

SLEEP AND METABOLISM

Effect of Slow Wave Sleep Disruption on Metabolic Parameters in Adolescents

Natalie D. Shaw, MD, MMSc^{1,2}; Andrew W. McHill, PhD^{3,4}; Michele Schiavon, PhD⁵; Tairmae Kangarloo, BS¹; Piotr W. Mankowski, BSc^{3,4}; Claudio Cobelli, PhD⁵; Elizabeth B. Klerman, MD PhD^{3,4}; Janet E. Hall, MD, MMSc^{1,2}

¹Reproductive Endocrine Unit, Department of Medicine, Massachusetts General Hospital, Boston, MA; ²Clinical Research Branch, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC; ³Division of Sleep and Circadian Disorders, The Brigham and Women's Hospital, Boston MA; ⁴Division of Sleep Medicine, Harvard Medical School, Boston MA; ⁵Department of Information Engineering, University of Padova, Padova, Italy

Study Objectives: Cross-sectional studies report a correlation between slow wave sleep (SWS) duration and insulin sensitivity (SI) in children and adults. Suppression of SWS causes insulin resistance in adults but effects in children are unknown. This study was designed to determine the effect of SWS fragmentation on SI in children.

Methods: Fourteen pubertal children (11.3–14.1 y, body mass index 29th to 97th percentile) were randomized to sleep studies and mixed meal (MM) tolerance tests with and without SWS disruption. Beta-cell responsiveness (Φ) and SI were determined using oral minimal modeling.

Results: During the disruption night, auditory stimuli (68.1 \pm 10.7/night; mean \pm standard error) decreased SWS by 40.0 \pm 8.0%. SWS fragmentation did not affect fasting glucose (non-disrupted 76.9 \pm 2.3 versus disrupted 80.6 \pm 2.1 mg/dL), insulin (9.2 \pm 1.6 versus 10.4 \pm 2.0 μ U/mL), or C-peptide (1.9 \pm 0.2 versus 1.9 \pm 0.1 ng/mL) levels and did not impair SI (12.9 \pm 2.3 versus 10.1 \pm 1.6 10⁻⁴ dL/kg/min per μ U/mL) or Φ (73.4 \pm 7.8 versus 74.4 \pm 8.4 10⁻⁹ min⁻¹) to a MM challenge. Only the subjects in the most insulin-sensitive tertile demonstrated a consistent decrease in SI after SWS disruption.

Conclusion: Pubertal children across a range of body mass indices may be resistant to the adverse metabolic effects of acute SWS disruption. Only those subjects with high SI (i.e., having the greatest "metabolic reserve") demonstrated a consistent decrease in SI. These results suggest that adolescents may have a unique ability to adapt to metabolic stressors, such as acute SWS disruption, to maintain euglycemia. Additional studies are necessary to confirm that this resiliency is maintained in settings of chronic SWS disruption.

Keywords: glucose, insulin, puberty, slow wave sleep

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Significance

Cross-sectional studies in children and adults and a small number of interventional studies in adults suggest that short slow wave sleep (SWS) duration and SWS fragmentation are risk factors for metabolic syndrome. The current studies, which represent the first SWS disruption studies with metabolic phenotyping to be conducted in children, demonstrate that a single night of SWS disruption does not diminish insulin sensitivity in pubertal children, challenging the existing dogma based on studies in adults. These results imply that adolescents may have a unique metabolic resiliency to acute sleep disruption; follow-up studies will be important in settings of more prolonged SWS restriction or disruption.

INTRODUCTION

Epidemiologic studies have demonstrated that both chronic short sleep and poor sleep quality are associated with an increased risk of obesity and type 2 diabetes mellitus (T2DM) in adults. Consistent with these cross-sectional observations, metabolic abnormalities have also been observed in adults after either acute sleep restriction or sleep fragmentation in a controlled laboratory setting.¹ Importantly, recent work has suggested that some of these effects are related to specific disruption of slow wave sleep (SWS). SWS is the deepest stage of sleep and is defined by the presence of low-frequency, high-amplitude waveforms known as slow waves or delta waves on electroencephalogram (EEG) tracings. Categorical sleep stage scoring has traditionally been used to identify SWS. However, use of spectral analysis to quantify slow wave activity (SWA) provides a more precise measure of sleep depth and quality. A connection between SWS and metabolism is supported by some, but not all,^{2–5} cross-sectional studies demonstrating an inverse correlation between the amount of SWS or SWA and body mass index (BMI),⁶ waist circumference,⁷ and hemoglobin A1C,⁸ and a positive correlation between SWS and lean body mass.⁹ Moreover, selective suppression of SWS with preservation of total sleep time decreases insulin sensitivity (SI) in adults.^{10,11} The strong association between disturbed sleep and metabolic abnormalities in adults has led to the hypothesis that poor sleep hygiene is a modifiable risk factor for

obesity and insulin resistance.¹ As sleep restriction (i.e., multiple nights with insufficient sleep) has reached epidemic proportions among teenagers,¹² it is important to determine the effects of reduced and/or disordered sleep on glucose homeostasis in pubertal children.

Two physiologic changes occur during puberty that may exacerbate the effect of reduced or fragmented sleep in adolescents. The first is that there is a more than 30% decline in SI during puberty, even among lean adolescents.¹³ The second is that, beginning at 11 to 12 y of age and continuing through late adolescence, there is a steep decline in the amount of SWS,^{14,15} the sleep stage now implicated in maintaining SI in adults.¹¹ Despite these dynamic changes in SI and sleep during normal adolescence, cross-sectional studies of sleep, body weight, and metabolism in teenagers have not consistently demonstrated an association between habitual sleep duration and body weight¹⁶ or SI.^{17–21} In studies in which sleep parameters were objectively determined via in-hospital polysomnography (PSG), however, investigators observed correlations between SWS and insulin secretion,²² SWS and SI,²³ and SWA and SI.²⁴ In the only interventional sleep study thus far in an adolescent population,²⁵ investigators observed a decrease in SI after 3 days of sleep restriction to only 4 h a night. Of interest, the total amount of time spent in SWS was preserved during this intervention, indicating the importance of further studies of the relationship between SWS and SI in this population.

To critically address the hypothesis that SWS is an important determinant of SI during puberty, we conducted SWS disruption studies using controlled auditory stimuli followed by a mixed meal tolerance test (MMTT) in a group of healthy adolescents.

METHODS

Subjects

Fourteen healthy children were studied. All subjects were pubertal as determined by Tanner staging and orchidometry, and all girls were premenarchal. Subjects had no signs or symptoms of diabetes and were not on any medications that influence glucose or insulin levels. One subject was on medications that may affect sleep and metabolism (methylphenidate and sertraline) but was not excluded because sleep parameters in the non-disrupted study fell within the normal range, the medication doses did not change between study visits, and exclusion of her data points did not change the results of any analyses. Subjects with known sleep disorders or suspected to have obstructive sleep apnea based on results of a validated sleep questionnaire²⁶ were excluded. Some polysomnographic and clinical data, including the characteristics of the study subjects, have been described previously.²⁷ The study was approved by the Partners Human Research Committee. Signed informed assent and consent were obtained from each subject and parent.

Experimental Protocol

Subjects underwent two PSG sleep studies spaced 2 mo apart, with and without SWS disruption in random order, in the Clinical Research Center of Massachusetts General Hospital, as previously described.²⁷ PSG was performed according to standard methodology using an EEG (total of six frontal, central and occipital leads), electro-oculogram (EOG), electromyogram (EMG), electrocardiogram (ECG), and pulse oximetry recordings (ALICE LE PSG system, Sleepware software, Phillips Respironics, Murrysville, PA). PSG recording began 10 min before lights out based on subject and parent reports of habitual bedtime (21:00–22:30) and continued until spontaneous awakening the following morning (05:00–07:30). An intravenous (IV) catheter was inserted upon admission and connected to a long line for remote blood sampling throughout the night.

During the SWS disruption night, auditory stimuli (3 sec 1,500 Hz tones at 40 dB increasing in 10-dB increments to a maximum of 100 dB followed by 18 sec of noise simulating a knock on the door at 75 dB) were delivered via a speaker placed at the head of the bed (iHome iP3 stereo speaker system, SDI Technologies, Inc, Rahway, NJ) for 8 h, as previously described.²⁷ Stimuli were delivered whenever at least two delta waves (≤ 4 Hz) appeared in a 15-sec PSG recording interval and were followed by tactile stimuli if the subject did not arouse. Subjects were allowed to sleep undisturbed after the 8-h intervention until spontaneous awakening the next morning. Two subjects repeated the disruption night because of insufficient disruption (250–280 min SWS) during the first visit related to technical difficulties.

All subjects ate dinner before lights out. Food and drink were not permitted from 21:00 until the MMTT was completed

the following morning. The MMTT was conducted within 1 h of final awakening to assess glucose tolerance. The MMTT is well tolerated by children, presents a more physiologic stimulus than either oral or IV glucose tolerance tests, and has been validated against hyperglycemic clamp studies.^{28,29} At time 0, subjects consumed a weight-based dose (6 cc/kg; maximum 360 cc) of Boost High Protein Nutritional Energy Drink [68% carbohydrate, 17% protein, and 15% fat] (Mead-Johnson) in under 5 min. Plasma glucose, insulin, and C-peptide levels were measured at time 0, 10, 20, 30, 60, 90, 120, 150, and 180 min. One subject did not tolerate the MMTT (nausea) during the disruption night so only fasting hormone levels were used in disrupted and non-disrupted sleep comparisons. Study nurses ensured that subjects remained awake for the duration of the MMTT.

We analyzed overnight heart rate variability (HRV), measured seated blood pressure and heart rate at the end of the study visit, and serum cortisol at 06:00–08:00, as previous studies have suggested that an increase in sympathetic tone or cortisol secretion during sleep disruption may be responsible for the ensuing insulin resistance.

Plasma glucose and cortisol were measured using the Architect Integrated ci8200 Chemistry and Immunochemistry Analyzer (Abbott Diagnostics, Abbott Park, IL), respectively, and plasma insulin and C-peptide were measured with the Roche E170 immunoanalyzer.

Data Analysis

Sleep Scoring

The sleep recordings were visually scored by a single registered PSG technician according to American Academy of Sleep Medicine criteria³⁰ in 30-sec epochs as stages of nonrapid eye movement (NREM; N1, N2, or N3), rapid eye movement (REM), or wake. The arousal index (AI) was defined as the number of arousals per hour of sleep. Sleep latency was defined as the duration of time between lights out and the first epoch of sleep, and sleep efficiency was defined as the percent of time in bed spent asleep. Wake after sleep onset was defined as minutes of wake after the first sleep epoch until natural awakening.

EEG Analysis

Spectral analysis was used to analyze total SWA in the 0.5–4 Hz range during stages N2 and N3 using SpectralTrainFig (<https://github.com/DennisDean/SpectralTrainFig>) within Matlab (MathWorks, Inc., version R2013b, Natick, MA). Artifacts were removed after being identified either within SpectralTrainFig or as any delta activity at least five times the average delta activity in N2 and N3 for the entire sleep opportunity. NREM episodes were defined as the SWA between epochs of REM sleep using a modified Feinberg and Floyd method.³¹ If the subject's sleep opportunity contained a "skipped" first REM episode, which is common in adolescents, an epoch of REM sleep was inserted (iREM) at either the first epoch of wake or at the nadir of SWA between visually identified NREM episodes (modified "Jenni and Carskadon criteria").³² The SWA in each NREM episode was expressed as a percentage of total night SWA to facilitate between-subject comparisons.

HRV Analysis

HRV was calculated using a continuous single-lead ECG recording from each 8-h sleep study. Kubios HRV software v2.2³³ was used to remove artifacts and to compute low-frequency band power (0.04–0.15 Hz), a marker of sympathetic activity, and high-frequency band power (0.15–0.4 Hz), a marker of vagal activity.

Assessment of Beta-Cell Function and Insulin Sensitivity

The homeostatic model assessment of insulin resistance (HOMA-IR), a surrogate estimate of basal SI, was calculated using the formula: fasting plasma insulin ($\mu\text{IU/mol}$) \times fasting plasma glucose (mmol/L)/22.5.³⁴ Beta cell responsiveness (Φ) and SI were assessed from serial insulin, C-peptide, and glucose measurements during the MMTT using the oral C-peptide and glucose minimal models, respectively.³⁵ These models have been validated using hyperglycemic clamp studies^{28,29} and radio-labeled glucose analogs in adults³⁶ and adolescents.³⁷ The C-peptide minimal model determines Φ to basal glucose levels (Φ_b) and to a nutrient load (Φ_l) and accounts for subject age, height, weight, and BMI. Total beta cell responsiveness to a meal (Φ) is partitioned into two components: a dynamic response (Φ_d , response to the acute rise in blood glucose during a meal), and a static response (Φ_s , response to the glucose level achieved above basal after a delay, T). SI, estimated from the glucose minimal model, reflects both insulin-mediated glucose disposal (tissue uptake) and inhibition of hepatic gluconeogenesis. The total disposition index (DI), which measures Φ in the context of the prevailing SI, is calculated as the product of Φ and SI.

Statistical Methods

Differences in sleep architecture, SWA, HRV, and metabolic parameters between the disrupted and non-disrupted nights were compared using analysis of covariance with a treatment (disruption versus no disruption) effect. The areas under the curve (AUC) for the glucose, insulin, and C-peptide responses to the MMTT were calculated using the trapezoidal rule. The relationships between sleep parameters and SI were assessed using Pearson correlation. Data that were not normally distributed were log-transformed for analysis. Data are expressed as mean \pm standard error [range] unless otherwise indicated, and $P < 0.05$ is considered significant.

RESULTS

Baseline Characteristics

Seven boys, ages 11.3–14.1 y, with testicular volumes of 4–15 mL (Tanner II–IV) and 7 premenarchal girls, ages 11.6–13.4 y, with Tanner II–III breasts participated (Table 1). Subjects were predominantly non-Hispanic Caucasians with a wide range of BMIs (29th to 97th percentile for age and sex). There were no changes in BMI percentiles or Tanner staging between study visits.

Standard Polysomnographic Metrics

During the non-disrupted sleep night, subjects slept for 8.2 ± 0.1 h and demonstrated normal sleep architecture with $32.7 \pm 2.5\%$

Table 1—Subject characteristics.

Subject	Sex	Age (y)	Race	BMI Percentile ^a	Pubertal Stage ^b
1	F	11.6	Cauc	29	III
2	F	12.3	Cauc	70	II
3	F	12.3	Cauc	73	III
4	F	12.3	AA	44	III
5	F	12.5	Cauc	53	III
6	F	13.2	Cauc	94	III
7	F	13.4	Cauc	68	III
8	M	11.3	Hisp	97	II
9	M	12.0	Hisp	92	II
10	M	12.2	Hisp	97	III
11	M	13.4	Cauc	37	IV
12	M	13.4	Cauc	37	IV
13	M	13.8	Cauc	46	IV
14	M	14.1	Cauc	78	IV

^a Age- and sex-adjusted BMI percentile > 85 is classified as overweight and > 95 is classified as obese in children. ^b Pubertal stage based on Tanner breast stage or testicular volume. All girls were pre-menarchal. Bold text indicates BMI percentiles classified as overweight or obese. BMI, body mass index; AA, African American; Cauc, Caucasian; Hisp, Hispanic.

of time spent in SWS (N3) (Table 2 and Figure 1A). Sleep efficiency, sleep latency, and AIs were consistent with normative data in adolescents³⁸ and there were no apneic episodes.

During the disrupted night, 68.1 ± 10.7 [20–120] auditory or tactile stimuli were delivered. These 8 h of disrupted SWS were followed by a brief duration of recovery sleep (23.9 ± 6.5 min) [0–72] before natural awakening. There was a $40.0 \pm 8.0\%$ [–11.5 to 84.0] decrease in %SWS ($P < 0.001$) relative to the non-disrupted sleep night, and the SWS that remained was fragmented into short bouts (1.3 ± 0.2 min [0.7–3.2] versus 7.1 ± 0.8 min [3.8–13.2] in the non-disrupted night; $P < 0.001$) due to frequent arousals (Table 2). SWS was replaced by lighter sleep (N1 and N2) with no change in %REM or %wake after sleep onset (Table 2, Figure 1B) such that the overall percentage of time spent in NREM was preserved (disrupted: $71.6 \pm 2.2\%$ [54.1–78.6] versus non-disrupted: $73.3 \pm 2.4\%$ [56.3–83.9], $P = 0.4$).

Slow Wave Activity

During the non-disrupted sleep night, total delta power was $2.7 \times 10^6 \mu\text{V}^2$, with SWA predominating in the first two NREM episodes (70%) [55–95] (Figure 1A), consistent with previous studies in this demographic.^{14,24} Sleep disruption decreased total delta power by $38.8 \pm 5.9\%$ [–6.5 to 65.9] (Figure 1B).

Beta Cell Function and Insulin Action

Subjects began the MMTT at 06:00–07:45 with no difference in timing between study visits. All subjects had normal (< 100 mg/dL) fasting blood glucoses the morning after the night of non-disrupted sleep (76.8 ± 2.4 mg/dL [57–91]) and disrupted sleep (80.6 ± 2.1 mg/dL [68–93]) with no difference

Table 2—Comparison of sleep parameters during the non-disrupted and disrupted sleep nights.

	Non-Disrupted Sleep Night	Disrupted Sleep Night	P
Sleep efficiency (%)	87.8 ± 3.0 (69.6–99.0)	85.8 ± 2.9 (59.6–96.9)	0.2
Sleep latency (min)	21.3 ± 3.5 (8.0–55.0)	30.7 ± 7.2 (1.0–77.0)	0.08
Arousal index (events/hour)	10.3 ± 0.7 (6.6–15.4)	22.7 ± 3.6 (1.2–59.0)	< 0.001
Sleep architecture			
N1 + N2 (%)	40.6 ± 2.1 (27.5–54.3)	51.6 ± 2.2 (38.0–63.1)	< 0.001
N3 (%)	32.7 ± 2.5 (20.1–54.7)	20.0 ± 2.8 (3.1–40.0)	< 0.001
REM (%)	14.5 ± 0.9 (7.0–17.9)	14.1 ± 1.4 (5.5–21.8)	0.8
WASO (%)	12.3 ± 3.0 (1.4–33.9)	14.2 ± 2.9 (3.0–40.4)	0.4
Slow wave activity ($\times 10^6 \mu V^2$)	2.7 ± 0.3 (0.9–4.5)	1.6 ± 0.2 (0.7–3.6)	< 0.001
LFn (%)	42.3 ± 3.0 (29.8–71.7)	53.1 ± 4.0 (29.6–88.6)	0.03
HFn (%)	57.5 ± 3.0 (28.2–70.1)	46.8 ± 4.0 (11.3–70.3)	0.03

Sleep disruption decreased the time spent in slow wave sleep (SWS, N3) and the total amount of slow wave activity (SWA, the electrophysiological correlate of SWS). Sleep became highly fragmented, as indicated by the elevated arousal index, and SWS was replaced by lighter sleep stages (stages N1 and N2) with no change in time spent in REM or wakefulness after sleep onset (WASO). Analysis of heart rate variability also demonstrated increased sympathetic activity (low-frequency [LF] power) and decreased vagal activity (high-frequency [HF] power) during the sleep disruption night compared with non-disrupted sleep. Data are presented as mean ± standard error (range). HF and LF spectral power are reported in normalized (n) units where $HF_n = 100 * HF / (HF + LF)$ and $LF_n = 100 * (LF / HF + LF)$. Bold text indicates statistically significant P values.

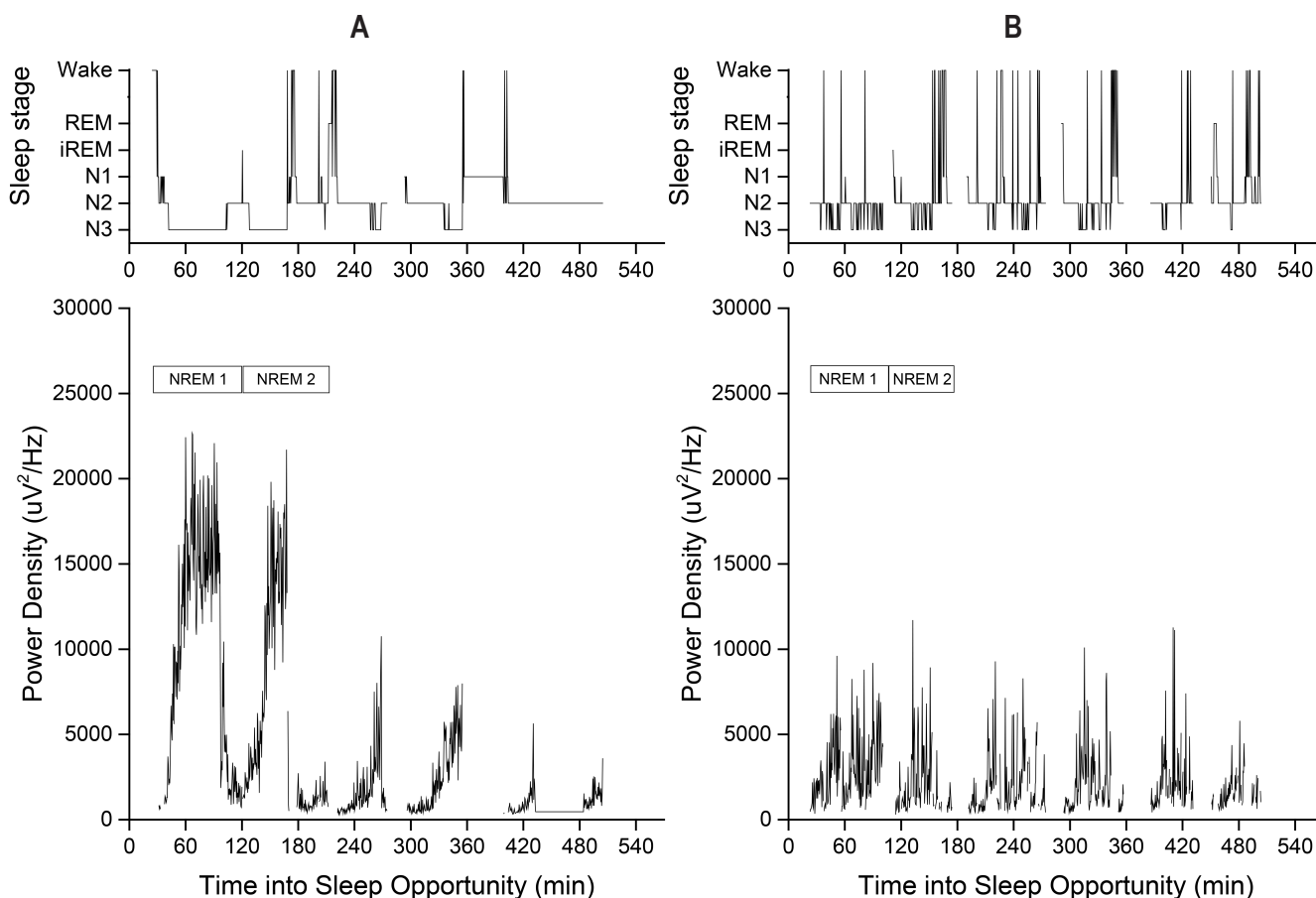


Figure 1—Sleep stages (wake, REM, inserted REM (iREM), N1, N2, and N3) (top) and slow wave activity (bottom) in a representative subject during 1 night of non-disrupted (A) and 1 night of disrupted (B) sleep. Visits were spaced 2 mo apart. Note the consolidated periods of deep sleep (N3) across the night during normal sleep (A) in contrast to the N3 fragmentation during the disrupted sleep night (B). The start and end of each of the first two NREM episodes are denoted in boxes above the slow wave activity in the bottom panels. NREM, nonrapid eye movement; REM, rapid eye movement.

between the two visits (Figure 2). Fasting insulin levels were also normal ($< 15 \mu\text{IU/mL}$) with the exception of one obese boy in whom fasting hyperinsulinemia ($> 20 \mu\text{IU/mL}$) was observed after both visits (non-disrupted $22.3 \mu\text{IU/mL}$, disrupted $25 \mu\text{IU/mL}$) and a second obese boy with fasting hyperinsulinemia ($21.6 \mu\text{IU/mL}$) restricted to the non-disrupted sleep visit. There were no differences in fasting glucose, insulin (non-disrupted: $9.2 \pm 1.6 [2.0\text{--}22.3]$, disrupted: $10.4 \pm 2.0 \mu\text{IU/mL} [4.2\text{--}35]$), or C-peptide (non-disrupted: $1.9 \pm 0.2 [1.0\text{--}3.3]$, disrupted: $1.9 \pm 0.1 \text{ ng/mL} [1.2\text{--}3.1]$) levels or in the corresponding postprandial AUCs between study visits (glucose AUC: $17,167.5 \pm 524.6 [13,275.0\text{--}19,920.0]$ versus $16,802.7 \pm 952.3 [3,675.5\text{--}36,126.0]$ [non-disrupted versus disrupted], insulin AUC $9,805.5 \pm 2,995.9 [3,985.5\text{--}46,702.5]$ versus $9,447.7 \pm 2,361.9 [3,675.5\text{--}36,126.0]$, C-peptide AUC $961.1 \pm 86.7 [650.0\text{--}1,912.5]$ versus $943.9 \pm 91.7 [667.0\text{--}1,938.5]$) (Figure 2).

Beta cell responsivity (Φ) to baseline glucose levels and to the MM challenge was maintained after SWS disruption (Figure 3A). SI, as determined by glucose minimal modeling and HOMA-IR (non-disrupted: $1.8 \pm 0.3 [0.4\text{--}4.3]$ versus disrupted $2.1 \pm 0.5 [0.8\text{--}7.8]$), was also preserved following one night of sleep disruption resulting in stable total disposition indices (DI) (Figure 3B). Results from both study visits were comparable to those previously reported in healthy adolescents with similar anthropometrics.³⁷

Correlations between Sleep Parameters and Insulin Sensitivity

A greater BMI percentile was associated with lower SI ($\beta = -0.2$, $P = 0.006$). There was no correlation between minutes spent in SWS and SI ($\beta = 0.04$, $P = 0.1$) or between percent change in SWS (min) and percent change in SI ($\beta = 0.03$, $P = 0.6$). A correlation between the percent change in delta power and the percent change in SI was explained by a single outlier. There was an interaction between the percent difference in SI between the two studies and SI during the non-disrupted night, such that only the most insulin sensitive subjects were vulnerable to the adverse metabolic effects of sleep disruption: the subjects in the highest SI tertile (SI range $16.5\text{--}31.3 \times 10^{-4} \text{ dL/kg/min per } \mu\text{IU/mL}$) demonstrated a 21–73% decrease in SI after sleep disruption, whereas the remaining subjects did not demonstrate a consistent response (Figure 3).

Biomarkers of Sympathetic Activation and Stress

ECG low-frequency power (sympathetic activity) increased by $29.6 \pm 11.3\% [-21.0 \text{ to } 147.5]$ and high-frequency power (vagal activity) decreased by $15.9 \pm 7.5\% [-34.6 \text{ to } 82.2]$ during the night of sleep disruption relative to non-disrupted sleep (Table 2). There were no differences in mean blood pressure (non-disrupted $67 \pm 6 \text{ mm Hg} [60\text{--}81]$ versus disrupted $79 \pm 2 [64\text{--}90]$), resting heart rate ($86 \pm 4 \text{ beats/min} [67\text{--}122]$ versus $86 \pm 4 [56\text{--}119]$), or cortisol levels ($7.8 \pm 0.8 \text{ mcg/dL} [2.6\text{--}11.5]$ versus $7.0 \pm 0.7 [3.2\text{--}11.8]$) in the morning after the two study nights.

DISCUSSION

The idea of sleep as a modifiable risk factor for obesity and insulin resistance is an attractive one that is supported by epidemiological literature and a small number of interventional

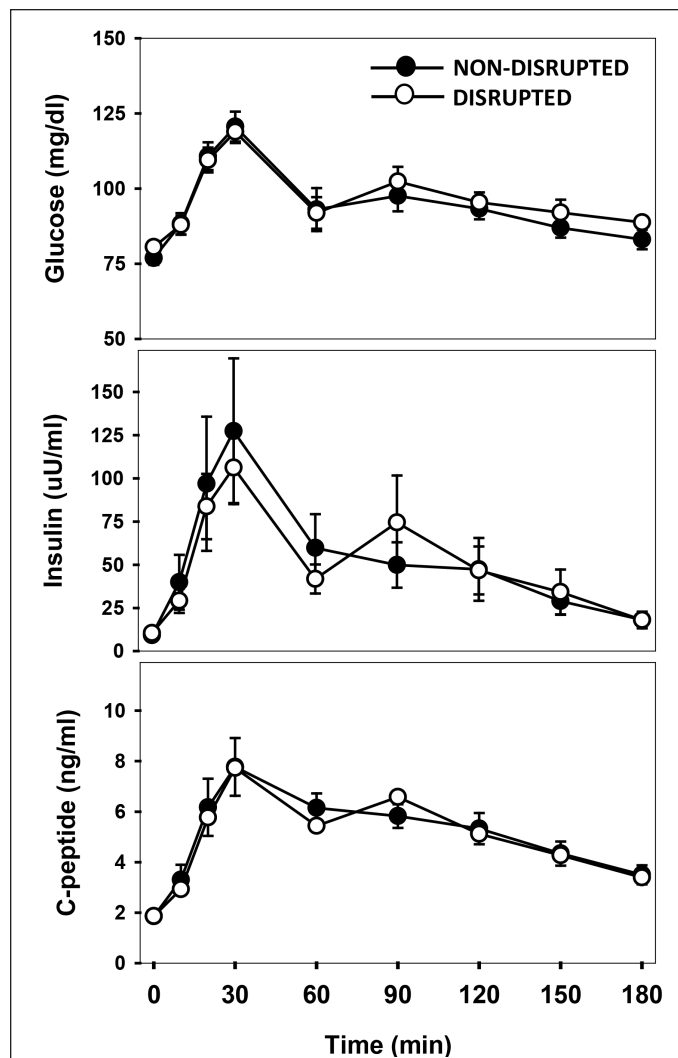
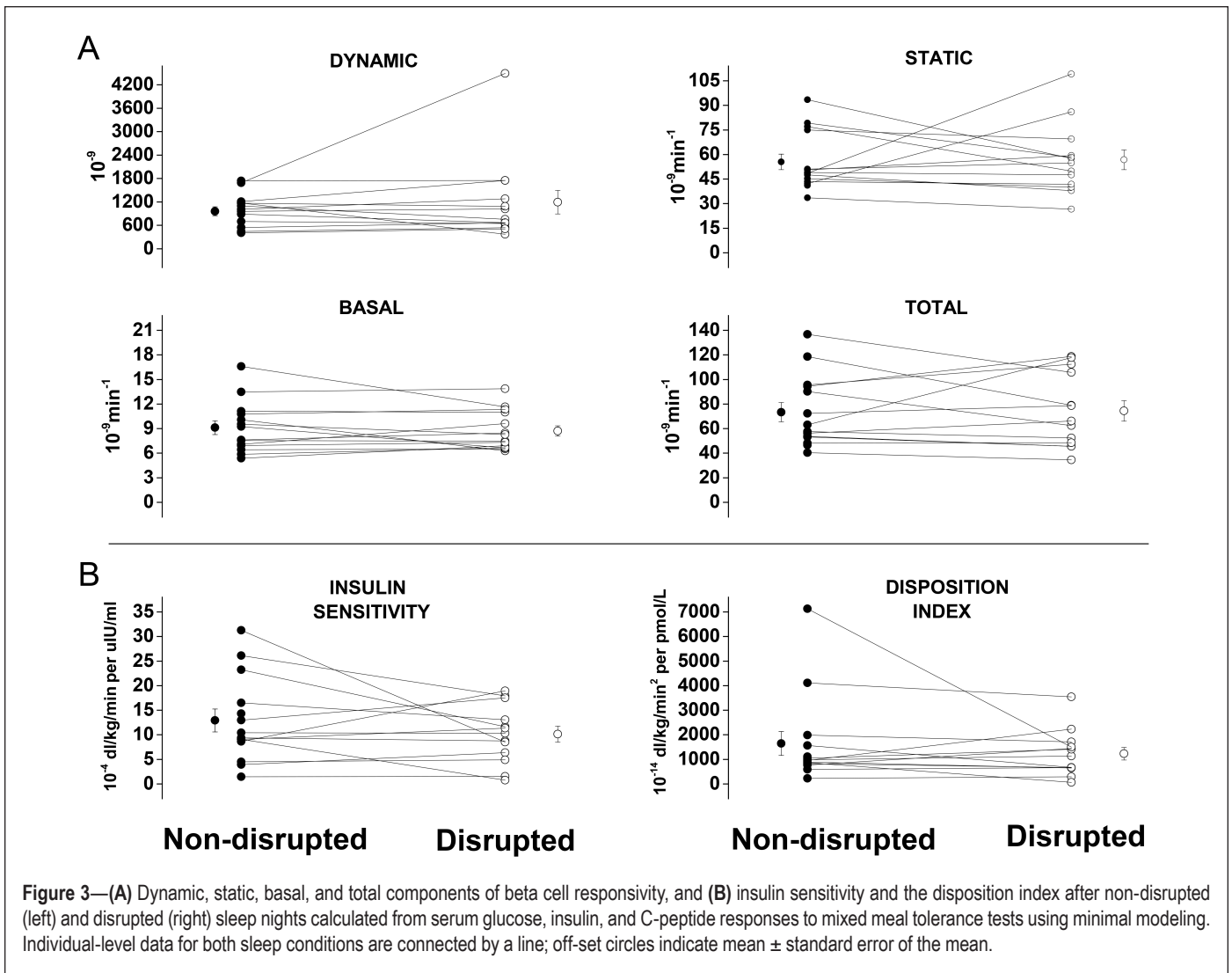


Figure 2—Slow wave sleep fragmentation in pubertal children did not alter fasting ($t = 0 \text{ min}$) glucose, insulin, or C-peptide levels or the responses to a mixed meal tolerance test ($t = 10, 20, 30, 60, 90, 120, 150, \text{ and } 180 \text{ min}$) performed within 1 h of waking (\circ) compared with results after a night of non-disrupted sleep (\bullet). Data are presented as mean \pm standard error.

sleep disruption studies. Most of these studies have been limited to adults, yet it is adolescents who would be predicted to be at the highest risk for insulin resistance and T2DM because of the “perfect storm” of chronic short sleep, a physiologic decline in SWS,^{14,15} and a physiologic peak in insulin resistance during puberty.¹³ The current studies were designed to fill this scientific gap by investigating the effect of SWS disruption on SI in pubertal subjects. Despite our prediction that adolescents would be more sensitive to SWS disruption than adults, we found that adolescents across a range of BMIs appear to be metabolically resilient to acute restriction and fragmentation of SWS.

Although previous interventional studies have demonstrated that even 2 to 3 nights of SWS or total sleep restriction results in metabolic derangements in adults, with effects on glucose homeostasis, orexigenic and anorexigenic hormones, food preferences, and portion sizes,³⁹ the research protocols



employed dramatically altered sleep structure to an extent unlikely to occur in everyday life. The single interventional study conducted in pediatric subjects also employed a severe sleep restriction protocol of 4 h of sleep for 3 days with no opportunity for daytime naps²⁵ in contrast to the current protocol in which sleep disruption was more modest and likely closer to naturalistic conditions. In addition, a meal of mixed macronutrients (the MMTT) was chosen over an oral or IV glucose tolerance test in the current studies to more closely emulate habitual nutrient intake while still providing a robust assessment of SI.

In the current studies, the adolescent subjects had lower SI and slightly higher beta cell responsiveness after 1 night of undisturbed sleep compared with results of MMTTs in normal weight adults,⁴⁰ consistent with the known increase in insulin resistance in adolescents. Greater baseline insulin resistance, however, did not translate into greater metabolic vulnerability to SWS disruption; basal SI, postprandial SI, and post-prandial beta cell responsiveness were maintained despite a 40% decrease in SWS. Most adolescents avert diabetes despite a profound decrease in SI during puberty.¹³ The decrease in SI is countered by a compensatory increase in insulin secretion⁴¹; however, the

beta cell response is quite modest and cannot fully explain the preservation of glucose homeostasis during puberty. Thus, adolescent physiology must invoke additional adaptive responses to defend against the physiologic increase in SI, perhaps at the level of the insulin receptor and downstream signaling pathways in the liver, muscle, and/or adipose tissue that have yet to be identified. The current studies suggest that a similar adaptive mechanism may help adolescents defend against the metabolic derangements associated with SWS disruption in adults.

The pathophysiologic links between SWS suppression and metabolic abnormalities in adults are unknown. One hypothesis is that these changes are mediated by increased sympathetic activity that induces insulin resistance by limiting peripheral blood flow, and hence insulin and glucose delivery, to skeletal muscle and adipose tissue.⁴² SWS is associated with a nadir in systemic sympathetic activity (SA)⁴³ and analyses of nocturnal HRV, a very sensitive marker of SA, suggest that severe SWS disruption in adults increases SA during the night.⁴⁴ Some^{11,44} but not all⁴⁵ studies in adults suggest that sympathetic hyperactivity may persist in the daytime after SWS disruption, although a study using muscle microneurography, which provides a direct readout of SA, found no evidence for increased

SA in the daytime after SWS disruption.⁴⁵ In the current study in adolescents, there was evidence of sympathetic hyperactivity during the night of sleep disruption, as seen in adults, but metabolic parameters were not disrupted. Other potential mediators of insulin resistance include increased cortisol, adipokines, and inflammatory cytokines; however, no SWS disruption studies^{11,44,45} including our own have thus far demonstrated clinically significant changes in any of these variables.

It is possible that our subjects remained insulin-sensitive not because of their unique pubertal physiology but because SWS was not suppressed to the same degree achieved in previous studies in adults. The current sleep disruption protocol was modeled after that of Tasali and colleagues,¹¹ who demonstrated that auditory-based sleep disruption decreases SWS by 90% in lean adult subjects. The same protocol applied to adolescent subjects in the current studies produced a more modest (40%) decrease in SWS, consistent with the greater homeostatic sleep drive that exists in early adolescence compared with adulthood.⁴⁶ Moreover, because subjects resumed SWS immediately after being aroused, a greater decrease in SWS may not be achievable in adolescents without inducing full awakenings. In light of Tasali et al.'s finding of a 25% decrease in SI after 3 nights of severe SWS disruption, it is possible that there is a dose-response relationship between SWS and glucose metabolism whereby SI is preserved if the amount of SWS remains above a certain threshold, a concept first introduced by Herzog et al.¹⁰ The increased homeostatic sleep drive among adolescents, however, also suggests that severe SWS disruption, to the extent achieved experimentally in adults, is unlikely to occur among teenagers in everyday life. We cannot exclude the possibility that metabolic abnormalities would manifest in adolescents after more prolonged sleep disruption, as may occur in sleep apnea or as a result of environmental noise.

It is of interest that only the most insulin-sensitive adolescent subjects demonstrated a consistent decrease in SI following SWS disruption. Previous studies that reported a decrease in SI after SWS suppression also differed from the current studies in that they included healthy, lean adults and specifically excluded the overweight or obese. In contrast, the adolescent subjects studied herein represented a wide range of BMIs (approximately one-third overweight or obese). Therefore, one explanation for the discrepant SI results is that SWS disruption only impairs SI in individuals who are highly insulin sensitive and has negligible effects in insulin-resistant individuals. This further suggests that SWS disruption may create insulin resistance by interfering with some of the same physiologic pathways that have already been inhibited by obesity such as endoplasmic reticulum stress, mitochondrial dysfunction, and ectopic lipogenesis mediated by inflammatory cytokines, free fatty acids, and nitric oxide.⁴⁷ Studies in transgenic mice have also demonstrated that obesity impairs the brain's ability to sense increased glucose or insulin levels and to respond appropriately by decreasing hepatic glucose output.⁴⁸ This finding is of particular interest because these glucose-sensing neurons (located in the arcuate nucleus) project to the ventrolateral preoptic nucleus,⁴⁹ which plays a critical role in promoting and maintaining NREM sleep.⁵⁰ Thus, the link between SWS

disruption and insulin resistance is likely to be multifactorial with effects on the liver, muscle, adipose tissue, and even the brain.

The current study has several limitations that may help inform the design of future studies in adolescents. The intensive nature of the research protocol limited the size of our sample and thus we were not able to tease out the effects of sex, pubertal stage, or BMI. Furthermore, data derived from a predominantly middle-class, Caucasian population may not be generalizable to minority groups who are at greater risk of T2DM and who may have different sleep patterns and sleep environments. Although we were careful to exclude subjects with sleep-disordered breathing and/or associated symptoms (e.g., snoring, excessive daytime sleepiness, or inattention and hyperactivity), we did not control for potential differences in sleep duration and quality in the 2 mo between study visits, which may have also been influenced by the time of year (e.g., school versus summer). Most importantly, this and other SWS disruption studies in adults cannot address whether chronic SWS disruption has the same detrimental metabolic effects as short-term disruption or if compensatory physiological changes develop over time. Of note, Broussard and colleagues recently demonstrated that 2 nights of recovery sleep was sufficient to reverse the metabolic abnormalities that developed after 4 nights of severe sleep restriction in healthy men.⁵¹ These results provide some reassurance that intermittent and short reprieves from chronic sleep restriction, and perhaps from sleep disruption, may be sufficient to avert T2DM.

In conclusion, we have now demonstrated that SI is preserved in adolescents following selective, acute SWS fragmentation. These data provide preliminary evidence that adolescents may be uniquely poised to respond to metabolic stressors, such as SWS disruption, to maintain euglycemia and further suggests that only those adolescents who are highly insulin sensitive, and therefore at low risk for T2DM, are susceptible to the metabolic insult of SWS disruption. Both interventional and observational studies of chronic sleep disruption in a larger group of adolescents are necessary to confirm and generalize these important findings.

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Address correspondence to: Natalie Shaw, MD, National Institute of Environmental Health Sciences, Building 101, A349, 111 TW Alexander Drive, Research Triangle Park, NC 27709; Tel: (919) 541-7798; Fax: (301) 451-5539; Email: natalie.shaw@nih.gov

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