 (2). Thus, the burden of childhood obesity in the US continues to increase and as more youth are affected, co-morbid conditions are becoming more common (1).

Short sleep duration and poor sleep quality are also common in the pediatric population, especially in teens (3). Whether or not these two increasingly prevalent pediatric problems are physiologically linked is a topic of investigation and associations between sleep characteristics and obesity are beginning to be better understood. This review explores the links between obesity and sleep, outlining what is known about the relationships between sleep characteristics, obesity, and cardiometabolic risk factors in youth with a particular focus on sleep duration and obesity, and obstructive sleep apnea (OSA) and its metabolic consequences.

OPTIMAL SLEEP

It is clear that the majority of youth are sleeping less than is recommended for optimal health. In 2015 the National Sleep Foundation released recommendations for optimal sleep durations for healthy individuals (4). It is recommended that school-aged children, ages 6–13 years, should target 9–11 h of sleep per night, while adolescents, ages 14–17 years, should target 8–10 h of sleep per night. However, according to a 2006 survey conducted by the National Sleep Foundation, actual self-reported sleep times were significantly less than 9 h in all adolescent age groups and progressively declined from early to late adolescence (5). Adolescents ages 11–12 years reported a mean sleep duration of 8.4 h, while adolescents ages 17–18 years reported just 6.9 h of sleep per night. This shortened sleep duration was largely due to later bedtimes and the adolescents were self-aware of their deficient sleep. This is particularly important because adolescents are not meeting their physiological needs for restorative sleep. Adolescent "sleep need," defined as the amount of sleep recorded when adolescents are given the opportunity to sleep for 10 h, is approximately 9 h and remains unchanged throughout adolescence (5).

SLEEP DURATION AND OBESITY

Sleep duration has been associated with obesogenic behaviors and obesity prevalence in both adult and youth populations. A study of 240 toddlers from low-income families demonstrated that decreased mean sleep duration from 9.2 to 8.5 h was significantly associated with obesity (6). Moderate-to-vigorous physical activity, measured with an Actical accelerometer, was positively related to sleep duration. While sleep characteristics such as co-sleeping, room sharing, later sleep onset time, increased sleep latency, and night awakenings did not correlate with obesity, they were associated with decreased sleep duration and obesogenic behaviors including less physical activity and poorer diet quality.

The precise mechanisms linking rapid eye movement (REM) sleep and obesity are incompletely understood, but may include decreased sleeping metabolic rate, and endocrine changes associated with decreased leptin and increased ghrelin levels promoting increased food consumption (7). Sleep deprivation is associated with decreased insulin sensitivity via alterations of the hormonal milieu including cortisol, ghrelin, leptin, growth hormone, and glucose tolerance (8, 9). These hormonal changes cause alterations in energy regulation, unhealthy food choices, increased food consumption, decreased physical activity, and perhaps a reduction in non-exercise activity thermogenesis.

Decreased REM sleep is observed in individuals with short sleep duration, and has been a proposed mechanism for the link between short sleep duration and increased weight status. A study by Liu et al. involving 335 youth examined sleep stages using polysomnography and found significant differences in overweight children compared to normal-weight children, as they slept less, had reduced sleep efficiency, decreased REM sleep time, activity and density, and longer latency to the first REM episode (7). After adjustment for demographics, pubertal status, and confounding medical conditions, 1 h less of REM sleep was associated with an approximately 2-fold increased odds of being overweight, and 1 h less of REM sleep was associated with an approximately 3-fold increased odds of being overweight.

Leptin and ghrelin are key hormones involved in appetite regulation (10). In their review article, Van Cauter et al. discussed neuroendocrine control of food intake (11). In sleep-restricted as opposed to well-rested individuals, leptin levels are decreased while ghrelin levels are increased resulting in subjective hunger. The amount of sleep restriction varied among the reviewed studies. Much less is known about leptin and ghrelin secretion and operation in children. Recent studies have shown conflicting results. While one study showed short sleep duration was associated with lower leptin levels, the other revealed short sleep duration was associated with higher leptin levels (12). **Table 1** summarizes some proposed mechanisms for poor sleep and obesity and some obesogenic behaviors seen in those with poor sleep.

The associations between sleep duration and obesity are thought to be mediated, at least in part, by diet quality. Less sleep is consistently linked with unhealthy dietary habits including larger portion sizes, increased perceived hunger, higher calorie food choices, and increased food and sugar-sweetened beverage intake (13, 14). Cespedes et al. examined 1,046 parental reports of sleep and diet in children from infancy to mid-childhood (15). Sleep was measured using a calculated sleep score based on parental reported sleep duration. Diet was measured using the Youth Healthy Eating Index (YHEI). Chronic sleep insufficiency was associated with lower YHEI score. Looking independently at each factor, children with less sleep and a less healthy diet had a higher BMI z-score. It is not known whether or not these were causal associations.

A systematic review and meta-analysis of 17 observational, cohort, cross-sectional, and case-control studies from 9 countries found convincing evidence of the link between shorter sleep duration and childhood obesity (9). Sleep durations of less than 9 h (children 10 years of age or older), less than 10 h (children between 5 and 10 years of age), and less than 11 h (children less than 5 years of age) were associated with a 58% increased risk of being overweight or obese. Each hour increase in sleep duration was associated with a 9% reduced risk of being overweight or obese. Interestingly, the decreased sleep duration (defined by

TABLE 1 | Proposed mechanisms and obesogenic behaviors in poor sleep and obesity.

Proposed mechanisms	Obesogenic behaviors

• Evening Chronotype
  ○ With early wake time
  ○ Social jetlag
• Bedtime Shift
• Hormonal Alterations
  ○ Leptin
  ○ Ghrelin
	• Unhealthy Food Choice
	  ○ Sugar-sweetened beverages
	  ○ Fast food
	  ○ High calorie snacks
	  ○ Excessive portion sizes
	• Increased food consumption
	• Increased perceived hunger
	• Food preoccupation

each studies' own criteria) and obesity association appeared to be stronger for boys than girls, with an odds ratio of 2.50 vs. 1.24 respectively (9), although the reason for this gender variance is not known and data from studies are conflicting (16–18). Cappuccio et al. published a meta-analysis reviewing the association between sleep duration and obesity in both youth and adults (19). Results in children correlated with previous findings (9), with an increased pooled odds of 1.89 for a shortened sleep duration associated with obesity (19). This association was also observed in adults from 12 countries and in various age groups from adolescence through late-adulthood, showing their results had a similar effect across populations and age groups.

Modifiable factors presumed to cause decreased sleep duration include evening exposure to electronic media, early school start times, academic workload, and caffeine consumption (3, 20). A systematic review of 33 studies to investigate the relationship between short sleep duration and potential mechanisms such as dietary habits, physical activity or lack thereof, screen time, and hormonal effects including, alterations in leptin and ghrelin levels, found evidence for associations of sedentary behavior, unhealthy dietary patterns, and insulin resistance with shorter sleep duration (8). Insulin resistance was assessed using one randomized controlled trial, one prospective cohort study, and one cross-sectional study, two with a negative association and one with a U-shaped association between sleep duration and HOMA-IR (homeostatic model assessment of insulin resistance). Conclusions for other mechanisms of physical activity, screen time, changes in leptin and ghrelin levels were indeterminate, attributed to lack of current evidence and need for more rigorous research.

SLEEP QUALITY AND OBESITY

Other indicators of sleep quality beside sleep duration have also been implicated as risk factors for obesity, although data are sparse. The National Sleep Foundation identified indicators of sleep quality for all age groups as: sleep onset latency, number of awakenings lasting less than 5 min, wake time after sleep onset, and sleep efficiency (ratio of total sleep time to time in bed) (21). Part of a cross sectional study by Quick et al. of 1,252 college students across nine U.S. universities evaluated sleep quality by self-report using the Pittsburgh Sleep Quality Index and weight status using two categories, normal weight (BMI less than 25) and overweight or obese (BMI 25 or greater) (22). Poorer sleep quality was significantly associated with being overweight or obese with an odds ratio of 1.07 (95% CI 1.01–1.13).

Fatima et al. published the first systematic review and meta-analysis looking at associations between sleep quality using the Pittsburgh Sleep Quality Index and overweight and obesity in youth (23). Poorer self-reported sleep quality, defined as higher sleep onset latency, more sleep disturbances, recurrent awakenings, and lower sleep efficiency, was associated with a higher odds of being overweight or obese (odds ratio of 1.46, 95% CI of 1.24–1.72), independent of sleep duration.

However, some studies have not found an association between sleep quality measures aside from sleep duration and weight status in younger children (24–26). When sleep quality measures of sleep latency, wake after sleep onset, sleep duration, and sleep efficiency were collected using a parental-reported sleep diary and objectively using wrist actigraphy, no association was found between sleep quality and body mass index, body fat percentage, or waist circumference (26). Of note, there was a low prevalence of overweight and obese subjects in this study, with only 7% having a BMI in this range.

SLEEP CHRONOTYPE AND OBESITY

Adolescent preferred sleep patterns lead to decreased sleep times, both because their biologic circadian rhythm shifts toward later sleep and wake times resulting in a "late chronotype" and because of competing interests to complete schoolwork and socialize during evening and nighttime hours (3). Chronotype is the sleep timing of an individual or that individual's propensity to sleep during a particular time. Early adolescence and puberty are biologically linked to later bed time and wake time preferences, known as an evening-type circadian rhythm or evening chronotype. Pubertal adolescents and young adults have a slower escalation in "drive-to-sleep" or lower sleep pressure, compared with their prepubertal counterparts. As sleep onset is delayed by both biological and societal influences and wake times tend to be fixed, sleep deprivation accumulates.

"Bedtime shift" describes later bed times on weekends compared to weekdays and may also affect the circadian rhythm as the circadian clock cannot adapt quickly to shifts in sleep onset. Independent of sleep duration, greater bedtime shift is linked to obesity severity (14). Circadian rhythm disorder or delayed sleep-phase syndrome is associated with daytime sleepiness and can lead to napping after school, ending ultimately with difficulty sleeping at night. In 186 adolescents, ages 12–17 years, sleep quality assessed using the self-reported Insomnia Severity Index, was not related to a higher BMI z-score (14). However, there was a significant association between a higher BMI z-score and a later weekend bedtime, and a greater bedtime shift. Ivers-Landis et al. evaluated self and parent-reported dietary, sleep duration, and sleep regularity parameters (bedtime shift, wake-time shift, and sleep duration shift) among 315 overweight and obese adolescents (27). Late bedtime shift and wake-time shift were associated with increased sugar-sweetened beverage consumption and food preoccupation.

Both biological influence and social influences that encourage later sleep times and decreased sleep duration are associated with obesity. Social jetlag describes the alterations in one's chronotype due to social obligations such as school, work, or other social events. Roenneberg et al. assessed social jetlag and obesity in a database of 64,110 primarily central European subjects, ages 16–65 years, who had completed the Munich ChronoType Questionnaire (28). Social jetlag, defined as the difference between mid-sleep point on free days vs. work days, significantly increased the odds of being overweight by 3.3 (95% CI 2.512–4.334). The association between a late chronotype

and BMI was studied in 511 young adolescents, ages 11–13 years, in the United Kingdom by Arora and Taheri in a cross-sectional study (29). A definitely evening chronotype, identified by the lowest score category on the self-reported Morningness Eveningness Questionnaire, represented 15.3% of the sample size and was positively associated with BMI z-score ($p < 0.01$).

Hulsegge et al. compared diet quality and quantity between 7,173 adult day workers and 683 adult shift workers using cross-sectional general population data from the European Prospective Investigation into Cancer and Nutrition-Netherlands cohort (30). Dietary intake was assessed using a food frequency questionnaire and dietary quality was assessed using the Mediterranean Diet Score and WHO-based Healthy Diet Indicator. Although shift workers had a similar diet quality to day workers, their energy intake was higher by 56 kcal/day (95% CI of 10–101). Shift workers with five or more night shifts per month had the highest energy intake, taking in an additional 103 kcal/day (95% CI of 29–176) than day workers consumed. These results suggest high energy intake may contribute to the metabolic disturbances (30), such as increased body weight, elevated systolic blood pressures, dysglycemia, type 2 diabetes, dyslipidemia, and cardiovascular disease, which have been linked with shift work (31).

SLEEP, GLUCOSE METABOLISM, AND CARDIOMETABOLIC RISK

Current evidence indicates sleep disturbances contribute to alternations in glucose metabolism and increased cardiometabolic risk (32–34). Similar to adults, poor self-reported sleep quality in youth has been associated with components of the metabolic syndrome, including dyslipidemia, higher blood pressure, and markers of insulin resistance (35–39). Alterations in leptin, ghrelin, and cortisol levels and increased sympathetic nervous system activity are thought to contribute to an atherogenic lipid profile (40). Qian et al. proposed that sleep fragmentation alters lipid metabolism via its effect on elevated cortisol levels, increased systemic inflammation, increased food intake, and obesity (41). Increased sympathetic nervous system activity secondary to insufficient sleep contributes to hypertension (38). Moreover, deficient deeper stages of sleep (REM and slow-wave sleep) are associated with an elevated morning blood pressure in obese adolescents, independent of weight status (36). If poor sleep is contributing to elevated blood pressures and metabolic syndrome in youth, interventions should be pursued, as the cumulative effects of these risk factors in obese youth are significant.

In 2016, the first study was published that investigated the association between sleep deprivation and insulin sensitivity performed via hyperglycemic clamp in 81 adolescents (32). Those with sleep deprivation, defined as less than 8 h of sleep per night, had a lower clamp-derived insulin sensitivity index, compared to those with sufficient sleep, defined as 8 h or more.

Other pediatric studies indicate obese children and adolescents who report less than 9 h of sleep per night have higher fasting insulin and HOMA-IR levels, and lower high-density lipoprotein cholesterol (HDL-C) levels (42). Interestingly, a U-shaped distribution was observed, as those with intermediate sleep durations, 9–10 h for 10–13 year olds and 8–9 h for 14–15 year olds, had the lowest HOMA-IR, higher HDL-C, and lower aspartate aminotransferase (AST). Those with higher or lower sleep durations had higher HOMA-IR consistent with greater insulin resistance. This U-shaped distribution has been demonstrated for sleep duration and hemoglobin A1c (HbA1c) and glucose levels during an oral glucose tolerance test (OGTT) as well (33, 43).

OBSTRUCTIVE SLEEP APNEA IN OBESE ADULTS WITH TYPE 2 DIABETES

A strong association between type 2 diabetes and OSA is seen in overweight and obese adults (44). The Sleep AHEAD (Action for Health in Diabetes) Study is an ancillary study measuring the prevalence of OSA in participants in the Look AHEAD study, a 16-center prospective trial examining the effects of an intensive lifestyle program in overweight and obese adults with type 2 diabetes (44). The Sleep AHEAD study demonstrated an alarmingly high prevalence of undiagnosed OSA in this population at 86% and an elevated average apnea-hypopnea index (AHI) of 20.5 events per hour. The only significant predictor of OSA was a higher waist circumference, but subjects with a higher BMI were more likely to have severe OSA (44). A meta-analysis of prospective cohort studies to evaluate the association between OSA severity and the risk for type 2 diabetes was performed by Wang et al. and included 6 prospective studies with almost 6,000 adult patients (45). Included studies used only objective measurements for the diagnosis of OSA and found moderate (AHI 15 to less than 30) and severe OSA (AHI 30 or greater) was an independent risk factor for type 2 diabetes in adults. Another meta-analysis of prospective and retrospective cohort studies in adults to assess the association between OSA and metabolic syndrome involved 10 studies with just over 2,000 patients (46). This meta-analysis revealed OSA was a significant risk factor for components of the metabolic syndrome, specifically higher systolic blood pressure, lower HDL, higher LDL (low-density lipoprotein), and higher triglyceride levels.

Importantly, the Sleep AHEAD study focused on the effect of weight loss on OSA (47). The two intervention groups in Look AHEAD and Sleep AHEAD are Intensive Lifestyle Intervention (ILI) and Diabetes Support and Education (DSE). ILI gives participants specific and detailed recommendations for portion control, daily caloric intake limits, and a physical activity requirement. Whereas DSE provides education and support on diet, physical activity, and social support, but without specific behavioral strategies (47, 48). Studies looking at the effects of weight loss on OSA in obese adults with type 2 diabetes are promising for the ILI group, as this group lost significantly more weight and had a significant decrease in the AHI after 1 year (48). At 1 year, three times more participants in the ILI than the DSE group had total OSA remission and severe OSA prevalence was halved in the ILI group. This effect of improved AHI in the ILI group was sustained even over the long-term period of 4 years

despite a 50% regain in weight, suggesting the benefits of this program are more than just weight loss alone (47).

Proposed mechanisms for an association between OSA and dysglycemia are multifactorial. OSA can lead to activation of the sympathetic nervous system, increased leptin and ghrelin levels contributing to increased appetite and food intake, decreased adiponectin level, oxidative stress, inflammation and obesity which all ultimately contribute to insulin resistance (49, 50). The relationship between OSA and type 2 diabetes may be explained by the stage of sleep when apneas and hypopneas are occurring, as these episodes are longer and have greater oxygen desaturations during REM vs. non-REM sleep, subsequently leading to greater sympathetic nervous system activity (51). These alterations may have an effect on insulin release and activity as an altered sympathetic/parasympathetic balance may affect hormones involved in glucose regulation (52). In a cohort of 115 adult subjects, only the REM AHI was associated with an increased HbA1c (52).

OBESITY AND METABOLIC CONSEQUENCES OF OSA IN YOUTH

In youth, the association between OSA and type 2 diabetes is less clear (33). Previous studies have shown inconsistencies across studies in youth, with small sample sizes and pubertal effects on insulin sensitivity affecting measures. A recent systematic review and meta-analysis of 10 studies by Patinkin et al. attempted to clarify relationships between OSA and metabolic risk markers in youth by focusing on studies with exclusively adolescent participants (53). OSA in adolescence was associated with dyslipidemia, hypertension, and insulin resistance as measured by HOMA-IR, as in adults.

A few pediatric studies have evaluated OSA and glucose homeostasis in obese youth (54–57) and have provided preliminary evidence of an association between OSA and lower insulin sensitivity with increased fasting plasma insulin and glucose levels, independent of BMI (33). Despite similar degrees of obesity, adolescents with moderate or severe OSA (AHI 5 or greater), as opposed to mild or no OSA, had significantly higher HOMA-IR ($p = 0.0497$) and fasting insulin levels ($p = 0.037$) (58). Redline et al. reported a cohort study in which 70% of adolescents with sleep-disordered breathing (SDB) were overweight and 59% of them met criteria for metabolic syndrome, notably elevated blood pressure, LDL, and fasting insulin levels, again independent of BMI (57). Only 16% of overweight adolescents without SDB had metabolic syndrome and after adjustment for age, race, sex, and prematurity, adolescents with SDB had an increased odds of 6.49 (95% CI 2.52–16.70) for metabolic syndrome compared to overweight adolescents without SDB.

A study from Beijing, involving 558 participants ages 14–28 years, demonstrated that even youth with high-risk for OSA measured by self-reported Berlin Questionnaire score had higher cardiometabolic risk including dyslipidemia, higher glucose levels during OGTT, elevated liver enzymes, non-alcoholic fatty liver disease, metabolic syndrome, and worse echocardiographic parameters, specifically higher interventricular septum thickness, left ventricular end diastolic diameter, and left ventricular posterior wall thickness (59).

However, there are also pediatric studies which have not reported a link between OSA and increased markers of metabolic risk. Erdim et al. did not find an association between OSA measured with overnight polysomnography and metabolic syndrome in 104 obese adolescents (60). AHI was 1 or greater in 47.2% of youth without metabolic syndrome and in 49% of youth with metabolic syndrome. In an attempt to focus on the association of metabolic syndrome and OSA in obese adolescents by minimizing confounding factors, they uniquely excluded patients with grade 3 or 4 adenoidal or tonsillar hypertrophy, a known common cause of OSA. Narang et al. found that intermittent nocturnal hypoxia, not OSA measured by AHI was associated with a higher fasting insulin level, more insulin resistance as measured by HOMA-IR, higher HbA1c, and increased AST and ALT after correction for waist-to-height ratio (61). These studies used different criteria for evaluating metabolic risk and therefore, larger studies with uniform outcome measures are needed.

A prospective, cross-sectional study by Hannon et al. of 57 obese adolescents with either normal glucose tolerance, dysglycemia, or type 2 diabetes showed those with dysglycemia or type 2 diabetes tended to have a higher AHI, but no linear relationship between glycemia and AHI was established (62). Another prospective, cross-sectional study by Shalitin et al. compared 11 obese youth with type 2 diabetes compared to 30 obese youth without diabetes who were BMI-SDS matched in order to evaluate the frequency and severity of OSA between these groups (63). The age range of youth for both groups was 6–21 years and mild OSA was defined as an AHI greater than 1, while moderate to severe OSA was defined as an AHI 5 or greater. There was no significant difference in the frequency or severity of OSA between obese patients with type 2 diabetes and those with normoglycemia. The power of these relatively small studies to detect a significant association has been limited. Nevertheless, if there is an association between OSA and poor glycemic outcomes and type 2 diabetes in youth, this would be important for intervention and prevention efforts in these obese youth. This calls for more support for large pediatric studies to clearly delineate the association between OSA and dysglycemia in obese youth. **Table 2** includes suggested areas in need of further research, especially in youth.

TABLE 2 | Areas for future research in sleep and obesity in youth.

- Sleep and Dyslipidemia
- Mechanisms to Determine Causality between Sleep and Obesity
- Chronotype and Obesity
- Obesity and risk for Type 2 Diabetes
- OSA and risk for Type 2 Diabetes
- Sleep Interventions to Improve Obesity and Cardiometabolic Risk

OSA AND TREATMENT EFFECT

A systematic review of outcomes of OSA treatment in obese youth showed that obese children are significantly more likely to have persistent OSA after adenotonsillectomy than normal-weight youth and consistent with results in adults, either behavior or surgical weight loss, significantly improves OSA in obese youth (64). However, studies are few and laden with limitations, demonstrating the need for more research into weight loss as a treatment option. Positive airway pressure is also effective, but adherence is a major challenge (64). A systematic review and meta-analysis of six studies assessed the effects of continuous positive airway pressure (CPAP) in adult patients with type 2 diabetes and OSA (65). Results showed improved insulin sensitivity, but no decrease in BMI or HbA1c level with at least 3 months of CPAP. Another adult study summarizing three randomized controlled trials with 149 total patients showed that CPAP withdrawal in patients with optimal compliance was associated with a return of OSA and clinically significant increases in blood pressure (66). In the CPAP withdrawal group compared to the CPAP continuation group, office systolic blood pressure, home systolic blood pressure, office diastolic blood pressure, and home diastolic blood pressure all significantly increased. In adults, improved CPAP adherence that involves more coverage of REM sleep, can improve HbA1c (51, 52).

SLEEP INTERVENTIONS AND HEALTH IN YOUTH

Although the association between poor sleep and obesity is clear, causation has yet to be determined. Preliminary studies in youth show some evidence that improved sleep may improve obesity risk (67). In fact, interventions such as earlier bedtimes, later wake times, and other healthy sleep behaviors could be low-cost (9). A pilot study using a sleep hygiene program involving 33 adolescents showed improvement in self-reported sleep quality and BMI z-scores, but not sleep duration measured by actigraphy (68). Sleep quality was measured using participant or parent questionnaires (Adolescent Sleep Hygiene Scale, Pittsburgh Sleepiness Scale, Sleep Disturbance Scale for Children, and Pediatric Daytime Sleepiness Scale). BMI z-scores decreased significantly from a baseline mean of 0.79 (*SD* 1.18) to a post-intervention mean of 0.66 (*SD* 1.19). While preliminary studies are showing potential, well-designed randomized controlled trials to assess if sleep interventions ultimately improve obesity and cardiometabolic health in youth are warranted.

CONCLUSION

Poor sleep and obesity are concurrent epidemics in youth. Insufficient sleep duration and poorer sleep quality are associated with a greater BMI and markers of cardiometabolic risk, including insulin resistance, dyslipidemia, and higher blood pressure in youth. Obese youth are more likely to have OSA linked with cardiometabolic risk, but a clear association between OSA and type 2 diabetes in youth has not been established. Whether sleep interventions can improve pediatric obesity and cardiometabolic health in youth is yet to be determined.

AUTHOR CONTRIBUTIONS

AG and TH contributed to concept and outline of the manuscript. AG wrote the first draft and sections of the manuscript. TH revised manuscript critically for important intellectual content. AG and TH contributed to manuscript revision, read, and approved submitted version. AG and TH agree to be accountable for all aspects of the work.

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Determinants of Slow-Wave Activity in Overweight and Obese Adults: Roles of Sex, Obstructive Sleep Apnea and Testosterone Levels

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Background: Slow-wave activity (SWA) in non-rapid eye movement (NREM) sleep, obtained by spectral analysis of the electroencephalogram, is a marker of the depth or intensity of NREM sleep. Higher levels of SWA are associated with lower arousability during NREM sleep and protect against sleep fragmentation. Multiple studies have documented that SWA levels are higher in lean women, compared to age-matched lean men, but whether these differences persist in obese subjects is unclear. Obstructive sleep apnea (OSA), a condition associated with obesity, is more prevalent in men than in women. Sex differences in SWA could therefore be one of the factors predisposing men to OSA. Furthermore, we hypothesized that higher levels of testosterone may be associated with lower levels of SWA.

Objective: The aim of the current study was to identify sex differences in the determinants of SWA in young and middle-aged overweight and obese adults.

Methods: We enrolled 101 overweight and obese but otherwise healthy participants from the community (44 men, 57 women) in this cross-sectional study. Participants underwent an overnight in-laboratory polysomnogram. The recordings were submitted to sleep staging and spectral analysis. Sex differences and the potential contribution of testosterone levels were evaluated after adjusting for age, body mass index and race/ethnicity.

Results: OSA was present in 66% of men and in 44% of women. After adjustment for differences in age, race/ethnicity and BMI, the odds ratio for OSA in men vs. women was 3.17 (95% CI 1.14–9.43, $p = 0.027$). There was a graded inverse relationship between the apnea-hypopnea index (AHI) and SWA in men ($\beta = -0.21$, $p = 0.018$) but not in women ($\beta = 0.10$, $p = 0.207$). In a multivariate regression model, higher testosterone levels were independently associated with lower SWA in men after controlling for age, race/ethnicity and apnea-hypopnea index ($\beta = -0.56$, $p = 0.025$).

Conclusion: Increasing severity of OSA was associated with significant decrease in sleep intensity in men but not in women. Higher testosterone levels were associated with lower sleep intensity in men. Men with higher testosterone levels may therefore have lower arousal thresholds and higher ventilatory instability in NREM sleep, and be at greater risk of OSA.

Keywords: obstructive sleep apnea, sex differences, slow wave sleep, slow wave activity, delta activity, testosterone, spectral analysis

INTRODUCTION

Slow-wave activity (SWA) in non-rapid eye movement (NREM) sleep, also known as delta activity, is a marker of the depth or intensity of NREM sleep. SWA may be quantified by spectral analysis of the electroencephalogram (EEG) in the low frequency range typical of slow waves (0.75–4.5 Hz) (1, 2). SWA measurements are highly reproducible from night to night in a given individual (3–5). Higher levels of SWA are associated with lower arousability during NREM sleep and protect against sleep fragmentation due to external or internal disturbances (6). Multiple studies have documented that SWA levels are higher in women, compared to age-matched men (7–9) but the majority of the findings were obtained in lean adults.

Observational studies have reported significant transient reduction of obstructive sleep apnea (OSA) severity during slow wave sleep (SWS), the deepest stage of NREM sleep, which is associated with higher levels of SWA (10, 11). Rigorous mechanistic studies examining the neuromechanical properties of the upper airway during NREM sleep have shown that the upper airway is indeed less collapsible during SWS (12, 13). A higher arousal threshold during SWS, which is partly determined by the intensity of NREM sleep, i.e., the level of SWA, has also been recognized as one of the factors leading to OSA improvement during SWS (14, 15).

Estimations of OSA prevalence have typically found that the disorder is much more common in men than in women, particularly in young and middle-aged adults (16, 17). Obesity is an important and well-recognized risk factor for OSA. However, women with OSA are typically heavier than men when matched for OSA severity (18–20). Thus, being a woman appears to offer a relative protection against the development of OSA, even in the presence of more severe obesity. The reasons for this sex disparity in OSA risk have not been clearly identified, and may lie in anatomical, neuronal, and/or hormonal differences (21). In particular, whether sex differences in SWA are also present in overweight and obese adults and are associated with differences in OSA presence and severity has not been systematically evaluated.

The potential role of testosterone levels, which are much lower in women than in men, in the regulation of SWA and in the risk of OSA is an important related question. Testosterone levels are known to be modulated by sleep duration, sleep restriction and sleep quality in men (22–24). Several small studies have examined the impact of OSA on testosterone levels in men and some have reported decreased testosterone concentrations in men with OSA compared to controls (25–27). On the other hand, multiple reports of exogenous testosterone administration triggering or worsening OSA have been published (28–32). However, it should be noted that participants received supraphysiologic doses of testosterone in all of these studies except one (32). In the latter study, the worsening of OSA severity was only transient (32). Conversely, pharmacologically induced reduction of testosterone in healthy men has been shown to increase breathing stability during NREM sleep, potentially leading to a reduction in the risk of OSA (33, 34), while a small study in premenopausal women demonstrated a decrease in breathing stability after <2 weeks of transdermal testosterone administration resulting in male testosterone levels (35).

The aim of the present study was therefore to examine sex differences in sleep architecture, assessed by visual scoring of all-night polysomnographic (PSG) laboratory recordings and quantitative EEG analysis, and to identify factors associated with SWA in overweight and obese men and women, with and without OSA. Additionally, we explored the possible contribution of endogenous testosterone levels to individual differences in SWA in both men and women.

PARTICIPANTS AND METHODS

Subjects aged 20–50 years with body mass index (BMI) >25 kg/m^2 were recruited from the community between 2008 and 2014 using written advertisements inviting participation in research studies on sleep and metabolism. Sleep complaints or symptoms of OSA were not used as selection criteria for the study. Exclusion criteria were: self-reported habitual sleep duration of <6 h per night or more than 10 h per night, chronic insomnia, any sleep disorder other than OSA, any prior or current treatment for OSA (upper airway surgery, CPAP therapy, oral appliances or supplemental oxygen), history of substance abuse, hypnotic or psychotropic medications, tobacco use, caffeine intake above 300 mg per day, alcohol intake above 10 drinks per week, pregnancy, hormonal therapy, liver disease, renal insufficiency, heart failure, cancer, chronic infectious diseases, neurological or psychiatric diseases, diabetes, shift work within the last 3 months and travel across more than two time zones in the 4 weeks before the sleep recording. All female participants were premenopausal and were not taking hormonal contraceptives.

Study participants had a physical examination, and a complete medical history was obtained. Height and weight were measured. Self-reported race/ethnicity was recorded. One hundred and thirty participants underwent an overnight in-laboratory PSG with a minimum recording time of 7.5 h. Twenty nine subjects were excluded from analysis due to artifacts in the EEG ($n = 22$), total sleep duration <5 h ($n = 5$) or recording missing from server ($n = 2$). EEG recordings devoid of significant artifacts and with total sleep time of at least 5 h were obtained in 101 participants (44 men and 57 women). The following morning, fasting blood samples were taken to measure total and free testosterone and sex-hormone binding globulin.

Assays

Plasma total testosterone was measured by a direct RIA kit (Coat-A-Count; Siemens Medical Solutions USA, Inc; Malvern, PA, USA) that has been validated against a liquid chromatography/tandem mass spectrometry method (36) with functional sensitivity of 10 ng/dl and normal range of 312–1240 ng/dl in men and 19–70 ng/dl in women. Total testosterone values were not obtained in 2 men and 1 woman. The plasma free testosterone concentration was computed as the product of total testosterone and percentage free testosterone, which was determined directly in plasma by competitive protein-binding assay with a sensitivity of 3 pg/ml (37, 38). Free testosterone levels were not obtained in 7 men and 1 woman. Sex hormone binding globulin (SHBG) was measured by an in-house assay based on a competitive protein binding procedure (37, 38). Intra-assay variation coefficients for testosterone, free testosterone and SHBG were 6, 6, and 19%, respectively, in men, and 12, 13, and 14%, respectively, in women.

Sleep Analysis

Sleep recordings were performed in the laboratory using a digital EEG acquisition system (Neurofax EEg-1100 A, Nihon Kohden, Tokyo, Japan). Lights-off time was tailored to match self-reported habitual bedtime (± 1 h) and total recording time was at least 6 h. Surface electrodes were used to collect the EEG signals and the acquisition montage included, in addition to a vertex central referential lead and one ground lead, two central EEG leads (C_3 and C_4), two occipital leads (O_1 and O_2), two mastoids leads (A_1 and A_2), one vertical and one horizontal electro-oculogram (EOG), one bipolar submental chin electromyogram (EMG), and one bipolar electrocardiogram (ECG). The presence or absence of OSA was evaluated by measuring oronasal airflow signal by thermal flow sensor and nasal pressure transducer, respiratory effort by thoracic and abdominal piezoelectric belts or resistance inductive plethysmography and arterial oxygen saturation by finger pulse oximetry. Limb movements were also recorded from one bipolar tibial EMG to exclude individuals with movement disorders. Polysomnographic recordings were visually scored in 30-s epochs by an experienced professional sleep technologist considering stages W, N1, N2, N3, and R in adherence with standardized criteria (39). Respiratory events, periodic limb movements and microarousals were scored according to established criteria (39, 40). The apnea-hypopnea index (AHI) was calculated as the total number of obstructive apneas and hypopneas per hour of sleep. Apneas were defined as total cessation of airflow for at least 10 s if respiratory effort was present. Hypopneas were defined as a decrease in nasal pressure signal of ≥30% of baseline, which was associated with either a ≥3% desaturation or an arousal. The presence of OSA was defined by an AHI ≥5 events/h. The microarousal index was defined as the number of microarousals per hour of sleep. The oxygen desaturation index (ODI) was defined as the number of oxygen desaturations ≥3% per hour of sleep.

During acquisition, all EEG signals were filtered between 0.3 and 35 Hz and digitally sampled and stored at 200 Hz with a 16-bit quantization range. Power spectral analysis of the EEG was performed on two central leads (C_3, C_4) re-referenced to the linked mastoids references (A_1 and A_2). All analyses were performed directly on the original recording files without data format conversion using the PRANA Software Suite (http://www.phitools.com, Strasbourg, France). After removal of muscular, ocular and movement artifacts by automatic and visual inspection, a fast Fourier transform using a 50% overlap between consecutive 4-s elementary windows was computed following the application of a Hanning taper and resulted in a spectral resolution of 0.25 Hz. The spectra of all elementary windows overlapping with artifacts were discarded prior averaging of the elementary spectra on a 30-s epoch basis in order to match the sleep/wake stage scores. When more than 50% of the elementary spectra windows were contaminated, averaging was skipped and the corresponding time series intervals replaced by missing vales in order to preserve continuity in the time series. Absolute power in the delta, theta, alpha and sigma frequency bands (0.75–4.5 Hz, 4.5–8.5 Hz, 8.5–12 Hz, and 12.5–15 Hz, respectively) were calculated by summing up powers of all frequency bins within each band, the lower bin being included and the upper one excluded. Finally, an average of spectral power in each frequency band was calculated using only the 30-epochs from NREM sleep during the first 6 h of sleep. Slow-wave activity in NREM sleep is equivalent to delta power in NREM sleep.

For illustrative purposes, the durations of NREM-REM cycles were normalized to account for individual differences, with each individual NREM period subdivided into 50 equal time bins and each REM period into 20 time bins, as previously described (41). NREM-REM cycles were defined according to the criteria of Feinberg and Floyd (42) and visually verified. For subjects with REM latency >120 min, the termination of the first cycle was identified as the first nadir of SWA. Three subjects had 3 NREM-REM cycles during the night of PSG, while all others had four cycles or more.

Statistical Analysis

Statistical analysis was performed using JMP version 8.0.2 (S.A.S Institute Inc., Cary, NC, USA) and confirmed with SPSS Statistics v20. All group data are expressed as means ± SEM for normally distributed variables, or median (interquartile range) for non-normally distributed variables. Transformation of raw data to achieve normal distribution was performed whenever

appropriate prior to statistical analysis. In particular, BMI, AHI and EEG spectral powers were transformed in all analyses using the natural log (Ln). Sex differences for demographic and hormonal characteristics were assessed by Student's *t*-test for continuous variables and chi-square test for categorical variables. Comparisons were considered statistically significant at $p<0.05$. Multivariate linear regression models adjusting for age, BMI, and race/ethnicity were fitted to examine sex differences in sleep stages, characteristics of OSA and spectral power in the different EEG frequency bands as well as the interaction sex *x* OSA and sex *x* AHI. When interactions were statistically significant, analyses were repeated separately in men and women. To identify the possible contribution of plasma levels of testosterone and SHBG to the observed sex differences, multivariate linear regression models including the hormonal variable in addition to age, BMI, AHI and race/ethnicity, were fitted separately in men and women.

RESULTS

Demographic Characteristics and OSA Prevalence

In the present cohort of 101 participants, women ($n = 57$) were significantly more obese than men ($n = 44$) (BMI 38.0 ± 1.1 vs. 34.4 ± 0.9 kg/m^2, $p = 0.021$) and more often African-American (74 vs. 50%, $p = 0.022$). The demographic characteristics of male and female participants, without and with OSA, are summarized in **Table 1**. Irrespective of sex, participants with OSA were significantly older than those without OSA (37 ± 1 vs. 31 ± 1 years, $p < 0.0001$). Consistent with previous reports (18–20), women with OSA had a higher BMI than men with OSA (median [IQR]: 38.7 [33.1–44.9] vs. 34.3 [32.1–36.8] kg/m^2, $p = 0.007$). Given these differences in demographics, subsequent analyses were performed adjusting for age, BMI and race/ethnicity. After adjustment, the odds ratio for OSA in men as compared to women was 3.17 (95% CI 1.14–9.43, $p = 0.027$).

Men with OSA showed a trend for higher total testosterone (422[358-551]vs. 394[318-63] ng/dl, $p = 0.095$) and SHBG (13[10-16] vs. 9[7-13]nM, $p = 0.070$) concentrations compared to men without OSA, but free testosterone levels did not differ between these two groups. No difference in total testosterone, free testosterone or SHBG levels were found between women with or without OSA.

Macro-Architecture of Sleep

Polysomnographic variables are presented in **Table 2**. Median (IQR) recording time in the 101 participants was 8 h 06 min (8 h 01–8 h 30). After adjustment, men with OSA had less N3 slow wave sleep compared to men without OSA (11[2-37] vs. 33[7-82]min, $p = 0.069$), although the difference did not reach statistical significance. Moreover, men with OSA had significantly less N3 slow wave sleep than women with OSA (11[2-37] vs. 50[25-65] min, $p < 0.001$). In contrast, in women, N3 slow wave sleep duration was not influenced by the presence of OSA (61[28-82] min in women without OSA vs. 50[24-85] min in women with OSA, $p = 0.822$). Finally, compared to women without OSA, women with OSA had less REM sleep (90 ± 6 vs. 106 ± 5 min, $p = 0.039$).

Sex Differences in Characteristics of OSA

Figure 1 illustrates sex differences in the characteristics of OSA for the entire cohort, after adjusting for age, BMI and race/ethnicity. Men and women had similar total and NREM AHI. However, men had a significantly greater AHI in REM sleep compared to women (24.4 ± 1.3 vs. 8.5 ± 1.3 events/hour, $p = 0.004$), as well as higher total ODI (4.4 ± 1.3 vs. 1.3 ± 1.3 events/hour, $p = 0.005$), NREM ODI (2.1 ± 1.5 vs. 0.6 ± 1.5 events/hour, $p = 0.041$), and REM ODI (7.7 ± 1.5 vs. 2.0

TABLE 1 | Demographics and hormonal values of the study population.

	Women no OSA	Women OSA	*p*-value	Men no OSA	Men OSA	*p* value
n	32	25		15	29	
Age (years)	31±1	35±1	**0.006**	30±2	38±1	**<0.0001**
BMI (kg/m^2)	35.6 (29.9–41.2)	38.7 (33.1–44.9)	**0.042**	31.9 (27.7–38.3)	34.3 (32.1–36.8)	0.501
Race/Ethnicity (%)			0.391			0.828
Non Hispanic	22	16		40	41	
White African	75	72		53	48	
American Hispanic	3	12		7	7	
Asian	0	0		0	3	
Total testosterone (ng/dl)	33 (25–50)	33 (22–49)	0.708	394 (318–463)	422 (358–551)	*0.095*
Free testosterone (pg/ml)	8 (6–11)	9 (5–12)	0.847	139 (117–169)	139 (116–166)	0.636
SHBG (nM)	26 (17–34)	22 (12–26)	0.160	9 (7–13)	13 (10–16)	*0.070*

Data are given as mean ± SEM for normally distributed continuous variables, and median (interquartile range) for non-normally distributed continuous variables. For non-normally distributed variables, the results of the statistical analysis reported were obtained after appropriate transformation to normality. To convert total testosterone to nmol/l, multiply by 0.0347; to convert free testosterone to pmol/l, multiply by 3.47.

BMI, body mass index; OSA, obstructive sleep apnea. Bold values indicate statistically significant p-values. Italic values indicate trends for statistical significance of p-value.

TABLE 2 | Polysomnographic variables.

	Women no OSA	Women OSA	*p*-value		Men no OSA	Men OSA	*p*-value		Sex × LnAHI interaction *p* value
			Unadj.	Adj.*			Unadj.	Adj.*	
Sleep period time (SPT, min)	465 (453–477)	468 (447–477)	0.587	0.749	490 (453–502)	471 (437–498)	0.721	0.994	0.979
Total sleep time (TST, min)	445 (427–460)	433 (399–455)	*0.053*	0.403	454 (410–491)	433 (404–470)	0.200	0.677	0.638
Sleep efficiency (%)	92 (87–97)	86 (84–93)	**0.041**	0.159	94 (86–96)	88 (83–94)	0.123	0.968	0.764
Sleep latency (min)	15 (11–22)	17 (10–26)	0.692	0.550	14 (7–22)	14 (10–25)	0.445	0.193	0.165
N1 (min)	24 (20–33)	28 (17–38)	0.997	0.707	31 (11–33)	40 (22–51)	**0.012**	0.108	**0.043**
N2 (min)	257 ± 7	258 ± 8	0.939	0.269	278 ± 9	269 ± 8	0.505	0.244	0.813
N3 (min)	61 (28–82)	50 (24–65)	0.563	0.822	33 (7–82)	11 (2–37)	**0.040**	*0.069*	*0.092*
REM (min)	106 ± 5	90 ± 6	**0.037**	**0.039**	106 ± 9	101 ± 6	0.619	0.811	0.424
Wake after sleep onset (min)	18 (9–34)	34 (19–59)	**0.023**	0.252	11 (6–32)	26 (11–53)	0.103	0.548	0.383

*Data are given as mean ± SEM for normally distributed continuous variables, and median (interquartile range) for non-normally distributed continuous variables. For non-normally distributed variables, the results of the statistical analysis reported were obtained after appropriate transformation to normality. *Adjusted for age, LnBMI and race/ethnicity.*
Sleep period time: interval between sleep onset and final morning awakening. Total sleep time: sleep period time minus duration of wake after sleep onset. Sleep latency: time from lights off to sleep onset. Sleep efficiency: total sleep time as percentage of the time allocated to sleep (i.e., the interval between lights off and lights on); N1, NREM stage 1; N2, NREM stage 2; N3, slow-wave sleep; REM, rapid eye movement sleep. Bold values indicate statistically significant p-values. Italic values indicate trends for statistical significance of p-value

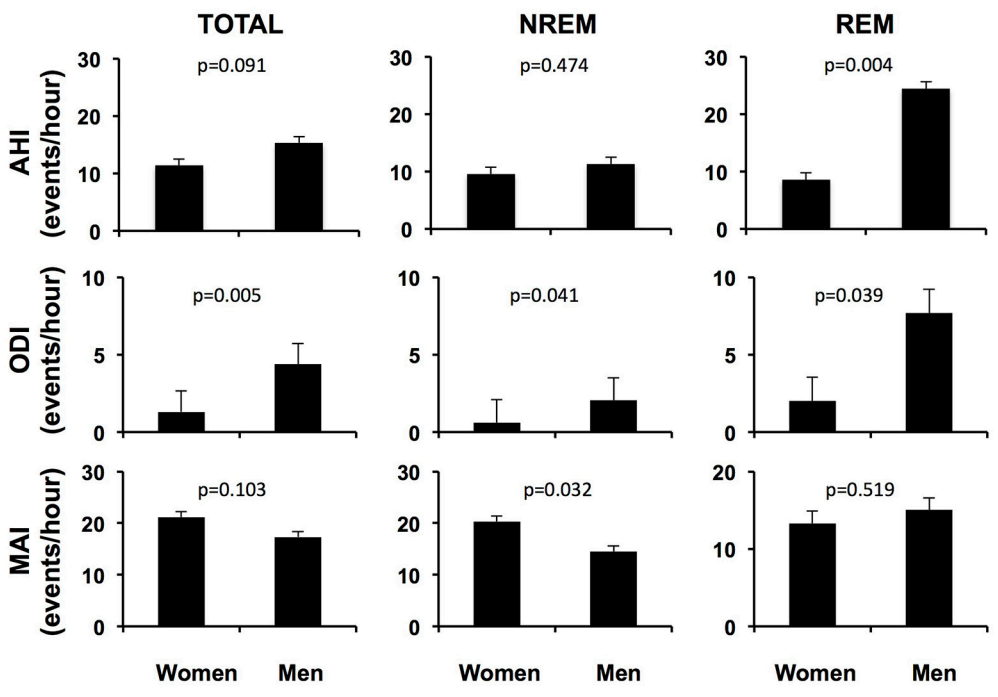

FIGURE 1 | Adjusted respiratory variables and microarousal index of men and women with OSA over the entire night, in NREM sleep and in REM sleep. *AHI* apnea-hypopnea index; *ODI* oxygen desaturation index; *MAI* microarousal index; *NREM* non rapid eye movement sleep; *REM* rapid eye movement sleep. Adjusted for age, BMI and race/ethnicity.

± 1.6 events/hour, $p = 0.039$). The percentage of sleep time spent below 90% oxygen saturation (T90%) was also higher in men than women (1.04 ± 2.34 vs. 0.07 ± 1.08%, $p = 0.005$), in both REM and NREM sleep. In contrast to variables quantifying hypoxemia, the overall microarousal index was similar in men and women. Although the microarousal index during NREM sleep was higher in women, it was still within the normal range.

Micro-Architecture of Sleep

Figure 2 illustrates the unadjusted profiles of SWA and theta activity normalized by NREM-REM cycles, in men and women with and without OSA. Profiles of alpha and sigma activity were similar in men and women, with and without OSA (not shown). In unadjusted analyses, the presence of OSA was associated with a 34% lower NREM SWA in men (non OSA 441 ± 51, *vs.* OSA 289 ± 24 μV^2; $p = 0.007$) but did not significantly impact NREM SWA in women (non OSA 479 ± 37, *vs.* OSA 521 ± 53 μV^2; $p = 0.582$). As summarized in **Table 3**, multivariate linear regression confirmed a sex-specific impact of OSA on NREM SWA as reflected by a significant sex *x* LnAHI interaction ($p = 0.001$). For NREM theta activity, a spectral power in an intermediate frequency range that is often contaminated by SWA, a trend for a greater impact of OSA in men than in women ($p = 0.066$ for sex *x* LnAHI interaction) was also present. In contrast, OSA had no significant influence on NREM alpha and sigma activity in either men or women, two power bands generated by neuronal systems distinct from those responsible for SWA.

To facilitate the interpretation of the impact of OSA severity on NREM SWA, we also fitted a linear regression model replacing LnAHI with AHI tertiles derived from the total of 101 subjects (first tertile or $T1_{AHI} < 2.3$; $T2_{AHI}$ 2.3–11.7; $T3_{AHI} > 11.7$ events/hour) after adjusting for age, race/ethnicity and BMI. A significant negative linear trend for adjusted SWA was clearly present in men (**Figure 3**; $\beta = -0.206$, $p = 0.018$) such that men in the highest tertile of AHI had SWA levels less than half of those found in men in the lower tertile of AHI. In contrast, in women there was no significant association between AHI tertiles and adjusted SWA ($\beta = 0.104$, $p = 0.207$). Similar results were obtained when subdividing the participants according to clinical cut offs of OSA severity (no [AHI < 5], mild [AHI 5–15] and moderate-severe OSA [AHI > 15], data not shown).

Determinants of NREM SWA in Men and Women

Because of the significant sex *x* LnAHI interaction present for SWA, we examined the determinants of NREM SWA separately in men and women, and explored the potential roles of circulating total and free testosterone levels as well as SHBG. The upper part of **Table 4** describes the results of four multivariate linear regression models predicting NREM SWA in men. Model 1 includes demographic characteristics only, and reveals that age ($p = 0.004$) and race/ethnicity ($p = 0.001$), but not BMI, are significantly associated with SWA levels in this cohort of 20–50 years old overweight and obese men. Introducing AHI (model

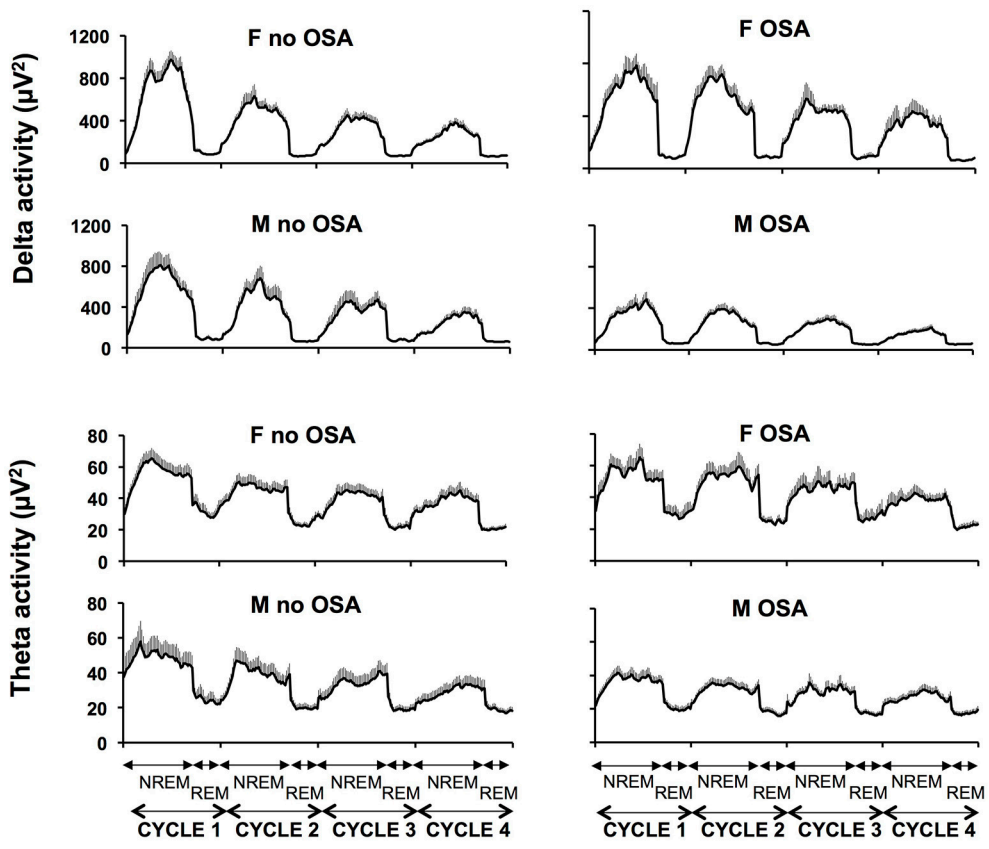

FIGURE 2 | Unadjusted mean profiles (+SEM) of absolute SWA (4 upper panels) and theta activity (4 lower panels) during the first four NREM-REM cycles in women without and with OSA (upper panels) and men without and with OSA (lower panels). *SWA* slow wave activity; *NREM* non rapid eye movement sleep; *REM* rapid eye movement sleep.

TABLE 3 | Impact of demographic characteristics and OSA on EEG spectral activity in NREM sleep (first 6 h of sleep).

	SWA (μV^2)		**Theta activity (μV^2)**		**Alpha activity (μV^2)**		**Sigma activity (μV^2)**	
R	0.654		0.483		0.322		0.344	
r^2	0.428		0.233		0.112		0.118	
r^2 adjusted	0.392		0.184		0.055		0.062	
p-value	**<0.0001**		**0.0003**		*0.075*		*0.060*	
	β (95% CI)	***p*–value**	**β (95% CI)**	***p*-value**	**β (95% CI)**	***p*-value**	**β (95% CI)**	***p*-value**
Sex	0.191 (0.103, 0.280)	**<0.0001**	0. 198 (0.087, 0.309)	**0.0006**	0. 116 (−0.013, 0.0.245)	*0.078*	0. 010 (−0.026, 0.224)	0.119
Age	−0.017 (−0.031, −0.002)	**0.024**	0.004 (−0.014, 0.022)	0.666	−0.001 (−0.021, 0.021)	0.981	−0.012 (−0.032, 0.008)	0.260
LnBMI	0.188 (−0.275, 0.652)	0.422	0.122 (−0.462, 0.706)	0.679	0.049 (−0.711, 0.757)	0.888	0.014 (−0.643, 0.671)	0.966
Race/Ethnicity	−0.237 (−0.324, −0.149)	**<0.0001**	−0.208 (−0.318, −0.098)	**0.0003**	−0.166 (−0.294, −0.038)	**0.012**	−0.207 (−0.330, −0.083)	**0.001**
LnAHI	−0.057 (−0.126, 0.012)	0.105	−0.008 (−0.096, 0.079)	0.851	0.018 (−0.084, 0.119)	0.203	0.012 (−0.086, 0.110)	0.816
Sex *x* LnAHI		**0.001**		*0.066*		0.119		0.798

β coefficients and p-values from multiple regression models with spectral parameters as dependent variable and sex, age, LnBMI, race/ethnicity, LnAHI and sex x LnAHI interaction as covariates. The results of the statistical analysis reported were obtained after log-transformation of spectral frequency bands.
NREM, non-rapid eye movement sleep; SWA, slow-wave activity; BMI, body mass index; AHI, apnea-hypopnea index. Bold values indicate statistically significant p-values. Italic values indicate trends for statistical significance of p-value

2) improved the percentage of variance (r^2) accounted for by the model; the association with age was no longer significant, while AHI ($p = 0.002$) was independently associated with SWA. The substitution of total testosterone levels for AHI (model 3) increased the r^2 further, and testosterone levels significantly and negatively correlated with SWA ($p = 0.005$). Since we found no association with BMI in models 1–3, BMI was dropped for the last model to maintain statistical power and we examined simultaneously the contributions of AHI and testosterone levels. SWA remained strongly negatively associated with testosterone ($p = 0.025$), while the strength of the association with age and AHI was considerably reduced. The left lower part of **Figure 3** illustrates the association between increasing tertiles of total testosterone levels and SWA in men, after adjusting for age, BMI and race/ethnicity (model 3).

Similar associations with testosterone were found when free, rather than total concentrations were used. After controlling for age, race/ethnicity, and AHI (model 4), free testosterone level was significantly associated with SWA in men ($p = 0.012$, data not shown). There were no significant associations with SHBG levels in any of the models.

The lower part of **Table 4** describes the results of the same four multivariate linear regression models predicting SWA in women. An association of NREM SWA with age and race/ethnicity was observed in all models, while no association between SWA and AHI was present. Testosterone levels were not a significant predictor but the beta coefficient was negative, similar to findings in men, and the significance level suggests the possibility that a trend may be found in a larger sample. The relationship between SWA and increasing tertiles of total testosterone is illustrated for the female participants in the left lower panels of **Figure 3**.

Of note, while model 4 accounts for nearly 50% of the inter-individual variability in SWA among men, the same covariates account for <20% of the variability among women. In this cohort of overweight and obese but otherwise healthy men and women ages 20–50 years, the degree of adiposity as assessed by the BMI was not associated with any measure of NREM sleep intensity and depth.

DISCUSSION

In this study, we performed a comprehensive and rigorous laboratory assessment of sleep quality in overweight and obese men and women recruited from the community. Our sample was not selected based on prior diagnosis or symptoms of OSA. We observed a high prevalence of OSA, which was present in two-thirds of the men and nearly half of the women. After adjustment for age, BMI and race/ethnicity, the odds ratio of having OSA was 3 times higher in men as compared to women. Our results also reveal that the sex differences in duration of SWS (43–46) and in intensity of NREM sleep by spectral analysis (8, 47) previously reported in lean populations are also present in overweight and obese adults with OSA. We found a differential impact of OSA on the intensity of NREM sleep in men vs. women. Indeed, after adjusting for potential confounders, there was a graded inverse relationship between the severity of OSA and SWA in men, but not in women. A previous cross-sectional study with a small sample size has shown that in men, the presence of OSA is associated with lower SWA (48), likely because of fragmentation and arousals induced by respiratory events. In women, the impact of OSA on SWA has not been previously characterized. A possible explanation for the observed sex differences in the impact of OSA

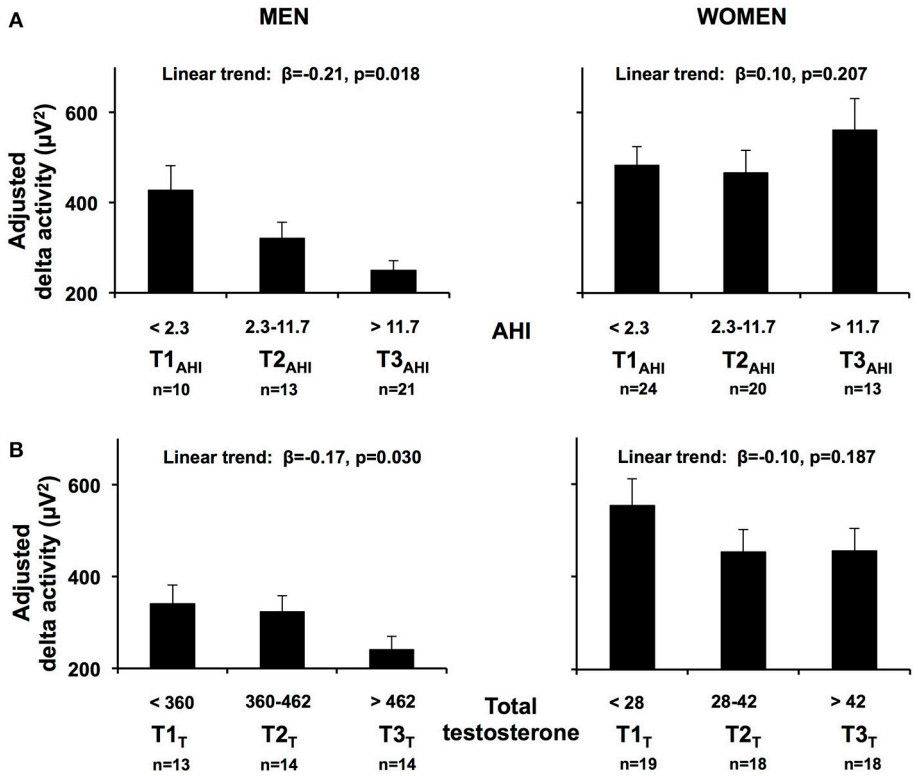

FIGURE 3 | (A) Adjusted mean SWA (+SEM) in NREM sleep, in the first 6 h of sleep according to AHI tertiles ($T1_{AHI}$, $T2_{AHI}$, $T3_{AHI}$) in men and women. **(B)** Adjusted mean SWA (+SEM) in NREM sleep, in the first 6 h of sleep according to total testosterone tertiles ($T1_T$, $T2_T$, $T3_T$) in men and women. Results obtained from linear regression models including AHI tertiles derived from the total of 101 subjects. Age and BMI were centered at their means. An inverse log transformation was applied to beta coefficients for each AHI tertile to convert from Ln[NREM SWA] to the standard values adjusted for age, BMI and race/ethnicity. Standard errors were obtained by inverse log transformation of the upper and lower 95% confidence intervals of the model estimates and by dividing the difference by four, for each tertile. *SWA*, slow wave activity; *AHI*, apnea hypopnea index.

on the intensity of NREM sleep could be that women tend to have REM-related OSA, i.e., the majority of the obstructive events during REM sleep, more often than men (49–51). However, this was not the case in our cohort and therefore would not explain our findings. Sex differences in NREM SWA have also been hypothesized to be due to difference in skull size and thickness, as well as skin thickness (52), but anatomic differences would also not explain the different impact of OSA on men's sleep compared to women's. Finally, differences in neuronal activity could exist between men and women. This has been suggested by a few studies assessing sex differences in the impact of aging on EEG activity, as well as findings in patients with affective disorders. In men, SWA as well as SWS decline after the third decade, while such a decline occurs later in women (8, 53, 54). Similarly, decreased levels of SWA have been reported in men with major depression, compared to controls, but this was not observed in women (55). The exact mechanisms underlying these differences have yet to be identified, but these and other studies suggest a greater "neural slow wave synchronization" ability in women than men (56). In our case, this could explain why women are able to reach SWS despite recurrent respiratory events as well as why OSA is less prevalent or less severe in premenopausal women despite higher levels of obesity when compared to men.

Another important novel finding is that, in our cohort of overweight and obese young to middle-aged men, higher testosterone levels were strongly associated with a lower intensity of NREM sleep. Similar results were obtained with free testosterone concentrations. This observation suggests that common genetic or non-genetic pathways may link the intensity of NREM sleep, a stable phenotype that is highly variable across individuals, and the set point of the hypothalamo-pituitary-gonadal axis. A potential consequence of this inverse association might be that high testosterone levels are associated with a lower arousal threshold and a greater vulnerability to respiratory instability. This interpretation is consistent with reports of increased severity of OSA following exogenous intramuscular testosterone administration (28–32), and further suggests that the exacerbation of OSA may occur through reductions in NREM SWA. It is noteworthy that the findings of our *cross-sectional* analysis are not in contradiction with the results of the few *intervention* studies that showed that CPAP treatment of OSA may increase testosterone levels (26, 57). Indeed, for any given male OSA patient in whom SWA has been fragmented by

TABLE 4 | Determinants of NREM slow wave activity (first 6 h of sleep) in men (top panel) and women (bottom panel).

MEN								
Variables	**Model 1**		**Model 2**		**Model 3**		**Model 4**	
r	0.579		0.695		0.708		0.728	
r^2	0.336		0.483		0.501		0.530	
r^2 adjusted	0.284		0.428		0.445		0.477	
p-value	**0.001**		**<0.0001**		**<0.0001**		**<0.0001**	
	β (95% CI)	***p* value**	**β (95% CI)**	***p* value**	**β (95% CI)**	***p* value**	**β (95% CI)**	***p* value**
Age	−0.029 (−0.048, −0.010)	**0.004**	−0.008 (−0.030, 0.013)	0.439	−0.029 (−0.047, −0.012)	**0.002**	−0.017 (−0.036, 0.002)	0.077
Race/Ethnicity	−0.250 (−0.386, −0.115)	**0.001**	−0.249 (−0.370, −0.128)	**0.0002**	−0.257 (−0.377, −0.137)	**0.0001**	−0.258 (−0.371, −0.145)	**<0.0001**
LnBMI	−0.300 (−1.198, 0.597)	0.503	0.233 (−0.635, 1.012)	0.590	−0.433 (−1.306, 0.440)	0.322		
LnAHI			−0.184 (−0.298, −0.071)	**0.002**			−0.106 (−0.224, 0.012)	0.077
LnT					−0.750 (−1.255, −0.244)	**0.005**	−0.560 (−1.043, −0.076)	**0.025**
WOMEN								
Variables	**Model 1**		**Model 2**		**Model 3**		**Model 4**	
R	0.462		0.475		0.503		0.489	
r^2	0.213		0.226		0.253		0.239	
r^2 adjusted	0.167		0.165		0.194		0.178	
p-value	**0.006**		**0.010**		**0.005**		**0.008**	
	β (95% CI)	***p* value**	**β (95% CI)**	***p* value**	**β (95% CI)**	***p* value**	**β (95% CI)**	***p* value**
Age	−0.0184 (−0.038, 0.001)	0.059	−0.022 (−0.043, −0.001)	**0.037**	−0.026 (−0.046, −0.006)	**0.014**	−0.029 (−0.051, −0.008)	**0.008**
Race/Ethnicity	−0.220 (−0.351, −0.090)	**0.001**	−0.222 (−0.353, −0.091)	**0.001**	−0.219 (−0.350, −0.088)	**0.002**	−0.29 (−0.339, −0.078)	**0.002**
LnBMI	0.358 (−0.206, 0.922)	0.208	0.242 (−0.376, 0.860)	0.435	0.427 (−0.129, 0.983)	0.130		
LnAHI			0.040 (−0.045, 0.125)	0.353			0.046 (−0.032, 0.124)	0.241
LnT					−0.253 (−0.534, 0.028)	0.077	−0.228 (−0.510, 0.055)	0.112

Results obtained after log transformation of NREM slow wave activity. NREM non-rapid eye movement sleep; BMI body mass index; AHI, apnea-hypopnea index; T, total testosterone. Bold values indicate statistically significant p-values.

repeated complete or partial obstruction of the upper airway, the restoration of sleep continuity, particularly during NREM sleep, should be associated with enhanced nocturnal testosterone release.

In our cohort, despite the fact that they were older and heavier, men with OSA had slightly higher total testosterone levels compared to men without OSA. SHBG was slightly higher as well in the former. SHBG is known to increase with age and this could explain our findings (58). No differences in free testosterone were present between groups, and results of the multivariate analysis were unchanged when free testosterone was used as a covariate in our models. Of note, most of our participants had moderate OSA, while studies that reported decreased testosterone levels in men with OSA *vs.* controls included a majority of patients with severe OSA (25–27). Furthermore, participants enrolled in those studies were older than our volunteers [average age over 50 in all but two studies (27, 59)]. In these studies, OSA and control participants were matched for either BMI (25) or age (26, 27). Furthermore, in all studies that examined testosterone levels in men with OSA (26, 27, 60–64) except

one (25), the average or median testosterone levels in the OSA group were in the normal range [>300 ng/dl (65)], suggesting that a majority of men with OSA do have normal testosterone levels.

We have to acknowledge the limitations of the present study. First, the cross-sectional nature of the study limits any assessment of causality. Second, the study protocol did not include a habituation night and we cannot exclude an impact of an unfamiliar sleeping environment on the sleep variables. However, this would have affected all groups equally. Third, our cohort is relatively small. Fourth, we could not systematically control for the menstrual phase in women (who were all premenopausal and off hormonal contraceptives). However, no impact of menstrual phase on NREM SWA levels has been detected in previous studies (66, 67). By not controlling for menstrual phase, we may also have underestimated the prevalence of OSA in women, since upper airway resistance is decreased in the luteal phase (68). Furthermore, we may have overestimated T levels in women as androgen concentrations have been shown to peak in the late follicular phase (69). Fifth, testosterone levels in women were measured by RIA, which is reported to be less accurate at low values. However, our assay has been validated against a liquid chromatography/tandem mass spectrometry method, with correlation coefficients of 0.97 for all samples (men and women), and 0.91 for samples obtained from women (36). Finally, our testosterone assay was performed on a single morning sample for each participant. However, our study was not aimed at diagnosing hypogonadism, which does requires measurement of testosterone levels on two separate occasions at least (65). Furthermore, only one morning sample was obtained in most other studies that examined the impact of OSA on androgen levels (26, 27, 60–64).

In summary, this cross-sectional analysis demonstrates a sex difference in the association between OSA severity and NREM SWA, as well as a robust negative association between total testosterone levels and intensity of NREM sleep in overweight and obese men. Further studies are needed to confirm and extend our results and elucidate the mechanisms linking circulating testosterone levels, SWA and OSA.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the University of Chicago Institutional Review Board. The protocol was approved by the University of Chicago Institutional Review Board. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

EVC: designed the protocol; LLM, KAT, and RL: recruited subjects and collected data; LLM, EVC, and BM: analyzed the data; LLM: drafted the manuscript; EVC, DAE, and BM: reviewed and edited the manuscript. EVC and BM are both the senior authors for this submission.

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SYSTEMATIC REVIEW
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Is Metabolic Rate Increased in Insomnia Disorder? A Systematic Review

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Background: Insomnia disorder is a highly prevalent health condition, affecting ~10–15% of the adult population worldwide. A central feature of insomnia is hyperarousal characterized as persistent and increased somatic, cognitive and cortical stimulation. Hyperarousal leads to a state of conditioned arousal that disrupts both sleep and daytime function. Research studies have shown increases in body temperature, heart rate, electroencephalographic activity, catecholamines, and oxygen consumption as a measure of metabolic rate. These findings provide evidence of increased physiological activation in insomnia however results are not consistent. The aim of the systematic review was to determine if metabolic rate in patients with insomnia is increased in keeping with the hyperarousal hypothesis.

Methods: We searched Pubmed, Web of Science, CINAHL, PsycINFO, EMBASE, and Scopus databases for observational and interventional studies that have measured metabolic rate in insomnia. Study characteristics were extracted and summarized and a risk of bias was performed for each of the studies.

Results: Two reviewers screened 963 abstracts with 35 articles of interest for full-text review. Four articles evaluating 75 participants were included in this systematic review. Two studies showed increased oxygen consumption across 24 h in insomnia patients compared with good-sleeping controls. One study which measured oxygen consumption at only a single timepoint showed no difference between insomnia patients and good-sleeping controls. A further study evaluating the effect of lorazepam on oxygen consumption in patients with chronic insomnia showed that lorazepam reduced metabolic rate during the night time only.

Conclusions: These findings show that metabolic rate appears to be increased across 24 h in line with the hyperarousal model of insomnia. However, these increases in metabolic rate in insomnia were minor compared to good-sleeping controls and the clinical significance is unclear. Larger, methodologically robust studies are required to confirm these findings and the effect of any increase in metabolic rate on sleep-wake disturbances or pathophysiology.

Keywords: metabolic rate, insomnia, hyperarousal, sleep disturbances, systematic review

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INTRODUCTION

Rationale

Insomnia disorder is a highly prevalent health condition, accounting for ∼10–15% of the adult population globally (1). It is diagnosed using subjective symptomology consisting of difficulty initiating sleep, maintaining sleep or early morning awakenings, or a combination of these, with concomitant daytime impairments for at least 3 nights per week and 3 months duration despite adequate opportunity to sleep (2). The disorder is complex with considerable heterogeneity and has shown to be highly persistent, with longitudinal studies showing insomnia symptoms present over 1 year (3–5). Insomnia negatively effects quality of life, mood (anxiety and depression), cognitive performance, and daytime functioning (6–9), with these symptoms driving treatment-seeking behavior (10).

The pathophysiological mechanisms responsible for insomnia disorder have yet to be fully elucidated. Several different models have been proposed. Spielman's 3-P model is the most prominent, using a diathesis-stress model to describe how insomnia develops and is maintained over time through predisposing, precipitating, and perpetuating factors (11). Behavioral and cognitive models have also evolved (12, 13) which incorporate conditioning and dysfunctional beliefs which promote negative perceptions of sleep. More recently, a neurobiological model of sleep-wake dysregulation caused by regional-specific neural activity that promotes wakefulness during sleep has been proposed (14). This model unites psychological aspects of insomnia to neurobiological mechanisms.

One aspect that has underpinned these models is the presence of somatic arousal in insomnia patients. Evidence has demonstrated that insomnia is characterized by persistent and increased somatic, cognitive, and cortical stimulation (14). This has been termed *hyperarousal* which is thought to be present over 24 h (both sleep and awake) leading to chronic sleep disruption and impairments in daytime function. There are a number of research studies that have shown increased cognitive and physiological activation in insomnia patients compared to good-sleeping controls. In particular, patients with insomnia have shown an increase in body temperature, heart rate, cortisol and catecholamines (15). Collectively, these findings suggest that 24-h metabolic rate will be elevated in insomnia patients compared to good-sleeping controls. The sleep-wake cycle and basal metabolism are intrinsically linked with pronounced reduction in body temperature occurring at sleep onset and about 15% reduction in metabolic rate during sleep (16). An elevation in metabolic rate in insomnia may affect sleep leading to greater hyperarousal and perpetuating insomnia symptoms.

Objectives and Research Question

We sought to determine whether metabolic rate is elevated in insomnia patients which would provide strong evidence of whole-body physiological hyperarousal. To date, there has been no systematic review of metabolic rate in insomnia patients. Therefore, the aim of the systematic review was to determine the metabolic rate of insomnia disorder patients.

METHODS

Search Strategy and Data Sources

An extensive and systematic search for studies on metabolic rate on insomnia populations was conducted using the following databases: PubMed, Scopus, Web of Science, CINAHL, Embase, and Psycinfo. The search terms selected were incorporated in the following Boolean expression: ("Metabolic rate" OR "Exercise capacity" OR "Energy metabolism" OR "Energy transfer" OR "Oxygen consumption" OR "Respiratory exchange ratio" OR "Oxygen utilization" OR "Energy utilization" OR "Energy expenditure" OR calorimetry) AND insomnia. The search terms were adapted when necessary to fit the specific search requirements of each database (see Supplementary Material). The primary search was undertaken on the 4th December 2017. No limitations were used in any database. After exporting articles into EndNote, duplicates were removed. Reference lists of relevant original and review articles identified through the search were searched for potential missed publications. The search was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement and documented using the PRISMA flow chart (17).

Our primary study question was whether or not metabolic rate, determined using gold-standard measurement (calorimetry), was increased in patients with untreated insomnia disorder compared with good-sleeping controls or compared to patients with insomnia undergoing treatment.

Study Selection and Data Extraction

Articles were evaluated against the following inclusion criteria:

1. The article had to contain original data (i.e., was not a review or editorial).
2. Population: diagnosis of insomnia disorder, ≥18 years old, non-shift-working, non-jet-lagged, otherwise healthy.
3. Compared to good-sleeping controls or post-intervention.
4. The primary or secondary outcome had to be the measurement of metabolic rate directly (e.g., calorimetry).
5. Type of study could be either observational case-control study or interventional.

In a first phase, the studies were independently reviewed by two authors (JC, MC) using the title and the abstract. Disagreements in abstract inclusion or exclusion were resolved by consensus with a third author (CG). In a second phase, full text articles were independently reviewed by the same authors (JC, MC) and disagreements were resolved by consensus with the third author (CG). Full text articles were selected using the selection criteria and included in the final inclusion list. The characteristics, measurements and outcomes of the selected studies were extracted in duplicate (JC, MC) into a table template. Results were tabulated as mean ± *SD* where possible. Metabolic rate results were reported as $\dot{V}O_2$ in ml/min. When these data were not available from an article, authors were contacted for the results or clarification.

Risk of bias was assessed for case-control studies using the National Institute for Health and Care Excellence methodology checklist (18). This checklist assisted with the assessment of

the studies' internal validity by methodically appraising the selection of cases and controls, confounding factors and statistical methods. Risk of bias in interventional studies was evaluated using the Cochrane collaboration's tool for assessing risk of bias (19). This tool assisted with the assessment of the studies' internal validity and detection of selection, performance, detection, attrition, or reporting bias.

RESULTS

Study Selection and Characteristics

The primary search identified 1,506 records from six databases [Pubmed (n = 479), Scopus (n = 438), Embase (n = 451), Web of Science (n = 99), Psycinfo (n = 27), and CINAHL (n = 12)] (see **Figure 1**). After removing duplicates there were 963 records to screen for titles and abstracts initially. Following abstract screening 928 articles were excluded. The full-texts of the remaining 35 articles were checked for eligibility and of these, 31 were excluded (see **Figure 1** for reasons). The four remaining articles (20–23) met the eligibility criteria and were included in this review, see **Table 1**. No further articles were identified through searching reference lists of reviews identified during the initial search nor the reference lists of the included articles. Authors were contacted directly when articles were missing data or information pertinent to the review (20–23). We received metabolic rate and some participant characteristic data (23). Unfortunately for the other studies (20–22) it was not possible to retrieve the standard deviations of waking metabolic rate measurements due to lack of access to the primary data. Due to a lack of information regarding variability within groups (e.g., standard deviations) from the four articles, we were unable to meta-analyse differences in metabolic rate between insomnia and good-sleeping controls.

Synthesized Findings

There were a combined total of 75 participants with and without insomnia disorder who underwent metabolic rate measurements in the four included articles. Three of the articles were case-control in design and used age, gender, and weight-matched good-sleeping controls (20, 21, 23). The other was a clinical trial assessing metabolic rate before and after treatment with two doses of lorazepam for insomnia (22). Participants in all studies were young to middle-aged (mean age ranged from 31 to 52 years), and were selected based on a healthy BMI. Three of the articles were published by the same research team (20–22) using the same indirect calorimetry technique to measure $\dot{V}O_2$ (Deltatrac). This method utilized a metabolic mask worn by the participants during eight 20-min periods between waking and bedtime, and continuously overnight. The fourth study used

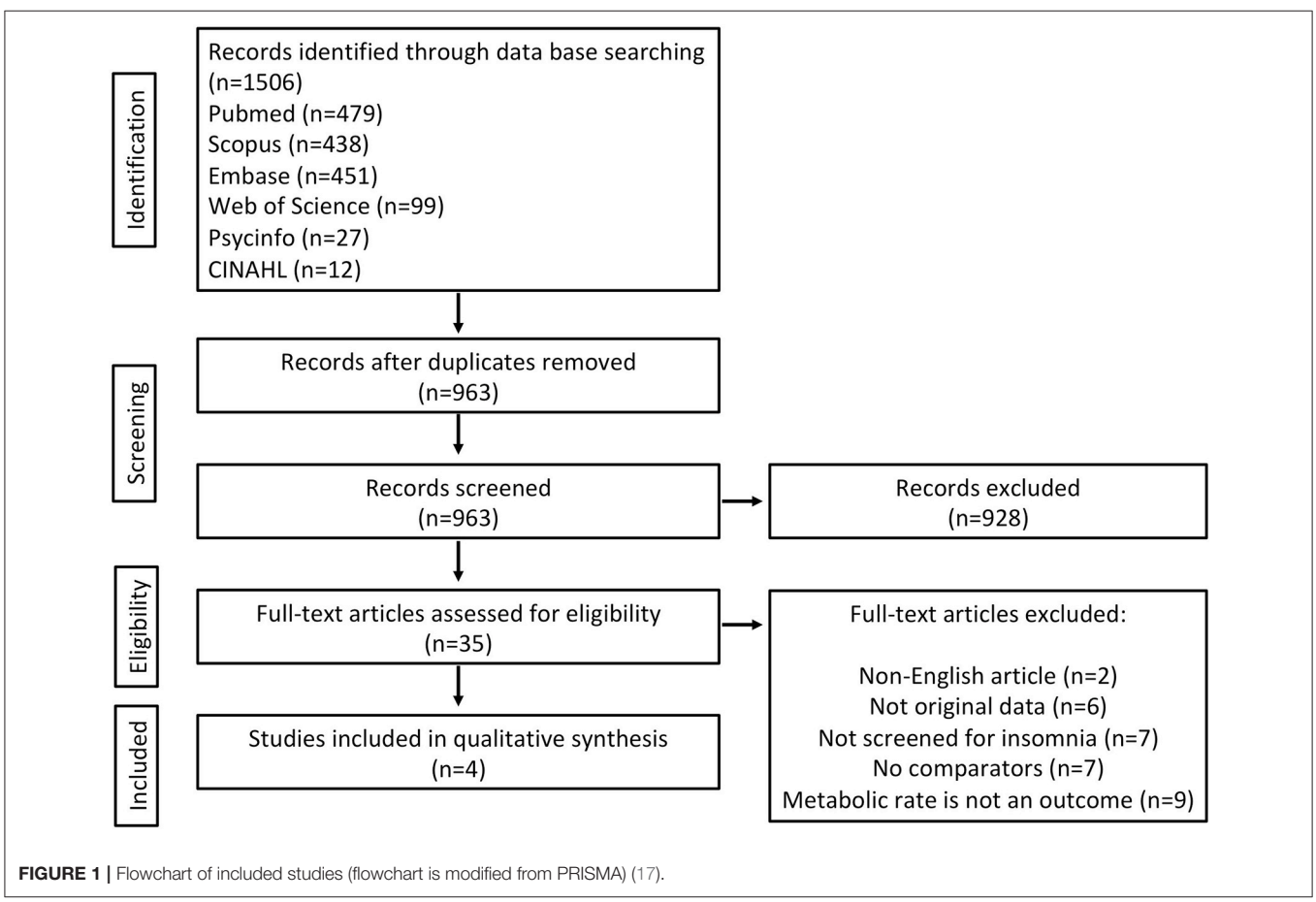

FIGURE 1 | Flowchart of included studies (flowchart is modified from PRISMA) (17).

TABLE 1 | Characteristics of identified studies.

References	Patient number, sex, age, BMI	Diagnostic criteria	Comparators number, sex, age, BMI	Case-match methodology	Sleep setting	Metabolic rate data capture	$\dot{V}O_2$ RESULTS CASES ml/min	$\dot{V}O_2$ RESULTS CONTROLS ml/min
				CASE-CONTROL STUDIES				
(20)	*n* = 10, sex unclear, Age 38.3 (7.1), selected for normal BMI	Screening questionnaire indicating sleep onset ≥45 min at least 4/7 OR were awake ≥60 min after falling asleep 4/7 and that this had existed for.≥ year	*n* = 10, sex unclear (but matched with cases), Age 38.6 (6.8), selected for normal BMI	Indicated normal sleep on questionnaire with self-reported SOL<30 min and WASO<30 min. Matched to a case by sex, age (±5 years), weight (±25 lb), and general TIB characteristics	Sleep center referrals and ads to the local community. Urban Dept Veterans Affairs Medical Center, Dayton Ohio, USA	SensorMedics Deltatrac Metabolic Monitor using a mask and metabolic cart. Waking metabolic data recorded for 20 min immediately after awakening after one night and 20 min after 6 MSLTs during the day and 20 min prior to lights out. Sleeping metabolic data was measured throughout the entire 2nd night's sleep. VO2 automatically averaged at the end of each minute.	Overall: nr Wake: 296 (no SD) Sleep: 266 (no SD)	Overall: nr Wake: 266 (no SD) Sleep: 256 (no SD)
(21)	*n* = 9, 2 female, Age 31.7 (8.4), BMI 23.7 (3.3)	Screening questionnaire indicating sleep onset ≥45 min at least 4/7 OR were awake ≥60 min after falling asleep 4/7 and that this had existed for ≥1 year. Patients who demonstrated SOL<30 min and SE>90% and overestimated SOL by ≥100% on PSG and self-reported SOL≥20 min on both PSG nights were considered *sleep misperception insomniacs*	*n* = 9, 2 female, Age 32.8 (6.2), BMI 25.0 (3.4)	Indicated normal sleep on questionnaire with self-reported SOL<30 min and WASO<30 min. Matched to a case by sex, age, weight	Urban Dept Veterans Affairs Medical Center, Dayton Ohio, USA	As for (20)	Overall: 304 (26) Wake: 331(no SD) Sleep: 277 (no SD)	Overall: 286 (34) Wake: 266 (no SD) Sleep: 266 (no SD)
(23)	*n* = 13, all female, Age 51.7 (8), BMI 22.7(2.6)	Primary chronic insomnia using DSM-IV criteria	*n* = 12, all female, Age 52.8 (9.9), BMI 22.4(1.5)	Age and BMI matched good-sleeping controls	Depression and Sleep Research University, Psychiatric Uni Clinic Basel, Switzerland.	Indirect calorimetry (Deltatrac II, Datex) was conducted in the awake state (around 8 a.m.) for at least 20 min	Overall: n/a; Wake: 178.8(16.46); Sleep: n/a	Overall: n/a; Wake: 184.2(18.85); Sleep: n/a
				INTERVENTIONAL STUDY			$\dot{V}O_2$ RESULTS Off drug	$\dot{V}O_2$ RESULTS on lorazepam
(22)	*n* = 12, 4 female, Age 36 (range 21–48), selected for normal BMI	Screening questionnaire indicating sleep onset ≥45 min at least 4/7 OR were awake ≥60 min after falling asleep 4/7 and that this had existed for ≥ year	Crossover design. All participants received placebo and active medication.	Lorazepam 0.5 mg and 1.5 mg vs. no drug	Sleep center referrals or advertisements in local newspaper, Urban Dept Veterans Affairs Medical Center, Dayton Ohio, USA	As for Bonnet 1995	Overall: 334 (no SD) Wake: nr Sleep: 299 (no SD)	0.5mg Overall: 334 (no SD) Wake: nr Sleep: 285 (no SD) 1.5 mg Overall: 338 (no SD) Wake: nr Sleep: 287 (no SD)

Data are mean (SD) unless other stated, BMI, Body Mass Index; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, 4th edition; SOL, sleep onset latency; WASO, wake after sleep onset; VO_2, volume of oxygen consumption; n/a, data not collected; nr, not reported.

a similar system (Deltatrac II) for measuring $\dot{V}O_2$, but collected this only during a single 20-min period around 8 a.m. (23).

Metabolic rate was greater in the insomnia group compared with the good-sleeping control group in two of the case-control design studies (20, 21). This finding was consistent when measured during both the day and night. In the first study, all measurements across the 24-h collection period were elevated in insomnia patients and 9 out of 10 of these reached statistical significance ($p < 0.01$) (20). In their second study, they compared patients with sleep-state misperception insomnia/paradoxical insomnia (those who feel like they are awake throughout the night, but physiological measurement of their sleep deem them to be asleep) to good-sleeping controls. They reported overall that there was a statistically significant difference between the groups ($p < 0.001$) and a difference both separately during the day and night ($p < 0.001$ and $p < 0.005$, respectively, see **Table 1** for mean differences) (21). In the case-control study by Seelig et al. (23) with all female participants only, $\dot{V}O_2$ measured in the morning was marginally greater in the control group compared with the insomnia group but this was not statistically significant (See **Table 1**). In the clinical trial participants, overnight $\dot{V}O_2$ was marginally decreased after taking lorazepam of either 0.5 or 1.5 mg dose ($p < 0.02$), but the daytime and 24-h values were the same in the no drug and lorazepam 0.5 and 1.5 mg conditions (22).

Risk of Bias

Overall, for the case-control studies, the risk of bias was evaluated as mixed (See Supplementary Material). All studies were shown to define clearly the case and control groups. However, two studies (20, 21) did not use a clinical diagnosis of insomnia disorder (for instance, DSM), but did report extensive questionnaire and diary data required for diagnosis of insomnia compared with good-sleeping controls (See **Table 1**). For the interventional study, there was no mention in the article regarding randomization, allocation concealment, or blinding, so it was unclear if this may have influenced the result (See Supplementary Material).

DISCUSSION

Summary of Main Findings

The systematic review identified four studies that measured metabolic rate directly in patients with insomnia. Metabolic rate was found to be increased during both day and night in patients with untreated insomnia in the studies that sampled $\dot{V}O_2$ across a 24 h period (20, 21). In contrast, when $\dot{V}O_2$ was measured at only one morning timepoint (∼8 a.m.), there was no difference between the insomnia and good-sleeping control groups (23). The final study compared $\dot{V}O_2$ across the 24 h on and off lorazepam treatment for insomnia, showed that lorazepam reduced $\dot{V}O_2$ during the night-time only (22). Overall these results indicate that metabolic rate across the 24-h period appears to be increased in insomnia patients when compared with age-, and gender-matched controls, which is consistent with hyperarousal model of insomnia. This result also aligns with recent findings that insomnia is associated with metabolic dysregulation compared with good-sleeping controls, suggesting that metabolic profiling may be a potential biomarker for disease risk in insomnia (24).

Methodological differences in the sampling period and study population, may account for observed $\dot{V}O_2$ differences between the studies. Seelig et al. (23) measured $\dot{V}O_2$ only during a single 20-min period shortly after waking, which may have been influenced by circadian variability, gender, and age-related differences from the studies by Bonnet and Arand (20–22). This study (23) only evaluated females who were on average about 15–20 years older than the participants in the other studies. Females on average have lower metabolic rates than men, and metabolic rate declines with age (25). It is unclear whether or not insomnia may affect metabolic rate differentially between males and females or across the lifespan. Additionally, the effect of benzodiazepines (lorazepam) on $\dot{V}O_2$ may have been mediated by the alteration to neuroendocrine stress response (26). The nocturnal $\dot{V}O_2$ was lower following administration of lorazepam, which may affect overnight cortisol secretion resulting in greater decrease in metabolic rate compared with the day. However, these results need to be interpreted with caution as the differences were marginal and the anxiolytic effects of lorazepam may have lowered overall $\dot{V}O_2$ during the night (22). The increased $\dot{V}O_2$ findings suggest that whole-body metabolic rate is elevated and supports the 24-h hyperarousal theory of insomnia (27). These data align with findings from a number of studies that have identified increased arousal in patients with insomnia across behavioral, cognitive, and autonomic nervous system domains (28). There is evidence of increased physiological activation in insomnia including heart rate, cortisol, body temperature, catecholamines, fast frequency electroencephaolography, and heart rate variability (15, 29). The findings of this review showing increased metabolic rate in insomnia aligns with this overall increased physiological activation. However, greater methodological rigor may be required to replicate and confirm the findings across these multiple physiological domains, as they are from small studies, and often are not repeated using similar methodologies enabling collation of data into meta-analyses (29).

It is possible that differences in metabolic rate between those with insomnia and good-sleeping controls could relate to different perceptions of a new environment, inducing different levels of a stress response (30). Sleep quality and quantity of people with insomnia may be affected by "first night effect," where sleep is impaired due to sleeping in a new environment for the first time (31). Conversely, other people with insomnia will experience "reverse first night effect" where sleep is improved due to the new environment that is often devoid of stimuli that promotes insomnia symptoms (32). As the recordings were measured during the first night in the laboratory, it is possible that this may have influenced the difference between good-sleeping control and insomnia groups.

What is interesting to note, is that $\dot{V}O_2$ measured using calorimetry during rest in these studies revealed different results compared with the broader literature using other measures of metabolic rate. A commonly used metabolic equivalent (METs) are calculated based upon diary data that derives a metabolic rate for different activities of daily living (e.g., time spent sitting,

sleeping, moderately exercising) (33). One study has shown that METs were not increased in insomnia compared with good-sleeping controls (34). In particular, males with insomnia [n = 40, on average overweight, BMI 29.6(SD 3.5)], their METs were lower than both normal weight (n = 48) and overweight (n = 75) good-sleeping controls. This raises the question, are patients with insomnia equally active as controls but their metabolic rate at rest is elevated compared to controls? The decreased activities of daily living could be a result of the behavioral components of insomnia, whereby patients feel less able to exercise or spend more time lying down as a result of their condition.

In two other studies, maximum oxygen uptake ($\dot{V}O_2$ peak) was used to examine exercise capacity, comparing those with insomnia symptoms from questionnaire and those without (34, 35). In both studies, $\dot{V}O_2$ peak was lower in those with insomnia symptoms, even after adjusting for age, sex, and other potential confounders. This shows that people with insomnia symptoms appear to have decreased exercise capacity. These results need to be explored in an otherwise healthy clinical insomnia population, but they suggest that maximum oxygen capacity may be lower in insomnia disorder compared to good-sleeping controls. In our review resting metabolic rate was elevated in insomnia compared to controls. Further research is required to determine if elevated resting metabolic rate coexists with decreased exercise capacity in diagnosed insomnia disorder and how this relates to disease risk profile.

LIMITATIONS

A major limitation of this review is that of the four articles identified in the final search, three were from the same research group within a single sleep center, suggesting their findings may not be representative of insomnia. As the only study completed by a different research team (23) showed no difference in metabolic rate between insomnia and good-sleeping control groups, any conclusions pointing toward higher metabolic rate in insomniacs comes from a single research team. Further studies from different teams, with a larger number of participants, taking into account the potential for first night effects would be required to confirm this finding. All of the studies had small to modest sample sizes, resulting in only 32 patients with insomnia disorder to be compared with 31 good-sleeping controls and only 12 participants who were measured before and after treatment with lorazepam. The clinical trial had high risk of bias due to unclear methods regarding randomization and blinding, and placebo effects are known to be strong in insomnia, potentially affecting the validity of the result (36). The participants in these studies were selected for healthy weight and were on average middle-aged. The results of these studies could therefore not be extrapolated to children or older adults, or those who are overweight. Data was also unable to be meta-analyzed as we were unable to retrieve all standard deviations. We believe the measurement of oxygen consumption in a whole room calorimeter may solve a lot of the methodological problems (37).

CONCLUSIONS

The results from a small number of studies suggest that metabolic rate appears to be increased in patients with insomnia disorder across 24-h in line with the hyperarousal model of insomnia. These findings need to be replicated in larger prospective studies. Clinical trials evaluating the effect of insomnia therapies, such as cognitive behavioral therapy, on metabolic rate would be useful in determining causality.

AUTHOR CONTRIBUTIONS

CG, JC, and MC contributed to the conception and design of the systematic review. JC and MC independently reviewed abstracts and papers and disagreements were resolved by consensus with CG. CH did the risk of bias. CG, JC, and MC wrote sections of the manuscript. DB and RG revised the manuscript and contributed with intellectual ideas. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2018.00374/full#supplementary-material

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REVIEW
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Sleep Extension in Short Sleepers: An Evaluation of Feasibility and Effectiveness for Weight Management and Cardiometabolic Disease Prevention

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Sleep duration has become increasingly recognized as an important influencer of health. Epidemiologic and observational studies have shown associations between short sleep duration and increased risk for chronic cardiometabolic disorders, including obesity, type 2 diabetes, and cardiovascular disease. These associations have led to investigations into the potential causal pathways through which short sleep may increase risk for these disorders. Clinical intervention studies have demonstrated that restricting sleep in normal sleepers has adverse health effects, including insulin resistance, and increased blood pressure. The totality of evidence points to negative health effects of short sleep and the recognition of sleep as a lifestyle behavior that may be targeted for disease prevention. It is well established that consistent, adequate sleep is associated with the lowest risk of obesity and cardiometabolic disorders. Yet, it is unclear whether increasing sleep in short sleepers can improve health. In today's society, it is common for individuals to deprive themselves of sleep during the work week, with the intent to sleep longer during the weekend, or have "catch-up sleep." Studies that have examined the health effects of extended sleep, post-sleep restriction, revealed some improvements in health outcomes. However, it is uncertain whether the improvements observed with catch-up sleep are sufficient to reverse the negative health effects of constant sleep restriction. Few intervention studies have been undertaken to determine whether extending sleep, long-term, in short sleepers is feasible and whether it can reduce the disease risk burden associated with short sleep duration. The purpose of this review is to highlight these studies and evaluate information related to the impact of sleep extension on risk factors for chronic cardiometabolic disorders. We discuss limitations of current research, including variability in participant characteristics and the extent to which sleep behaviors are modified and monitored. Although the evidence-base for benefits of sleep extension

is still in the early stages, studies to date indicate that prolonging sleep, in short sleepers, may improve cardiometabolic risk. Finally, our review calls attention to areas that require further study and for larger scale studies of behavior modification to establish the health effects of sleep extension in short sleepers.

Keywords: sleep extension, cardiometabolic, health, sleep duration, prevention

INTRODUCTION

Sleep duration has become increasingly acknowledged as an important factor in overall health status, and sleep deficiency has begun to be recognized as a potential modifiable risk factor for certain chronic conditions. Current research has shown that short sleep duration (SSD), defined as <7 h/night, is associated with an increased risk of obesity, metabolic disorders, and CVD (1–4). This association may be U-shaped, however, as there is also evidence that longer sleep duration, >9 h/night, is associated with adverse health effects (5). However, in today's "24/7" society, short sleep duration is more prevalent that long sleep duration. According to data from the National Health Interview Survey, the prevalence of very short sleep (<5 h) and short sleep (5–6 h) has increased from 1.7 to 2.4% and 19.7 to 26.7%, respectively, from 1977 to 2009 (6). Concurrently, long sleep (>9 h) decreased from 11.6 to 7.8% (6). This rise in the prevalence of short sleepers has permitted epidemiological evaluations of the influence of sleep duration on cardiometabolic risk factors. The data from these studies suggest a relation between sleep duration and obesity, hypertension, type 2 diabetes, and overall mortality (7, 8). These results have led to intervention studies to further examine the causal implications of these findings.

There are several mechanisms by which SSD may be related to obesity: metabolic changes affecting appetite-regulating hormones (9); low physical activity; and increased food intake without comparable change in energy expenditure (10, 11). Leptin is an adipocyte derived hormone that plays in important role in energy homeostasis (12). Its effect on energy balance regulation and SSD has been extensively studied, but with mixed results (13–15). Multiple clinical studies have shown that sleep restriction, in healthy, normal sleepers (7–9 h), leads to increases in daily energy intake (16–18) sufficient to lead to weight gain if sustained over time (18). On the other hand, there are conflicting reports regarding the impact of sleep restriction on energy expenditure (11, 17, 18). Nonetheless, it seems that any change in energy expenditure due to sleep restriction is either insufficient to offset the increase in energy intake or contributes to the positive energy balance.

Increased blood pressure (BP) and inflammatory markers, such as interleukin-6 (IL-6), are associated with systemic inflammation and decreased cardiovascular health (19). There are several clinical intervention studies examining the impact of sleep restriction, for periods ranging from a few days to a few weeks, on cardiometabolic risk factors (2–4, 20). When examining metabolic health, studies have demonstrated that sleep restriction reduces whole body insulin sensitivity and insulin resistance at a cellular level (21, 22). However, evidence linking SSD to adverse cardiometabolic risk does not de facto imply a benefit of sleep extension on health outcomes. In this review, we describe the cardiometabolic effects of sleep restriction, and we highlight and critically evaluate current interventions that aim to extend the duration of sleep in short-sleepers as a means of improving cardiometabolic risk factors. These studies are summarized in **Table 1**. We also address opportunities for future research in this area.

Abbreviations: BP, blood pressure; CVD, cardiovascular disease; SSD, short sleep duration; TIB, time in bed.

ENDOCRINE AND CARDIOMETABOLIC EFFECTS OF RECOVERY SLEEP AFTER SLEEP RESTRICTION

In today's society, it is common for individuals to decrease their sleep during the work week, and to attempt to "catch-up" by sleeping longer on the weekends. It is reported that about 56% of Americans sleep less during the work week compared to non-work days (29). Several clinical trials have reproduced this scenario to understand the health effects of short term sleep recovery. Van Leeuwen et al. (30) tested the effects of sleep restriction and subsequent recovery sleep on glucose, leptin, and satiety in healthy men. Participants were sleep restricted for 5 days with a time in bed (TIB) of 4 h/night followed by a 2-day sleep recovery of 8 h TIB. Leptin levels were elevated during sleep restriction and remained elevated during the recovery period, compared to baseline, but no differences were found in subjective satiety measures throughout the experiment in both groups. It is possible that leptin remained elevated because an 8 h TIB was not an adequate amount of time to allow sufficient recovery sleep after 5 nights of <4 h sleep (31). Faraut et al. (31) investigated the effects that napping and recovery sleep had on the immune and inflammatory systems of healthy young men. Participants were restricted to 2 h TIB for one night followed by either an 8 h recovery night, a 30-min nap mid-day plus an 8 h recovery night, or a 10 h recovery night. The control group slept 3 consecutive nights of 8 h, with no changes in immune or inflammatory parameters throughout the experiment. Leukocyte counts increased in all sleep restricted groups compared to baseline, but this increase persisted in the 8 h recovery condition, while the numbers decreased in the nap + 8 h recovery and the 10 h recovery conditions. There was only a significant increase in myeloperoxidases in the sleep restriction +8 h recovery group; this increase was significant during both the restriction and

TABLE 1 | Summary of clinical studies included in the present review that investigated the health effects of sleep extension.

Study Ref	Intervention	Participants	Duration (Weeks)	Methods	Sleep as an outcome	Intervention group results
Logue et al. (23)	Weekly group counseling sessions	25 overweight and obese adults	12	Group 1: diet and exercise counseling Group 2: diet and exercise counseling plus sleep education starting week 4	No Primary outcome: weight loss	Group 2 lost more weight compared to Group 1 (change of −5 vs. −2%) No difference in sleep quality
Tasali et al. (24)	1 individual counseling session at baseline to extend sleep and prescribe a sleep schedule	10 overweight adults	2	All subjects: increase TIB to 8.5 h/night, monitored with actigraphy No comparison group	Yes Extending sleep will improve appetite and decrease cravings	14% decrease in overall appetite and a 62% decrease in the desire for sweet and salty foods
Al Khatib et al. (25)	1 individual counseling session at baseline to extend sleep and prescribe a sleep schedule	41 healthy, normal weight adults	4	Sleep extension group: extend sleep by 1–1.5 h/night, monitored with actigraphy Control group	Yes Extending sleep will improve weight maintenance and cardiometabolic health	Reduced intake of free sugars (−9.6 g) compared baseline
Haack et al. (26)	Both groups received sleep hygiene information at baseline Extension group was prescribed individualized sleep schedules	22 adults with prehypertension or type 1 hypertension	6	Sleep extension group: extend sleep by 1 h/night, monitored with actigraphy Control group	Yes Extending sleep lowers BP	Decreased average systolic and diastolic beat-to-beat BP from baseline to endpoint (14 ± 3 vs. 8 ± 3 mmHg)
McGrath et al. (27)	Weekly individual sleep counseling	134 adults with elevated BP	8	Sleep intervention group: 60 min counseling session using Sleepio Standard care group	No Primary outcome: reduce mean 24 h BP	Reported improved sleep quality, no changes in BP
Leproult et al. (28)	1 individual counseling session at baseline to extend sleep and prescribe a sleep schedule	16 healthy adults	6	All subjects: extend sleep by 1 h/night, verified by actigraphy No comparison group	Yes Extending sleep will improve metabolic health	Improved insulin-to-glucose ratios

recovery periods compared to baseline. The control and other 2 sleep restriction groups experienced no changes in inflammatory markers.

Spiegel et al. (32) used a more appropriate recovery sleep episode of 12 h TIB for 7 nights after participants were sleep restricted for 6 nights to 4 h TIB. In this group of healthy men, leptin levels during sleep restriction were 19% lower compared to the sleep recovery phase, suggesting that increasing sleep, following a period of drastically restricted sleep, may reverse the potential negative effects of short sleep on leptin levels. However, baseline leptin levels were not measured, and it is therefore unclear whether recovery sleep returned leptin levels to their baseline state or increased leptin levels. Difference between these two studies may have been due to the different lengths of the study or the duration of the recovery sleep period. The study by Spiegel et al. (32) had longer restriction and recovery phases than that of Van Leeuwen et al. (30), possibly allowing leptin to adapt to these changes in sleep. Another study (13) incrementally restricted sleep in 14 women for 4 nights, resulting in a decrease in leptin levels, with accompanying increases in energy intake and body weight. After 2 nights of recovery sleep, averaging 9.35 h/night, all of these parameters had returned to baseline.

In addition to energy balance outcomes, studies have also examined cardiometabolic risk factors in response to sleep extension. Van Leeuwen et al. (30) observed increases in fasting insulin and insulin-to-glucose ratio after sleep restriction, with both returning to baseline after the 2-night recovery period. Pejovic et al. (33) observed increases in 24 h plasma IL-6 levels following 6 nights of 6 h TIB. These levels returned to baseline after 3 nights of 10 h recovery sleep.

All of the interventions noted herein had limited short sleep restriction and sleep recovery periods, with no more than 7 days of recovery sleep. Despite the mixed results, these preliminary findings are encouraging and provide a basis for the potential reversibility of adverse health effects caused by sleep restriction through sleep extension. These studies have provided justification for examining sleep extension sleep, in short sleepers, as a means to provide endocrine and cardiometabolic health benefits. These investigations were undertaken to determine whether benefits would also be observed among chronic short sleepers, rather than sleep-restricted adequate sleepers, as included in the studies described above.

IMPACT OF SLEEP EXTENSION ON BODY WEIGHT

Short-term recovery sleep studies suggest that the increase in weight and energy intake associated with sleep restriction may be ameliorated by extending sleep. Recent studies further tested this hypothesis but employed longer interventions in an outpatient setting. Logue et al. (23) performed a 12-week randomized controlled trial in overweight and obese adults, examining the effectiveness of lifestyle interventions on weight loss and sleep. The participants were randomized into 2 groups, each receiving weekly 60-min counseling sessions. Both groups received diet and exercise counseling, but the second group received additional sleep-related information starting at the week 4 session. Body weight was measured at each session and sleep quality and efficiency were assessed using the Pittsburgh Sleep Quality Index and the Sleep Timing Questionnaire, respectively, at weeks 0, 6, and 12. Food frequency questionnaires were collected at weeks 0 and 12. The results showed that both groups lost weight, but the group receiving additional sleep counseling lost more weight compared to the group only receiving diet and exercise counseling (−5% change from baseline vs. −2% change from baseline, respectively). In addition, both groups reported improved sleep efficiency, but no data regarding sleep duration were reported. These findings provide some evidence that following sleep recommendations, in addition to diet and exercise, may lead to greater weight loss than diet and exercise alone. However, given that both groups perceived improvements in sleep efficiency, it is unclear whether the sleep recommendations were instrumental in effecting greater weight loss. Indeed, there were several potential concerns with this study that should be noted: (1) the group receiving the additional sleep counseling ate significantly fewer added fats and sweets at baseline compared to the control group; when controlling for this, statistical significance disappeared; (2) retention rate was low, with approximately 54% of participants completing the study; (3) sleep was not assessed objectively and change in sleep duration as a result of the intervention could not be ascertained. These limitations decrease the impact of the study.

Another study (24) investigated the effects of behavioral counseling in combination with 2 weeks of sleep extension. Ten overweight adults, reporting sleeping <6.5 h/night at baseline, were provided a single baseline behavioral counseling session on sleep hygiene and asked to lengthen their TIB to 8.5 h/night. An additional counseling session was provided after the first week of the intervention if the study team deemed it necessary. Prescribed bedtimes and wake times were given to individuals based on their preferred schedules. Wrist actigraphy was used to objectively measure sleep throughout the study, and appetite was assessed at baseline and endpoint using visual analog scales. At the end of the 2-week intervention, participants had increased their sleep duration by an average of 1.6 h compared to baseline (7.1 vs. 5.6 h). Additionally, there was a 14% decrease in overall appetite and a 62% decrease in the desire for sweet and salty foods. These results are encouraging given that a recent meta-analysis concluded that the increase in energy intake after sleep restriction is approximately 385 kcal/d, (18) which is sufficient to lead to weight gain if sustained over time. Furthermore, the increased consumption was accompanied by a significant increase in fat intake. Although the study lacked a control group, (24) the increase in sleep duration associated with the decrease in appetite is promising and should be followed by controlled trials to determine if increasing sleep duration from short to adequate reduces food intake, improves dietary habits, and leads to improved body weight over time. It is also important to note that the study did not assess actual food intake and was only 2 weeks long, a period too short to observe effects on body weight. Therefore, unanswered questions remain as to the influence of sleep extension on body weight.

There have been several mechanisms put forth to explain the increase in energy intakes observed with sleep restriction. One proposition is that sleep restriction leads to changes in appetite-regulating hormones. Similar to the recovery sleep studies, the effects sleep restriction has on these hormones, particularly leptin, are varied (13–15). Another is that sleep restriction stimulates neuronal networks associated with reward, which increases the salience of foods (34). Despite divergent opinions on the mechanism, there is strong consensus that sleep restriction leads to increased food intake (17, 18, 35). Thus, extending sleep could potentially reverse this increase in food intake. In a study performed by Al Khatib et al. (25) participants reported a reduced intake of free sugars compared to a control group after 4 weeks of 1–1.5 h/night sleep extension. This trial investigated the feasibility of sleep extension, via behavioral counseling, to affect body weight and cardiometabolic health. Forty-one healthy adults with body mass index 18.4–24.9 kg/m^2 who were self-reported short sleepers (5–7 h/night) were enrolled. The goal for the sleep extension group was to lengthen sleep duration by 1–1.5 h/night for 4 weeks. This was attempted by providing a single sleep consultation at baseline to design and implement a personalized strategy with prescribed bed times and wake times. There were 4 in-person visits throughout the study, 2 at baseline and 2 at endpoint, to assess outcomes related to energy balance (food intake, energy expenditure, and body composition) and cardiometabolic risk factors (blood biomarkers and BP). Wrist actigraphy was used as an objective measure of sleep duration to verify participants' adherence to sleep recommendations. At the end of the intervention, the sleep extension group had significantly increased their sleep duration by 21 min, whereas the control group reduced their sleep by 11 min. However, only 3 of 21 participants in the sleep extension group were able to reach the weekly sleep goal of 7–9 h of sleep/night. Possibly due to the minimal improvements in sleep duration in the sleep extension group, there was no significant difference in body composition or cardiometabolic risk factors between groups after the 4-week period. Additionally, relative to baseline, the sleep extension group experienced poorer sleep quality, as measured by actigraphy, compared to the control group. However, the sleep extension group reported improved perceived sleep quality compared to the control group, as reflected by a decrease in Pittsburgh Sleep Quality Index score. The decrease in sleep quality when measured objectively may have been the result of a longer TIB and may diminish over time.

Overall, these studies examining the impact of sleep extension on body weight regulation suggest that interventions including sleep education plus a prescribed sleep extension schedule have the potential to lead to improved body weight and food choices. However, several limitations hinder definitive statements and recommendations to lengthen sleep duration for weight management at this time. All of these studies are limited by their small sample size, with the intervention groups ranging from 10 to 21 subjects, and variability in participant characteristics. Additionally, while each provided some type of sleep education and counseling, the content was unique to each study and the extent of counseling (duration and intensity) varied between studies.

IMPACT OF SLEEP EXTENSION ON INFLAMMATION AND BLOOD PRESSURE

Clinical interventions have provided evidence that restricting sleep is associated with increased inflammatory markers (2) and BP (20) which are associated with cardiovascular disease (CVD). Recently, several clinical trials have examined if sleep extension could help alleviate these risks. Haack et al. (26) performed a 6-week randomized controlled trial studying 22 adults with prehypertension or type 1 hypertension who slept 7 h/night or less. After 2 weeks of actigraphy recordings to verify short sleep, participants were randomized to a sleep extension group, where they were instructed to increase their TIB by 1 h and were prescribed individualized bed times and wake times, or to a sleep maintenance group, who kept their habitual bedtimes and wake times. Both groups received instructions on how to improve sleep hygiene and, at baseline and endpoint, 24 h BP was monitored and a fasting blood sample was taken. As hypothesized, at the end of the 6-week intervention period, the sleep extension group had an increase in daily sleep duration of 35 ± 9 min. Additionally, the systolic and diastolic beat-to-beat BP average over the 24 h recording significantly decreased in the sleep extension group from baseline to endpoint by 14 ± 3 and 8 ± 3 mmHg, respectively. Decreased beat-to-beat BP variability is associated with CVD, end organ damage, and vascular elasticity (36, 37). However, there were no changes in overall BP or inflammatory markers.

McGrath et al. (27) also examined adults with elevated BP who had self-reported difficulties sleeping. Within an 8-week trial, 134 participants who were self-reported poor sleepers were randomized to either standard care or sleep intervention. The intervention group received weekly lifestyle sessions using a tool called Sleepio. Sleepio is an online platform that provides education on sleep hygiene and cognitive behavioral therapy and has been shown to improve sleep quality in patients with insomnia (38). The study investigated whether there was a difference in 24 h systolic BP, 24 h diastolic BP, peak and mean diurnal and nocturnal systolic BP and diastolic BP, and sleep quality between the two groups. At the end of the 8 weeks, the intervention group reported improved sleep quality, but there was no difference between the intervention and standard care groups in BP or other CVD-related measures. Participants did not provide data on sleep duration at baseline. Additionally, no objective measure of sleep was obtained throughout the study, nor was there a sleep extension component. For these reasons, the trial may not have produced the same improvements observed previously, (26) despite its longer intervention period and larger sample size. Although the study by McGrath et al. (27) showed no significant results regarding BP, the improved beat-to-beat BP average observed by Haack et al. (26) is encouraging and warrants further investigation into the role sleep extension may have in modulating CVD risk.

IMPACT OF SLEEP EXTENSION ON GLUCOSE AND INSULIN SENSITIVITY

Insulin resistance is associated with numerous diseases including obesity, metabolic syndrome, and type 2 diabetes mellitus (39). Sleep may be a potential modifiable risk factor for these disorders (1). There is extensive research describing the negative effects sleep restriction on glucose levels and insulin sensitivity, (21, 22, 40) however there is much less information available on the effects of sleep extension on these outcomes. In fact, we could find only one study that investigated the effects of sleep extension on glucose and insulin (28). The goal of that study was to increase sleep duration by 1 h, every night, for 6 weeks. Actigraphy was used to verify that participants were short sleepers, sleeping <7 h/night. Participants were given instructions on proper sleep hygiene and individualized sleep schedules at the beginning of the study, but no other lifestyle intervention was included. Compliance was verified every 2 weeks during the intervention. Participants successfully extended their TIB and sleep duration throughout the intervention compared to their habitual sleep, reaching an increase of 54 ± 33 min in the first 2 weeks, 48 ± 31 min over the second 2 weeks, and 44 ± 34 min over the last 2 week. At the end of the 6-week intervention, there was a significant improvement in the insulin-to-glucose ratio, indicating lessened insulin resistance. As with other studies of this type, there was no comparison group, and there was high inter-individual variation in the amount of additional sleep that was obtained.

CONCLUSION

There are limited clinical trials investigating the effects of sleep extension on health. The studies that have been conducted vary greatly in study length, participant characteristics, and intervention types. Two of the studies discussed did not use an objective measure of sleep to examine sleep quality and duration, providing only subjective data (23, 27). Additionally, while each study provided some type of sleep education, the two studies that did not objectively measure sleep were the only ones to provide continuous counseling sessions throughout the intervention period (23, 27). The other trials only included one counseling session at the start of the intervention, with the session content varying from study to study. The studies that used actigraphy to monitor sleep provided participants with specific sleep schedules, prescribing an extension in TIB

of 1–1.5 h/night, (25, 26, 28) with the exception of one study that imposed a TIB of 8.5 h/night (24). It is worth noting that the trials that utilized objective measurements and individualized sleep extension interventions achieved greater benefits compared to the ones that utilized counseling sessions alone. Also, each study had different inclusion criteria, with two trials not requiring that participants be short sleepers at baseline (23, 27). Due to these study differences, it is difficult to provide recommendations regarding the impact of sleep extension on health.

Furthermore, it is unclear whether sleep quality improves as a result of sleep extension. Participants reported significant improvements of sleep quality in only one trial, (27) in which sleep was not a primary outcome and objective measures were not used. Two of the three studies that utilized objective measures of sleep reported no change in sleep quality, (26, 28) while the other (25) found that the sleep extension group experienced decreased sleep quality compared to the control group. However, this decline could be due to a period of adjustment that potentially would resolve over time and should be monitored in future trials. To that effect, it may be important for future studies to include a run-in period since Cizza et al. (41) have reported improvements in sleep duration of 15–30 min depending on measurement type, objective vs. subjective, respectively, during a waiting period between screening and randomization.

In addition to assessing sleep quality, future sleep extension studies would benefit from being performed exclusively in short sleepers, including larger sample sizes, measuring sleep duration and compliance using objective tools, and providing a prescribed sleep schedule in conjunction with counseling sessions throughout the intervention. In light of the decline in the average sleep duration among US adults, paired with the knowledge that short sleep increases the risk of obesity, CVD, and diabetes, it is encouraging to know that increasing sleep is a feasible endeavor that may be utilized in the future as a behavioral intervention to help alleviate these health burdens. However, standardized, longer-term, randomized intervention trials are needed to verify these preliminary findings.

AUTHOR CONTRIBUTIONS

TP, BA, and M-PS-O contributed to the conception and organization of the review. TP wrote the first draft, TP and M-PS-O wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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REVIEW
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The Bidirectional Relationship Between Obstructive Sleep Apnea and Metabolic Disease

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Obstructive sleep apnea (OSA) is a common sleep disorder, effecting 17% of the total population and 40–70% of the obese population (1, 2). Multiple studies have identified OSA as a critical risk factor for the development of obesity, diabetes, and cardiovascular diseases (3–5). Moreover, emerging evidence indicates that metabolic disorders can exacerbate OSA, creating a bidirectional relationship between OSA and metabolic physiology. In this review, we explore the relationship between glycemic control, insulin, and leptin as both contributing factors and products of OSA. We conclude that while insulin and leptin action may contribute to the development of OSA, further research is required to determine the mechanistic actions and relative contributions independent of body weight. In addition to increasing our understanding of the etiology, further research into the physiological mechanisms underlying OSA can lead to the development of improved treatment options for individuals with OSA.

Keywords: sleep apnea, leptin, glucose, diabetes, obesity, insulin, metabolism, disordered breathing

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OBSTRUCTIVE SLEEP APNEA: CLINICAL PRESENTATION AND PRECLINICAL MODELS

Obstructive sleep apnea (OSA) is a common sleep disorder classically characterized by apneic events leading to intermittent hypoxia and sleep fragmentation. OSA is most commonly found in obese, middle age men (6). Obesity is strongly associated with OSA (7) with approximately 40–70% of the obese population diagnosed with OSA (1, 2). Unfortunately, OSA has broad detrimental effects on health ranging from increased daytime sleepiness to a 4-fold increase in mortality (1). As the name implies, OSA derives from obstruction of the airway. While the cause of obstruction varies between individuals, common obstructions occur due to abnormal anatomy [e.g., narrow airway, enlarged tonsils (8)], obese anatomy [e.g., increased fat storage in pharyngeal tissue (9, 10)], and/or decreased neuromuscular tone (11). During a polysomnography evaluation in the sleep laboratory, an individual with OSA experiences periods of breathing reduction (hypopnea) or cessation (apnea) coincident with respiratory effort. The severity of an individuals' apnea and hypopnea is defined by the apnea-hypopnea index (AHI). An individual with mild OSA experiences 5–15 apnea-hypopnea events per hour, whereas those with moderate or severe OSA experience 15–30 or >30 events/h, respectively (12). Apneic events lead to reductions in blood oxygen saturation, and over the course of the night, present as intermittent hypoxia (IH) (13). It is estimated that an individual with severe OSA may reach blood oxygen saturation levels as low as ∼76% (14) and it is widely regarded that these drops in oxygen play a key role in many of the downstream disease states associated with OSA. Reduction in blood oxygen and elevations in blood carbon dioxide are sensed by chemoreceptors in the brain and carotid bodies, which trigger brief microarousals

and result in sleep fragmentation (15). These repeated microarousals are believed to contribute to Excessive Daytime Sleepiness (EDS), another characteristic of OSA. EDS, as scored by the Epworth Sleepiness Scale, measures an individuals' perceived sleepiness. Higher levels of EDS are associated with an increased risk of falling asleep at work or driving, and is associated with decreased life satisfaction (15). In a large sleep study, 76% of individuals with severe OSA exhibited EDS, and 56% of individuals with mild or moderate OSA exhibited EDS (16). In addition to apneic events, an individual with OSA exhibits a blunted hypercapnic ventilatory response (HCVR) and a blunted hypoxic ventilatory response (HVR) (17), demonstrating impaired chemosensitivity. Interestingly, blunted HCVR (18) and HVR (19, 20) are observed in some obese patients without OSA, most often those with obesity hypoventilation syndrome, suggesting that impaired chemosensitivity may occur before the onset of apneic events.

In contrast to OSA, central sleep apnea (CSA) is defined by the cessation of air flow *without* perceived respiratory effort (21). Like OSA, individuals with CSA may exhibit multiple apneas throughout the night. While CSA effects <5% of individuals referred to the sleep clinic (22), an increased risk for CSA is observed in individuals with compromised chemoreception. For example, CSA is found in ∼24% of chronic opioid users (23) due to opioid-induced impairments to the carotid bodies and hypoglossal nerve signaling (23). Interestingly, ∼13–20% of individuals diagnosed with OSA exhibit central apneas as well (24, 25). In particular, individuals with type 2 diabetes (T2D) have an increased chance of experiencing both OSA and CSA (i.e., mixed apnea) (26). Increased recognition of mixed apneic events has led to an emerging hypothesis which postulates that OSA and CSA share common mechanisms of action (22).

Currently, continuous positive airflow pressure, or CPAP, is the most effective and widely used treatment for OSA (27). By delivering a continuous flow of air, CPAP actively keeps the airway open and can improve the AHI of OSA patients an average of ∼13 events/h (28). Despite the dramatic improvement in AHI from CPAP treatment, compliance is low. Only 39–50% of users will use CPAP (29) for the recommended minimum of at least 4 h per night for 5 days per week (30). Thus, improved treatment strategies for OSA are needed.

Yet, despite the prevalence of OSA, the serious health risks, and the inadequate treatment options, we have a poor understanding of how sleep apnea develops. While clinical studies have been instrumental in laying the foundation of OSA research, basic science approaches using rodent models have enabled investigators to explore the etiology of OSA. Initially, the English bulldog was used as a naturally occurring model of OSA which exhibited snoring, sleep disordered breathing, and daytime sleepiness (31). While it was first believed that the apnea of the English bulldog was occurring solely due to abnormalities of the upper airway (e.g., narrow nares and enlarged soft palate), these anatomical features only accounted for a subset of apneic events. Indeed, during sleep studies, English bulldogs displayed apneic events *without* respiratory effort, representative of central sleep apnea (31). While the English bulldog was a good initial model, and mirrored humans by exhibiting naturally occurring OSA (23), it also experienced apnea in a lean state. To better account for the obesity observed in many OSA individuals, lean and obese Yucatan miniature pigs were utilized as another naturally occurring model of OSA (32). Similar to the English bulldog, obese pigs experienced mixed apneic events, however, lean pigs did not experience any apneic events (32). These data suggested that obesity may be a key factor contributing to sleep apnea. While Yucatan miniature pigs were a naturally occurring model of OSA, further mechanistic studies were difficult owing to sheer size of the animals and lack of available genetic tools.

Currently, much of the mechanistic hypotheses involving OSA are tested in rodent models. The rodent offers superior capabilities in behavioral and genetic manipulation, allowing more detailed investigation into the mechanisms leading to the metabolic consequences of OSA. To examine sleep apnea in rodent models, researchers have modeled two main characteristics of OSA: sleep fragmentation and intermittent hypoxia (IH). In general, sleep loss and decreased sleep quality without the presence of OSA is associated with obesity, impairments in glucose regulation, and reductions in insulin sensitivity (33). While IH is associated with many of these same outcomes, IH often leads to weight loss instead of weight gain, perhaps due to the observed increases in circulating leptin (see leptin section below). Since IH mirrors both the oxygen desaturation as well as the microarousals associated with OSA (15), many of the mechanistic hypotheses on OSA and metabolism have sprouted from IH studies. A wealth of data indicates that chronic IH results in profound impairments in cardiometabolism similar to those experienced by individuals with OSA, including hypertension (34), ventricular hypertrophy (35), insulin resistance, and hyperlipidemia (36, 37). Using whole-body plethysmography, researchers have also observed similarities between the chemosensitivity of obese rodents (measured via the ventilatory responses to hypercapnia and hypoxia, with and without IH) to that of individuals with OSA (38, 39). In this way, the ventilatory responses of rodents presents itself as another measure analogous to the physiology of individuals with OSA. Diet-induced obese rodents can also be used alongside lean controls to determine the effect of obesity on ventilation parameters and IH-induced outcomes. Indeed, just as in humans (18), diet-induced obesity leads to a depressed ventilatory response in rodent models (38, 40). Using a combination of clinical and rodent studies, investigators can significantly increase our understanding of the etiology of sleep apnea.

THE ETIOLOGY OF OSA AND ITS BIDIRECTIONAL RELATIONSHIP WITH METABOLIC DISEASE

In some individuals, OSA etiology is clearly associated with anatomical obstruction. For example, most OSA diagnosed in children is due to enlarged tonsils and is treated with tonsillectomy (41). However, many clinical evaluations for OSA do not reveal any obvious anatomical obstructions (42, 43). In the

absence of a clear anatomical obstruction, much of the etiological theory on OSA has focused on one of its most profoundly associated factors: obesity.

Multiple studies have shown a clear, positive association between obesity and AHI (7). More specifically, increased visceral obesity (44) and neck circumference (45) have been linked to OSA. While it is generally accepted that obesity is an important prerequisite for OSA, the hypothesized mechanisms by which obesity contributes to OSA vary widely. Indeed, the relative contributions of an individuals' physical weight vs. an individuals' metabolic physiology in the development of OSA is an active area of debate (46–48).

Traditionally, the strong association between obesity and OSA led many to conclude that OSA occurred due to increased fat mass mechanically restricting airflow. Specifically, increased fat deposits in the tongue and/or larger pharyngeal tissue (9, 10, 49) were hypothesized to be too heavy for the reduced muscular tone normally experienced during rapid-eye movement (REM) sleep and thus, the tissue's increased physical weight lead to an obstruction the airway and apnea or hypopnea. Increased physical mass also affects lung mechanics, reducing functional residual capacity and tidal volume (50). For the remainder of this review, we refer to mechanisms supporting this hypothesis as *weight-dependent* (**Figure 1**). However, focusing only on physical body weight as an underlying mechanism to OSA does not explain why only a subset of obese individuals have sleep apnea (51). Nor does physical body weight alone explain why lean individuals develop sleep apnea (52). Nevertheless, the association between obesity and OSA suggests that these variables may be related to each other in ways that go beyond the physical mechanical weight of fat.

There are two alternative explanations to the strong association between obesity and OSA. The first of which is that OSA is leading to obesity and metabolic dysfunction (**Table 1**). Indeed, OSA-associated IH and sleep fragmentation have been repeatedly found to induce and exacerbate cardiometabolic disease (91). This directional hypothesis is generally accepted and well-reviewed [see (53, 77, 92)]. Therefore, we only highlight key studies supporting this hypothesis in this review. Instead, we focus on a second intriguing possibility, that obese physiology and not physical weight *per se* leads to the development of OSA (**Table 2**).

Emerging hypotheses postulate that physiological components of obesity, including glycemic control, insulin action, and leptin signaling, contribute to the development of OSA. It's possible that obese physiology leads to greater reductions in pharyngeal dilator muscle tone and results in increased chance of obstruction during sleep (114). This greater reduction in muscle tone may be due to chronically increased muscle activity, due to increased autonomic response, and/or histological changes to the muscle tissue itself via inflammatory pathways (49). Alternatively, obese physiology may be leading to disordered breathing and increased central sleep apnea via decreased chemosensitivity (18–20). Given the increased risk of mixed apneic events observed within type 2 diabetics, this latter observation is particularly interesting. Research on this

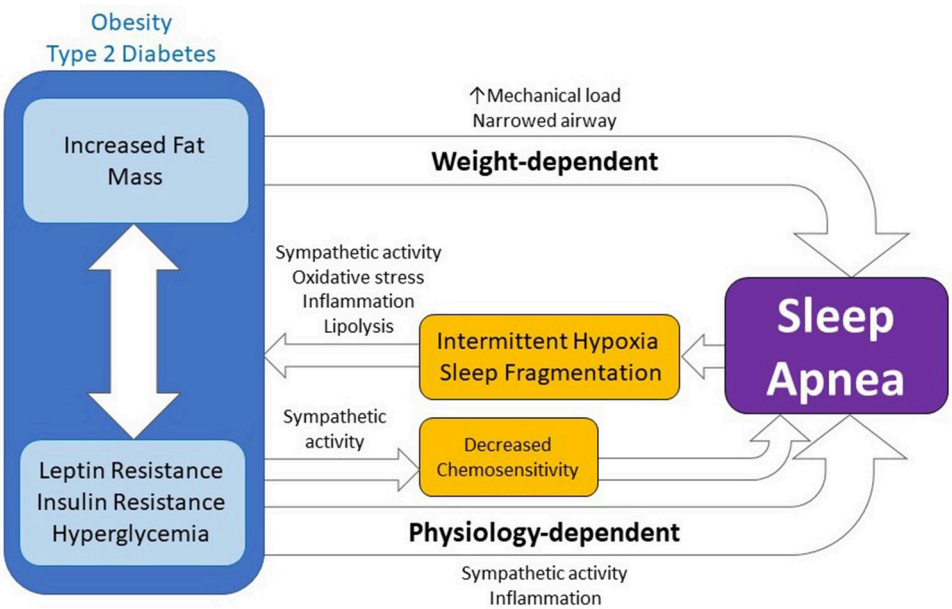

FIGURE 1 | The bidirectional relationship between obstructive sleep apnea and metabolic disease. Sleep apnea results in intermittent hypoxia and sleep fragmentation which lead to and exacerbate obesity and type 2 diabetes by increasing sympathetic activity, oxidative stress, inflammation, and lipolysis. Moreover, metabolic disease can lead to, or exacerbate, sleep apnea through weight-dependent and physiology-dependent mechanisms. While weight-dependent mechanisms are a function of the physical increase in body mass or fat mass (e.g. increased mechanical load, narrowed airway), physiology-dependent mechanisms are physiological changes coincident with obesity or diabetes which go on to influence chemosensitivity and sleep apnea either directly or via action on sympathetic activity, inflammation, or other mechanisms.

TABLE 1 | Summary of presented evidence that obstructive sleep apnea and its components are associated with decreased glycemic control, insulin resistance, increased leptin, and decreased chemosensitivity.

Model	Results	References
Obstructive sleep apnea (human)	↓ Hypoxic ventilatory response ↓ Hypercapnic ventilatory response ↓ Glycemic control ↑ Insulin resistance ↑ Leptin	(17, 52–66)
Type 2 diabetes + Obstructive sleep apnea	↓ Glycemic control ↑ Apnea-hypopnea index ↑ Central sleep apnea ↑ Insulin resistance	(26, 54–57, 67–75)
Sleep fragmentation	↓ Glycemic control ↑ Insulin resistance ↑ Leptin	(33, 76)
Intermittent hypoxia	↓ Glycemic control ↑ Insulin resistance ↑ Leptin ↓ Chemosensitivity	(36, 37, 77–89)
Obesity + Intermittent hypoxia	↑ Insulin resistance ↓ Hypoxic ventilatory response ↓ Hypercapnic ventilatory response	(39, 77, 90)

TABLE 2 | Summary of presented evidence that the manipulation of glycemic control, insulin, and leptin are associated with increased apneic events and decreased chemosensitivity.

Model	Results	References
Metabolic surgery	↓ Apnea-hypopnea index	(93, 94)
Type 2 diabetes (poor glycemic control, insulin resistance)	↑ Apnea-hypopnea index ↑ Central sleep apnea	(26, 53, 71, 74, 75, 95)
Streptozotocin-treatment (destroys pancreatic β-cells)	↓ Apnea-hypopnea index ↓ Hypoxic ventilatory response ↓ Hypercapnic ventilatory response	(96, 97)
Type 1 diabetes (insulin deficient)	↑ Apnea-hypopnea index ↑ Central sleep apnea	(98, 99)
Polycystic ovary syndrome (insulin resistance)	↑ Apnea-hypopnea index	(100–102)
Metformin treatment (insulin sensitizer)	↓ Apnea-hypopnea index ↑ Chemosensitivity	(96, 97, 103)
Leptin impairment (leptin and/or leptin receptor deficiency)	↓ Hypoxic ventilatory response ↓ Hypercapnic ventilatory response	(104–108)
Lipodystophy (low leptin, insulin resistance)	↑ Apnea-hypopnea index	(109–113)

front is on-going and it's possible that other mechanisms by which obese physiology impacts sleep apnea may soon be defined. Collectively, we refer to mechanisms that support these hypotheses as *physiology-dependent* or *weight-independent* (**Figure 1**).

Teasing apart the relative contribution of physical, weight-dependent mechanisms from physiological-dependent mechanisms is inherently difficult due to the close relationship between obesity and its associated changes in glycemic control, insulin action, and leptin signaling. For example, obesity is strongly associated with glucose dysregulation and weight loss alone can substantially improve fasting glucose and glucose tolerance within individuals with T2D (115). In the context of OSA, weight loss through dieting can also substantially improve AHI (116). However, it is unclear if these dramatic improvements in apneic symptoms are from weight-dependent or physiological-dependent mechanisms, as dieting both reduces physical body weight and improves glucose metabolism.

A unique way to partition the effect of weight loss from substantial changes in metabolic physiology has utilized data from bariatric surgical procedures. Bariatric surgical procedures, such as the Roux-en-Y Gastric Bypass (RYGB), the vertical sleeve gastrectomy (VSG), and the laparoscopic adjustable gastric band (LAGB) lead to significant, sustained weight loss and improvements in glucose regulation (117). However, RYGB and VSG are unique among bariatric surgical procedures in that glucose metabolism is improved through both weight-dependent and weight-independent mechanisms. In fact, due to their ability to improve glucose regulation in part through weight-independent mechanisms, RYGB and VSG are sometimes referred to as metabolic surgeries (118). This contrasts with the metabolic improvements following LAGB which parallel total weight loss without additional improvements from weight-independent means (117, 118). Following metabolic surgeries, improvements in glucose tolerance can occur quickly, before significant weight loss occurs (117, 118). In some cases, individuals can discontinue their diabetic medication before being discharged from the hospital (117). To determine how OSA may be affected by metabolic improvements independent of weight loss, it would be ideal to quantify OSA on a time scale before significant weight loss occurs. However, most polysomnography following bariatric surgical procedures occurs 6 months to 1 year post-operatively and thus after significant weight loss is achieved. However, quantifying EDS, closely related to OSA, can be done without polysomnography. In one study, individuals undergoing RYGB showed resolution of EDS symptoms within 1 month, accompanied by only marginal weight loss (119). While it is tempting to speculate that sleep apnea too may be improved on a time scale indicative of weight-independent mechanisms, this question remains unanswered. While EDS is associated with OSA, there is also an independent relationship between obesity and sleep. Overweight individuals are more likely to exhibit increased sleepiness during the day independent of OSA (120, 121). Moreover, decreased sleep duration and sleep quality has been linked to increases in BMI and metabolic dysfunction (122). Therefore, improvements in EDS following bariatric surgery could be a result of small to moderate changes in body weight and/or improvements in metabolic physiology independent of sleep apnea. Alternatively,

directly comparing OSA outcomes following metabolic surgeries such as RYGB and VSG vs. weight-loss surgeries such as LAGB can provide insight into the relative contributions of weight-dependent and physiology-dependent mechanisms in the etiology of OSA. A number of comparative studies have reported that OSA resolution 1-year after RYGB or VSG is approximately double that of individuals undergoing LAGB (93, 94). Furthermore, other studies have shown that LAGB has no better OSA resolution compared to diet-induced weight loss, despite more weight loss attained via LAGB (123). Given the added weight-independent metabolic benefits following RYGB and VSG, these data suggest that some component of obese physiology and not body weight itself, may be involved in the etiology of OSA.

To better address how obese physiology may impact disordered breathing, investigators have incorporated preclinical animal models. Indeed, the preclinical setting allows researchers to systemically manipulate glycemic control, insulin sensitivity, and/or leptin and examine their specific contributions to disordered breathing. While we address each of these variables in detail in the sections below, a commonality among these experiments is the use of high-fat diets to induce obesity within the animal models. Similar to humans, diet-induced obesity leads to a depressed hypercapnic ventilatory response (40) and a restrictive ventilatory pattern (39) in mice. Importantly, since diet-induced obesity alone leads to both increased physical weight and metabolic syndrome, a more detailed approach (such as including weight as a covariate or using weight-matched controls) must be used to specifically determine how obese physiology contributes to disordered breathing. Moreover, the addition of high-fat diets has also been found to exacerbate the metabolic consequences of IH. Obese, high-fat fed mice exposed to chronic IH demonstrate further detriments in insulin resistance (39, 90), suggesting that obesity itself or obese physiology may exacerbate OSA disease outcomes.

GLYCEMIC CONTROL

A prominent characteristic of obese physiology is an impairment in glycemic control. Clinical association studies and randomized control trials have evaluated the relationship between OSA and glycemic control with mixed results. In support of an association between apnea and glycemic control, a recent pilot study found that the combination of respiratory events and nocturnal awakenings could predict variability of fasting blood glucose in T2D patients (67). Nocturnal hypoxemia has also been independently associated with the development of impaired glycemic control (54) and T2D in healthy individuals (55) and worsened glycemic control in individuals with T2D (68). Moreover, with the use of continuous glucose monitoring, T2D individuals with OSA have been shown to exhibit peaks in circulating glucose levels temporally following blood oxygen desaturation (69). Taken together, these studies demonstrate that OSA, and in particular nocturnal hypoxemia, likely leads to elevated glucose levels. In non-diabetic individuals, daily, 24-h rhythms in circulating glucose variability have been associated with OSA severity (56), suggesting that the association between OSA and improper glucose control may precede T2D. Whether circulating glucose levels directly impact disordered breathing or OSA is less clear. It would be informative to explore if individuals with recurrent hypoglycemia or nocturnal hypoglycemia are at increased risk for OSA and/or have reduced chemosensitivity (124). While unexplored, this information could advance our understanding of the involvement of glycemic control and/or glucose sensing in the development of sleep apnea.

In animal models, simulation of OSA using chronic IH has greatly advanced our knowledge of how OSA may impact disease states via cyclic drops in blood oxygen. Rodents exposed to chronic IH have increased gluconeogenesis in the liver (78–80), fasting hyperglycemia, and decreased glucose tolerance (81). Acute, 3-h, exposures of IH in healthy humans also leads to an increase in circulating glucose levels before noticeable changes to insulin sensitivity (125). Indeed, much of the effects on glycemic control from OSA may be attributed to IH (126). Moreover, altering metabolic state prior to IH impacts the outcome, indicating a bidirectional relationship between glycemic control and IH. For example, fasting can mitigate some cardiovascular consequences of IH, including the activation of glycogen synthase in the myocardium (127). Additionally, treatment with a lipolysis inhibitor ameliorates hyperglycemia and glucose intolerance induced by IH in mice (81), highlighting an important role for the adipose tissue and lipolysis in many of the downstream consequences of IH and perhaps OSA (77, 128). Taken together, it is likely that circulating and fasting glucose is increased by OSA and that elevated glucose before the theoretical onset of OSA is likely to exacerbate the cardiometabolic outcomes of OSA.

Another way to explore the relationship between glycemic control and OSA is by intervention and treatment studies. One would hypothesize that if alterations in glucose were downstream of OSA, then treatment of OSA alone would improve glycemic control. While there are randomized controlled studies which support this hypothesis (129), others report no improvement in glycemic control with CPAP use (130). One possibility for these conflicting results is the presence of existing glycemic impairment. For example, in a recent study, higher glycemic variability was associated with sleep disordered breathing in both T2D and non-diabetic individuals, however CPAP treatment only improved glycemic variability in those *without* T2D (57). Similarly, a meta-analysis concludes that CPAP may prevent the development of T2D in non-diabetic individuals (131), again pointing to the effectiveness of CPAP on glycemic control before T2D develops. However, withdraw from CPAP in both obese T2D and non-diabetics leads to an increase in nocturnal glucose without affecting glucose tolerance, production, or insulin (132), suggesting that CPAP use is leading to a reduction in glucose. Together, these data point to the likelihood that glucose impairment is downstream of OSA in non-diabetic individuals, but it remains to be elucidated the relationship between glycemic control and OSA within those with T2D.

One possible mechanism linking glucose dysregulation and OSA is via autonomic dysfunction. T2D leads to autonomic dysfunction and this directly affects respiratory control and cardiac outcomes consistent with the presentation of OSA (133).

This mechanism is supported by impaired autonomic activity observed in individuals with central hypoventilation syndrome which exhibit sleep disordered breathing, hypoglycemia and hyperinsulinemia (134). Sympathetic activity is also directly involved in modulating fasting hyperglycemia following exposure to IH (135), pointing to the ability of the sympathetic system to modulate glucose metabolism in addition to respiratory outcomes (133). Chronic IH has also been observed to increase tonic and reactive afferent chemoreceptor outputs from the carotid body which in turn effects catecholamine to modulate the autonomic nervous system (82–84) and leads to fasting hyperglycemia (136) and hypertension (84). An interesting area of research positions the carotid bodies as key integrators of glucose metabolism, OSA, and autonomic function. Glomus cells in the carotid bodies sense oxygen, carbon dioxide, and glucose. Interestingly, oxygen and glucose signals can potentiate one another, leading to scenarios where dysregulation of glucose may lead to a dysregulation of O_2 and CO_2 sensing which in turn may affect breathing (137). Addition of 2-deoxy-d-glucose (2DG; a glucoprivic agent) in the drinking water of rats can prevent phrenic long-term facilitation, a form of respiratory motor plasticity, suggesting that alterations in glucose sensing can directly alter breathing (138). Work in this field is ongoing and shows great potential in elucidating bidirectional pathways between glucose control and disordered breathing via the sympathetic system.

INSULIN

A key player in glucose metabolism and tightly linked to obesity, insulin action has also been investigated in the context of OSA (95, 139). OSA is correlated with an increased risk of T2D (53) and within the diagnosed OSA population, approximately 15–30% exhibit symptoms of T2D (140, 141). Moreover, a meta-analysis of longitudinal studies concludes that the relative risk ratio of an individual with moderate/severe OSA developing T2D is 1.63 (95% CI: 1.09–2.45) compared to an individual without significant apneic events (58). Within the T2D population, reportedly 58–86% of individuals also present with OSA (70–72). Moreover, in individuals with existing T2D, a dose-dependent relationship is found between worsening glycemic control and the severity of OSA independent of obesity (68, 73, 74). If autonomic neuropathy is present alongside T2D, the individual is at an increased risk for mixed apneic events due to the degradation of respiratory neurons resulting in overall decreases in chemoreception and increased HCVR (75). While these statistics may suggest that T2D precedes the development of OSA, this hypothesis has not been supported by clinical longitudinal studies (142) or meta-analysis (143). Instead, the clinical data point to the likelihood that OSA exacerbates existing T2D through an insulin-related mechanism.

Within non-diabetic and T2D individuals, insulin resistance appears to be more closely tied with OSA than fasting hyperglycemia or glucose variability. Clinical association studies have generally found that insulin resistance is independently associated with OSA (52, 59–64), however data undermining this association, particularly from early clinical studies are present (144, 145). Much of the research exploring the relationship between insulin and OSA has been pioneered in rodent models utilizing IH. Indeed, chronic IH exposure leads to insulin resistance in lean rodents and exacerbates insulin resistance in diet-induced obese models (39, 77, 80, 90). IH can also affect β cell function, leading to augmented basal secretion and reduced glucose-stimulated insulin secretion (80, 146). Much of insulin resistance induced by IH has been attributed to elevated sympathetic activity (147–149), as pharmacological or surgical methods used to block the sympathetic response prevent the development of IH-induced insulin resistance (136, 150). Indeed, individuals with OSA demonstrate increased sympathetic nerve activity (151). Additionally, IH is observed to increase pancreatic oxidative stress and reduce β3-adrenergic receptor mediated insulin secretion (152). A possible mediator between elevated sympathetic activity and insulin resistance following IH may be increased lipolysis. Increased sympathetic outflow contributes to lipolysis, which in turn leads to elevated free-fatty acids and finally insulin resistance (153). This hypothesis is supported by data from animal models where pharmacological inhibition of lipolysis prevents IH-induced decreases in insulin sensitivity (81). A recent clinical study further demonstrated that lipoprotein abnormalities observed in OSA individuals are more directly related to insulin resistance than OSA severity itself (154). Upstream of lipolysis, hypoxia-inducible factor-mediated transcription (e.g., HIF-1α, HIF-2α) may play an important role in linking oxygen desaturation induced by IH with lipolysis (155–157) and/or insulin resistance (158).

While mounting evidence supports the conclusion that IH leads to insulin resistance, research on how decreased insulin sensitivity may lead to the development of OSA is scant due in part to the challenging experimental designs. One such way to specifically manipulate insulin is with the drug streptozotocin (STZ). STZ leads to apoptosis of pancreatic beta cells and, when given in low to moderate doses, is used as a model of T2D, reflecting insufficient insulin action and hyperglycemia. Interestingly, STZ-induced T2D (e.g., STZ-T2D) rats have marked reductions in ventilatory control, including reductions in the HCVR and the HVR, as well as increased incidents of apnea (96, 97). Insulin or metformin treatment can substantially improve disordered breathing in STZ-T2D rats (96, 97), suggesting that insufficient insulin action may contribute to the development of sleep apnea. However, it is possible that observed changes in chemoreception and disordered breathing are secondary to STZ-induced decreases in peripheral sympathetic activity (159) as opposed to insulin action *per se*. Along these lines, STZ-T2D rats exposed to chronic IH exhibit an attenuation in fasting hyperglycemia and mitigated (160) or improved (161) insulin resistance, perhaps reflecting the inability of IH to stimulate a sympathetic system dampened by STZ treatment. Notably, this effect in STZ-T2D rodents is distinct from IH's effect in diet-induced obese T2D animals, which experience an exacerbation in insulin resistance (39, 77, 80, 90).

If insulin action were central to the pathogenesis of sleep apnea, one might expect insulin deficient, Type 1 Diabetic (T1D) individuals to have a higher incident of disordered breathing. In support of this conclusion, children with T1D exhibit more total apneic events and increased CSA, associated with hyperglycemia and autonomic dysfunction (98, 99). Conversely, individuals with T1D are also at risk for a rare syndrome presenting with disordered breathing and hypoglycemia. Dead-in-bed syndrome is believed to occur due to initial bouts of nocturnal hypoglycemia associated with excessive hypotonia of the airway followed by IH, breathing depression, and finally cardiac arrhythmia (162). While these two conditions are distinct in insulin action, they share a common result on sympathetic function. Indeed, chemoreceptors at the carotid bodies are known to respond to elevated insulin with sympathetic activation (163, 164) while hyperglycemic events also cause autonomic dysfunction (99). These data suggest that it may not be insulin action *per se* associated with disordered breathing, but insulin's effect on the autonomic system. Beyond T1D and T2D individuals, other disease states associated with insulin resistance have increased risk of exhibiting disordered breathing. Women with polycystic ovary syndrome (PCOS) exhibit insulin resistance and are 30 times more likely to exhibit OSA compared to women without PCOS (100). Moreover, the insulin resistance displayed by PCOS individuals predicts OSA independent of obesity (101, 102). Hyperinsulinemia and hypoglycemia is also present in individuals with congenital central hypoventilation syndrome (CCHS), a syndrome associated with impairments in chemosensitivity and sleep disordered breathing due to a mutation in the *PHOX2B* gene (134). Individuals with CCHS also exhibit dysregulation to their autonomic nervous system which likely contributes to both their metabolic and disordered breathing phenotype (134). Taken together, these clinical studies suggest that insulin resistance may be an important contributing factor in OSA pathogenesis. However, it is difficult to determine the isolated role of insulin action as alterations in autonomic nervous system activity and/or chemosensitivity are often occurring simultaneously.

In most randomized clinical trials, CPAP treatment improves short-term insulin resistance (165), however the impact of CPAP on long-term insulin resistance is unknown (77). Long-term improvements in insulin action due to CPAP would support the hypothesis that OSA leads to or exacerbates insulin resistance and undermine the hypothesis that insulin resistance itself was leading to OSA. Echoing the latter, a recent randomized, placebo-controlled pilot study reported that manipulating insulin sensitivity via treatment with pioglitazone did not affect OSA (145). However, data from rodent models complicate these findings. In non-obese, high-fat diet fed rats, metformin treatment increased insulin sensitivity and prevented the development of sleep apnea independently of body weight (103). This discrepancy may be due to the specific type of apnea being studied. In rats, central apneic events occurring with relatively higher frequency than in the general human population. If this is true, further research into differentiating between obstructive, central, and mixed apneic events may yield differential contributions of insulin resistance.

Overall, ample evidence demonstrates that insulin resistance is associated with OSA independent of obesity, and that the cyclic bouts of hypoxia experienced by OSA individuals may be key to exacerbating insulin resistance. However, evidence demonstrating that insulin action alone leads to or exacerbates OSA is limited. One possibility is that insulin resistance is one of many factors affecting sleep disordered breathing and requires coincident impairments in the autonomic nervous system, glycemic control, or others (see leptin in the following section) to generate the conditions necessary for promoting OSA.

LEPTIN

Leptin is a satiety hormone released by and in proportion to adipose tissue stores. The robustly positive relationship between leptin and body fat makes leptin an obvious confound when speculating on the root cause of OSA. In general, as leptin increases with fat mass, it acts as an anti-obesity hormone. However, too much leptin can lead to leptin resistance wherein the anti-obesity properties are no longer triggered. Indeed, treating obese individuals with peripheral leptin fails to reduce body weight (166). However, leptin resistance may not impact all of leptin actions. For example, even in obese individuals, leptin's action on sympathoexcitatory actions is maintained (167). It is possible that elevated leptin and/or leptin resistance observed in obesity may be contributing to OSA.

In non-T2D individuals, clinical studies have identified a positive association between OSA and leptin independent of body fat (61, 65). Though a causal relationship has not been defined, there is also evidence that both leptin resistance (168, 169) and OSA increase with aging (144). Healthy pre-menopausal women have significantly higher circulating leptin levels compared to men independent of body weight (168) and are also significantly less affected by OSA (0.6% of pre-menopausal females vs. 3.9% of males) (170), suggesting that increased leptin signaling or elevated leptin may be protective of OSA. However, this effect appears to be absent in post-menopausal women (171). Based on these association studies like these, if leptin action is involved in OSA, then the involvement of other endocrine systems including sex-hormones and insulin resistance may be important co-contributors to OSA.

Recently, accumulating evidence points to leptin action upstream of disordered breathing. Clinical data from individuals with obesity hypoventilation syndrome suggest that leptin resistance contributes to a reduction in HCVR and HVR likely via an impaired chemosensitivity (172). Leptin deficient *ob/ob* mice exhibit a disordered breathing phenotype (104), including a reduction in HCVR (105), and treating *ob/ob* mice with leptin improves ventilation within 3 days, before significant weight loss occurs (105). The obese Zucker rat, which lacks leptin receptors, also exhibit a decreased HVR (106) however maintain a stable upper airway during sleep (173). Leptin resistant New Zealand Obese mice exhibit inspiratory flow limitation, suggestive of sleep disordered breathing (107). These rodent data are partially recapitulated in individuals with lipodystrophy which exhibit chronically low levels of leptin

(109, 110) and are at a greater risk to the development of OSA (111), suggesting that insufficient leptin action may lead to OSA in humans. However, lipodystrophic individuals also have increased fat deposits around the neck and exhibit characteristic insulin resistance (112, 113), making it difficult to determine the individual contribution of leptin on apneic events independent of physical body weight or other physiological variables such as insulin.

Leptin action may also be instrumental in downstream signaling of OSA. IH has been shown to lead to a significant increase in leptin levels in both rodents and humans (37, 85–89). Similar increases in leptin are observed in OSA patients (66) and in those with shortened sleep (76). CPAP treatment in OSA individuals tend to decrease leptin levels independent of body weight, however this is not consistently observed in all studies (128). As many patients lose weight with CPAP, noting changes in body fat specifically (174), is particularly important to consider when reflecting on leptin action. When exposed to IH, rodents with deficient leptin signaling have exacerbated insulin resistance (175) and increased cardiovascular impairments including endothelial dysfunction (176). Leptin treatment prior to IH reduces insulin resistance and hyperlipidemia and improves endothelial relaxation and vascular stiffness in *ob/ob* mice (175, 177). Most intriguingly, leptin treatment can mitigate IH-induced hyperlipidemia and cardiovascular outcomes in lean, wild type (177) suggesting that a boost in leptin signaling may prevent downstream cardiometabolic consequences of IH. As these studies focus on peripheral leptin treatment, it is unclear if leptin is acting primarily on peripheral or central targets. However, recent evidence that manipulation of specific neuronal leptin receptors can lead to tachypnea and a decreased HCVR (108) supports the hypothesis that neuronal leptin signaling may contribute to disordered breathing.

Given the role of leptin in ventilatory drive and the increases observed following IH, leptin may be acting by way of a counterregulatory mechanism in an attempt to improve disordered breathing. Some have proposed leptin is directly controlled by hypoxia (86). However, leptin's tight relationship with other key players in OSA, including obesity and insulin sensitivity (178), especially in T2D individuals (179), make it difficult to draw specific conclusions about the role of leptin in OSA. Key areas of leptin's involvement in OSA require further exploration, including leptin's action in chemosensitive regions, and the synergistic role of leptin, insulin, and other hormones on downstream cardiometabolic outcomes associated with OSA.

CONCLUSIONS AND FUTURE DIRECTIONS

The strong association between obesity, OSA, and T2D has led many to speculate about the bidirectional relationship between metabolic disease and OSA. A wealth of clinical studies suggests that OSA can exacerbate T2D, and animal studies have echoed this conclusion demonstrating that rodents exposed to IH show impairments in glycemic control, insulin resistance, and altered leptin levels. With the aid of these animal models, a number of mechanistic hypotheses have been posed which link OSA to the metabolic syndrome, including an elevation in sympathetic tone, increased lipolysis, inflammation (180), and reductions in chemosensitivity. A more debated hypothesis positions the physiological components of obesity, including glucose, insulin, and leptin signaling as key contributors to the etiology of OSA. While it is becoming clear that elements beyond the physical weight of body fat may be leading to OSA, the field is largely undecided on which factor(s) are critical to OSA's etiology. Novel hypotheses on this aspect of the directional relationship would do well to consider the synergistic relationship between insulin and leptin at the foundation for healthy and disordered breathing. Other new avenues of research show great promise in increasing our understanding of OSA and the relationship to cardiometabolic diseases. Emerging evidence that the gut microbiota is altered following IH (181), for example, elucidates a novel, potential link between OSA, glucose metabolism, and the gut (182). The involvement of the circadian biology with OSA and sleep disordered breathing also shows great promise (183). OSA individuals exhibit a circadian dysregulation of cortisol (184), and treatment with melatonin has been found to mitigate IH-induced hyperglycemia (185), insulin resistance, and microvascular damage (186). Research in these fields are on-going and may revel exciting new information about OSA etiology.

Advancing our knowledge on the etiology of OSA may lead to novel treatment strategies. Currently CPAP is the most effective and widely used treatment for individuals with OSA (27). Despite its low compliance (29), CPAP treatment modestly improves blood pressure (151), attenuates heart failure (187), and improves cardiac function (188, 189) and can significantly reduce mortality due to cardiovascular diseases (190). CPAP can also improve AHI (191) and blood oxygenation in individuals presenting predominantly with CSA (192). CPAP is unique in that it not only targets physical obstructions but also alleviates a brain-central failure to breathe. Indeed, the success of CPAP reflects the heterogenous nature of sleep apnea with both anatomical and neuronal underpinnings. For comparison, surgical treatments such as the Uvulopalatopharyngoplasty (UPPP) target anatomical obstructions and success rates are heavily dependent on degree of anatomical obstruction (193). Whereas drugs targeting the brain improve OSA (194), but not to the extent as CPAP. For example, fluoxetine (Prozac), a selective serotonin reuptake inhibitor commonly used to treat depression, in combination with ondansetron, improves apneic events by ∼40% (195). Similarly, acetazolamide, a carbonic anhydrase inhibitor used to treat glaucoma and other conditions, has been shown to improve central sleep apnea and oxygen saturation (196). Taken together, the current treatment data supports a growing hypothesis that OSA involves more than physical anatomical obstructions and implicates a physiological component in the development of apneic events. Especially in the cases of mixed apneic events, more common in those with T2D (26), it becomes critical to understand the etiology of sleep apnea in order to effectively treat it beyond physical and anatomical obstructions.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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The Relationship Among Morningness-Eveningness, Sleep Duration, Social Jetlag, and Body Mass Index in Asian Patients With Prediabetes

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Background: Circadian system is known to influence energy metabolism. Recent evidence suggested that evening preference could be associated with higher body mass index (BMI). Moreover, evening preference is known to be associated with insufficient sleep duration and greater social jetlag, both described to be associated with obesity. This study aimed to explore whether morningness-eveningness was directly associated with BMI or its effect was transmitted through sleep duration or social jetlag in patients with prediabetes.

Methods: A total 2,133 patients with prediabetes were enrolled. Morningness-eveningness was assessed using a Composite Scale of Morningness (CSM). Average weekly sleep duration and sleep timing were obtained, and social jetlag was calculated. BMI was calculated by weight (kg)/height^2 (m^2). A mediation analysis was performed based on two pathways, i.e. CSM→sleep→duration→BMI and CSM→social jetlag→BMI. A sequential equation model was used to estimate the direct and indirect effects of CSM on BMI.

Results: Mean (SD) age and BMI were 63.6 (9.2) years and 25.8 (4.0) kg/m^2. For CSM→sleep duration→BMI pathway, every one point decrease in CSM (more evening preference) was associated with a decrease in sleep duration by 0.054 h (95% CI 0.043–0.066), whereas sleep duration was negatively associated with BMI (coefficient = −0.156, 95%CI −0.288, −0.024). Mediation analysis indicated that a change in CSM (from 90th to 10th percentile, more evening preference) was associated with a decrease in sleep duration and an increase in BMI by 0.102 kg/m^2 (95% CI 0.015, 0.207). In addition, this change in CSM was directly associated with an increase in BMI by 0.511 kg/m^2 (95%CI 0.030, 0.952). The CSM→social jetlag→BMI pathway analysis revealed that social jetlag was not significantly associated with BMI. A subgroup analysis in those aged ≤60 years (n = 784) revealed that each hour increase in social jetlag was associated with an increase in BMI by 0.56 kg/m^2 (p = 0.026) while CSM and sleep duration were not.

Conclusion: In patients with prediabetes, more evening preference was directly associated with higher BMI and indirectly through insufficient sleep duration, while social jetlag did not mediate the relationship between CSM and BMI. In those ≤60 years, only greater social jetlag was associated with higher BMI. These data could inform further interventional studies to reduce BMI in this high risk group.

Keywords: prediabetes, circadian, body mass index, eveningness, sleep duration, social jetlag

INTRODUCTION

Diabetes is a global health problem. In the United States, 9.4% of the population, or 30.3 million people, were estimated to have diabetes in 2015 (1). The world's heaviest burden of diabetes, however, is in the Western Pacific Region, with 159 million people having diabetes in 2017, with the number expected to rise by 15% by 2045 (2). Prediabetes, a condition in which blood glucose levels are elevated but do not yet meet a criteria for diabetes, is a precursor which markedly increases the risk of developing type 2 diabetes and cardiovascular disease (1). Diabetes prevention with intensive lifestyle interventions has been shown to significantly reduce the risk of diabetes progression (3). In the Diabetes Prevention Program, exercise and weight loss of 7% in patients with prediabetes resulted in a 58% reduction in the risk of developing diabetes, confirming the crucial role of adiposity in abnormal glucose metabolism (3). Therefore, identifying novel factors influencing adiposity in prediabetes patients could lead to interventions to prevent diabetes in this high risk group.

The circadian system, controlled by the master circadian clock located in the suprachiasmatic nuclei of the hypothalamus, is known to play a major role in regulating daily rhythms of metabolism, sleep/wake cycle, feeding behavior, and hormonal secretions (4). There is evidence that circadian disruption or circadian misalignment has detrimental effects on energy metabolism. Experiments utilizing forced-desynchrony protocols which the participants ate and slept on a recurring 28-h day have been shown to result in reduced resting metabolic rate and leptin levels (5, 6). Night shift work, often associated with chronic circadian misalignment, has been shown to be a risk factor for developing obesity (7). This could be partially due to insufficient and/or poor-quality sleep, well-known risk factors for obesity (8, 9) often observed in shift workers. In addition, alterations in meal timing itself can affect circadian regulation (10). In non-shift working population, milder forms of circadian misalignment can be observed, such as in those with evening preference.

The time of day during which individuals prefer to sleep or perform daily activities denotes morningness-eveningness or chronotype. Individuals with evening preference, typically with a later bedtime than those with morning preference, often have a greater degree of circadian misalignment between behavioral rhythms and the endogenous central circadian clock (11). More evening preference has been shown to be associated with greater social jetlag, which is a phenomenon resulting from shifting sleep timing between work days and free days resembling traveling across time zones (12). Emerging evidence from studies in adolescents as well as in general population suggested that evening preference (or late bedtime) and social jetlag were associated with increased adiposity (13–15), although some found this relationship in overweight individuals only (14). Whether evening preference is associated with overweight/obesity in patients with prediabetes, a group at high risk for developing diabetes, has not been previously explored.

Therefore, this study aimed to explore the contribution of morningness-eveningness preference to body mass index (BMI) of patients with prediabetes. By employing mediation analyses, we further examined if this association is mediated through factors known to be associated with both eveningness and obesity, including insufficient sleep duration and social jetlag, or whether there is a direct contribution of morningness-eveningness preference to BMI.

MATERIALS AND METHODS

This cross-sectional study utilized the baseline data from the cohort study of prediabetes patients, which has been conducted since October 2014 at the outpatient clinic of Department of Family Medicine, Ramathibodi Hospital, Bangkok, Thailand. Prediabetes patients aged ≥18 years were recruited. Criteria used for diagnosis prediabetes were fasting plasma glucose (FPG) between 100 and 125 mg/dl (5.6–6.9 mmol/L) or hemoglobin A1c (HbA1c) between 5.70 and 6.49% (38.80–47.44 mmol/mol) (16). Patients were excluded if they had any of following: FPG ≥126 mg/dl (≥7.0 mmol/L), HbA1c level ≥6.5% (48.0 mmol/mol), or were shift workers. The study's protocol was approved by the Ethical clearance Committee of Ramathibodi Hospital, Mahidol University. All participants signed written informed consent.

Data Collection

Demographic data (i.e., age, sex, educational level), family history of diabetes mellitus in first degree relatives, history of smoking (never or current/past) and alcohol consumption (never or current/past) were collected by trained interviewers. Depressive symptoms, previously shown to be related to overweight/obesity (17), were assessed using the Thai version of the Center for Epidemiologic Studies-Depression (CESD) Scale (18). Underlying diseases (i.e., hypertension, dyslipidemia, and chronic kidney disease defined as estimated glomerular filtration rate <60 ml/min/1.73 m^2) and date of diagnosis of prediabetes were reviewed from patient's medical records by investigating physicians (TA, ST, and DL). Height and weight were measured

with a digital scale (Seca 284, CA, U.S.A., precision to 0.1 cm and 0.1 kg) at the date of enrollment by trained staff. BMI was calculated by weight (kg)/height^2 (m^2).

Morningness-Eveningness Assessment

Morningness-eveningness preference was assessed using the validated Thai version of the Composite Scale of Morningness (CSM) (19). The CSM consists of 13 questions regarding the preferred time individuals would like to wake up and go to bed, preferred time for physical and mental activity, and subjective alertness. The total score ranges from 13 (i.e., extreme eveningness) to 55 (i.e., extreme morningness).

Subjective Sleep and Social Jetlag Assessment

Participants were interviewed to collect the data of sleep characteristics including sleep duration and sleep quality, as well as social jetlag. Sleep duration was obtained by the question of "During the past month, how many hours of actual sleep did you get at night?" This question was asked separately for weekdays and weekends. Average sleep duration was then calculated as [(sleep duration on weekdays*5) + (sleep duration on weekend*2)]/7.

To assess sleep quality over the previous month, we utilized the Pittsburgh Sleep Quality Index (PSQI) score, also validated in Thai (20). A modified PSQI score was created by removing the sleep duration component to assess sleep quality independently from sleep quantity (21). Higher scores reflect poorer sleep quality.

The participants were also asked about their usual bedtime, wake-up time, and sleep onset latency on weekdays and weekends over previous month. Mid sleep time of weekdays and weekends were estimated from these information as the midpoint between sleep onset and wake time. Social jetlag (in hours) was then calculated by the absolute difference between mid-sleep time on weekdays and weekends.

Dietary Assessment

Information about all food and beverages intake in the past 24 h before the date of interview were collected by 24-h food recall. Time of the day (presented in 24-h clock time), types and portion of food intake including beverages and snack of each meal were collected. Total daily calorie intake was calculated by dieticians using a Thai food database (INMUCAL-Nutrients V.3, Institute of Nutrition, Mahidol University, Bangkok, Thailand).

Physical Activity Assessment

The physical activity of study's participants was assessed by Global Physical Activity Questionnaire (GPAQ) version 2 (22). This questionnaire asked about the time that participants spent for vigorous- and moderate-intensity activities according to work, travel to, and from places, and recreational activities. The intensity of physical activities was measured as Metabolic Equivalents (METs) that one MET was equivalent to a caloric consumption of 1 kcal/kg/h, and four and eight METS were assigned to the time spent in moderate and vigorous activities, respectively. Total physical activity for each participant was then calculated by summation of MET values of work, travel to and from places, and recreational activities.

Statistical Analysis

Characteristics of the participants were presented as mean (standard deviation, SD) or median (range) for continuous data and as frequency and percentage for categorical data. Univariate linear regression analysis was applied to assess the association between study factor (i.e., CSM score) and mediators (i.e., sleep duration and social jetlag) and BMI outcome. In addition, associations between study factor, mediators, BMI, and other covariables, including demographic variables (i.e., age, sex, educational level), risk behavior (i.e., smoking and alcohol use), depressive symptoms, modified PSQI, dietary parameters (i.e., breakfast and dinner time, and total daily calorie) and physical activity were assessed.

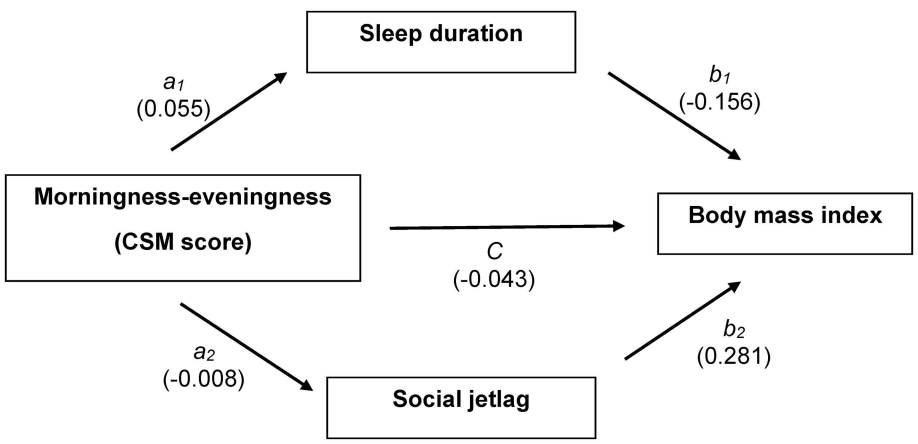

FIGURE 1 | Causal association diagram between CSM score and body mass index with multiple mediation models; CSM score is an independent variable, sleep duration and social jetlag are mediators, and body mass index is an outcome. Coefficient for each path is shown.

Mediation analysis for continuous data was then applied to assess the direct effect of morningness-eveningness preference (CSM score) on BMI and indirect effects of CSM score on BMI mediated by sleep duration and social jetlag.

Causal pathways among CSM score, sleep duration, social jetlag, and BMI were constructed as illustrated in **Figure 1**. According to these pathways, three equations were constructed as follows.

Mediation model for sleep duration: path a_1

$$Sleepduration_i = a_0 + a_1 CSM_i + \sum_k e_k z_k \quad (1)$$

Mediation model for social jetlag: path a_2

$$Socialjetlag_i = b_0 + a2CSM_i + \sum_k e_k z_k \quad (2)$$

Outcome model for BMI: paths b_1, b_2, and c'

$$\begin{aligned} BMI_i &= c_0 + b_{1j} Sleepduration_j + b_{2j} Socialjetlag_j + c' CSM_i \\ &\quad + \sum_l e_k z_k \quad (3) \\ z_k &= confounders \end{aligned}$$

Sleep duration was regressed on CSM score (a_1) as shown in Equation (1). Social jetlag was then regressed on CSM score (a_2) as shown in Equation (2). Finally, BMI was fitted on sleep duration mediator (path b_1), social jetlag mediator (path b_2), and CSM score as direct effect (path c) as shown in Equation (3). The three equations were adjusted for confounding factors, which were significantly associated with each equation including age, sex, educational level, smoking, and alcohol use, modified PSQI score, CESD score, dinner, and breakfast time. Product coefficient method was applied to estimate the average causal mediation effect (ACME) of sleep duration ($a_1 b_1$) and social jetlag ($a_2 b_2$).

In addition, two sensitivity analyses were performed. Because dietary recall is subjected to bias especially with regards to caloric intake, we performed analyses excluding caloric consumption data. We also analyzed the participants according to age since social jetlag is known to be more prevalent in younger age (14). Since our population's mean age was 63.6 years, we divided them into >60 years or ≤60 years according to Thai's definition of elderly (23).

A bootstrap analysis with 1,000 replications was used to estimate ACMEs and their 95% confidence interval (CI) using bias-corrected bootstrap technique. All analyses were performed using STATA version 14. $P < 0.05$ was considered as significant level for all tests.

RESULTS

A total of 2,133 prediabetes patients were eligible for this study. Baseline characteristics of participants are presented in **Table 1**. Means (SD) age and BMI of participants were 63.6 (9.2) years and 25.8 (4.0) kg/m^2. Percentages of female, current/past smoker, and alcohol user were 65.7, 24.6, and 47.3%, respectively.

TABLE 1 | Demographic data, morningness-eveningness, sleep characteristics, and dietary parameters (n = 2,133).

Factor	Result (SD; range)
DEMOGRAPHICS	
Age (years)	63.6 (9.2; 32–92)
Female	1,401 (65.7)
Body mass index (kg/m^2)	25.8 (4.0; 12.36–54.96)
Educational level	
Primary or less	738 (34.6)
Secondary school	607 (28.4)
College or higher	788 (37)
SMOKING HISTORY	
Never	1,608 (75.4)
Current/past	525 (24.6)
ALCOHOL USE	
Never	1,124 (52.7)
Current/past	1,009 (47.3)
Family history of diabetes	832 (39.0)
Hypertension	1,457 (68.4)
Dyslipidemia	1,877 (88.2)
Chronic kidney disease	105 (4.9)
CESD score*	6 (4.91; 0–46)
Physical activity*	128.57 (269.76; 0–4,080)
MORNINGNESS-EVENINGNESS, SOCIAL JETLAG, AND SLEEP CHARACTERISTICS	
Composite scale of morningness (CSM) score	46.6 (4.8; 21–55)
Social jetlag* (h)	0 (0.46; 0–4)
SUBJECTIVE SLEEP ASSESSMENT	
Sleep duration (h)	7.0 (1.3; 1–11)
Modified PSQI	6.9 (2.0; 3–14)
DIETARY PARAMETERS	
Breakfast time (hh:min)	08:03 (01:06; 03:00–12:00)
Dinner time (hh:min)	18:00 (01:13; 12:30–00:00)
Total daily calories	1,027.9 (410.8; 172–3,918)

*CESD, Center for Epidemiologic Studies-Depression; PSQI, Pittsburgh sleep quality index. *Median.*

Around 40% of participants had family history of diabetes in first degree relatives. Most participants had dyslipidemia (88.2%) and hypertension (68.4%) but only 4.9% had chronic kidney disease. Mean (SD) of CSM (morningness-eveningness preference) score was 46.6 (4.8). Average sleep duration was 7.0 (1.3) h and modified PSQI scores were 6.9 (2.0). Medians and ranges of social jetlag and CESD score were 0 (0–4) h and 6 (0–46). Dietary recalls revealed that means breakfast and dinner times were 08:03 (1:06) and 18:00 (1:13), respectively, and total daily calorie intake was 1,027.9 (410.8). Physical activity was 128.57 MET (0–4,080).

Morningness-Eveningness, Mediators (Sleep Duration, and Social Jetlag) and BMI

Univariate regression analyses were performed (see **Table 2**). These revealed that more evening preference (lower CSM score), greater social jetlag and shorter sleep duration were associated

TABLE 2 | Univariate regression analysis between morningness-eveningness, sleep characteristics, demographic data, and body mass index.

Variables	BMI		Sleep duration		Social jetlag	
	Coefficient	*p*-value	Coefficient	*p*-value	Coefficient	*p*-value
MORNINGNESS-EVENINGNESS, SOCIAL JETLAG, AND SLEEP CHARACTERISTICS						
CSM score	−0.080	<0.001	0.053	<0.001	−0.011	<0.001
Social jetlag	0.429	0.008	0.037	0.491	–	–
Sleep duration	−0.239	<0.001	–	–		
Modified PSQI	0.055	0.168	0.027	0.040	−0.007	0.158
DEMOGRAPHICS						
Age	−0.076	<0.001	0.009	0.001	−0.010	<0.001
Male	−0.392	0.014	0.241	<0.001	0.018	0.327
BMI	–	–	−0.239	<0.001	0.429	0.008
Educational level	−0.316	0.045	−0.011	0.836	0.069	<0.001
Smoking	−0.236	0.180	0.213	<0.001	0.045	0.031
Alcohol use	0.085	0.576	0.181	<0.001	0.032	0.072
CESD	0.007	0.628	−0.022	<0.001	0.0004	0.798
Physical activity	−0.0005	0.096	9.67e−06	0.916	−0.00002	0.529
DIETARY PARAMETERS						
Breakfast time	0.054	0.419	0.052	0.017	0.008	0.300
Dinner time	−0.092	0.129	−0.055	0.007	0.022	0.002
Total daily calories	−0.0005	0.004	0.0002	0.877	0.00006	0.004

CESD, Center for Epidemiologic Studies-Depression; CSM, composite scale of morningness; PSQI, Pittsburgh sleep quality index.

with higher BMI. In addition, younger age, being female, and lower educational level were associated with higher BMI. Higher physical activity was associated with lower BMI, but the result was not statistically significant. Total daily calories, but not meal timing, were associated with BMI.

For sleep duration, the results revealed that more evening preference, along with lower modified PSQI score, younger age, being female, non-smoking, non-alcohol use and greater depressive symptoms were significantly associated with shorter sleep duration. In addition, earlier breakfast time and later dinner time were also associated with shorter sleep duration.

For social jetlag, more evening preference (lower CSM score) was significantly associated with greater social jetlag. In addition, younger age, higher educational level, smoking, and later dinner time were also associated with a greater amount of social jetlag.

Mediation Analysis

Because more evening preference was associated with both mediators (i.e., shorter sleep duration and greater social jetlag) and higher BMI, and both mediators (i.e., shorter sleep duration and greater social jetlag) were associated with higher BMI, we further explored if the association between CSM score and BMI was mediated by sleep duration and/or social jetlag. Two mediation equations for sleep duration and social jetlag and one outcome equation for BMI were constructed, adjusting for age, sex, smoking, alcohol use, educational level, modified PSQI, CESD, breakfast time, dinner time, and total daily calories. Their coefficients adjusted for confounding factors are illustrated in **Table 3**.

The results from the mediation equation of sleep duration showed that every one unit increase in CSM score (more morning preference) was significantly associated with an increase in sleep duration by 0.055 h (95% CI: 0.043, 0.066). For the equation of social jetlag mediator, the result suggested that increased CSM score (more morning preference) was significantly associated with decreased social jetlag with the coefficient of −0.008 (95% CI: −0.012, −0.004).

The results of the BMI outcome model (**Table 3**) revealed that increased sleep duration was significantly associated with decreased BMI, with a coefficient of −0.156 (95% CI: −0.288, −0.024). More morning preference (higher CSM score) was also significantly associated with lower BMI ($p = 0.024$). Social jetlag, however, was not significantly associated with BMI ($p = 0.132$).

A bootstrap with 1,000 replications was then applied to estimate the ACMEs of CSM score on BMI mediated by sleep duration ($ACME_1$), social jetlag ($ACME_2$) and to estimate the direct effect of CSM on BMI (c'), see **Table 4**. The results revealed the $ACME_1$ was significant, i.e., that every one unit increase in CSM score (more morning preference) would be associated with an increase in sleep duration, which then significantly decreased BMI by 0.0085 kg/m^2 (95% CI: 0.0167, 0.0003). However, the association between CSM score and BMI mediated by social jetlag was not significant ($ACME_2 = -0.0022$, 95% CI: −0.0056, 0.0011). In addition, we found that there was also a direct effect of CSM on BMI such that every one unit increase in CSM score (more morning preference) was associated with a decrease in BMI by 0.043 kg/m^2 (95% CI: −0.080, −0.005).

Additional analyses were performed excluding caloric intake from the models. The results were similar. Bootstrap analysis revealed that the $ACME_1$ was significant, i.e., that every one unit increase in CSM score (more morning preference) would be associated with an increase in sleep duration, which then

TABLE 3 | Multiple mediation analysis of CSM score and body mass index.

Equations	Factor	Coefficient	SE	*Z*	*P*	95% CI
Sleep duration	CSM score	0.055	0.006	9.20	<0.001	0.043, 0.066
	Age	0.001	0.003	0.46	0.644	−0.005, 0.008
	Male	0.145	0.081	1.79	0.074	−0.014, 0.303
	Education	0.019	0.057	0.32	0.746	−0.094, 0.131
	Smoking	0.066	0.087	0.76	0.449	−0.105, 0.236
	Alcohol use	0.120	0.065	1.86	0.063	−0.007, 0.248
	CESD score	−0.015	0.006	−2.50	0.012	−0.026, −0.003
	Physical activity	−0.0001	0.0001	−0.98	0.327	−0.0003, 0.0001
	Modified PSQI	0.040	0.014	2.77	0.006	0.012, 0.069
	Breakfast time	0.084	0.024	3.50	<0.001	0.037, 0.131
	Dinner time	−0.038	0.021	−1.77	0.076	−0.080, 0.004
	Total daily calories	−0.00003	0.0001	−0.40	0.690	−0.0002, 0.0001
Social jetlag	CSM score	−0.008	0.002	−3.74	<0.001	−0.012, −0.004
	Age	−0.009	0.001	−8.14	<0.001	−0.011, −0.007
	Male	−0.0004	0.029	0.01	0.990	−0.057, 0.056
	Education	0.047	0.021	2.28	0.023	0.007, 0.087
	Smoking	0.024	0.031	0.77	0.439	−0.037, 0.086
	Alcohol use	0.010	0.023	0.45	0.654	−0.035, 0.056
	CESD score	−0.0001	0.002	−0.06	0.952	−0.004, 0.004
	Physical activity	−0.00002	0.00003	−0.58	0.565	−0.0001, 0.00005
	Modified PSQI	−0.005	0.005	−0.96	0.335	−0.015, 0.005
	Breakfast time	0.012	0.009	1.33	0.182	−0.005, 0.028
	Dinner time	0.009	0.008	1.10	0.272	-0.007, 0.024
	Total daily calories	0.00003	0.00002	1.10	0.271	−0.00002, 0.00007
BMI outcome	Social jetlag	0.281	0.187	1.51	0.132	−0.085, 0.647
	Sleep duration	−0.156	0.067	−2.31	0.021	−0.288, −0.024
	CSM score	−0.043	0.019	−2.26	0.024	−0.079, −0.006
	Age	−0.076	0.010	−7.68	<0.001	−0.095, −0.056
	Male	−0.181	0.252	−0.72	0.473	−0.674, 0.313
	Education	−0.555	0.179	−3.10	0.002	−0.905, −0.204
	Smoking	0.065	0.271	0.24	0.810	−0.466, 0.596
	Alcohol use	0.278	0.202	1.38	0.168	−0.117, 0.674
	CESD score	−0.028	0.019	−1.49	0.135	−0.064, 0.009
	Physical activity	−0.0005	0.0003	−1.46	0.146	−0.001, 0.0002
	Modified PSQI	0.070	0.045	1.56	0.120	−0.018, 0.158
	Breakfast time	0.003	0.075	0.04	0.966	−0.143, 0.149
	Dinner time	−0.162	0.067	−2.42	0.016	−0.293, −0.030
	Total daily calories	−0.0005	0.0002	−2.55	0.011	−0.0009, −0.0001

significantly decreased BMI by 0.008 kg/m^2 (95% CI: −0.016, −0.0006). The association between CSM score and BMI mediated by social jetlag was not significant. There was also a direct effect of CSM on BMI such that every one unit increase in CSM score (more morning preference) was associated with a decrease in BMI by 0.042 kg/m^2 (95% CI: −0.079, −0.004).

The distribution of CSM score was explored with 10th, 50th, and 90th percentile of 40, 47, and 52, respectively. A difference in CSM score between 90th and 10th percentile in our cohort (12 points, more evening preference) was associated with a decrease in sleep duration and an increase in BMI by 0.102 kg/m^2 (95% CI 0.015, 0.207), and was directly associated with an increase in BMI by 0.511 kg/m^2 (95%CI 0.030, 0.952). Percentages of mediation effects of sleep duration and social jetlag on BMI were 15.94 and 4.20%, respectively, while the direct effect by CSM contributed 79.86% (**Table 4**).

Subgroup Analysis by Age (≤60 vs. >60 Years)

Mediation analysis in participants aged ≤60 years is shown in **Table 5**. The BMI outcome model revealed that sleep duration and CSM score were not associated with BMI. However, greater social jetlag was significantly associated with BMI. Every 1 h increase in social jetlag was associated with higher BMI by 0.56 kg/m^2 (95% CI: 0.06, 1.06).

TABLE 4 | Causal association effects between CSM score and body mass index.

Parameter	Model	Pathway	Beta	95% CI
$ACME_1$	Sleep duration model	CSM→ Sleep duration→BMI (a_1b_1)	−0.0085	−0.0167, −0.0003
$ACME_2$	Social jetlag model	CSM→Social jetlag→BMI (a_2b_2)	−0.0022	−0.0056, 0.0011
Direct effect	BMI model	CSM→BMI (c′)	−0.043	−0.0797, −0.0054
Total effects			0.0533	0.0175, 0.0891
PERCENTAGE OF DIRECT AND MEDIATION EFFECTS OF CSM ON BMI				
Percent mediation effect through sleep duration			15.94	
Percent mediation effect through social jetlag			4.20	
Direct effect			79.86	

ACME, average causal mediation effect.

Mediation analysis in participants aged ≥60 years is shown in **Table 6**. The BMI outcome model revealed that increased sleep duration was significantly associated with decreased BMI, with a coefficient of −0.283 (95% CI: −0.439, −0.126). More morning preference (higher CSM score) was also significantly associated with lower BMI ($p = 0.008$). Social jetlag, however, was not significantly associated with BMI ($p = 0.962$). A bootstrap with 1,000 replications was then performed (**Table 7**). The results revealed the $ACME_1$ was significant, i.e., that every one unit increase in CSM score (more morning preference) would be associated with an increase in sleep duration, which then significantly decreased BMI by 0.016 kg/m^2 (95% CI: −0.028, −0.005). However, the association between CSM score and BMI mediated by social jetlag was not significant ($ACME_2 = 0.00007$, 95% CI: −0.003, 0.003). In addition, we found that there was also a direct effect of CSM on BMI such that every one unit increase in CSM score (more morning preference) was associated with a decrease in BMI by 0.059 kg/m^2 (95% CI: −0.106, −0.013).

DISCUSSION

In this large cohort of patients with prediabetes who were non-shift workers, we demonstrated that more evening preference was independently associated with higher BMI, after adjusting for multiple covariates. This was mainly due to a direct relationship (estimated at 80%) and also was partly mediated by shorter sleep duration (estimated at 16%). This was especially true for our participants who were older than 60 years. In this group, while greater social jetlag was associated with more evening preference, it was neither significantly associated with BMI after adjusting for confounders, nor did it mediate the relationship between morningness-eveningness and BMI. For participants aged ≤60 years, social jetlag was a predominant predictor of BMI while evening preference did not play a significant role. This could possibly be due to the finding that social jetlag was greater in younger group in our study ($p = <0.001$) which was similar to previous report (14).

In our cohort, a difference in CSM score between 90th and 10th percentile (more evening preference) was associated with an increase in BMI by 0.102 kg/m^2 mediated through sleep duration, and was directly associated with an increase in BMI by 0.511 kg/m^2. In those aged ≤60 years, 1 h increase in social jetlag was associated with an increase in BMI by 0.556 kg/m^2.These effect sizes could be clinically significant, as in the Diabetes Prevention Program, 7% weight loss (approximately a 2.2 kg/m^2 reduction in BMI in this population with baseline weight of 94.2 kg) by diet and exercise in participants with impaired glucose tolerance resulted in a 58% reduction in the risk of developing diabetes during a follow up of 2.8 years (3). Our results highlight the relationship between circadian preference, social jetlag, sleep duration, and adiposity, further supporting the role of circadian regulation on BMI. These results could inform further interventional studies to reduce BMI in this patient group who are at high risk of developing diabetes. Whether circadian preference and social jetlag represent risk factors for future diabetes development requires further follow up of this cohort.

Mechanisms underlying the relationship between circadian regulation and energy metabolism were elucidated in well-controlled experiments inducing circadian misalignment. After 10 days of forced-desynchrony in 10 healthy participants, leptin levels decreased by 17% and a daily cortisol rhythm reversed (5). In a separate experiment combining sleep restriction and circadian misalignment for 3 weeks, mimicking night shift work, leptin profile was observed to be slightly decreased while ghrelin profile slightly increased, along with an 8% reduction in resting metabolic rate (6). Furthermore, a 6-day inpatient simulated night shift protocol led to a 3% decrease in total energy expenditure as measured by a whole-room calorimeter (24). These data are supported by emerging evidence from population-based studies focusing on the role of evening preference, typically associated with mild form of circadian misalignment, and overweight/obesity. In a study of 511 adolescents, evening types had significantly higher BMI z-scores than morning types (15). Furthermore, evening preference has been shown to be associated with weight gain (25) or failed attempts to lose weight (26, 27). In the National Weight Control Registry, morning chronotype was associated with weight loss maintenance (26). Among 252 severely obese adults undergoing bariatric surgery, those who were evening-type had significantly higher BMI and higher weight regain 4 years after surgery than those who were morning-type (27). The results from the current study are in agreement with these data and provide significant evidence of the relationship between evening preference and BMI in patients with prediabetes,

TABLE 5 | Multiple mediation analysis of CSM score and body mass index in participants age ≤60 years ($n = 784$).

Equations	Factor	Coefficient	SE	*Z*	*P*	95% CI
Sleep duration	CSM score	0.051	0.010	5.32	<0.001	0.032, 0.069
	Male	0.180	0.126	1.42	0.155	−0.068, 0.428
	Education	0.034	0.092	0.37	0.712	−0.146, 0.214
	Smoking	0.078	0.132	0.59	0.553	−0.180, 0.337
	Alcohol use	0.084	0.101	0.83	0.405	−0.114, 0.282
	CESD score	−0.014	0.008	−1.71	0.088	−0.031, 0.002
	Physical activity	0.00006	0.0001	0.40	0.688	−0.0002, 0.0004
	Modified PSQI	0.046	0.022	2.06	0.040	0.002, 0.090
	Breakfast time	0.121	0.037	3.25	0.001	0.048, 0.195
	Dinner time	−0.050	0.033	−1.52	0.129	-0.115, 0.015
	Total daily calories	−0.00009	0.0001	−0.83	0.407	−0.0003, 0.0001
Social jetlag	CSM score	−0.014	0.005	−3.15	0.002	−0.024, −0.005
	Male	0.007	0.061	0.12	0.905	-0.113, 0.127
	Education	0.141	0.044	3.17	0.002	0.054, 0.228
	Smoking	0.002	0.064	0.03	0.977	-0.123, 0.127
	Alcohol use	0.041	0.049	0.84	0.400	-0.055, 0.137
	CESD score	−0.001	0.004	−0.35	0.723	-0.010, 0.007
	Physical activity	−0.00002	0.00007	−0.33	0.742	−0.0002, 0.0001
	Modified PSQI	−0.015	0.011	−1.38	0.168	−0.036, 0.006
	Breakfast time	0.006	0.018	0.31	0.757	−0.030, 0.041
	Dinner time	0.026	0.016	1.65	0.099	−0.005, 0.058
	Total daily calories	0.00006	0.00005	1.23	0.220	−0.00004, 0.0002
BMI outcome	Social jetlag	0.556	0.255	2.18	0.029	0.056, 1.06
	Sleep duration	0.061	0.123	0.50	0.619	−0.181, 0.303
	CSM score	−0.039	0.034	−1.15	0.251	−0.105, 0.027
	Male	−0.328	0.437	−0.75	0.453	−1.185, 0.529
	Education	−0.257	0.319	−0.80	0.421	−0.883, 0.369
	Smoking	0.268	0.456	0.59	0.557	−0.626, 1.162
	Alcohol use	0.404	0.349	1.16	0.247	−0.280, 1.088
	CESD score	−0.009	0.029	−0.30	0.764	−0.067, 0.048
	Physical activity	−0.0001	0.0005	−0.26	0.798	−0.001, 0.0001
	Modified PSQI	0.072	0.078	0.92	0.357	−0.081, 0.225
	Breakfast time	0.057	0.130	0.44	0.659	−0.197, 0.312
	Dinner time	−0.105	0.115	−0.91	0.361	−0.329, 0.120
	Total daily calories	−0.0004	0.0004	−1.17	0.242	−0.001, 0.0003

a group which BMI is an important predictor of diabetes development.

Those with more evening preference may have certain behaviors which contribute to the relationship between eveningness and BMI. Evening types were described to be associated with insufficient sleep duration (28, 29), the findings confirmed in our study. This could possibly be due to preferred later sleep timing with the need to wake up earlier than desired to conform to the general society's schedule. The mechanisms linking insufficient sleep and increased obesity risk have been well-characterized in experimental sleep restriction studies, including alterations in appetite regulating hormones (30–32), increased hunger/appetite and unhealthy food consumption (33–35), and little or no change in energy expenditure which could not compensate for increased caloric intake (36–38). The results from these experimental studies are well-supported by epidemiological studies linking short sleep to obesity (39). In a meta-analysis of over 600,000 adults, each hour of shorter sleep duration was associated with 0.35 kg/m^2 change in BMI (39). Insufficient sleep may also hinder the effectiveness of weight loss. In an experiment involving 10 overweight adults for 14 days, those sleeping 8.5 h had a greater loss of fat-free body mass than those assigned to 5.5 h time in bed (40). Emerging data suggested that adequate sleep may be beneficial in weight loss. In a study of 10 overweight adults with short habitual sleep duration (<6.5 h), home sleep extension for 2 weeks (by 1.6 h) was associate with a 14% decrease in overall appetite and a 62% decrease in desire for sweet and salty foods (41). In another study of 123 obese/overweight individuals who underwent a low calorie diet intervention for 14–24 weeks, longer self-reported sleep duration

TABLE 6 | Multiple mediation analysis of CSM score and body mass index in participants age >60 years ($n = 1,358$).

Equations	Factor	Coefficient	SE	*Z*	*P*	95% CI
Sleep duration	CSM score	0.058	0.008	7.74	<0.001	0.043, 0.073
	Male	0.138	0.105	1.31	0.191	−0.068, 0.344
	Education	0.019	0.073	0.26	0.795	−0.125, 0.163
	Smoking	0.053	0.116	0.46	0.645	−0.173, 0.280
	Alcohol use	0.132	0.084	1.57	0.116	−0.033, 0.298
	CESD score	−0.015	0.008	−1.82	0.069	−0.031, 0.001
	Physical activity	−0.0002	0.0001	−1.63	0.103	−0.0005, 0.00004
	Modified PSQI	0.037	0.019	1.93	0.053	−0.0005, 0.074
	Breakfast time	0.061	0.031	1.97	0.049	0.0003, 0.122
	Dinner time	−0.033	0.028	1.19	0.235	−0.089, 0.022
	Total daily calories	3.47*e*−06	0.00009	0.04	0.968	−0.0002, 0.0002
Social jetlag	CSM score	−0.005	0.002	−2.60	0.009	−0.009, −0.001
	Male	−0.015	0.028	−0.51	0.607	−0.070, 0.041
	Education	0.008	0.020	0.41	0.681	−0.030, 0.047
	Smoking	0.039	0.031	1.27	0.204	−0.021, 0.100
	Alcohol use	−0.010	0.023	0.46	0.648	−0.055, 0.034
	CESD score	−0.0002	0.002	−0.07	0.946	−0.005, 0.004
	Physical activity	−0.00002	0.00003	−0.58	0.565	−0.0001, 0.00005
	Modified PSQI	0.0009	0.005	0.18	0.856	−0.009, 0.011
	Breakfast time	0.020	0.008	2.39	0.017	0.004, 0.036
	Dinner time	−0.005	0.008	−0.71	0.475	−0.020, 0.009
	Total daily calories	0.00002	0.00002	0.83	0.405	−0.00003, 0.00006
BMI outcome	Social jetlag	−0.014	0.297	−0.05	0.962	−0.596, 0.568
	Sleep duration	−0.283	0.080	−3.54	<0.001	−0.439, −0.126
	CSM score	−0.059	0.023	−2.63	0.008	−0.104, −0.015
	Male	−0.204	0.309	−0.66	0.509	−0.810, 0.402
	Education	−0.569	0.215	−2.65	0.008	−0.991, −0.147
	Smoking	−0.027	0.340	−0.08	0.937	−0.693, 0.639
	Alcohol use	0.282	0.248	1.14	0.256	−0.204, 0.768
	CESD score	−0.045	0.024	−1.85	0.065	−0.093, 0.002
	Physical activity	−0.0005	0.0004	−1.22	0.222	−0.001, 0.0003
	Modified PSQI	0.057	0.056	1.03	0.304	−0.052, 0.167
	Breakfast time	−0.025	0.092	−0.27	0.787	−0.205, 0.155
	Dinner time	−0.175	0.083	−2.11	0.035	−0.337, −0.012
	Total daily calories	−0.0004	0.0003	−1.67	0.095	−0.0009, 0.0001

and better sleep quality were associated with greater fat mass loss measured by a dual-energy x-ray absorptiometry (42). A recent randomized study explored the effects of caloric restriction with or without sleep restriction for 8 weeks (43). While both groups lost similar amount of weight, sleep restriction group (average sleep reduction of 169 min/week) lost significantly less proportion of total mass lost as fat (43). Our data, derived exclusively from prediabetes patients, supported these previous findings and suggested that sleep extension should be explored as an adjunct to diet and exercise in reducing diabetes risk.

Besides sleep duration, other behaviors associated with more evening preference could be contributing to increased BMI. Greater social jetlag, often seen in those with more evening preference, was described to be associated with higher BMI in those with baseline BMI $\geq$ 25 kg/m^2 in a large population based study of more than 60,000 individuals in Europe (14). Our study, with participants' mean BMI of 25.8 kg/m^2, revealed that social jetlag, a marker of circadian misalignment, was associated with BMI in those aged $\leq$60 years. This was likely due to greater social jetlag in younger age group. This result was in agreement with a recent study of participants with non-communicable chronic diseases (mean age 55 years) which revealed the association between social jetlag and being overweight (44). In older participants, despite the association between social jetlag and evening preference, social jetlag was not an independent predictor of BMI after adjusting for other covariates, thus other factors could play a role. Meal timing, an important input of the circadian system, could be a factor, as delayed or mistimed meals could lead to alterations and uncoupling between the central and peripheral oscillators (10, 45). Consuming food at a later time

TABLE 7 | Causal association effects between CSM score and body mass index in participants age >60 years.

Parameter	Model	Pathway	Beta	95% CI
$ACME_1$	Sleep duration model	CSM → Sleep duration → BMI (a_1b_1)	−0.016	−0.028, −0.005
$ACME_2$	Social jetlag model	CSM → Social jetlag → BMI (a_2b_2)	0.00007	−0.003, 0.003
Direct effect	BMI model	CSM → BMI (c′)	−0.059	−0.106, −0.018
Total effects			0.076	0.031, 0.121
PERCENTAGE OF DIRECT AND MEDIATION EFFECTS OF CSM ON BMI				
Percent mediation effect through sleep duration			22	
Percent mediation effect through social jetlag			0	
Direct effect			78	

ACME, average causal mediation effect.

of the day was shown to be associated with higher BMI (46), development of obesity (47) and less weight loss in response to weight loss therapy (48, 49). In our cohort, however, later dinner time was associated with lower BMI. This could possibly be due to recall bias of the dietary information, or that the relationship between meal timing and individual circadian timing is more important than the meal time as expressed by clock time itself. This was supported by a recent study in 110 young adults which found that consumption of food during the circadian evening (as assessed by dim-light melatonin onset) was associated with higher BMI while the clock hour of food consumption had no effects (50). Besides dinner time, breakfast is another important factor as evening types were associated with breakfast skipping or morning anorexia (51, 52). Less caloric consumption at breakfast or breakfast skipping were shown to be related to increased adiposity (53, 54) and sleep-wake irregularity (55).

In addition to social jetlag and meal timing, evening types generally prefer later sleep timing. This could be associated with greater exposure of light at night leading to circadian disruption. Experimental exposure to blue light 2 h before bedtime in young men led to decreased energy expenditure during the following morning (56). Population based studies have also supported the association between light at night and overweight/obesity (57, 58). Sleep time in our cohort, while correlated with CSM, was not an independent predictor of BMI (data not shown) but the information on light exposure at night was not available. Lastly, more evening preference has been shown to be associated with unhealthy diet, which could contribute to obesity in a long term (15, 59). It is likely that a combination of these factors, rather than any alone, contributes to overweight/ obesity in those with more evening preference. Whether comprehensively targeting these behaviors associated with more evening preference will reduce BMI and possibly future diabetes risk, and the contribution of each factor, is a subject of future research.

Given that the study was performed only in Thai population, some differences from other population should be noted. According to the CSM cutoff, our population mostly had morning preference (a cutoff of 44) (60). It is known that geographic location, likely due to temperature and sun light exposure, is related to circadian preference with countries closer to the equator being more morning (61, 62). Alternatively, a cutoff based on population has been suggested using 10th and 90th percentile for evening and morning preference, respectively (60). Even though our population had relatively more morning preference, we did see a relationship between CSM and BMI across CSM continuum. Another aspect which could be culturally related is meal timing. Breakfast and dinner timing in this cohort are typical of our population which differ from some others such as Spain which typical lunch time is 3 p.m. (48) or in the United States that eating intervals often extend beyond 15 h (63). These differences could play a role our results on relationship between meal timing and BMI.

Our study has the strengths of enrolling a large number of participants with prediabetes, along with comprehensive assessments of circadian preference, sleep and dietary intake. However, there are limitations. The study was conducted at one medical center in Thailand, and thus may not reflect findings in a general population. Sleep assessments, although obtained through validated questionnaires, were subjective. Dietary recall is subject to imprecision such as participants' ability to recall their food intake and timing, and a tendency to underreport which is a well-known phenomenon (64). An average of only 1,027 calories per day could be possibly related to the attempt of the participants to limit their food intake prior to their doctor's visit when most of the assessments occurred. However, excluding the caloric information did not alter the results of our analyses. In addition, the information on light exposure, especially at night, is not available in this study.

In summary, in patients with prediabetes, more evening preference was directly associated with higher BMI and indirectly through insufficient sleep duration. These data supported the importance of circadian regulation in energy metabolism and could inform further interventional studies to reduce BMI in this high risk group.

AUTHOR CONTRIBUTIONS

TA planned the study, collected and analyzed the data, wrote and edited the manuscript. DL and ST collected the data and reviewed/edited the manuscript. AT planned the study, analyzed the data, and reviewed/edited the manuscript. SR planned the study, contributed to discussion, wrote and edited the manuscript.

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The acyl-CoA Synthetase, *pudgy*, Promotes Sleep and Is Required for the Homeostatic Response to Sleep Deprivation

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The regulation of sleep and the response to sleep deprivation rely on multiple biochemical pathways. A critical connection is the link between sleep and metabolism. Metabolic changes can disrupt sleep, and conversely decreased sleep can alter the metabolic environment. There is building evidence that lipid metabolism, in particular, is a critical part of mounting the homeostatic response to sleep deprivation. We have evaluated an acyl-CoA synthetase, *pudgy* (*pdgy*), for its role in sleep and response to sleep deprivation. When *pdgy* transcript levels are decreased through transposable element disruption of the gene, mutant flies showed lower total sleep times and increased sleep fragmentation at night compared to genetic controls. Consistent with disrupted sleep, mutant flies had a decreased lifespan compared to controls. *pdgy* disrupted fatty acid handling as *pdgy* mutants showed increased sensitivity to starvation and exhibited lower fat stores. Moreover, the response to sleep deprivation is reduced when compared to a control flies. When we decreased the transcript levels for *pdgy* using RNAi, the response to sleep deprivation was decreased compared to background controls. In addition, when the *pdgy* transcription is rescued throughout the fly, the response to sleep deprivation is restored. These data demonstrate that the regulation and function of acyl-CoA synthetase plays a critical role in regulating sleep and the response to sleep deprivation. Endocrine and metabolic signals that alter transcript levels of *pdgy* impact sleep regulation or interfere with the homeostatic response to sleep deprivation.

Keywords: acyl-CoA synthetase, sleep deprivation, sleep regulation, lipid metabolism, sleep fragmentation, Drosophila, lifespan

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INTRODUCTION

There is an increasing recognition that lipid metabolism contributes to sleep and wakefulness regulation and the response to sleep deprivation. The involvement of lipids supports the energy hypothesis of sleep regulation, in which energy is required to perform the restorative actions of sleep as well as to carry out waking activities (1). In addition, mobilized lipids can act as signaling molecules and change the composition of membranes within the cell (2). Mutations that alter free fatty acid mobilization reduce sleep rebound and protect the animal from the cognitive deficits usually seen with sleep deprivation (3). In addition, knockdown of two presumptive acyl-CoA

synthetases reduces the homeostatic response to sleep deprivation and had metabolic phenotypes (4). Mutations in fatty acid binding protein result in reductions of sleep in flies and mammals (5, 6). In mammals, TRIB1 has been linked with population differences in sleep regulation (7) and mutations in fatty acid metabolism alters REM sleep (8). Plasma levels of lipid-related molecules are altered through sleep deprivation (9), and free fatty acids are elevated during sleep restriction (10). Lipid metabolism genes are found as genes that are the most changed in response to sleep deprivation in unbiased array or network analyses (11, 12). Thus lipid metabolism, and in particular free fatty acid handling plays an important, yet ill-defined, role in sleep regulation.

Acyl-CoA synthetases (ACSs) are enzymes that attach a CoA moiety to fatty acids to activate them. The resulting acyl-CoA molecule is an "activated" fatty acid that is retained in the cell, and the acyl-CoA can go on to fulfill energy needs, carry out signaling properties, or other functions within the cell or in other cells (2). Each organism has multiple ACSs with specific organ and cellular expression patterns (13). ACSs also have substrate specificity, in which different chain length fatty acids are conjugated to CoA by different ACSs (13). Knockout and knockdown data from mice reveal increases and decreases in lipid storage likely resulting from defects in the dynamics of lipid mobilization (14–16). In flies, mutations in the ACS, *bubblegum* (*bgm*), result in a severe neurodegeneration phenotype that can be rescued by fatty acid consumption (17). In flies, knockdown of two ACSs reduces the homeostatic response to sleep deprivation (4). Thus, ACSs play crucial role in energy and metabolic management and are responsive to changing physiologic conditions.

The ACS *pudgy* (*pdgy*) is upregulated under starvation conditions to continue to fulfill energy needs in the face of decreased energy consumption (18). Transcript levels of pdgy also vary between sleep and wake states in the fly (11). In this manuscript, we used a behavioral genetics approach to assess whether *pdgy* impacts the response to sleep deprivation and whether there were accompanying metabolic changes. We found that decreasing the expression of *pdgy* eliminated the homeostatic response to sleep deprivation. In contrast to the other ACSs observed before, *pdgy*, reduced baseline sleep and increased the fragmentation of the animal. Rescue of *pdgy* in a mutant background restored the sleep phenotypes. These results suggest that *pdgy* regulates sleep through the activation of fatty acids. Given that endocrine factors can alter the transcript levels of ACSs, our data suggest a mechanism by which endocrine factors can alter sleep regulation and lipid mobilization in response to sleep deprivation.

MATERIALS AND METHODS

Flies and Husbandry

Flies were reared in standard laboratory conditions, such light:dark schedule, standard food (yeast, sucrose, corn syrup, molasses, and agar), 25°C and 50% humidity. The *UAS-Pdgy*^{RNAi} line was obtained from the Vienna *Drosophila* Resource Center (19). *Actin-GAL4/CyO* (*Act-GAL4*), *pdgy*^{BG02662}, *pdgy*^{EY02124} (20), *Mi{PT-GFSTF.0}pdgy*$^{MI04730-GFSTF.0}$, and flies to mobilize the transposon were obtained from the Bloomington Stock Center (Bloomington, Indiana). *pdgy*^{BG02662} has a 8,447 bp transposon inserted in the 5′ untranslated region and *pdgy*^{EY02124} has a 10,908 bp transposable element within the coding region of the *pdgy* gene (21). Background controls were generated by mobilizing the P-element using the Δ*2-3* version of the transposase to extract the transposable element, termed "revertants." The *Mi{PT-GFSTF.0}pdgy*$^{MI04730-GFSTF.0}$ uses a Minos transposable element that integrates into an intronic region with a splice acceptor such that the GFP tag is expressed in the protein under the control of endogenous enhancers and repressors (22). The *Fat body-GAL4* (*FB-GAL4*) was generously provided by Ronald Kuhnlein and the *UAS-pdgy*^{wt} was generously provided by Aurelio Teleman.

Sleep Measurements, Sleep Deprivation, and Starvation

Sleep in flies was measured using the Trikinetics *Drosophila* Activity Monitor (DAM) system. Activity counts were converted into sleep using the protocol developed previously (23) in which data was collected in 1 min bins and 5 min of inactivity as the empirically-derived definition of sleep (23, 24). Sleep architecture and sleep metrics were calculated using an in-house program (23–25).

Sleep deprivation was accomplished using the geotaxis method as described in (3). Briefly, 3-5 day old female flies were loaded into 65 mm tubes with food on one end and an air permeable plug on the other end. After 2 full days of baseline, flies were sleep deprived using the SNAP device. Flies were deprived of sleep for 12 h between ZT12 (lights out) to ZT0 (lights on) at which point flies were released into recovery where they remained unperturbed for 48 h. Increases in sleep were calculated from the second day of baseline and presented as a percentage of sleep lost.

Starvation experiments were carried out similarly, in which flies were allowed 2 days of baseline sleep on normal food and then put onto 1% agar and water during the starvation period. For starvation tolerance assays, flies were loaded into the DAMS system for continuous monitoring and which provided activity resolution to the nearest hour to identify when the fly died.

Real-time Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from 20 fly heads using Trizol (Invitrogen, Carlsbad, CA) and cleaned of contaminating DNA using DNAse I. cDNA synthesis was performed in quadruplicate using Superscript (Invitrogen, Carlsbad, CA), according to manufacturer protocol. Equal amounts of cDNA were used as a starting material to amplify *RP49*. cDNA from reverse transcription reactions with comparable RP49 levels were compared. Expression values for *RP49* were used to normalize results between groups. For flies maintained on an LD schedule, both experimental and untreated controls, were collected at the same circadian time, ZT0-1 for sleep deprived or starved animals. Relative levels of transcript were determined using the

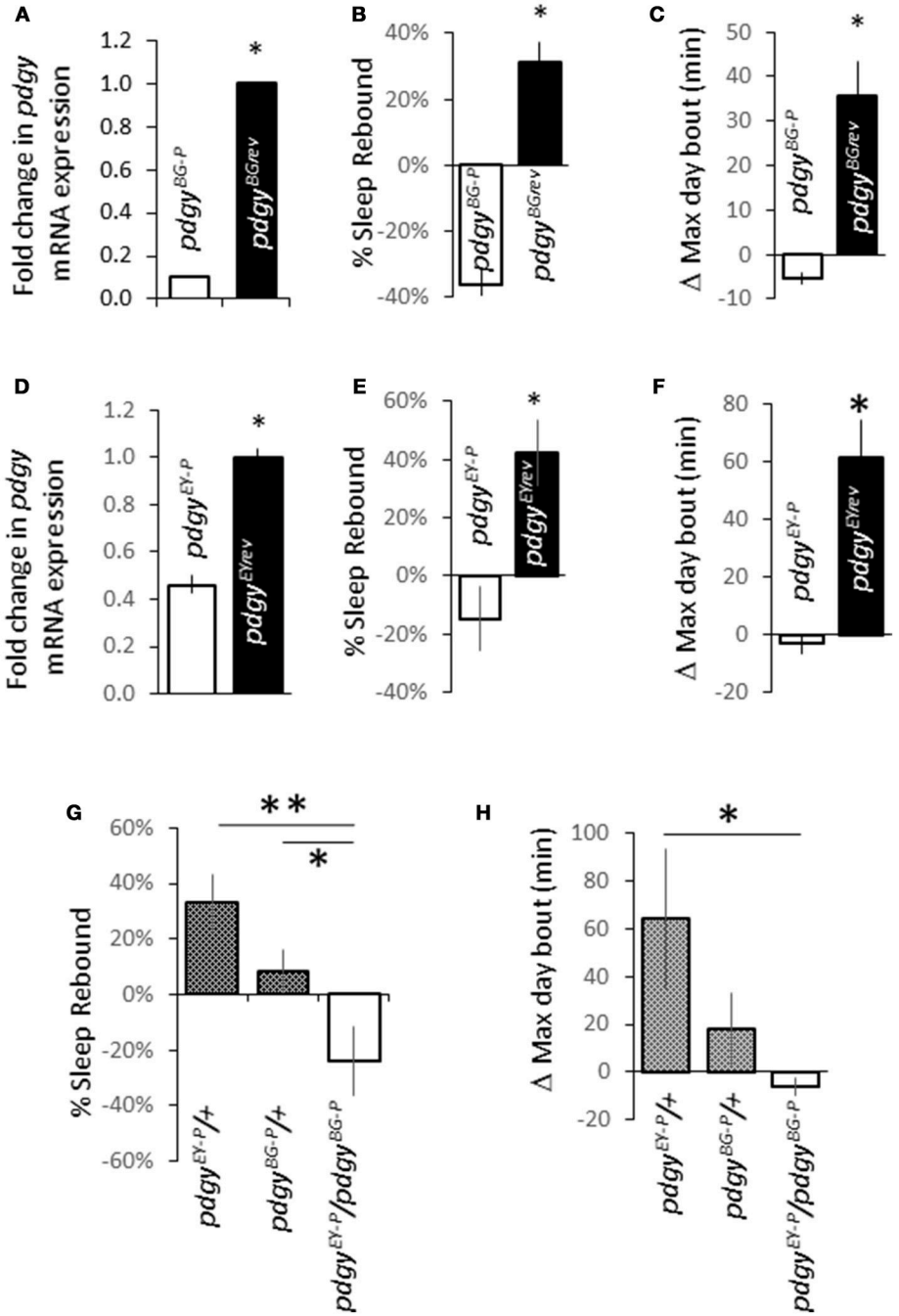

FIGURE 1 | Disruption in *pdgy* expression alters the response to sleep deprivation. **(A)** Levels of *pdgy* mRNA are decreased in $pdgy^{BG02662}$ ($pdgy^{BG-P}$) flies compared to the background control, $pdgy^{BGrev}$ ($n = 3$ groups of 5 pooled flies for each genotype, $p < 0.05$ by Students *t*-test). **(B)** The amount of sleep recaptured after sleep deprivation is significantly decreased when *pdgy* is disrupted ($n = 49$ $pdgy^{BG-P}$ and $n = 39$ $pdgy^{BGrev}$). **(C)** The individual change in the maximum day bout was increased in the $pdgy^{BGrev}$ compared to the $pdgy^{BG-P}$. **(D)** A second P-element near the $pudgy^{BG-P}$ insertion and in a different background, $pdgy^{EY02124}$ ($pdgy^{EY-P}$) has decreased levels of *pdgy* RNA levels compared to the background controls, $pdgy^{EYrev}$. **(E)** The sleep homeostatic response is muted in $pdgy^{EY-P}$ ($n = 40$) compared to the background control $pdgy^{EYrev}$ ($n = 43$). **(F)** The average increase in the maximum day bout is increased between the $pdgy^{EY-P}$ and the $pdgy^{EYrev}$. **(G,H)** To determine if there was something in the background responsible for the sleep phenotype in the background of the two strains of flies, we ran a complementation test. $pdgy^{EY-P}/+$ ($n = 16$) and $pdgy^{BG-P}/+$ ($n = 14$) showed a positive response to sleep deprivation while the $pdgy^{EY-P}/pdgy^{BG-P}$ ($n = 13$) heterozygote has a significantly lower sleep rebound **(G)** and maximum day bout on the day following sleep deprivation **(H)**. $^{*}p < 0.05$, $^{**}p < 0.01$.

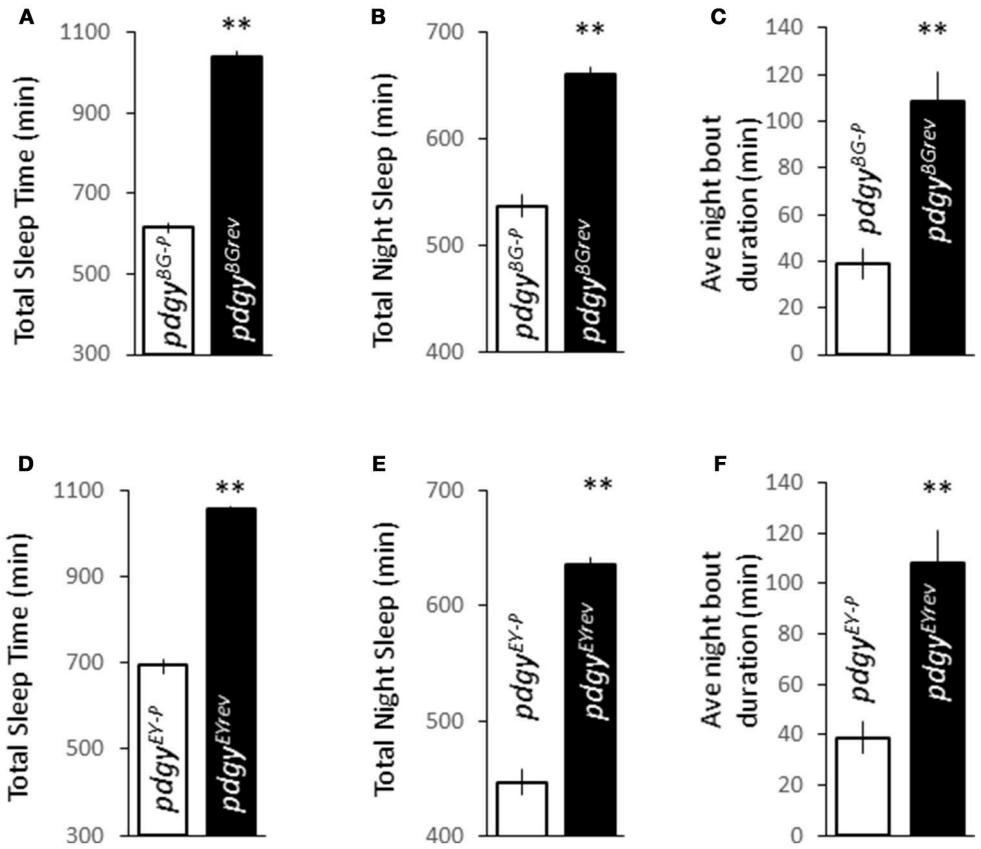

FIGURE 2 | Disruption in *pdgy* expression alters baseline sleep characteristics. **(A–C)** Flies with decreased pdgy expression, $pdgy^{BG-P}$ ($n = 84$), show reductions in sleep with sleep times over 24 h sleep duration throughout the night **(B)**, **(A)** and sleep consolidation as measured by average night bout duration **(C)** compared to background control $pdgy^{BGrev}$ ($n = 63$). **(D–F)** Total sleep time **(D)** nighttime sleep duration **(E)**, and night and consolidation **(F)** are decreased in flies with decreased *pdgy* ($pdgy^{EY-P}$) ($n = 89$) compared to background controls ($pdgy^{EYrev}$) in a second background ($n = 68$). $^{**}p < 0.01$ by Student's *t*-test.

$\Delta\Delta$ method as described (26) to determine the relative levels of transcript between genotype or experimental flies.

Longevity Assay

Three-day-old flies were randomly assigned to one of 3 vials of 10 flies. Flies were counted and transferred onto new food every 3 times per week. Flies alive were expressed as a percentage of the original starting number of flies. Lifespan curves were analyzed using Kaplan-Meier analysis to determine significant differences.

For peroxide tolerance, individual flies were loaded into the DAMS system. After 2 days on normal food, the flies were put onto normal food containing 1% hydrogen peroxide (27) and monitored continuously until the animal died. Deaths were visually confirmed.

Triglyceride Measurements

For each genotype, 10 female flies were frozen and stored at −80°C. Lipid measurements were carried similar to (3). Briefly, flies were weighed and homogenized in a 2:1 (methanol:chloroform) solution to extract the lipids (28). The MeOH:chloroform is evaporated using the speed vac, and the lipids were re-suspended in the starting reagent for Infinity (ThermoElectron, Waltham, MA) triglyceride reagent and triglyceride levels detected using the colorometric detection according to the manufacturer's specifications. Lipid levels are quantified using a standard curve of known triglyceride run in parallel.

Heart Rate

Heart rate was measured according to the methods laid out in (29). We anesthetized flies with 1 mL of FlyNap (Carolina Biological Supply) for 10 min (30). FlyNap does not affect heart function (31). Flies were attached to a slide with the dorsal side up by affixing their extended wings using double-stick tape. Flies were lit from below to see the heart contraction under 200X magnification. Contractions were counted over a 15 s period. The average of 5 separate observations per fly were used. There was a 15 s interval in between observation periods. The experimenter was blinded to genotype and counted the beats per 15 s in the fly.

Resting Metabolic Rate

Resting metabolic rates were determined using a Sable Systems International CA-10 (Las Vegas, NV USA). Virgin female flies per genotype were collected using light CO_2 anesthesia and put

into vial until use. Metabolic rates were measured by using groups of 30, 5–7 day old flies at rest within flow-through respirometry chambers. Dry, CO_2-free air was passed through the 10-ml glass respirometry chambers at 50 mL/min and then dried again and passed through a Li-Cor 6251 carbon dioxide analyzer (Lincoln, NE). Metabolic rates were measured for 5 min using constant respiromentry. The metabolic rate was the stable rate established. Within each run, seven experimental chambers containing flies were sampled in a sequential fashion by using a computer-controlled valve system. Genotypes were assigned randomly to the chambers for each run. One additional chamber was kept empty and sampled before and after each experimental chamber to remove any baseline drift. Temperature was measured by using a thermocouple within the empty chamber. Analog signals from the flow meter, carbon dioxide analyzer, and thermocouple were converted to digital and recorded on a computer (Sable Systems). After CO_2 levels were measured, the weight of the flies was measured on a microbalance to normalize levels to tissue weight.

Immunolocalization

Tissue from the Mi{Mic} flies inserted in *pdgy* were dissected and fixed in 4% paraformaldehyde in PBS for 1 h. Tissues was washed, blocked with normal goat serum, and then incubated overnight in 1:100 anti-GFP primary antibody (R&D Biosystems, Minneapolis, MN) at +4°C. After washing in PBS-T, the samples were incubated overnight in 1:100 Alexa Fluor 488 secondary antibody (Thermo Fisher, Eugene, Oregon) overnight at +4°C, and then washed and mounted in Vectashield. Images were taken using an Olympus IX51 inverted microscope at 600X total magnification using a UPLFLN 60X NA 1.25 objective and an MPlanFLN 10X NA0.30. FITC (EX 482/35 506DM EM 536/40) filter were used (Brightline). Images were captured with a Hamamatsu ORCA285 CCD camera. Shutters, filters, and camera were controlled using SlideBook software (Intelligent Imaging Innovations, Denver, CO).

Data Analysis and Statistics

Where not otherwise stated, groups were analyzed by Student's *t*-test, in the case of comparing 2 samples and an ANOVA analysis for more than 2 groups with a *post hoc* Student's *t*-test to determine which groups are different.

RESULTS

We identified 2 independent P-element insertions within or near the first exon of the *pdgy* gene that could disrupt transcription or function, $pdgy^{BG02662}$ ($pdgy^{BG-P}$) and $pdgy^{EY02124}$ ($pdgy^{EY-P}$). We chose these two particular P-elements because they were created in two different backgrounds and were unlikely to have common mutations that might result in aberrant phenotypes (20). We first measured the RNA levels in the P-element compared to the revertant control. The revertants, $pdgy^{BG02662rev}$ ($pdgy^{BGrev}$) and $pdgy^{EY02124rev}$ ($pdgy^{EYrev}$) were generated by precisely excising the P-element to restore function in the same background as the P-element mutant. Both the $pdgy^{BG-P}$ (**Figure 1A**) and the $pdgy^{EY-P}$ (**Figure 1D**) had lower *pdgy* transcript levels compared to the revertants. To determine if

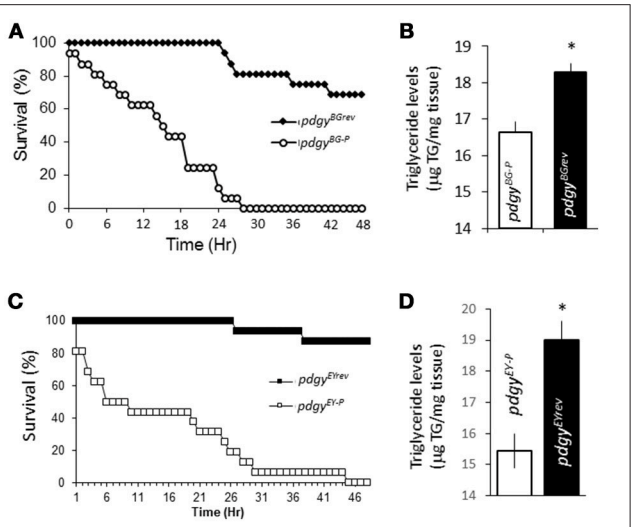

FIGURE 3 | *pdgy* mutants display metabolic phenotypes. **(A)** Under starvation conditions, the $pdgy^{BG-P}$ displayed decreased survival compared to background controls, $pdgy^{BGrev}$. **(B)** $pdgy^{BG-P}$ had decreased lipid stores compared to background controls. **(C,D)** Starvation phenotypes compared to its background control ($pdgy^{EYrev}$) as the $pdgy^{BG-P}$ **(D)** and $pdgy^{EY-P}$ shows a similar triglyceride phenotypes. $^*p < 0.05$.

pdgy impacts the response to sleep deprivation, we deprived flies of sleep for the 12 h primary sleep period from ZT13-ZT0. After sleep deprivation, both *pdgy* P-element alleles did not exhibit a sleep rebound after losing a full night's sleep. The background controls, $pdgy^{BG2rev}$ (**Figure 1B**) and $pdgy^{EYrev}$ (**Figure 1E**), had a large compensatory sleep rebound that were in the range of normal (23). Both the $pdgy^{BGrev}$ (**Figure 1C**) and the $pdgy^{EYrev}$ (**Figure 1F**) had increased average maximum day bout from ZT0-ZT12, an indicator of increased consolidation of sleep (32). The metric is compared the value from the baseline day before sleep deprivation to the same period the day after sleep deprivation (the 12 h post sleep deprivation). The increase in the maximum day bout indicates greater consolidation of sleep in the post-deprivation period, which is a property of the homeostatic response to sleep deprivation. Thus, the P-element mutations have an impaired ability to carry out a sleep rebound.

We then determined if the 2 P-elements or backgrounds complemented one another to rescue the phenotype. Heterozygous expression of each of the P-elements exhibited a rebound whereas the $pdgy^{BG-P}/pdgy^{EY-P}$ heterozygous animal exhibited a significantly negative rebound (**Figure 1G**). The change in the maximum day bout was increased between the $pdgy^{EY02124}/+$ and the $pdgy^{BG02662}/pdgy^{EY02124}$ but not a statistical difference between the $pdgy^{BG02662}/+$, though the outcross showed a positive change and the heterozygote had a decrease in the max day bout (**Figure 1H**). Thus the sleep rebound phenotype is due to the *pdgy* locus and genetically supports the conclusion that both alleles have decreased levels of *pdgy* that do not compensate for each other.

In the course of the sleep deprivation experiments, we observed that the flies with the transposon insertion sleep

considerably less. In fact they had a decreased overall duration (**Figures 2A,D**), with a striking decreased in night duration (**Figures 2B,E**), the fly's most consolidated sleep period. In addition, these flies were significantly more fragmented than their background controls (**Figures 2C,F**). Unlike mutations in other lipid metabolism genes in *Drosophila*, *pdgy* reduces baseline duration and consolidation in the primary sleep period of both the $pdgy^{BG-P}$ and $pdgy^{EY-P}$ mutants.

We then confirmed whether our *pdgy* hypomorphs showed metabolic phenotypes compared to the background controls. We started by determining the fly's starvation sensitivity. After 48 h of starvation, ∼75% of the $pdgy^{BGrev}$ were still alive while the $pdgy^{BG-P}$ mutant flies had completely died after approximately 27 h (**Figure 3A**). The same relationship was observed with the $pdgy^{EYrev}$and $pdgy^{EY-P}$ flies (**Figure 3C**). These results suggest that there is a decreased levels of energy stores or a decreased ability to mobilize those resources under starvation conditions. We directly measured the triglyceride levels. In our hands, the $pdgy^{BG-P}$ had decreased triglyceride levels compared to the $pdgy^{BGrev}$ (**Figure 3B**) and the relationship was similar between the EY mutant and background control as well (**Figure 3D**). Thus, it appears that both the $pdgy^{BG-P}$ and $pdgy^{EY-P}$ had decreased triglyceride levels compared to the revertants and less available resources to mobilize. Though these results do not preclude an inability to properly mobilize or utilize liberated free fatty acids.

Both decreased sleep durations and fragmented sleep have been associated with adverse consequences related to inadequate sleep. We assessed whether lifespan was decreased in the mutants. $pdgy^{BG-P}$ had a 50% survival at ∼33 days and a final lifespan to 59 days while the $pdgy^{BGrev}$ had a 50% survival at 42 days and final survival at 81 days (**Figure 4A**). A similar phenotype was observed in $pdgy^{EY-P}$ as the 50% survival was at 38 days and the genotype survived till 59 days while $pdgy^{EYrev}$ had a 50% survival at day 50 and a final survival for 95 days (**Figure 4B**). These results are consistent with previous results from inadequate sleep.

Another consequence of poor sleep is that there are increases in heart rate. We evaluated heart rate in the flies. Heart rate was elevated in both sets of mutants compared to their revertant controls at 9-11 days old (**Figures 4C,E**). We were interested if the phenotype would progress as the flies aged because the consequences of low sleep duration and sleep fragmentation would continue to build up. In flies that were 22–25 days old, both sets of mutants still had an elevated heart rate, but the phenotype did not appear to get worse with increased time (**Figure 4D,F**). The increased heart rate is consistent with phenotypes associated with inadequate sleep. We reasoned that the similar phenotypes might have similar compensatory responses to in other ACSs. Both hypomorphic lines showed increased transcript levels of *bgm* and *hll* compared to revertant controls, indicating that there are modifications in lipid processing enzymes in response to decreased levels of *pdgy* (**Figures 4G,H**).

Since the flies displayed a decreased lifespan, we wanted to determine if the flies were generally weaker than the background flies. When the flies were challenged with hydrogen peroxide, flies with the P-element integrated into the genome were able to tolerate the infusion of a compound known to increase cellular stress throughout the animal (33). We evaluated young mutant flies and the background revertants (**Figure 5A**). To our surprise, the P-element mutants appeared to be more resilient to the infusion of peroxide compared to the background revertants at 5 days old. It is possible that with age, the P-elements would have shown an increasing susceptibility with age. We then tested middle age and older flies as well. In each case, the P-element flies were more resilient to peroxide treatment than the background controls (**Figures 5B,C**). Interestingly, the peroxide had the intended result as the number of hours that the population survived on peroxide decreased with age but the relationship between the genotypes was not changed. These results indicate that the P-element flies are not inherently weaker than the background controls.

We evaluated whether the resting metabolic rate was different between the *pdgy* mutants and background controls (**Figure 5D**). Interestingly, the metabolic rate was lower in the *pdgy* mutants, perhaps reflecting the decreased ability to appropriately process fatty acids. In addition, these data suggest that the respiration rate is not higher in the mutant flies.

The P-element mutations suggested a decreased level of *pdgy* was responsible for the decreased duration and increased sleep fragmentation. We used an independent knockdown technique guided by the bipartite GAL4-UAS system to decrease levels of *pdgy* (34). We knocked down *pdgy* throughout the animal using the ubiquitous driver *Actin-GAL4* (*Act-GAL4*). We confirmed knockdown as levels of *pdgy* transcript were about 20% in the *Act-GAL4>UAS-*$pdgy^{RNAi}$ of what they were in the background controls (**Figure 6A**). When the animal was sleep deprived, the control lines, *Act-GAL4/+* and the *UAS-*$pdgy^{RNAi}$*/+* flies showed a significantly higher rebound in sleep rebound compared to the experimental line, *Act-GAL4>UAS-*$pdgy^{RNAi}$ (**Figure 6B**). The maximum day bout was lower in the knockdown flies compared to both controls (**Figure 6C**). We then wanted to determine if a more localized knockdown in the fat body would alter the rebound using the *Fat body-GAL4* (*FB-GAL4*), an enhancer trap that has been shown to express in the adult fly fat body (35). When *pdgy* is reduced using *FB-GAL4*, the rebound to sleep deprivation was again reduced below the background lines, indicating that the knockdown of *pdgy* in the fat bodies may contributes to the sleep rebound phenotype (**Figure 6D**). When max day bout was assessed, it was lower in the knockdown flies compared to the *FB-GAL4/+* background flies but did not reach significance in compared to *UAS-*$pdgy^{RNAi}$*/+* flies (**Figure 6E**). Though, the *UAS-*$pdgy^{RNAi}$*/+* flies showed an increase while the knockdown flies showed a decrease in max day bout, which is consistent with how the sleep rebound was expressed. With both drivers, we did not observe the baseline sleep phenotype, possibly because the knockdown was not strong enough or in the appropriate cells.

As a final confirmation that *pdgy* was a critical part of the sleep rebound, we employed a rescue strategy in which the wild-type *pdgy* transcript was expressed ubiquitously in an otherwise *pdgy* hypomorphic animal. We put both *Act-GAL4* and *UAS-*$pdgy^{wt}$, generously provided by Aurelio Teleman (18), into the $pdgy^{BG-P}$ background. The rescue flies exhibited increase night sleep (**Figure 7A**) and increased

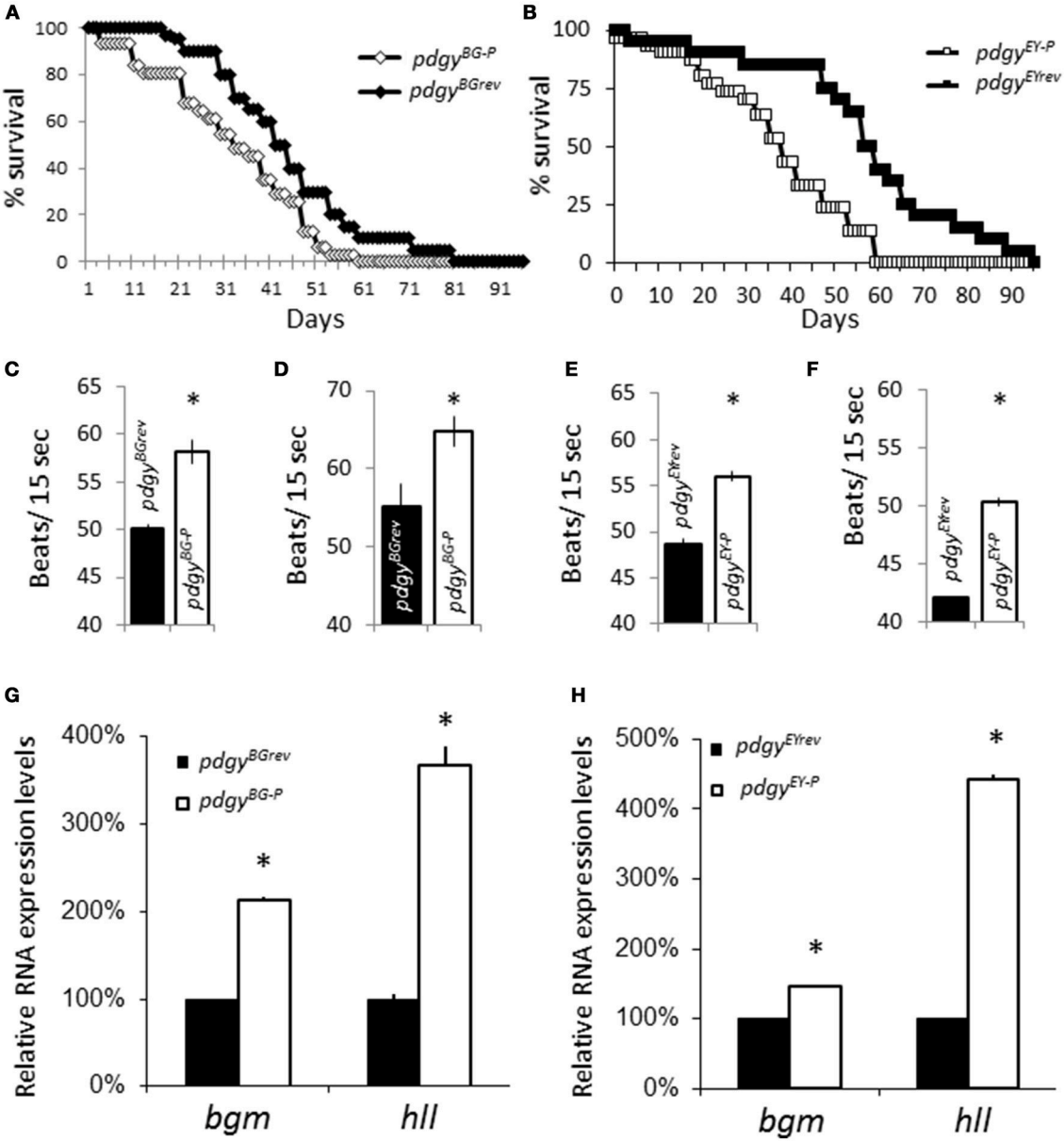

FIGURE 4 | Consequences of the *pdgy* mutants. **(A)** Lifespan measure for the $pdgy^{BG-P}$ ($n = 30$) compared to the revertant control, $pdgy^{BGrev}$ ($n = 30$). **(B)** Lifespan measure for the $pdgy^{EY-P}$ ($n = 30$) compared to the revertant control, $pdgy^{EYrev}$ ($n = 30$). $pdgy^{BG-P}$ has increased heart rates compared to background controls at **(C)** 9–11 d.o. and **(D)** 22–25 d.o. A similar phenotype is observed in $pdgy^{EY-P}$ compared to background controls at **(E)** 9–11 d.o. and **(F)** 22–25 d.o. **(G)** Levels of the ACSs *bubblegum* (*bgm*) and *heimdall* (*hll*) are elevated in the *pdgy* hypomorph ($pdgy^{BG-P}$ in **G**) and ($pdgy^{EY-P}$ in **H**) compared to the revertant control. $^*p < 0.01$.

night time consolidation (**Figure 7B**) compared to flies that did not carry the rescue construct. In addition, rescue flies had a homeostatic response to sleep deprivation whereas mutant flies did not (**Figures 7C,D**). Thus, these data indicate that rescuing *pdgy* restores more wild-type sleep traits.

Since knockdown using *FB-GAL4* did not phenocopy the *pdgy* hypomorphs, we wanted to determine if there was *pdgy* expression elsewhere in the animal. We took advantage of a set of flies in which an EGFP tag has been integrated into the *pdgy* gene through random integration. This allows us to visualize where the *pdgy* gene is expressed endogenously. This technique comes with inherent caveats, but it has also revealed novel expression patterns and generated interesting hypotheses when an antibody is unavailable, as it is with *pdgy*. Using this technique, we observed faint *pdgy* expression in the fat bodies. We did observe expression in the Malpighian tubules (**Figures 8A–D**) as well as throughout the gut with particular localization in the cardia of the gut (**Figures 8E–F**). In addition, we observed expression in the Malpighian tubules (**Figure 8B**). Thus, the

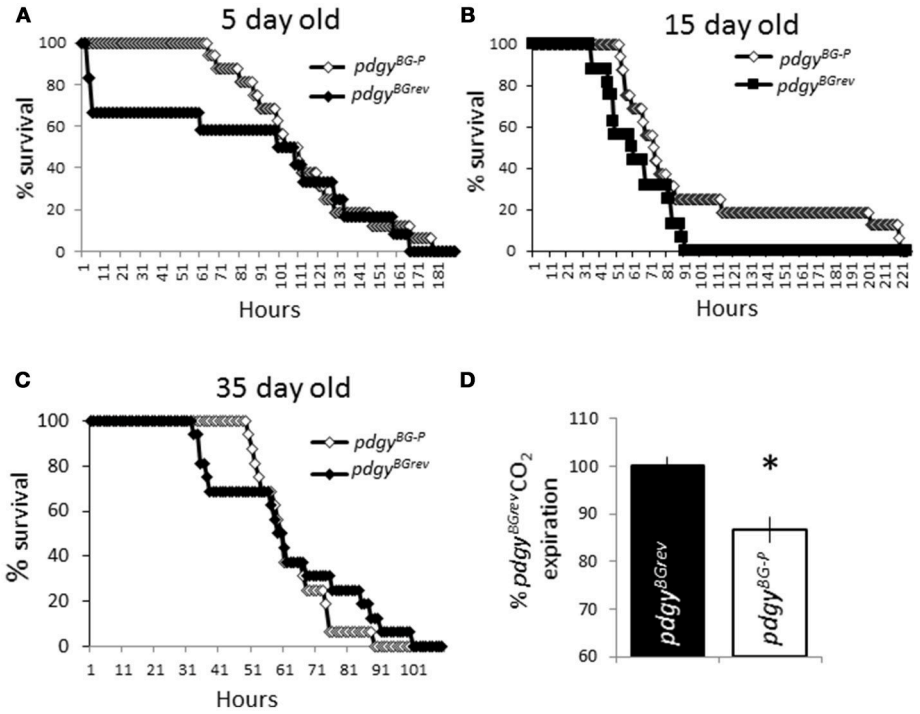

FIGURE 5 | *Pdgy* mutants are not generally unfit flies. $pdgy^{BG-P}$ and $pdgy^{BGrev}$ were put on peroxide in their food different ages, 5 d.o. **(A)**, 15 d.o. **(B)**, and 35 d.o. **(C)**. $n = 16$ for each condition. **(D)** We measured levels of CO_2 expired as an indicator of respiration. Levels of CO_2 are displayed as percent of control. $^*p < 0.05$.

expression of *pdgy* may be more widespread in the fly and it is possible these organs could play a role in the response to sleep deprivation.

DISCUSSION

Lipid metabolism has become a critical regulator of sleep and the response to sleep deprivation. The role of metabolism is taking a more prominent role in governing sleep regulation and the response to sleep deprivation (1, 36–38). During sleep there is very small drop in energy expenditure (39), suggesting that there is a substantial energy requirement, even during sleep. In microarray studies, lipid metabolism genes have elevated transcription in the brain in both mammals and flies (11, 40). In humans, the brain is active during sleep, which will consume energy, especially during rapid eye movement (REM) sleep (41, 42). In addition, energy consumption continues throughout the body during sleep, resulting in only a modest decrease in energy consumption compared to waking (39). Energy may play a critical role because both waking activities and the restorative activities of sleep require energy to carry out their functions (1). Thus, molecules and enzymes that control energy metabolism may regulate sleep and wake cycles and disruptions or changes in metabolic handling and the signals, such as endocrine signals, may have an impact on sleep behavior.

We have been evaluating the role that lipid metabolism plays in regulating the response to sleep deprivation (3). Through a microarray that compared waking that induced damage to the organism compared to waking that does not result in performance and health decrements, we identified several lipid metabolism genes, including the ACSs *bgm* and *heimdall* (*hll*) (4). One lipid metabolism gene, the ACS *pdgy*, was not identified, whereas *pdgy* (identified as *CG9009*) had been identified as a sleep regulatory gene (11). It remains unclear if *pdgy* plays a role in sleep regulation or the response to sleep deprivation.

Here, we have demonstrated that the acyl-CoA synthetase, *pdgy*, plays a critical role in the response to sleep deprivation. This is consistent with the role that other lipid metabolism genes in the fly (3, 4). For example, mutations in ACSs, such as *bgm* and *hll*, result in decreases in the sleep rebound. Knockdown of *hll* also has a lipid phenotype as lipid levels were lower but retained the distribution in mutant flies as measured by lipidomics (4). In contrast with other ACSs, we also demonstrated that *pdgy* also reduced the night sleep duration and increased fragmentation, both of which decrease the restorative ability of sleep. *Pdgy* (or *CG9009* as it was previously designated) had been identified as a gene that increased its transcription levels with sleep deprivation. (11). Thus, we provide strong evidence that *pdgy* is involved in sleep regulation and the response to sleep deprivation.

Pdgy has been identified as an ACS (21), metabolic enzymes that activate fatty acids. These fatty acids can act as signaling molecules, fulfill cellular functions or transferred into the mitochondria for oxidative phosphorylation to generate energy (**Figure 9**). In that study, the authors hypothesized that the $pdgy^{BG-P}$ had a difficulty utilizing stored triglycerides. The

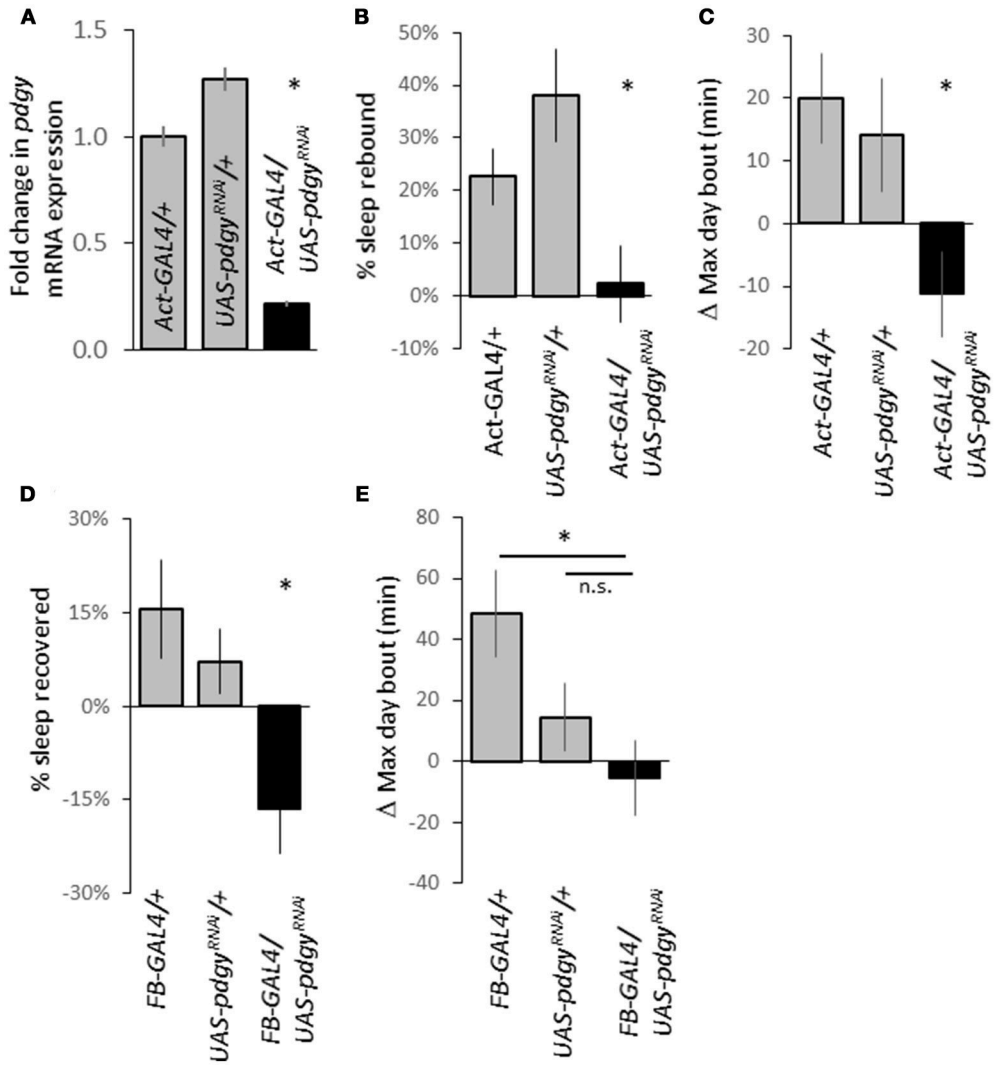

FIGURE 6 | *Pdgy* knockdown results in a decreased rebound. **(A)** We confirmed knockdown using real-time qPCR as RNA levels were decreased in *Act-GAL4>UAS-pdgy*^{RNAi} compared to background controls. **(B)** Ubiquitous *pdgy* RNAi knock down (*Act-GAL4>UAS-pdgy*^{RNAi}) reduced the response to sleep deprivation compared to the background controls, *Act-GAL4/+* and *UAS-pdgy*^{RNAi}*/+*. N = Act 50, UAS = 38, Act/UAS = 40). **(C)** An increase in the maximum day bout was suppressed when pdgy was knocked down ubiquitiously. **(D)** Knockdown in the fat body (*FB-GAL4>UAS-pdgy*^{RNAi}) also decreased the response to sleep deprivation compared to background controls FB-GAL4/+ and *UAS-pdgy*^{RNAi}*/+*. **(E)** The change in the maximum day bout in *FB-GAL4>UAS-pdgy*^{RNAi} compared to background controls. For **(D,E)**, *N = FB-GAL4/+*=29, *UAS-pdgy*^{RNAi}*/+* =53, *FB-GAL4/UAS-pdgy*^{RNAi}=32). *$p < 0.05$.

results presented here agree with those conclusions. In the starvation experiments, $pdgy^{BG-P}$ and $pdgy^{EY-P}$ died in about 24 h without food, whereas the majority of the revertants were still alive after 48 h of starvation. In addition, we found a triglyceride phenotype, suggesting that *pdgy* is involved in lipid metabolism. Where the manuscripts do not match is in the precise metabolic outcomes. In Xu et al., the $pdgy^{BG-P}$ had elevated triglycerides compared to the controls, and in a starvation assay, the $pdgy^{BG-P}$ flies exhibited increased starvation resistance. In our study, we also examined another P-element mutation $pdgy^{EY-P}$ also show a metabolic phenotype similar to the $pdgy^{BG-P}$. Since these mutations were generated in two different backgrounds, the BG lines were made in an isogenized line while the EY lines were made in a different background that were more conducive to P-element integration (20), these two lines do not carry the same background mutations and gene expression levels. In addition, when the two P-element lines were crossed to each other, there was no complementation, again suggesting that pdgy was responsible for the sleep phenotype. Therefore, the phenotypes observed in the $pdgy^{BG-P}$ and $pdgy^{EY-P}$ are likely due to the *pdgy* gene and not second-site mutations for 2 different lines.

On its face these results may seem incompatible, but the relative differences may be due to how each of the mutants were generated. In Xu et al., they outcrossed the BG mutant five times to a white mutant, which in theory exchanges the background

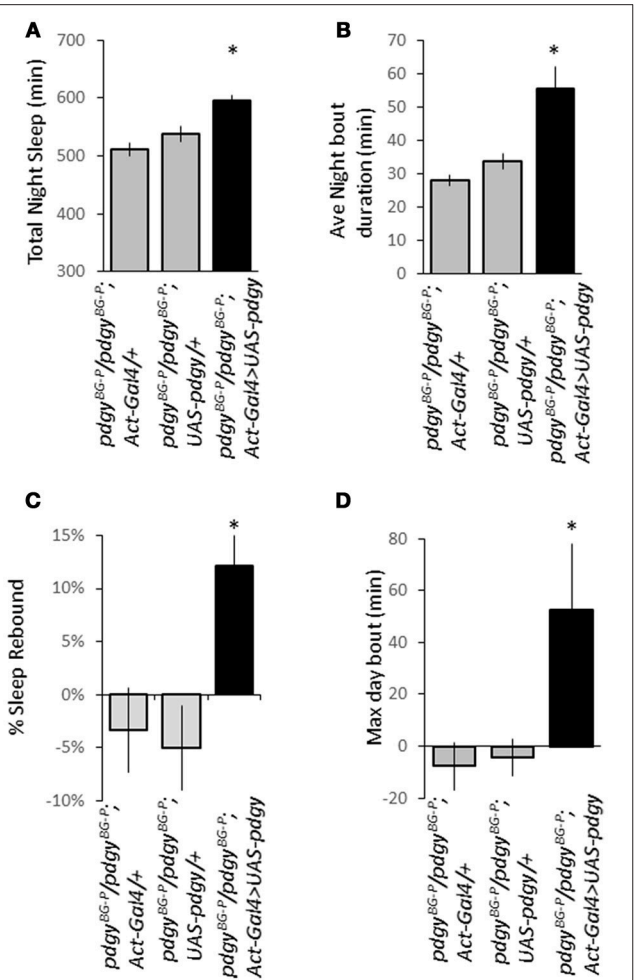

FIGURE 7 | Rescue of pdgy in the $pdgy^{BG-P}$ background restores sleep homeostasis, duration, and consolidation. Re-introduction of the wild-type version of *pdgy* (dark bars) restores the duration of night time sleep **(A)** and increases the average night bout duration as a measure of sleep consolidation **(B)** compared to the driver (*Act-GAL4/+*) and the responder (*UAS-pdgy*) both in gray. (For both **A,B**, $n = 63$ for Act-GAL4/+, 64 for UAS-pdgy/+, and 61 for Act-GAL4>UAS-pdgy). **(C)** Rescue also restored a significant rebound after sleep deprivation. **(D)** The maximum day bout is increased on the first recovery day after sleep deprivation. For **(C,D)**, $n = 29$ for Act-GAL4/+, 26 for UAS-pdgy/+, and 30 for Act-GAL4>UAS-pdgy. $^*p < 0.05$.

around the P-element for the background on the white-eyed flies. The white-eyed mutant is then the comparator genotype. This method standardizes the background and make it easier to compare between different mutants. The white-eyed mutant background is not a preferable background, just a standardized background with all of the mutations and differences that occur in the white background. Our approach was to precisely excise the P-element to restore *pdgy* transcript levels in the same background that the original mutation was in. This is a common method to restore function in flies. Therefore, our comparator group is different than their comparator group and triglyceride levels would be relatively different. The metabolic system is complex and may have complex interactions. Therefore, in the Xu et al. experiments, the background may allow enough of the lipids to be processed to permit starvation resistance whereas in the *pdgy* revertant background may not allow lipid utilization that supports survival under starvation conditions.

These results highlight how the difference in genetic context can lead to serious consequences or not so serious consequences. One example of where this has occurred is with the CLOCK mutant mouse. In one case, the impact of the mutation was increased weight gain, lipids dysregulation and cholesterol possibly due to the lack of circadian rhythms (43). Yet when that same mutation was moved into a second background, the opposite phenotype was observed (44). In both cases, circadian rhythms were disrupted, but the consequences were different in the different circumstances. These results may have a clinical implication as shift workers, who often have misaligned circadian rhythms, have an increased likelihood to be obese (45). Interestingly, understanding the differences between the two backgrounds may provide insights into possible treatment options to prevent lipid dysregulation in people with circadian disruptions.

We have gone on to demonstrate using other genetic methods that *pdgy* plays a role in the response to sleep deprivation. The sleep deprivation phenotype was replicated using both knockdown and rescue indicating that *pdgy* is necessary and sufficient to play a role in this process. The baseline sleep phenotype was replicated in the rescue experiments. We hypothesize that the knockdown was not strong enough in the appropriate cells to impact the sleep duration and fragmentation phenotypes, but that presents an opportunity to identify cells responsible for these phenotypes. Moreover, the phenotypes were observed in 2 different P-element lines with 2 different backgrounds. In sum, the genetics strongly implicates the *pdgy* in sleep regulatory pathways.

In the fly disruptions in the metabolic system have been shown to alter the response to sleep deprivation. Presumably, the energy demand is created to carry out the restorative aspects of sleep. At a cellular level, the restorative role that sleep plays will require energy to convert and/or dispose of the damage generated during waking activities. Under most normal circumstances that energy is limited (1). Therefore during sleep deprivation the body cannot satisfy the energy demands of both restoration and waking functions and must leave some things undone. Consistent with this hypothesis, levels of circulating free fatty acids are increased with sleep deprivation, consistent with an increase in available energy with sleep deprivation (10). These incomplete functions may underlie the deleterious consequences of waking. Mutations that decrease triglyceride lipase activity inhibit the response to sleep deprivation. Moreover, a mutation in the gene *Lipid storage droplet-2* (*LSD-2*), results in increased triglyceride lipase activity and a blunted sleep rebound (3). Interestingly, starved flies (both the $cycle^{01}$ mutant and the w^{1118} line) and *LSD-2* mutant flies were able to learn after the same amount of sleep loss as sleep deprived flies that showed learning impairments. What these two conditions have in common is that they both have elevated levels of lipolysis that could sustain increased energy needs. In other animals there are temporary periods in which sleep is disrupted, but function is maintained. Female orcas and dolphins that have

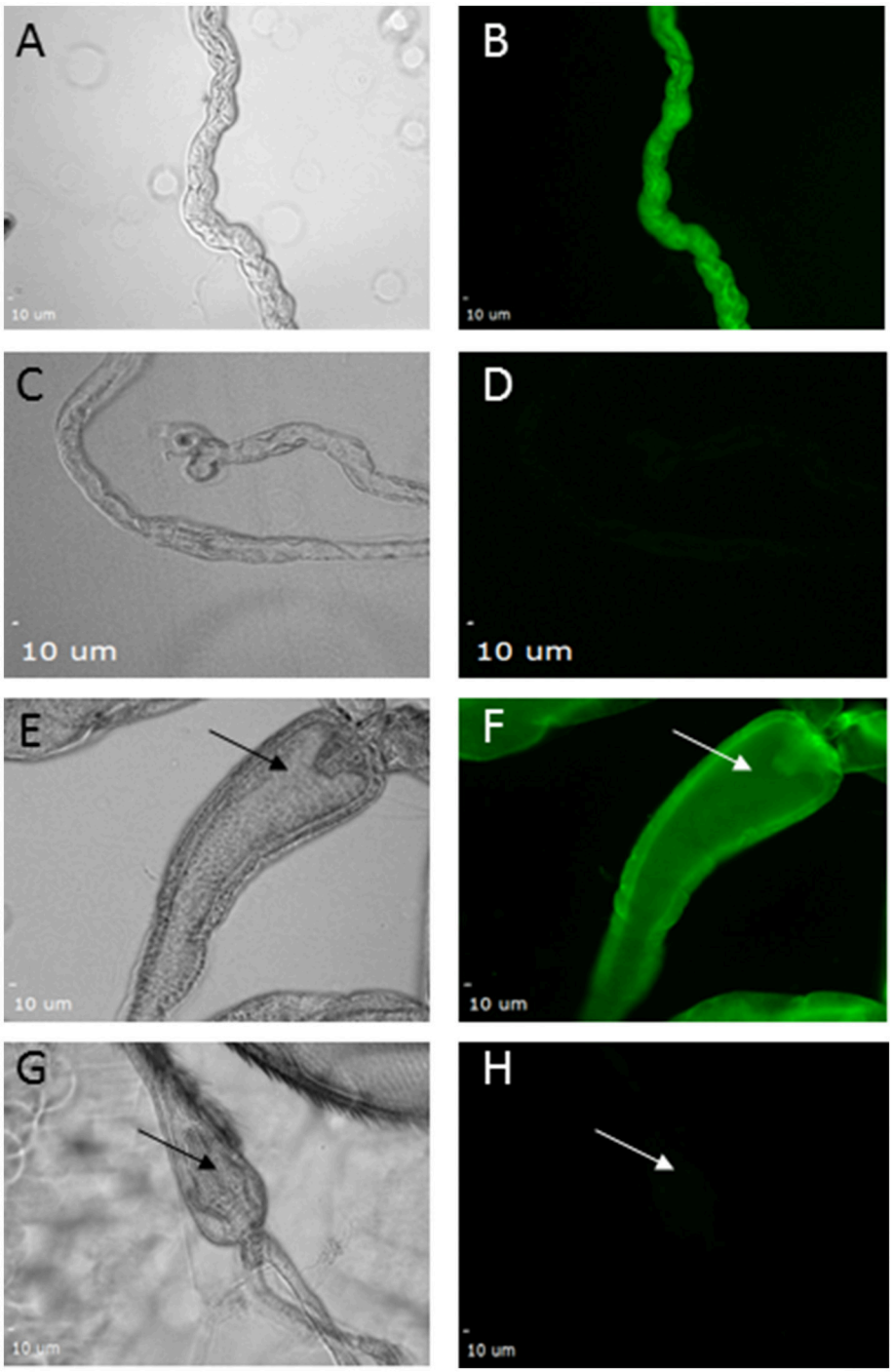

FIGURE 8 | Expression of pdgy was detected in the gut and Malpighian tubules using an enhancer trap line. GFP expression was detected in the Malpighian tubules **(A–D)** and in the gut **(E–H)**. Bright field images show Malpighian tubules **(A,C)**. Fluorescence expression was detected in **(B)** when the secondary antibody was excited by wavelengths in the green wavelengths as opposed to a control **(D)** with a secondary antibody not excited or emitting in this same wavelength. GFP expression was also expressed in the gut. Shown here is the recognizable portion of the gut, the cardia. Expression was seen in **(F)** but not in **(H)** with the same conditions as above. Bright field images showing the field **(E,G)**. Arrows point to the cardia in each image.

just given birth remain awake with their calves for approximately a month without a subsequent increase in sleep to compensate (46). White-crowned sparrows retain cognitive function during the migratory season, despite substantial sleep deprivation (47). Cognitive function is not retained if the sleep loss is outside of the migratory period. Sandpipers are able to maintain reproductive

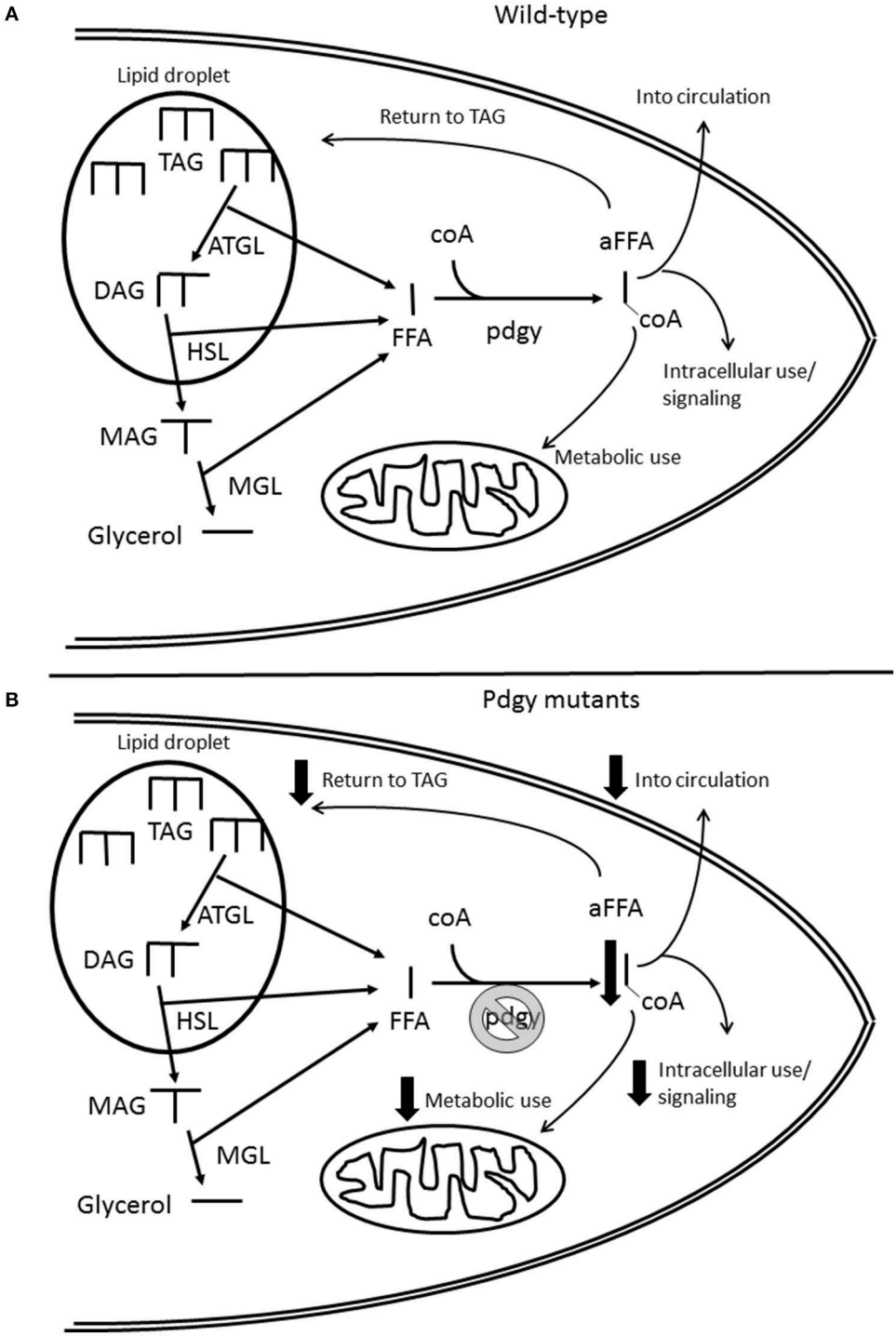

FIGURE 9 | Schematic of the role of pdgy in cellular lipid metabolism. **(A)** Under normal conditions, the adipose triglyceride lipase (ATGL) releases free fatty acids (FFA) and diacylglycerides (DAG) from the lipid droplet. Another FFA is released from DAG by hormone sensitive lipase (HSL) and another FFA is released from the Monacylglyceride (MAG) by monoglyceride lipase (MGL) leaving glycerol. Pdgy then adds a coA molecule to the FFA to activate it (aFFA). The aFFA then is used for metabolic, may be reinserted into the lipid droplet, cell signaling or protein post-translational modifications, or distribution to other tissues through the circulation. **(B)** The absence or reduction of pdgy would result in less FFA coverted to aFFA. This mutation would potentially reduce the FFA available to carry out necessary functions within the cell and throughout the organism. Another consequence may be the increase in the levels of FFA within the cell.

fitness in the face of sleep deprivation (48). This possibility of increased lipolysis is consistent with all of the natural conditions in which sleep deprivation is not accompanied by impairments from sleep loss. These data support the need for lipid metabolism to support sleep and the response to sleep deprivation though the effects are a complex process and may depend on the genetic and environmental context (49). The *pdgy* sleep phenotypes observed here are consistent with other lipid metabolism mutants that do not permit a homeostatic response to sleep deprivation.

We evaluated potential consequences of short sleep duration and increased fragmentation sleep in the P-elements. In other situations, short sleep increases cardiovascular effects, including heart rate potentially due to increases in the sympathetic system (50, 51). Moreover, short sleep duration has been associated with shorter lifespan in flies and humans (52–54). The phenotypes observed, elevate heart rate and decreased longevity, were consistent with the consequences of inadequate sleep. These occurred in both P-element mutants, but these flies were not more sensitive to stresses as measured by exposure to hydrogen peroxide. These consequences were not observed in either the RNAi knockdown experiment or the rescue experiments in the $pdgy^{BG-P}$ background. The GAL4/+ in the BG background showed the decreased lifespan, but the UAS/+ did not shown the decrements in lifespan on the same timeline as the rescue experimental line. This despite the fact that sleep duration was decreased and sleep was fragmented in the background lines, but was normal in the rescue line. This result argues against that sleep loss and fragmentation result in a reduced lifespan phenotype. Alternatively, the rescue expression of *pdgy* may provide protection under these circumstance. Another possibility is that in the P-element background, the lack of proper fatty acid utilization results in the observed phenotypes.

We used an enhancer trap line to localize pdgy within the fly. Enhancer traps integrate an exogenous piece of DNA into the animal's DNA. Once present, expression of the GFP falls under the control of local enhancer and repressors to guide expression. For pdgy, the MI{MIC} line is integrated within the pdgy gene and thus likely reflects endogenous expression. Our results demonstrated strong expression in the cardia and throughout the gut as well as expression in the Malpighian tubules, the kidney-like organ in the fly. We didn't see strong expression in the adipose tissue, though we did not test it under starvation conditions where there is likely a large upregulation of pdgy in the fat bodies (18). In mammals, ACSs have been found in the intestinal epithelial cells (55), supporting the interpretation that MI{MIC} expression mimics endogenous expression. Another possibility is that the MI{MIC} expression responds to only part of the enhancer control. As we did not see complete phenocopy between the RNAi knockdown in the fat body and the P-element hypomorphs. Therefore it is possible that this expression pattern may contribute to sleep regulation.

The fact that lipid metabolic enzyme *pdgy* can alter sleep regulation has a two broader implications. First, this is further evidence that lipid metabolism plays a critical role in sleep regulation under baseline conditions and after sleep deprivation. Energy management appears to be an obvious route to impact sleep regulation, but this does not rule out a contribution by lipid signaling. Secondly at the physiological level, endocrine signals change the expression of metabolic enzymes. For example, both growth hormone and insulin alter the transcription for lipogenesis (56). Given that there are numerous ACSs that likely have specificity for chain lengths of fatty acids, not all of these changes would have the same impact on sleep regulation as *pdgy* it will alter the cellular metabolic environment. In fact, starvation alters the metabolic environment and increases wakefulness (3, 57–59). Therefore, when the hormonal environment changes because of dietary conditions or because of disease, one of the consequences could be that the fatty acid mobilization and activation enzymes are changed and that those changes may impact sleep regulation and the response to sleep deprivation. In addition, inadequate sleep can alter the endocrine environment (37, 60–62). Thus, there is a direct connection between the endocrine system to sleep regulation through metabolic enzymes.

AUTHOR CONTRIBUTIONS

MT designed experiments, ran experiments, analyzed data, and wrote the manuscript. NK, JL, CF, and TH all designed and performed experiments, and analyzed the subsequent data.

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Oxyhemoglobin Saturation Overshoot Following Obstructive Breathing Events Mitigates Sleep Apnea-Induced Glucose Elevations

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Background: Obstructive sleep apnea (OSA) and nocturnal hypoxia are associated with disturbances in glucose regulation and diabetes. Temporal associations between OSA, oxygenation profiles and glucose have not been well-described. We hypothesized that oxyhemoglobin desaturation during apneic events and subsequent post-apnea saturation overshoot predict nocturnal glucose.

Methods: In 30 OSA patients who underwent polysomnography while subjected to CPAP withdrawal, we characterized S_PO_2 swings by frequency, desaturation depth, and overshoot height relative to baseline. We examined the associations between frequently sampled glucose and S_PO_2 swings during the preceding 10 min. We developed multi-variable mixed effects linear regression to examine the independent associations between glucose and each level of these S_PO_2 swings, while controlling for OSA severity.

Results: Desaturation depth was not associated with glucose ($p > 0.05$). In contrast, overshoot was associated with glucose in a dose-dependent manner. Each S_PO_2 peak that did not rise to within 1% of baseline was associated with incremental glucose elevations of 0.49 mg/dL ($p = 0.01$), whereas peaks that exceeded baseline by >1% were associated with glucose reductions of 0.46 mg/dL. Overshoot remained an independent predictor of glucose after adjustment for mean S_PO_2 and OSA severity ($p > 0.05$).

Conclusions: Vigorous S_PO_2 improvements after apneic events may protect patients against OSA-related glucose elevations.

Keywords: intermittent, hypoxia, metabolism, automated, phenotype

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INTRODUCTION

Obstructive sleep apnea (OSA) is a highly prevalent disease (1–3), which is associated with disturbances in glucose regulation including risks of type 2 diabetes (4–9). Intermittent closure of the upper airway in OSA causes hypoxia, sleep fragmentation and large intrathoracic pressure swings. Hypoxia may be an important stimulus for impaired glucose metabolism. In high altitude residents who are chronically exposed to ambient hypoxia, oxyhemoglobin saturation is associated with increased fasting glucose and glucose intolerance (10, 11). Investigators have also demonstrated that OSA-induced hypoxia was associated with glucose intolerance (7, 12).

Furthermore, exposure to sustained or intermittent hypoxemia causes glucose intolerance and insulin resistance in human and animal experiments (13–16). In a recent study, we sampled blood at 20-min intervals during full polysomnography in CPAP-adherent OSA patients after discontinuing CPAP for 3 nights. During acute exposure to OSA, we observed that dynamic glucose elevations were closely preceded by periods of hypoxia, as assessed by the frequency of desaturations or median oxyhemoglobin saturation (S_PO_2) (17). These findings suggest rapid effects of OSA-related hypoxia on plasma glucose levels during sleep. Similarly, in a recent cross-sectional study in high altitude residents who are chronically exposed to hypoxia, we found that mean nocturnal S_PO_2 is associated with elevated hemoglobin A1c, independent of sleep apnea severity and daytime S_PO_2, indicating that nocturnal hypoxia contributes significantly to worsening overall glucose control (18). Nevertheless, the temporal associations between respiratory disturbances, oxygenation swings, and acute alterations in plasma glucose have not been well-described.

In OSA patients, the evolution of oxygenation over the course of the night is highly variable. During sleep, upper airway obstruction leads to falls in ventilation and subsequent oxyhemoglobin desaturation. At the termination of apneic events, arousals from sleep and improvements upper airway patency can result in transient increases in ventilation and oxygenation. The frequency and height of these S_PO_2 peaks can be influenced by several factors including ventilatory responses to gas exchange disturbances during apneic events (19), arousability, sympathetic activation, and co-morbid cardiopulmonary disease. These physiologic factors may not be fully reflected by traditional measures of sleep apnea and hypoxia severity including apnea hypopnea index (AHI) and time spent with $S_PO_2 < 90\%$ (T90). Therefore, dynamic oxygenation characteristics including frequency and depth of desaturation and subsequent correction may provide additional provide additional insight into metabolic risk in OSA patients.

We hypothesized that greater degrees of oxygen desaturation are associated with higher glucose, while greater oxygenation improvements after apneic events may protect against these elevations. To examine this hypothesis, we developed an automated approach to characterize oxygenation profiles in OSA patients by quantifying the frequency and height of periodic S_PO_2 nadirs and peaks. We then examined the associations between frequently sampled nocturnal plasma glucose levels and hypoxia, OSA severity, and dynamic S_PO_2 swings.

METHODS

The present study is a *post-hoc* analysis of a recent interventional trial of CPAP withdrawal. The recruitment, sleep study recording and blood sampling methods have been previously described (17). Briefly, CPAP-adherent patients with moderate-to-severe sleep apnea (AHI $\geq$ 20) underwent in-laboratory attended polysonography, with and without CPAP. During these studies, we measured glucose by sampling venous blood every 20 min. Because nocturnal glucose levels varied greatly and were distributed in a bimodal pattern between patients with and without diabetes, we limited our current analysis to non-diabetic patients. This study was approved by the Johns Hopkins Institutional Review Board, and all subjects provided informed written consent.

Data Analysis

Definitions

Oxyhemogloblin saturation (S_PO_2) is dynamic and can vary about a baseline. Thus, we characterized oxygenation profiles by the frequency and amplitude of oxygen oscillations relative to baseline. Because the presence of sleep disordered breathing affects oxyhemoglobin saturation, we defined the baseline saturation as the mean S_PO_2 during treatment with CPAP, which resulted in stable respiratory patterns and oxygenation. This method also accounts for differences in lung function or co-morbidities that may also affect S_PO_2. **Figure 1** illustrates the relationship between oxygenation in an OSA patient with repetitive apneic episodes, and the CPAP-treated baseline S_PO_2, which approximates oxygenation during stable breathing.

To detect acute changes in oxygenation, we applied an automated peak detection algorithm (Matlab, Natick, MA) to identify local S_PO_2 minima and maxima during the 10-min windows preceding blood draws (**Figures 1B,C**). Apneic events lead to falls in oxygenation and local oxygenation minima ($\triangledown$). At the termination of apneas and hypopneas, increases in ventilation cause sharp rises in oxygen, resulting in local S_PO_2 maxima (Δ), that occasionally exceeded, or overshot, the baseline. Desaturation depths and overshoot heights of these minima and maxima, respectively, were calculated relative to the CPAP baseline. In **Figure 1B**, S_PO_2 fell from a baseline S_PO_2 of 95% to nadirs of between 89 and 91%, representing desaturation depths of 4–6%. Each of these desaturations was followed by incomplete correction of oxyhemoglobin saturation, to S_PO_2 levels of 1–2% below baseline (negative overshoot). In contrast, the frequent desaturations in **Figure 1C** were followed by over-correction of oxygen, exceeding the baseline by 1–3%. We analyzed our data in 10-min windows because sensitivity analyses demonstrated that model performance progressively decayed with increasing windows of 15 and 20 min. Additional sensitivity analyses were performed with progressively increasing lag times between S_PO_2 parameters and glucose.

Analytic Methods

Our primary outcome was nocturnal glucose levels. Our repeated measures design allowed us to examine within- and between-subject changes in glucose, in association with variations in oxygenation and OSA severity. To minimize confounding from periods of wakefulness, we censored glucose measurements that were preceded by >30 s (5%) of wakefulness within 10 min of blood sampling. The 30-s threshold was chosen because blood sampling may occur in the middle of a 30-s scoring epoch, which would reduce the number epochs from 20 to 19. We compared glucose levels during periods with high and low overshoot heights and desaturation depths, using median

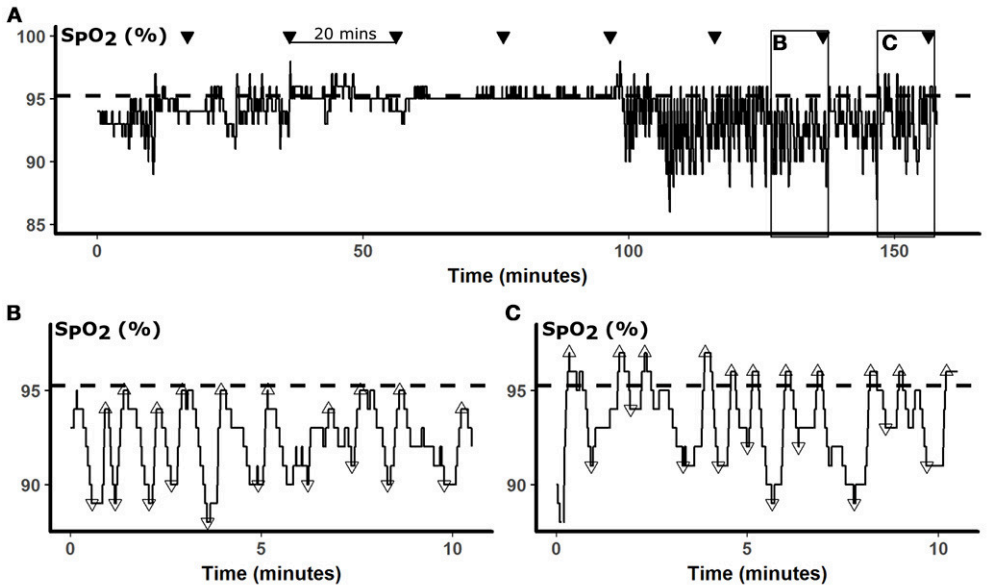

FIGURE 1 | Data analysis. In **(A)**, a representative 160-min S_PO_2 is presented. The baseline S_PO_2 calculated from the mean S_PO_2 while treated with CPAP is represented by the dashed horizontal line. During sleep studies, glucose was measured every 20 min (▼). To examine S_PO_2 predictors of glucose, we analyzed oximetry during the 10 min preceding each glucose measurement, as illustrated by boxes in **(A)** and shown in greater details in **(B,C)**. S_PO_2 nadirs (▽) and peaks (Δ) were identified by automated peak detection. The desaturation depth and subsequent overshoot were calculated relative to the baseline. Panel **(B)** illustrates a period of frequent desaturations, which were followed by only partial restoration of oxygenation, resulting in negative overshoot. In contrast, Panel **(C)** illustrates repetitive desaturations were followed peaks that exceeded the baseline, resulting in positive S_PO_2 overshoots, despite desaturations were of similar depths.

overshoot (0.3%) and desaturation depth (5%) as the cutoffs. We stratified these analyses by periods with greater or less than the median number of apneic episodes in 10 min (7, which corresponds to an AHI of 42 episodes/h). In these analyses, we modeled glucose as a function of the density of apneic episodes (<7 vs. ≥ 7), high vs. low mean overshoot or desaturation depth, and their interaction with mixed-effects linear regression models. To further examine the association between nocturnal glucose levels and the frequency and degree of overshoot and desaturation, we developed univariable mixed-effects linear regression models of glucose at each point as functions of the number of overshoots of <-1, ≥ -1 and <0, ≥ 0 and <1, and $\geq 1\%$ during the preceding 10 min. Similar analyses were performed with desaturations at cutoffs of ≥ 4 and <5, ≥ 5 and <6, ≥ 6 and <7, and $\geq 7\%$ cutoffs. To account for the effects of hypoxia on nocturnal plasma glucose, we developed multivariable regression models to adjust for mean S_PO_2 preceding the glucose measurements. We then performed sensitivity analyses to examine the effect of including glucose measurements in the results. Data analyses were performed in R (www.r-project.org) with the "Linear Mixed-Effects Models using 'Eigen' and S4" package.

RESULTS

Subject Baseline Characteristics

Baseline subject characteristics are presented in **Table 1**. Thirty subjects met criteria for inclusion. The majority of subjects were male. On average, our subjects were middle aged and obese. OSA was present to a severe degree, with time with $S_PO_2 < 90\%$ for almost 20 min, on average.

TABLE 1 | Demographic and polysomnographic characteristics.

Characteristic	Mean (SD)
N	30
Male sex [*n* (%)]	21 (70.0)
Observations per subject	10.77 (4.34)
Age (years)	48.83 (10.43)
BMI (kg/m^2)	37.03 (7.88)
Sleep apnea severity (AHI) (events/h)	46.57 (29.18)
Time with $S_PO_2 < 90$ (minutes)	17.09 (19.13)
Mean S_PO_2 (%)	93.18 (2.01)

Associations Between Desaturation Depth and Overshoot and Plasma Glucose During Periods of High and Low Apnea Density

Figure 2A illustrates glucose after periods of low and high densities of apneic events (greater than or less than 7 events per 10 min, respectively), with low and high mean desaturations. Glucose was higher after periods with greater falls in saturation regardless of event frequency (>7 vs. <7 apneas or hypopneas per period), although this difference did not exceed the threshold for statistical significance ($p = 0.09$). Neither independent

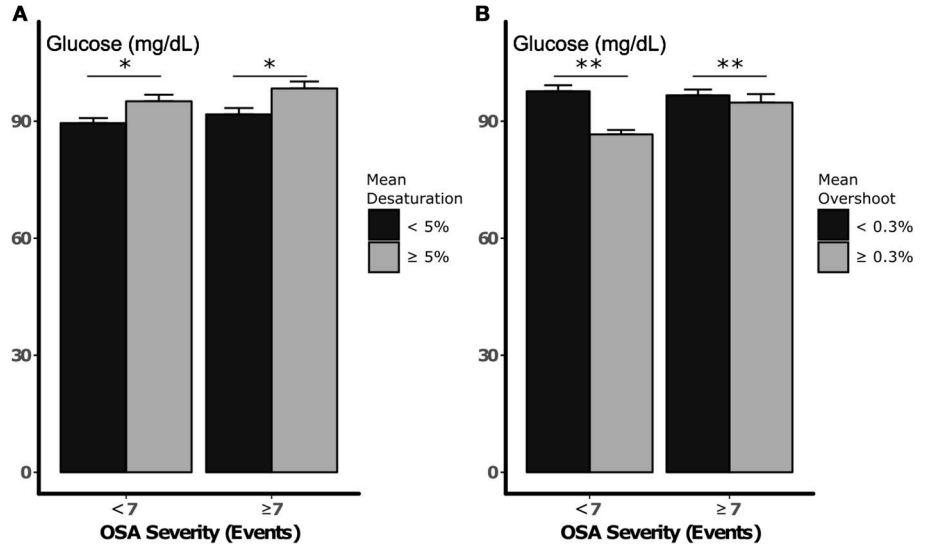

FIGURE 2 | Nocturnal glucose vs. desaturation depth **(A)** and S_PO_2 overshoot **(B)**, during periods of low and high sleep apnea density. Error bars represent standard errors. *$p < 0.10$ and **$p < 0.05$, respectively, for independent association between mean desaturation depth and S_PO_2 and nocturnal glucose. Statistical analysis did not demonstrate an independent or interactive association between sleep apnea density and glucose during sleep.

association between sleep apnea density and glucose nor an interaction between desaturation and sleep apnea density was detected ($p = 0.48$ and 0.52, respectively). On the other hand, greater compared to less overshoot was independently associated with a 4.9 mg/dL reduction in glucose ($p = 0.01$, **Figure 2B**). In these analyses, an independent association between sleep apnea density and glucose was not observed, nor was there an interaction between sleep apnea density and S_PO_2 overshoot ($p = 0.88$ and 0.34, respectively). Sensitivity analysis with inclusion of periods of decreased sleep efficiency did not significantly alter these results.

Independent Associations Between Nocturnal Glucose and Desaturation and Oxygenation Overshoot Frequency at Varying Levels of Intensity

The association between desaturation frequency at varying levels and nocturnal glucose is illustrated in **Figure 3A**. We did not observe a significant association between the depth of desaturation and glucose. Nevertheless, all beta coefficients were similar and approached the threshold for significance ($p = 0.07$) when all desaturation levels were pooled prior to analysis (see $supplement). In contrast, overshoot height predicted glucose in a dose-dependent manner (**Figure 3B**). At negative levels of S_PO_2 overshoot (i.e., when S_PO_2 did not return to within 1% of the baseline after an apneic episode), each S_PO_2 peak during the preceding 10 min was associated with an incremental increase in glucose of 0.49 mg/dL. With greater degrees of overshoot, the incremental change in glucose associated with each apneic event progressively fell. In fact, vigorous overshoots that exceeded baseline saturation by ≥1% were associated with reductions in glucose of 0.46 mg/dL ($p = 0.03$). In multi-variable models that incorporated all levels of S_PO_2 overshoot, very low levels of overshoot (<−1%) were independently associated with increases in glucose (**Table 2**). In these models, we observed a trend between ≥1% overshoot above the baseline and reductions in glucose ($p = 0.07$). The associations between ≥1% overshoot and lower glucose was significant after adjustment for traditional metrics of sleep-disordered breathing, including sleep disordered breathing frequency and mean S_PO_2. The associations between partial oxygenation correction (<−1% overshoot) with increased glucose was not significant with these adjustments because of collinearity with mean S_PO_2. These findings suggest that the degree of oxygenation overshoot, but not desaturation depth, after apneic events is a robust predictor of nocturnal glucose.

DISCUSSION

In the present study, we examined OSA-related hypoxia profiles and their association with glucose during sleep. The novel finding of the study is that the pattern of hypoxia is an important predictor of the overall effect of hypoxia on nocturnal glucose level. Greater S_PO_2 overshoot was dose-dependently associated with lower glucose, independent of mean S_PO_2 or the frequency of sleep apnea events. As expected, acute hypoxia was also dynamically associated with elevated plasma glucose. Interestingly, there was no relationship between the frequency of OSA events or desaturation depth after these events, and nocturnal plasma glucose levels. Taken together, OSA characterized by nocturnal hypoxia and infrequent and incomplete restoration of oxygenation reflects a phenotype that may be more vulnerable to nocturnal glucose elevation.

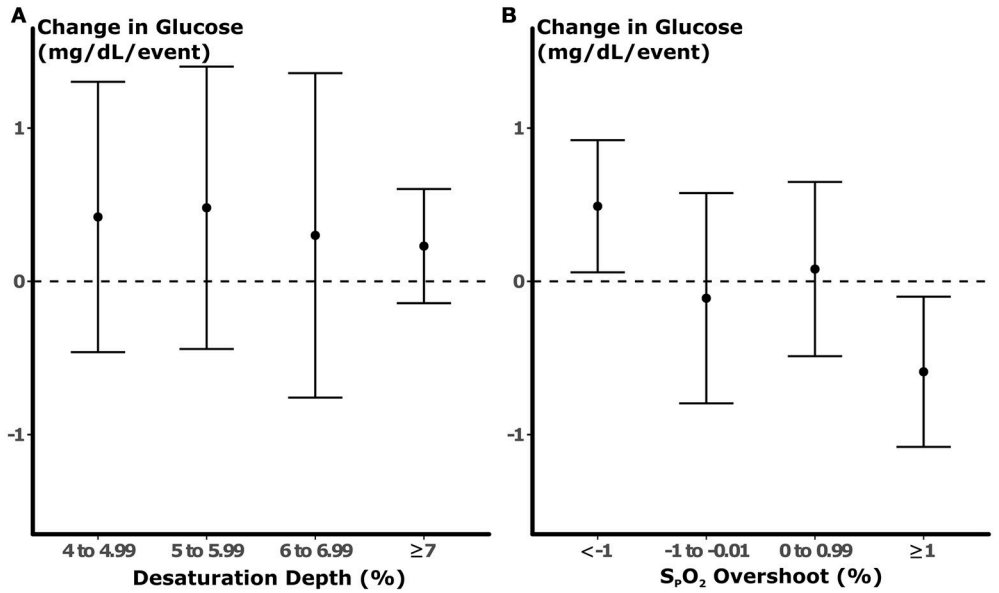

FIGURE 3 | Results of univariable models of glucose. Each point represents the change in glucose (±95% confidence interval) predicted by the number of desaturation **(A)** or overshoot **(B)** during the preceding 10 min prior to blood sampling. No statistically significant association was observed between desaturation depth and glucose. In contrast, each apneic episode that was followed by partial normalization of oxygenation was associated with an increase in glucose by 0.49 mg/dL. With increasing degrees of S_PO_2 overshoot, the changes in glucose related to each event fell, and S_PO_2 peaks that exceeded baseline by >1% were associated with reductions in glucose.

TABLE 2 | Regression models of associations between plasma glucose and S_PO_2 overshoot and sleep disordered breathing severity.

	Uni-variable models		Multi-variable model 1		Multi-variable model 2	
Predictor	**Beta**	***p*-value**	**Beta**	***p*-value**	**Beta**	***p*-value**
S_PO_2 Overshoot						
<−1%	0.49	0.01	0.42	0.04	0.24	0.33
−1 to (−0.01)%	−0.11	0.77	−0.01	0.67	–	–
0 – 0.99%	−0.14	0.54	−0.00	0.99	–	–
≥1%	−0.46	0.03	−0.40	0.07	−0.46	< 0.05
Mean S_PO_2	−0.89	0.005	–	–	−0.63	0.08
Apneas and hypopneas	0.10	0.36	–	–	0.10	0.40

Conversely, large S_PO_2 overshoot may blunt the impact of sleep disordered breathing on nocturnal glucose in OSA patients.

Several mechanisms may explain the links between oxygenation profiles and OSA-related glucose disturbances. First, episodic oxygen restoration may mitigate nocturnal hypoxia related to apneic events. Frequent intermittent oxygen rises may be indicative of frequent arousals, which shorten apnea and hypopnea duration (20–22). In addition, large inspiratory efforts preceding brisk S_PO_2 rises could also increase oxygen stores in the lungs and diminish subsequent desaturations. Second, vigorous overshoot could be a marker of arousal and/or lighter sleep, which limits the likelihood of developing additional apneic events and metabolic disturbances (23). In fact, the association between S_PO_2 overshoot of >1% and glucose lowering did not attenuate with adjustment for mean nocturnal S_PO_2, suggesting that S_PO_2 overshoot may reduce plasma glucose through mechanisms independent of hypoxia. Alternatively, the degree of hypoxia during apneic events and subsequent overshoot could be influenced by comorbidities (24–28). For example, patients with visceral obesity or cardiopulmonary dysfunction may exhibit both blunted S_PO_2 rises and higher glucose excursions after apneic episodes.

In contrast to S_PO_2 overshoot, desaturation depth was not related to the degree of glucose change. Further analyses with commonly clinically reported metrics of hypoxia severity including time with S_PO_2 < 90% also did not predict nocturnal glucose (data not shown). The present study also did not demonstrate an association between OSA events and dynamic nocturnal glucose changes. These findings imply that existing

metrics of OSA severity and related hypoxia do not adequately predict the glycemic impact of sleep disordered breathing. In contrast to previous approaches to characterizing nocturnal hypoxia, we implemented a readily deployable, automated algorithm that focuses on a novel dimension of the oxygenation profile that captures the additional physiologic responses to sleep disordered breathing. Our study demonstrates that frequency and degree of oxygenation overshoots is an important predictor of OSA-related metabolic sequelae, independent of traditional measures of sleep apnea severity.

Our study has several limitations, which are worth considering when interpreting the results. First, the observational nature of our study limits causal inferences. Nevertheless, we demonstrated that alterations in mean S_PO_2 and S_PO_2 overshoot occurred before changes in nocturnal glucose. Moreover, S_PO_2 overshoot predicted nocturnal glucose changes in a dose-dependent manner. These findings are consistent with the notion that hypoxia causes glucose elevations, which can be mitigated by intermittent improvements in oxygenation. Second, our method defines baseline S_PO_2 from polysonography while treated with CPAP. The requirement for stable respiratory patterns on a separate night may limit the ability to deploy our methods to estimate metabolic risk in a clinical setting. CPAP may also increase lung volumes and improve baseline S_PO_2 (29). Nevertheless, low lung volumes and attendant S_PO_2 reductions may be part of the causal pathway in the pathogenesis of OSA-related hyperglycemia. Third, we examined the relationship between oxygenation and glucose profiles in non-diabetic patients with moderate-to-severe OSA. Additional studies are required to determine the generalizability of these methods to patients with diabetes and/or mild disease by AHI criteria.

CONCLUSIONS

In summary, our study demonstrated that intermittent restoration of S_PO_2 was independently associated with dynamic nocturnal glucose reductions. In contrast, reductions in mean S_PO_2 but not the nadir S_PO_2 after apneic events predicted glucose elevations. These findings indicate that nocturnal hypoxia is an important determinant of OSA-related glucose disturbances. Our findings further imply that interventions that improve oxygenation in OSA patients may mitigate nocturnal glucose excursions. Additional studies are required to examine the role of oxygen to prevent worsening glucose control, and prospectively validate the use of oxygenation profile analysis to predict metabolic risk in OSA.

AUTHOR CONTRIBUTIONS

LP, AS, and JJ contributed to the data analysis and interpretation, and manuscript preparation.

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A Pilot Randomized-Controlled Trial on the Effect of CPAP Treatment on Glycemic Control in Gestational Diabetes: Study Design and Methods

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Background: Gestational diabetes (GDM) is associated with adverse short- and long-term maternal and fetal outcomes. Observational data support a link between sleep-disordered breathing (SDB) during pregnancy and GDM. However, it is unknown whether treatment of SDB with continuous positive airway pressure (CPAP) improves glucose control in this patient population. In addition, CPAP adherence and feasibility as a treatment option in pregnancy is unknown. This pilot randomized, controlled trial aims to primarily determine the feasibility of CPAP treatment in pregnant women with SDB and GDM. This study is also investigating the effect of SDB treatment on 24-h glucose profiles as an exploratory outcome.

Objectives: To describe the study methodology in this ongoing study of pregnant women with GDM and SDB.

Patients and Methods: Pregnant women with GDM and SDB defined by apnea-hypopnea index (AHI) $\geq$10 (Chicago Scoring Criteria) on level 2 polysomnography are randomized to either auto titrating CPAP (experimental group) or a nasal dilator strip (control group) until delivery. The primary outcome, objectively-assessed adherence to CPAP, is measured over the course of the treatment period using device-specific software. Recruitment and retention rates will be calculated to assess the feasibility for planning future trials. Twenty-four hour glucose profiles are measured over a 72-h period using the continuous glucose monitoring (CGM) system, before and after the intervention.

Conclusion: The results of this study will be highly informative to determine whether CPAP is a feasible treatment for pregnant women with GDM and SDB, a specialized population at risk for substantial comorbidity. The trial results will ultimately be useful in planning future SDB treatment trials in pregnancy and GDM.

The study is registered on clinicaltrials.gov (NCT02245659).

Keywords: sleep apnea, diabetes, CPAP (continuous positive airway pressure), gestational diabetes, pregnancy

INTRODUCTION

Gestational diabetes (GDM) is glucose intolerance that is first recognized during pregnancy (1). The prevalence of GDM doubled between 1994 and 2002 in a multi-ethnic U.S. population (2), possibly related to the global epidemic of obesity. Current estimates of prevalence range from 8 to 16%, depending on the study population and diagnostic criteria used (3, 4). Maternal hyperglycemia can lead to both short-term and long-term adverse outcomes for both the mother and baby, including increased rates of preeclampsia, perinatal mortality, cesarean section, neonatal metabolic abnormalities, macrosomia and resulting birth injuries (5). In addition, in the long-term, a history of GDM in women is associated with an increased risk of developing cardiovascular disease (6–8), type 2 diabetes (9, 10) and non-alcoholic fatty liver disease (NAFLD) (11, 12). Although the majority of GDM cases arise during pregnancy and resolve after delivery, long-term follow-up studies demonstrate that ∼20–50% of women progress to type 2 diabetes, a risk which has doubled over the past 10 years (13–16). Moreover, there is also an increased risk for developing metabolic syndrome and being overweight later in life among offspring exposed to GDM (17–20). Thus, prevention and improved management of GDM may improve outcomes for both the mother and offspring.

Sleep-disordered breathing (SDB) is prevalent in 17–45% of pregnant women by the third trimester (21–25), depending on the diagnostic cut-offs used, degree of obesity, and comorbidities present in the study population. SDB is characterized by breathing pauses during sleep, which results in sleep disruption from frequent arousals and intermittent hypoxia. Similar to polysomnography-based estimates of SDB prevalence, symptoms of SDB are reported in 14–35% of pregnant women by the 3rd trimester (26–28). In addition to weight gain, pregnancy-specific physiological changes that include upper airway narrowing, vascular congestion, nasal congestion and decreased functional residual capacity (29, 30) are hypothesized to increase the risk of SDB. In several studies that have adjusted for obesity, SDB in the non-pregnant population is associated with glucose intolerance and type 2 diabetes (31, 32). Although the specific mechanisms of this relationship have yet to be clearly defined, pathways involving increases in sympathetic drive, cortisol, and inflammation, are likely involved (33, 34). Extrapolating from the non-pregnant literature, SDB may also represent a novel risk factor for GDM (21, 31, 35). Two recent meta-analyses of observational studies demonstrated an increased risk of GDM in maternal SDB (36, 37) (adjusted odds ratios of 1.86–3.06). More recently, publication of the nuMoM2b cohort sleep substudy, which assessed SDB in pregnancy in the largest cohort to date ($n = 3{,}705$), revealed similar results between the presence of SDB in pregnancy and the risk of GDM (odds ratios ∼3) (38).

Despite the accumulating evidence that gestational SDB is associated with GDM, the data are limited by the biases inherent in observational studies, particularly with possible residual confounding from obesity. Performing controlled interventional studies with treatment of SDB is the next essential step to determine whether there is an associated improvement in outcomes. Our ultimate objective is to perform a large-scale, multi-center randomized trial evaulating the effects of CPAP treatment of SDB on glycemic control in GDM. To date, a few studies, mostly uncontrolled and all with small sample sizes, have investigated whether treatment of SDB with CPAP improves blood pressure among patients with gestational hypertension or preeclampsia [Reviewed in (21)]. In a recently published trial of CPAP vs. no CPAP for 2 weeks in women with GDM, of the 15 women on CPAP, only 7 were adherent (minimum usage 4h/night for > 70% of nights) (39). However, adherence to CPAP for a longer duration during pregnancy and its effect on 24-h glucose profiles is unknown.

Several CPAP trials in non-pregnancy that have failed to demonstrate improvements with respect to metabolic outcomes have also been limited by poor CPAP adherence [reviewed in (31)]. Optimizing CPAP use, on the other hand, has improved cardiometabolic outcomes (40–45), particularly in in-laboratory proof-of-concept studies with 8 h a night of CPAP use in patients with prediabetes (46) and type 2 diabetes (47). In pregnancy, CPAP adherence may be further affected by the unique sleep complaints that are prevalent in pregnancy, including less deep sleep and more frequent nocturnal awakenings (48). In order to appropriately power large, multi-center trials in pregnant women with SDB for improving cardiometabolic outcomes, the adherence to CPAP among pregnant women needs to first be established. As such, the primary aim of this pilot trial is to assess the adherence to CPAP in pregnant women with GDM, a unique population at risk for significant comorbidity. The secondary aims are to collect pilot data on glycemic control in the form of 24-h continuous glucose monitoring (CGM) as well as other relevant outcomes to allow planning and sample size calculations for a future, multi-center trial with the primary aim of evaluating the effects of SDB treatment on glycemic control in GDM.

METHODS

Trial Protocol

This is an unblinded, randomized-controlled parallel study. **Figure 1** demonstrates the protocol design and testing at each visit. Recruitment started in March 2015 at two centers in Montreal, Canada (McGill University Health Centre and Centre Hospitalier Universitaire Sainte-Justine).

Participants

Participants are screened for eligibility if they are pregnant women aged ≥18 years referred to the GDM clinic between >20 and <34 weeks gestational age with a diagnosis of GDM. Participants receive a diagnosis of GDM prior to their first study visit based on a positive non-fasting screening 50-g glucose load (24–28 weeks gestation) of >11.1 mmol/L. If this test is abnormal but not in the diabetic range (7.8–11.1 mmol/L), then a fasting, standard, 75-g oral glucose tolerance test (OGTT) is ordered. GDM is defined at our center with either an abnormal 50-g screening test with level ≥11.1 mmol/L or from one of the following from the 75-g OGTT: (1) fasting glucose ≥5.1 mmol/L, (2) 1-h glucose ≥10.0 mmol/L, or (3) 2-hr glucose ≥8.5 mmol/L (49), all in the absence of pre-existing or pregestational diabetes. Screening questions on snoring, witnessed apneas or

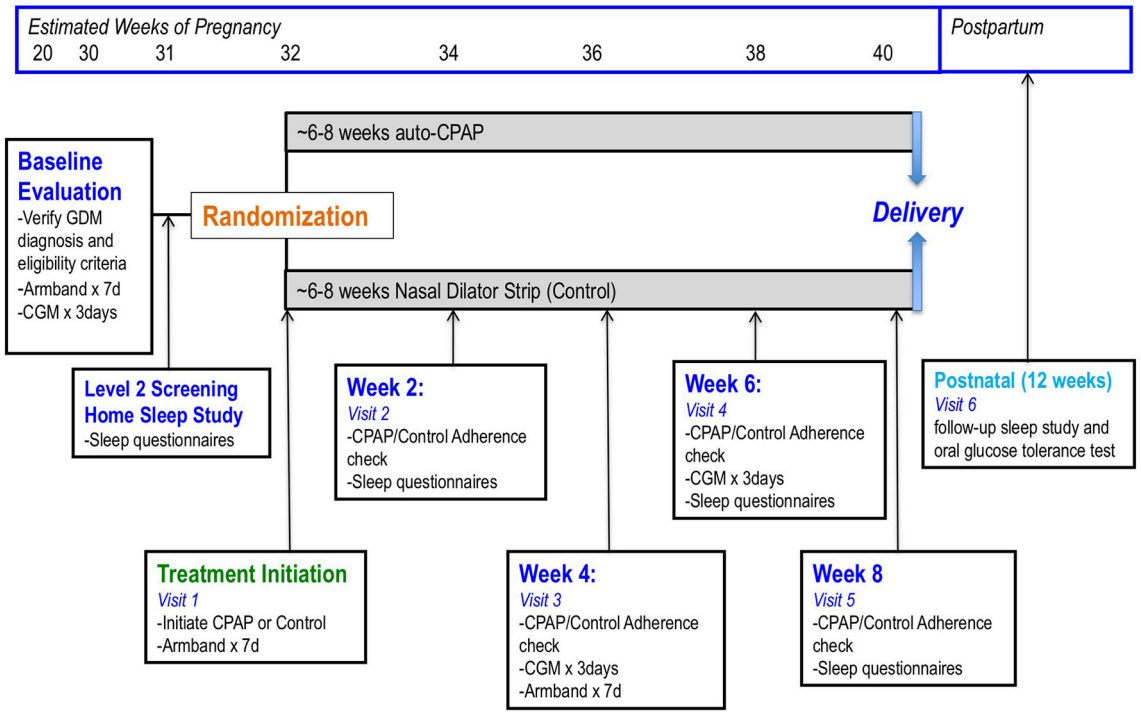

FIGURE 1 | The trial protocol involves a baseline evaluation that initially confirms the diagnosis of gestational diabetes (GDM). This testing usually takes place between 24 and 28 weeks of gestational age. During the baseline visit, participants wear an Armband, consisting of an accelerometer, for 1 week in order monitor activity levels and sleep-wake patterns. Insertion of a continuous glucose monitoring (CGM) device for 72 h takes place at this visit. Within 1–2 weeks, a home sleep study to screen for sleep-disordered breathing (SDB) is organized. If participants are eligible for the study, randomization to either auto-titrating continuous positive airway pressure (CPAP) or nasal dilator strip (control group) takes place. Participants follow-up with the study nurse every 2 weeks to verify adherence to therapy and complete study measurements. Post-partum, participants are invited back for a repeat sleep study ∼12 weeks after the study is completed and also complete an oral glucose tolerance test as part of routine follow-up with the endocrinologist.

other symptoms of SDB are not used in the selection process. Participants are not eligible if they had multiple pregnancy, current cigarette smoking or alcohol consumption, chronic renal disease, cardiovascular disease, stroke, active psychiatric disease, active malignancy, HIV infection, Hepatitis C or B, prior treatment for SDB, occupation involving shift work or travel across time zones, or inability to provide informed consent.

Within 1 week of the initial GDM clinic visit, participants are scheduled for a one-night level 2 screening home polysomnogram. A sleep technologist sets up the complete polysomnogram (Titanium unit, Medcare, Natus Inc., Mississauga, ON) in the participant's home in the evening and verifies all signals. Following the sleep recording, a driver picks up the device from the participant's home and returns it to our sleep laboratory. Data from the recorder is downloaded and studies are scored by a Registered Polysomnographic Technologist with review by one of the study sleep physicians (SP, RJK). Standard quality assurance measures for scoring reliability are applied including ensuring a minimum of ∼4 h of total sleep time. In addition, oximetry, electroencephalography (EEG), and nasal cannula signals are verified for adequate quality, which is necessary for accurate scoring of respiratory events. Sleep-wake state, arousals and periodic limb movements are scored in accordance with current AASM criteria (50); respiratory events are scored using standard Chicago criteria (51). A diagnosis of SDB establishing eligibility for randomization is made based on the presence of an apnea-hypoponea index (AHI) ≥10 based on Chicago respiratory scoring criteria. Since pregnancy is generally characterized by milder SDB and lesser degrees of oxygen desaturation (21, 52–55), the more sensitive Chicago scoring criteria were chosen for diagnosis of SDB.

Potential participants in whom sleep studies demonstrate severe SDB (AHI ≥30 events/h) with significant sleepiness (ESS ≥15), or significant oxygen desaturation regardless of symptoms (4% oxygen desaturation index ≥30 or sustained hypoxia <80%) are excluded and sent for urgent evaluation at the Sleep Clinic at our institution.

Randomization

Eligible participants are randomized using web-based randomization with permuted blocks of varying size (Dacima software, Montreal, Quebec).

Treatment Interventions

Experimental Group

Nightly CPAP treatment until delivery: In the participants randomized to the active intervention arm, auto-titrated CPAP (auto-CPAP) is started within 2 weeks of the initial GDM

clinic visit following a positive sleep study for SDB. Active arm participants receive an individualized 1 h session on set-up, mask-fitting and preference, and potential side-effects (e.g., dry mouth, nasal congestion). A variety of nasal masks are initially tried for comfort during set-up, but if mouth-breathing, or intolerance to the nasal mask occurs, an oronasal mask is fitted. Education, troubleshooting of side effects occurs every 2 weeks, and more often as necessary by telephone or in-person visits. Download of the CPAP adherence data (adherence, leaks, pressure settings, efficacy with residual AHI) occurs either by Wi-Fi using available software or by interrogation of the memory chip at specific visits every 2 weeks (**Figure 1**) (56). All participants assigned to CPAP are encouraged to use the device for the remaining period of pregnancy (∼8 weeks) for as many hours during sleep as possible.

Control Group

Significant uncertainty exists on the optimal control group for CPAP studies. In particular, in pregnant women, a sham CPAP group may likely interfere with sleep quality and may shorten sleep duration. These effects may worsen glucose control and bias the results in favor of the active intervention arm. For these reasons, nightly nasal dilator strips are being tested in this population as a possible control. The strips (Breathe Right®, GlaxoSmithKline, Brentford, UK) mechanically pull the lateral nasal walls outward, causing nasal passage dilatation, thereby easing breathing and reducing snoring, but not SDB, in the general population (57). Since nasal strips have not been evaluated thoroughly in pregnancy, we are assessing for adherence in a way that is analogous to a pill count, by monitoring for leftover strips. Side effects and tolerability is also queried during each clinic visit. Control arm participants also complete a level 2 home polysomnogram 2 weeks after starting the nasal strip to determine if any improvements in respiratory parameters occurred.

Follow-Up Visits

Antenatal

The GDM clinics at the recruitment sites routinely follow patients with GDM every 2–4 weeks from diagnosis until delivery. During these clinic visits, participants receive their routine care from their endocrinologist, nurse, maternal-fetal-medicine specialist and dietitian. Participants are then seen by our research team during the same visit if possible to verify and record CPAP adherence, download CGM and capillary blood glucose measurements when appropriate, and assess sleep habits through questionnaires. **Figure 1** indicates the specific tests and evaluations that are performed at each scheduled visit.

Postnatal

After delivery, neonatal and obstetrical outcomes are abstracted through chart review. All study participants are referred to the Sleep Clinic between 1 and 3 months post-partum for assessment of persistent SDB symptoms and a repeat sleep study. Participants also have a follow-up in the GDM clinic with an OGTT to assess for post-partum diabetes and prediabetes. Treatment with CPAP is offered to participants in both groups after delivery until SDB is reassessed at the postnatal Sleep Clinic visit.

Outcomes

1. The primary outcome in this study is to objectively determine the adherence to CPAP among women with GDM and SDB. Acceptable adherence is defined by mean usage $\geq$4 h/night for at least 70% of nights, the conventional threshold for acceptable CPAP adherence in the general non-pregnant population (40–42, 58). Secondary analyses will examine predictors of CPAP adherence (i.e., demographic and SDB severity variables).
2. As a secondary outcome, the suitability of nasal dilator strips as a possible control intervention for a future large-scale, multi-centered RCT in GDM will be assessed. Patient adherence, changes in indices of SDB as assessed by level 2 polysomnography, and changes in sleep quality and daytime sleepiness (questionnaire-based assessments) will be measured.
3. Recruitment and retention rates will be computed at the end of the trial. This will be important in planning a future large scale RCT.
4. The feasibility of measuring glucose levels using CGM in CPAP vs. control groups is also being assessed. CGM provides in-depth information on glucose levels that cannot be obtained from routine blood glucose measurements. We use the well-validated iPro2® (Medtronic, Northridge, CA) CGM (59, 60), a small, minimally invasive device with a painless, subcutaneous sensor measuring interstitial glucose levels every 5 min. These levels are comparable to venous blood glucose measurements (61–63). A trained technician inserts the CGM device subcutaneously in the arm or abdomen, which takes measurements over 3 days to take into consideration day-to-day variability (64). CGM has been used in pregnant subjects previously and is well-tolerated (65). The CGM is blinded so participants cannot see glucose values and will be not be burdened by alarms or sensor inaccuracy. To ensure accuracy of the measurements, CGM requires calibration against capillary blood glucose measurements that are measured as part of standard of care 4 times/day by the participant (61). Insulin requirements are documented at each visit using self-reported insulin doses in patient logbooks. Participants are asked to maintain their usual diet and exercise patterns during this time. Glucose levels between the treatment and control groups will be compared.

Other Assessments

Sleep Quality and Duration

Short sleep duration and sleep quality are also assessed, as these can be additional factors contributing to glucose dysregulation (66, 67). The Pittsburgh Sleep Quality Questionnaire (PSQI) (68) Berlin Questionnaire (69) and Epworth Sleepiness Scale (70) are administered at baseline, and again at weeks 2 and 6 after treatment initiation. Sleep time is objectively measured with the Sensewear® Armband (BodyMedia), which uses an actigraphy analysis to record movements for estimating sleep-wake activity. This Armband is worn for 1 week both at baseline and 1 month

after treatment initiation. The pilot study is intended to assess the feasibility of performing these measurements in the pregnant GDM population with SDB.

Activity and Diet Assessments

Dietary habits, including self-reported total energy intake and macronutrients consumed (e.g., fats, protein, carbohydrates) are assessed through routine clinically-administered dietary logs that are monitored by the clinic dietician every 2 weeks. Body weight is recorded at each visit. Moreover, we also measure levels of physical activity through the Sensewear® Armband analysis, which is validated for and measures levels of physical activity (steps, average mets, and energy expenditure) (71, 72). The Armband is worn for 1 week at baseline and after treatment initiation as indicated in **Figure 1**.

Maternal-Fetal Outcome Data

The medical records for each participant is reviewed to obtain data regarding the perinatal course, including details on labor and delivery, post-partum complications, and the course and complications in the newborn.

Sample Size

The assumption underlying the calculation of the sample size was that for CPAP to be an acceptable treatment option in pregnant women with GDM, the adherence should be similar to that observed in the non-pregnant population. Interventional studies with CPAP in non-pregnant populations have demonstrated a wide range of adherence rates [46–83% (73, 74)], with a few recent trials demonstrating adherence rates of ≥60–70% (40, 41). We estimate that 30 participants (CPAP group) will be needed to observe a CPAP adherence rate of 70% with a confidence interval of width of 0.34 or less with probability > 90%. In **Table 1**, the confidence interval width for CPAP adherence is estimated based on various sample sizes that may be achieved with the pilot trial, with a probability >90%.

Data Analysis

Between-group differences will be assessed for statistical significance with the Student's *t*-test for normally distributed variables and the non-parametric Mann-Whitney test for non-normal distributions. Continuous variables will be reported as mean ± standard deviation. Median and interquartile ranges will be used for non-normally distributed variables. The primary outcome assesses CPAP adherence, which will be reported as % acceptable adherence rates with associated 95% confidence intervals (using conventional definition of ≥4 h/night for >70% of nights) in participants randomized to the CPAP intervention group. Adherence in the control group will be calculated as the proportion of nights the Breathe Right strips are used (total number of strips used from box over period of treatment nights). Secondary analyses will include the predictors of CPAP acceptable adherence in this population (e.g., demographic and SDB severity variables, SDB sleep symptoms). Recruitment rates will be calculated as the number of participants randomized at each site over the enrolment period. Retention rates will be 100 minus the percent of participants that dropped out after randomization over the period of the trial. Missing data will be analyzed by multiple imputation.

For examination of between-group differences in glycemic control, an intention-to-treat analysis is planned. At baseline and follow-up assessments, mean (standard deviation) 24 h, daytime, and nighttime glucose values will be computed as well as time that glucose levels are above 7.8 mmol/L (hyperglycemia). Between-group differences in change in glycemic control will be calculated with 95% CI. Secondary analyses will estimate linear regressions and adjust for relevant covariates (age, sex, BMI, baseline AHI, baseline glucose).

To minimize bias in interpreting results, the statistician and researchers involved in outcome assessment during data collection and analyses will be blinded to treatment arm allocation.

TABLE 1 | Confidence interval widths for achieving CPAP adherence rates of 70% based on sample size per group achieved in pilot trial.

Sample size	Confidence interval width, with probability >0.90
20	0.36
25	0.36
30	0.34

DISCUSSION

In light of observational data demonstrating robust associations between SDB and the risk of GDM and gestational hypertension (22, 36–38, 75) interventional controlled trials are necessary to assess the direct impact of SDB treatment on improving outcomes related to GDM. Several cardiometabolic trials in non-pregnancy with equivocal or negative results have had barriers in adequate CPAP adherence (76, 77), while other studies ensuring full CPAP compliance have demonstrated improvement in glucose levels using overnight or 24-h glucose monitoring (46, 47, 78). This pilot trial in SDB and GDM therefore focuses on CPAP adherence as the primary outcome.

Although the glycated hemoglobin (HbA1c) is the conventional measure of glucose in clinical practice in patients with type 2 diabetes, this measure does not capture changes that may occur specific to time of day (i.e., nocturnal vs. daytime), post-prandial measurements, general levels of glycemic variability throughout the day, and time spent in hyperglycemia. Moreover, since HbA1c reflects glycemia over several weeks, potentially important changes in glucose levels over a shorter period of time, as may occur during pregnancy, may be missed. CGM has been recently used in clinical trials in patients with T2DM to more specifically characterize the effect of newer therapies on various aspects of glucose control (79, 80). Of note, maintaining normal glucose levels with 24-h monitoring in GDM has been shown to result in improved maternal and fetal outcomes (81).

The results of this trial will provide novel data on the feasibility and acceptance of CPAP treatment of SDB among women with GDM and will provide valuable preliminary findings on which

aspects of glucose control, if any, are improved by CPAP in pregnancy. These data will be essential to planning future, larger RCTs to definitively evaluate the impact of SDB treatment during pregnancy on maternal and infant outcomes.

ETHICS AND CONSENT

All participants provide written, informed consent in order to undergo the screening sleep study and protocol. The study is approved by the Research Ethics Board of the McGill University Health Centre and Centre Hospitalier Universitaire Sainte-Justine. All adverse events are reported by the study PI (SP) to the Research Ethics Board using standard procedures. Appropriate steps are immediately taken to deal with adverse events including sending affected participants for medical evaluation as indicated.

AUTHOR CONTRIBUTIONS

SP conceived of the study design, managed and led the study, wrote the manuscript and was involved in all editing. SM and KD helped with study design and editing of manuscript. NG and RG helped with study design and participant recruitment. LL, AO, AK, and GT helped with study conduct. AB helped with study design and statistical analyzes. ER helped with study design and participant recruitment. KD helped with study design, and editing. RK helped with study design, study conduct, and editing.

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