



## Original Contribution

# Association of Nocturnal Melatonin Secretion With Insulin Resistance in Nondiabetic Young Women

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Exogenous melatonin ameliorates insulin resistance in animals, while among humans, polymorphisms in the melatonin receptor gene are associated with insulin resistance. We aimed to investigate the association of endogenous nocturnal melatonin secretion with insulin resistance in humans. We analyzed the association between endogenous nocturnal melatonin secretion, estimated by measuring the main melatonin metabolite, 6-sulfatoxymelatonin, from the first morning urinary void, and the prevalence of insulin resistance based on fasting blood samples collected in a cross-sectional study of 1,075 US women (1997–1999) without diabetes, hypertension, or malignancy. Urinary 6-sulfatoxymelatonin level was standardized to urinary creatinine level; insulin resistance was defined as an insulin sensitivity index value (using the McAuley formula) less than 7.85. Logistic regression models included adjustment for age, body mass index, smoking, physical activity, alcohol intake, dietary glycemic index, family history of diabetes mellitus, blood pressure, plasma total cholesterol, uric acid, and estimated glomerular filtration rate. Higher nocturnal melatonin secretion was inversely associated with insulin levels and insulin resistance. In fully adjusted models, the odds ratio for insulin resistance was 0.45 (95% confidence interval: 0.28, 0.74) among women in the highest quartile of urinary 6-sulfatoxymelatonin:creatinine ratio compared with women in the lowest quartile. Nocturnal melatonin secretion is independently and inversely associated with insulin resistance.

circadian rhythm; cross-sectional analysis; diabetes mellitus, type 2; insulin resistance; melatonin; 6-sulfatoxymelatonin; premenopause

Abbreviations: aMT6s:Cr, 6-sulfatoxymelatonin:creatinine; CI, confidence interval; CV, coefficient of variation; ISI, insulin sensitivity index; MET, metabolic equivalent of task.

Production and secretion of melatonin by the pineal gland follows a diurnal pattern, peaking 3–5 hours after darkness and declining precipitously prior to awakening (1). This diurnal pattern of melatonin secretion, together with the widespread location of melatonin receptors in the body, including  $\beta$  cells of the pancreatic islets, allows melatonin to play a role in the entrainment of the body's physiological activity to the diurnal rhythm (2).

Several lines of evidence suggest that melatonin may play a role in glucose metabolism. In vitro, prolonged exposure of  $\beta$  islet cells to melatonin (mimicking a sleep period) increases  $\beta$ -cell glucose sensitivity (3, 4). In animal studies, dietary melatonin supplementation in diabetes-prone rats reduced insulin

levels and improved insulin sensitivity (5, 6). Genome-wide association studies in humans identified polymorphisms in the melatonin receptor type 1B gene (*MTNR1B*) that were significantly associated with fasting glucose and hemoglobin A<sub>1c</sub> levels in nondiabetic persons, and also with gestational and type 2 diabetes (7–9). Finally, small cross-sectional studies have shown that melatonin levels are lower in persons with metabolic syndrome and persons with diabetes than in healthy persons (10–12). However, whether or not melatonin secretion is related to insulin or glucose metabolism in nondiabetic humans is unknown. Thus, we investigated the association between nocturnal melatonin secretion and the presence of insulin resistance in a cross-sectional analysis

comprising 1,075 healthy women aged 32–52 years from Nurses' Health Study II (13).

## MATERIALS AND METHODS

### Study population

Nurses' Health Study II is a longitudinal cohort study that began in 1989 when 116,430 female registered nurses in the United States returned a mailed questionnaire that ascertained information on health-related behaviors and medical diagnoses. Since 1989, participants in this cohort have returned repeat questionnaires every 2 years that update information on lifestyle, diet, and medical events. Follow-up of women in Nurses' Health Study II has exceeded 90% of the eligible person-time since its inception. Between 1997 and 1999, 29,616 women submitted blood and urine samples by overnight mail with a cold-pack. These samples were processed, aliquoted, and stored at  $-130^{\circ}\text{C}$ .

Of the 29,616 nurses who provided biological samples, plasma levels of insulin and triglycerides were previously determined for 1,500 women whose blood samples were collected in the fasting state to study associations with hypertension (14). Additional characteristics of these 1,500 women included a body mass index (weight (kg)/height (m)<sup>2</sup>) less than 30 and no prior history of hypertension, diabetes, cardiovascular disease, or cancer.

For the current study, we included only the 1,091 women whose urine samples were from the first morning void and who had serum measurements of insulin and triglycerides to allow calculation of insulin sensitivity. We excluded those women with a low urinary creatinine concentration ( $<30$  mg/dL, below the sensitivity of the assay) and women who had nonphysiological melatonin levels (more than 40 times the median value). The final study population included 1,075 women; 96% were premenopausal, 3% were postmenopausal, and 1% were unsure of their menopausal status. The Partners Institutional Review Board reviewed and approved this study, including the fact that all participants provided implied consent by virtue of voluntarily returning mailed questionnaires and biological specimens.

### Ascertainment of melatonin secretion

Nocturnal melatonin secretion was estimated by measuring the concentration of the major metabolite of melatonin, 6-sulfatoxymelatonin, in a urine specimen from the first morning void. Urinary 6-sulfatoxymelatonin level has been widely used as an estimate of melatonin secretion in over 300 scientific studies. First-morning-void urinary 6-sulfatoxymelatonin level standardized to urinary creatinine level correlates well with cumulative nocturnal melatonin secretion (Spearman's correlation coefficient = 0.76) (15), and the correlation is independent of renal function (16). This allows for accurate estimation of nocturnal melatonin secretion in epidemiologic studies (17–19). To account for the concentration of urine, 6-sulfatoxymelatonin (aMT6s) levels were standardized to urinary creatinine (Cr) levels for all analyses (i.e., urinary aMT6s:Cr ratio).

Urinary concentrations of 6-sulfatoxymelatonin were measured at Brigham and Women's Hospital's diagnostic laboratory using an enzyme-linked immunosorbent assay (ALPCO Diagnostics, Windham, New Hampshire); the interassay coefficient of variation (CV) for this assay was 13%. Urinary creatinine was measured in the same laboratory by a modified Jaffé method (interassay CV = 6%) (18).

### Ascertainment of insulin sensitivity index

Insulin and triglyceride levels were measured using radioimmunoassay and standard enzymatic methods, respectively. The CVs were 10.4% for insulin and 14.1% for triglycerides. The insulin sensitivity index (ISI), or glucose disposal rate ( $M$ ) corrected for free-fat mass (i.e., MFFM), was calculated using the McAuley formula:

$$\frac{\text{MFFM}}{I} = e^{[2.63 - 0.28(\text{insulin}) - 0.31 \ln(\text{TG})]},$$

where insulin and triglycerides (TG) are the fasting plasma levels of insulin (mIU/L) and triglycerides (mmol/L), respectively. This estimation of ISI has been verified within many population groups, including persons with and without type 2 diabetes and persons with metabolic syndrome, and across ethnic groups (20–24). Insulin resistance was defined as a categorical variable by the lowest quartile of insulin sensitivity, consistent with other studies using the McAuley formulae (20–23). In the population studied, the lowest quartile of insulin sensitivity corresponded to persons with ISI values less than 7.85.

### Ascertainment of covariates

Age, body mass index, and smoking status were determined from a questionnaire completed at the time of urine and blood sample submission. Physical activity was self-reported on the biennial questionnaire immediately prior to biological sample collection and was expressed as metabolic equivalent of task (MET) scores per week. These questionnaire-derived data on physical activity are highly correlated with activity diaries ( $r = 0.79$ ) (25). Alcohol consumption and carbohydrate intake were self-reported on a semiquantitative food frequency questionnaire that was returned prior to submission of the urine specimen. This food frequency questionnaire has been extensively validated; the correlation coefficients for correlation between alcohol intake and energy-adjusted carbohydrate intake based on the food frequency questionnaire and on dietary records were 0.90 for alcohol and 0.61 for energy-adjusted carbohydrate intake (26, 27). Information on carbohydrate intake was used to determine the glycemic index as previously described (28–32). Family history of diabetes mellitus was ascertained from the original 1989 questionnaire.

Plasma was also assayed for uric acid by means of a uricase oxidation assay (CV = 3.4%), creatinine by means of a modified Jaffé method (CV = 6.5%), and total cholesterol by means of a standard esterase-oxidase method (CV = 5.3%). Estimated glomerular filtration rate was determined using the

Chronic Kidney Disease Epidemiology Collaboration equation (33).

Participation in shift work was determined from the 2001 questionnaire, which collected information on the number of months during which the nurse had worked rotating night shifts in the 2 years prior to blood and urine collection. Working rotating night shifts was defined as working at least 3 nights per month. Nurses who replied that they had worked at least 3 nights per month for 1 or more months in the 2 years prior to blood and urine collection were classified as rotating shift workers (34).

### Statistical analyses

Because the continuous baseline variables, including the ISI, were not normally distributed, we performed nonparametric testing with the Kruskal-Wallis test to examine baseline differences in continuous covariates across quartiles of urinary aMT6s:Cr ratio. Differences in categorical variables across quartiles of urinary aMT6s:Cr ratio were assessed with the  $\chi^2$  test.

The association of nocturnal melatonin secretion with insulin resistance was analyzed first with urinary aMT6s:Cr ratio as a continuous variable and then with these values divided into quartiles to look for a nonlinear relationship. Because of a substantial right skew in the distribution of urinary aMT6s:Cr ratio, these values were log-transformed using the natural logarithm whenever urinary aMT6s:Cr ratio was analyzed as a continuous variable.

ISI was dichotomized, with insulin resistance defined as an ISI value less than 7.85. We employed logistic regression to examine the association of urinary aMT6s:Cr ratio with dichotomous insulin resistance. In other analyses, ISI was examined as a continuous variable, and associations with urinary aMT6s:Cr ratio were analyzed with linear regression. We generated 2 hierarchical multivariable models: model 1, adjusted for age and body mass index; and model 2, adjusted for age, body mass index, smoking status, physical activity, dietary glycemic index, alcohol intake, family history of diabetes mellitus, systolic and diastolic blood pressure, plasma levels of uric acid and total cholesterol, and estimated glomerular filtration rate. The variables included in the multivariable models were chosen because of their known association with insulin resistance and/or diabetes or their theoretical effects on urinary melatonin concentration.

We also performed several secondary analyses. First, we repeated our multivariable analyses after restricting the data to participants who had not worked night shifts in the 2 years prior to blood and urine collection, and later restricting the analysis to only those who had worked night shifts in the 2 years prior to collection. We also repeated our analysis after redefining insulin resistance as  $ISI < 6.3$ , the definition of insulin resistance used in the original paper deriving the McAuley index (20). We analyzed the possibility of a nonlinear association between urinary aMT6s:Cr ratio and insulin resistance by fitting restricted cubic splines; tests for nonlinearity used the likelihood ratio test, comparing the model containing only the linear term with the model containing both linear and cubic spline terms. We repeated the analysis using urinary 6-sulfatoxymelatonin level without

normalization by urinary creatinine. All statistical analyses were performed with SAS, version 9.2 (SAS Institute, Inc., Cary, North Carolina).

## RESULTS

### Baseline characteristics

The baseline characteristics of the study population as a whole and by quartile of urinary aMT6s:Cr ratio are displayed in Tables 1 and 2. The median urinary aMT6s:Cr ratio was 47.3 ng/mg (5th–95th percentile range, 15.1–109.8). The median ISI value was 9.5 (5th–95th percentile range, 6.0–15.2). The median age was 43 years (5th–95th percentile range, 36–49), and the median body mass index was 24.1 (5th–95th percentile range, 19.7–29.1). While urinary 6-sulfatoxymelatonin level increased across quartiles of increasing urinary aMT6s:Cr ratio, there was no significant change in urinary creatinine level between quartiles, demonstrating that variation in the ratio was driven by variation in urinary 6-sulfatoxymelatonin and not urinary creatinine. Across quartiles of increasing urinary aMT6s:Cr ratio, the ISI and estimated glomerular filtration rate were higher, while fasting insulin, proportion of insulin resistance, body mass index, and plasma uric acid levels were all lower. While there was a statistically significant difference in physical activity between quartiles, no trend across increasing quartiles was observed. Age, blood pressure, smoking status, dietary glycemic index, plasma triglycerides, total cholesterol, prevalence of shift work, and family history of diabetes mellitus did not demonstrate statistically significant differences between quartiles of urinary aMT6s:Cr ratio.

### Melatonin and insulin resistance

The association of nocturnal melatonin secretion with insulin resistance is displayed in Table 3. Women whose urinary aMT6s:Cr ratio was in the highest quartile ( $>67.6$  ng/mg) had a crude odds ratio for insulin resistance of 0.42 (95% confidence interval (CI): 0.28, 0.63) compared with those whose urinary aMT6s:Cr ratio was in the lowest quartile ( $<32.3$  ng/mg). After adjustment for age, body mass index, smoking status, physical activity, dietary glycemic index, alcohol intake, family history of diabetes mellitus, systolic and diastolic blood pressure, plasma uric acid level, total cholesterol, and estimated glomerular filtration rate, the odds ratio for insulin resistance in the highest quartile of urinary aMT6s:Cr ratio compared with lowest quartile was 0.45 (95% CI: 0.28, 0.74). Repeating the analysis with urinary aMT6s:Cr ratio as a continuous variable (log-transformed) also demonstrated a statistically significant association between higher nocturnal melatonin secretion and a lower prevalence of insulin resistance in fully adjusted models (per 1-unit increase in log urinary aMT6s:Cr ratio, odds ratio = 0.74, 95% CI: 0.57, 0.95).

We performed a sensitivity analysis in which we repeated our logistic regression analyses after excluding from the model all women who had worked rotating night shifts during the previous 2 years. After these exclusions, the fully adjusted odds ratio for insulin resistance among women in the highest quartile of urinary aMT6s:Cr ratio compared

**Table 1.** Baseline Characteristics (Median Values) of US Women From the Nurses' Health Study II Cohort, by Quartile of Urinary 6-Sulfatoxymelatonin<sup>a</sup>:Creatinine Ratio, 1997–1999

Covariate	Total Population ( <i>n</i> = 1,075)		Quartile of Urinary aMT6s:Cr Ratio								<i>P</i> Value <sup>b</sup>
			1 ( <i>n</i> = 268)		2 ( <i>n</i> = 269)		3 ( <i>n</i> = 269)		4 ( <i>n</i> = 269)		
	Median	5th–95th <sup>c</sup>	Median	5th–95th	Median	5th–95th	Median	5th–95th	Median	5th–95th	
6-Sulfatoxymelatonin, ng/mL	47.9	12.8–156.0	24.6	7.4–51.9	41.3	18.7–489.6	58.0	27.3–126.0	90.2	43.3–288.0	<0.0001
Urinary creatinine, mg/dL	107	48–217	110	47–214	110	50–219	102	48–225	104	50–209	0.86
aMT6s:Cr ratio, ng/mg	47.3	15.1–109.8	23.8	8.5–31.4	38.9	32.9–46.4	55.9	48.1–66.1	86.4	69.3–180.6	<0.0001
Insulin sensitivity index (McAuley formula)	9.5	6.0–15.2	9.2	6.0–14.8	9.3	5.8–13.9	9.7	5.7–15.0	10.0	6.4–16.9	0.0003
Age, years	43	36–49	44	36–49	43	36–49	43	36–49	43	35–49	0.05
Body mass index <sup>d</sup>	24.1	19.7–29.1	24.5	19.7–29.2	24.1	20.2–29.9	24.3	19.7–29.0	23.5	19.2–29.1	0.02
Physical activity, METs/week	12.2	0.9–57.1	13.1	0.9–56.2	11.0	0.9–51.6	14.9	1.0–70.6	11.0	0.8–51.9	0.04
Systolic blood pressure, mm Hg	120	100–140	120	100–140	120	100–140	120	100–140	120	100–140	0.90
Diastolic blood pressure, mm Hg	70	60–87	70	60–87	70	60–87	70	60–87	70	60–87	0.37
Glycemic index	53.8	47.8–59.0	53.8	47.3–59.2	53.6	47.9–58.9	53.7	47.8–59.1	54.2	48.3–59.7	0.21
Alcohol consumption, g/day	1.6	0.0–34.9	1.8	0.0–13.0	1.8	0.0–17.0	1.8	0.0–25.3	1.1	0.0–14.0	0.04
Total cholesterol, mg/dL	186	141–245	186	144–245	186	137–246	188	144–244	184	139–242	0.69
Insulin, IU/mL	4.6	1.2–13.2	5.0	1.4–15.3	5.1	1.4–12.7	4.4	1.3–15.2	4.1	0.9–10.2	0.0001
Triglycerides, mg/dL	78	41–189	83	41–199	80	45–190	77	41–173	74	39–182	0.18
Uric acid, mg/dL	3.9	2.7–5.6	4.0	2.8–5.6	3.9	2.7–5.6	3.8	2.7–5.5	3.9	2.5–5.6	0.04
Estimated glomerular filtration rate, <sup>e</sup> mL/minute/1.73 m <sup>2</sup>	92.6	70.6–112.2	89.5	70.8–111.4	92.4	70.6–111.6	93.0	69.4–112.3	94.0	71.5–114.6	0.003

Abbreviations: aMT6s:Cr, 6-sulfatoxymelatonin:creatinine; CI, confidence interval; MET, metabolic equivalent of task.

<sup>a</sup> 6-Sulfatoxymelatonin is the major metabolite of melatonin.

<sup>b</sup> *P* values were calculated by means of the Kruskal-Wallis test.

<sup>c</sup> 5th–95th percentile range.

<sup>d</sup> Weight (kg)/height (m)<sup>2</sup>.

<sup>e</sup> Estimated glomerular filtration rate was determined using the Chronic Kidney Disease Epidemiology Collaboration equation (33).

**Table 2.** Baseline Characteristics (Frequencies) of US Women From the Nurses' Health Study II Cohort, by Quartile of Urinary 6-Sulfatoxymelatonin<sup>a</sup>:Creatinine Ratio, 1997–1999

Covariate	Total Population (n = 1,075)		Quartile of Urinary aMT6s:Cr Ratio								P Value <sup>b</sup>
			1 (n = 268)		2 (n = 269)		3 (n = 269)		4 (n = 269)		
	No.	%	No.	%	No.	%	No.	%	No.	%	
Insulin resistance <sup>c</sup>	268	25	87	32	74	28	62	23	45	17	0.0002
No shift work in past 2 years	893	83	217	81	219	81	220	82	228	85	0.65
Family history of diabetes mellitus	166	15	37	14	41	15	51	19	38	14	0.32
Smoking status											
Current smoker	54	5	15	6	17	6	10	4	12	4	0.52
Past smoker	246	23	62	23	62	23	59	22	63	23	0.98

Abbreviation: aMT6s:Cr, 6-sulfatoxymelatonin:creatinine.

<sup>a</sup> 6-Sulfatoxymelatonin is the major metabolite of melatonin.

<sup>b</sup> P values were calculated by means of the  $\chi^2$  test.

<sup>c</sup> Insulin resistance was defined as the lowest 25% of insulin sensitivity index values, as measured by the McAuley formula (20).

with the lowest was 0.45 (95% CI: 0.28, 0.78). A similar analysis that included only those women who had worked night shifts during the previous 2 years was limited by small numbers. However, the fully adjusted odds ratio for insulin resistance among night-shift-working women in the highest quartile of urinary aMT6s:Cr ratio compared with the lowest was 0.27 (95% CI: 0.05, 1.40). In an additional sensitivity analysis, we repeated our logistic regression analysis with insulin resistance defined as ISI < 6.3. Using this definition, the fully adjusted odds ratio for insulin resistance among women in the highest quartile of urinary aMT6s:Cr ratio compared with the lowest was 0.38 (95% CI: 0.16, 0.93). In the analysis that employed restricted cubic splines, a linear association between urinary aMT6s:Cr ratio and insulin resistance was confirmed ( $P = 0.02$ ); visual inspection of the cubic spline model did not reveal a threshold effect for the association of urinary aMT6s:Cr ratio with insulin resistance.

Consistent with an inverse association with insulin resistance, higher nocturnal melatonin secretion was directly associated with ISI. Using multivariable linear regression analysis,

every 1-unit increase in log-transformed urinary aMT6s:Cr ratio was associated with a 0.0023-point higher ISI ( $P < 0.001$ ).

## DISCUSSION

In 1,075 nonobese young women without hypertension or type 2 diabetes, higher nocturnal melatonin secretion, as measured by urinary 6-sulfatoxymelatonin, was independently associated with greater insulin sensitivity and a lower prevalence of insulin resistance. These associations were robust, and a strong association was observed after inclusion of multiple potential confounders and after the exclusion of rotating shift workers.

Laboratory studies suggest that melatonin may have a beneficial effect on glucose metabolism. As an example, diabetes-prone rats given melatonin supplementation were less likely to develop diabetes and its associated metabolic derangements in comparison with genetically identical animals not provided with melatonin (5). While caution must be applied in extrapolating from the metabolic response to exogenous melatonin in

**Table 3.** Odds Ratios for Insulin Resistance in US Women From the Nurses' Health Study II Cohort According to Urinary 6-Sulfatoxymelatonin<sup>a</sup>:Creatinine Ratio, 1997–1999

	Continuous <sup>b</sup> Urinary aMT6s:Cr Ratio (per 1-Unit Increase)		Quartile of Urinary aMT6s:Cr Ratio							
			1 (Referent)		2		3		4	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Unadjusted model	0.68	0.54, 0.84	1	0.79	0.54, 1.14	0.62	0.43, 0.91	0.42	0.28, 0.63	
Adjusted model 1 <sup>c</sup>	0.75	0.59, 0.94	1	0.84	0.56, 1.25	0.65	0.43, 0.97	0.48	0.31, 0.74	
Adjusted model 2 <sup>d</sup>	0.74	0.57, 0.95	1	0.84	0.54, 1.30	0.68	0.44, 1.07	0.45	0.28, 0.74	

Abbreviations: aMT6s:Cr, 6-sulfatoxymelatonin:creatinine; CI, confidence interval; MET, metabolic equivalent of task; OR, odds ratio.

<sup>a</sup> 6-Sulfatoxymelatonin is the major metabolite of melatonin.

<sup>b</sup> Urinary aMT6s:Cr ratio was log-transformed for the linear regression analysis because of the nonnormal distribution of the raw measurements.

<sup>c</sup> Adjusted model 1 included age and body mass index.

<sup>d</sup> Adjusted model 2 included age, body mass index, physical activity (in METs), dietary glycemic index, daily alcohol intake, total cholesterol, family history of diabetes mellitus, smoking status, uric acid, estimated glomerular filtration rate, and systolic and diastolic blood pressure.



rodents, a night-active species, to that in humans, a day-active species, additional evidence supporting a beneficial metabolic effect of melatonin comes from in vitro studies. Pancreatic  $\beta$  islet cells exposed in vitro to melatonin for a prolonged period, mimicking the conditions of normal sleep, demonstrated increased sensitivity to glucose, while only brief melatonin exposure impaired glucose sensitivity (3, 35). Furthermore, genetic variants in *MTNR1B* expressed by  $\beta$  islet cells of the pancreas were strongly associated with glucose metabolism and type 2 diabetes in large-scale human genetic studies (7–9, 36). These findings, taken together with the presence of insulin receptors in the pineal gland, indicate a homeostatic relationship between insulin and melatonin that, if disrupted, could contribute to insulin resistance and development of type 2 diabetes (37).

Cross-sectional human studies have shown that patients with metabolic syndrome and diabetes have lower endogenous nocturnal melatonin levels than healthy persons (10–12, 37). These studies were limited by small sample sizes and by potential confounding from circadian rhythm disruption, which is seen more frequently in subjects with diabetes than in the general population and has been associated with reduced nocturnal melatonin production (38, 39). In the larger of the 2 studies, subgroup analysis demonstrated that nocturnal melatonin levels were lower only in those diabetics with proliferative retinopathy (10). Given the importance of the retina in regulation of melatonin secretion and the presence of melatonin receptor 1B on the retina, it is possible that the lower nocturnal melatonin levels were due to the presence of the retinal lesions.

Nocturnal melatonin secretion has been shown to be disrupted by sleep disorders (39). Thus, our finding that lower nocturnal melatonin secretion is associated with insulin resistance may be a potential mechanism explaining the previously described relationship between disruption of normal sleep pattern and incidence of diabetes. Specifically, nondiabetic men who reported sleeping less than 5 hours per night were twice as likely to develop diabetes as those who reported sleeping 7 hours per night (40, 41).

Our study had limitations. First, because it was cross-sectional, we were unable to draw any conclusions about the direction of the association; it is possible that insulin resistance, through hyperinsulinemia, causes increased activation of insulin receptors in the pineal gland, thus suppressing melatonin secretion. However, genetic studies suggest that alterations in melatonin signaling produce insulin resistance, rather than a mechanism operating in the other direction. Second, we did not directly sample serial overnight plasma levels of melatonin. However, first-morning-void urine measurements of 6-sulfatoxymelatonin normalized to creatinine provide reliable estimates of overnight cumulative melatonin production, and this estimate is independent of renal function (15, 16). Third, we did not use the euglycemic hyperinsulinemic clamp technique in our participants, which is the gold standard for assessing insulin sensitivity. Further, we were unable to analyze alternative measures of insulin sensitivity, such as homeostasis model of assessment of insulin resistance or the Quantitative Insulin Sensitivity Check Index, because blood glucose measurements in these blood samples were unreliable; as we noted above, nurses returned their blood sam-

ples by overnight mail, by which time the glucose had been extensively metabolized by red and white blood cells. Nevertheless, the McAuley index is highly correlated with insulin sensitivity as measured by the clamp technique (area under the receiver operating characteristic curve = 0.83), compares favorably with homeostasis model of assessment of insulin resistance, and has been successfully employed in multiple studies (20–23, 42). Fourth, our definition of insulin resistance was somewhat arbitrary; however, our definition was consistent with that used in other studies, and our results were robust when we employed the same definition as that used in the original McAuley report (20). In addition, we found a consistent strong association between melatonin secretion and insulin sensitivity as a continuous variable. Fifth, our study population was limited to nonobese women; furthermore, most participants were white. Thus, it is unknown whether our findings can be applied to men, to obese persons, or to other racial groups. Sixth, in this observational study, information on many of the baseline covariates used in the analyses was self-reported on biannual questionnaires; potential misreporting of this information could have produced residual confounding. However, the validity of this questionnaire-based information has been demonstrated. Finally, as with all observational studies, we cannot exclude the possibility that our findings were confounded by factors we could not ascertain.

In conclusion, small decreases in nocturnal melatonin secretion are associated with increased insulin resistance in nonobese, nondiabetic young women. Further work is needed to validate this association in other populations, to perform a prospective analysis to assess whether melatonin secretion can predict the incidence of diabetes, and to study whether or not melatonin supplementation may modify glucose metabolism in persons with insulin resistance or diabetes.

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