Melatonin Secretion and the Incidence of Type 2 Diabetes

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Importance Loss-of-function mutations in the melatonin receptor are associated with insulin resistance and type 2 diabetes. Additionally, in a cross-sectional analysis of persons without diabetes, lower nocturnal melatonin secretion was associated with increased insulin resistance.

Objective To study the association between melatonin secretion and the risk of developing type 2 diabetes.

Design, Setting, and Participants Case-control study nested within the Nurses' Health Study cohort. Among participants without diabetes who provided urine and blood samples at baseline in 2000, we identified 370 women who developed type 2 diabetes from 2000-2012 and matched 370 controls using risk-set sampling.

Main Outcome Measures Associations between melatonin secretion at baseline and incidence of type 2 diabetes were evaluated with multivariable conditional logistic regression controlling for demographic characteristics, lifestyle habits, measures of sleep quality, and biomarkers of inflammation and endothelial dysfunction.

Results The median urinary ratios of 6-sulfatoxymelatonin to creatinine were 28.2 ng/mg (5%-95% range, 5.5-84.2 ng/mg) among cases and 36.3 ng/mg (5%-95% range, 6.9-110.8 ng/mg) among controls. Women with lower ratios of 6-sulfatoxymelatonin to creatinine had increased risk of diabetes (multivariable odds ratio, 1.48 [95% CI, 1.11-1.98] per unit decrease in the estimated log ratio of 6-sulfatoxymelatonin to creatinine). Compared with women in the highest ratio category of 6-sulfatoxymelatonin to creatinine, those in the lowest category had a multivariable odds ratio of 2.17 (95% CI, 1.18-3.98) of developing type 2 diabetes. Women in the highest category of melatonin secretion had an estimated diabetes incidence rate of 4.27 cases/1000 person-years compared with 9.27 cases/1000 person-years in the lowest category.

Conclusions and Relevance Lower melatonin secretion was independently associated with a higher risk of developing type 2 diabetes. Further research is warranted to assess if melatonin secretion is a modifiable risk factor for diabetes within the general population.

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MELATONIN IS A PINEAL hormone under the control of the biological clock, which is located in the hypothalamus and regulated by light exposure.[1] Secretion of melatonin follows a diurnal pattern, typically peaking 3 to 5 hours after sleep onset when it is dark, with almost no production occurring during daylight.[2] Melatonin receptors have been found throughout the body in many tissues including pancreatic islet cells, reflecting the widespread effects of melatonin on physiological functions such as energy metabolism and the regulation of body weight.[3-5]

Several lines of evidence suggest that melatonin may have a role in glucose metabolism. Ingestion of melatonin had a protective effect against the onset of diabetes in diabetes-prone rats with improvements also seen in the animals' cholesterol and triglycerides levels vs controls.[6-8] In several large genome-wide association studies, single nucleotide polymorphisms in the type B melatonin receptor (MTNR1B) were associated with higher levels of fasting glucose, higher levels of hemoglobin A_{1c}, and increased incidence of gestational and type 2 diabetes.[9-11] Among these single nucleotide polymorphisms, those that cause loss of function of the melatonin receptor were associated with the highest incidence of type 2 diabetes.[5] While the effect of endogenous melatonin on glucose metabolism in humans is unknown, the animal data and human genetic studies suggest that either low melatonin secretion or reduced melatonin signaling can impair insulin sensitivity and lead to type 2 diabetes.

A prospective association between melatonin secretion and type 2 diabetes, however, has not been reported. Thus, we performed a nested, case-control study among women participating in the Nurses' Health Study to investigate the independent associa-

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tion of melatonin secretion and the incidence of type 2 diabetes.

METHODS

Population and Study Design

We performed a case-control study nested within the Nurses' Health Study.[12] The Nurses' Health Study began in 1976 when 121 701 registered nurses aged 30 to 55 years returned an initial questionnaire. On this and subsequent biennial questionnaires, health status, medications, dietary intake, and lifestyle factors including smoking history, physical activity, and sleeping patterns were ascertained. In addition to completing questionnaires, blood and first morning urine samples were provided by 18 743 women between 1999 and 2000.

Participants returned these samples with a cold pack by overnight mail, and then the samples were aliquoted and stored in liquid nitrogen until the time they were assayed. Women were eligible for the current study if they provided an adequate first morning urine sample (urinary creatinine >30 mg/dL), fasting blood sample, and were without a history of malignancy or diabetes in 2000. This study was approved by the institutional review board at the Brigham and Women's Hospital; all participants provided implied consent by virtue of voluntarily returning biological specimens and mailed questionnaires.

Cases and Controls

From eligible women, cases were defined as those participants who had a confirmed diagnosis of type 2 diabetes (as defined below) after the 2000 questionnaire. Based on preliminary data in a small sample, we estimated that 370 matched case-control pairs would provide more than 80% power to detect an odds ratio (OR) of at least 1.6 between the lowest and highest tertiles of melatonin with a 2-sided significance threshold of .05.

Accordingly, we selected 370 participants who developed type 2 diabetes between 2000 and 2012. Controls were selected by risk-set sampling from participants without type 2 diabetes at the time of each case diagnosis. Controls were matched 1 to 1 with cases within 1 year of age, within 1 month of blood collection, and by self-reported race.

Ascertainment of Diabetes

Participants who self-reported diabetes on the biennial questionnaires were asked to complete a supplementary questionnaire to ascertain results of diagnostic blood glucose levels and use of medications for hypoglycemia. Details from the supplementary questionnaire were reviewed to ensure that the diagnosis of diabetes satisfied the criteria specified by the American Diabetes Association.[13] The validity of these supplementary questionnaires was established in the Nurses' Health Study by an endocrinologist, blinded to the questionnaire results, who reviewed the medical records of 62 women with self-reported diabetes; the endocrinologist confirmed the diagnosis of diabetes in 61 cases (98.4%).[14]

Nocturnal Melatonin Secretion

Melatonin secretion was estimated by measuring the concentration of its major metabolite, 6-sulfatoxymelatonin, in a first morning void urine specimen, and normalized to urinary creatinine. The first morning void concentration of 6-sulfatoxymelatonin normalized to urinary creatinine (ratio of 6-sulfatoxymelatonin to creatinine) has been used extensively to estimate overnight melatonin secretion. For example, 2 studies[15,16] in populations similar to the current study have shown that the urinary ratio of 6-sulfatoxymelatonin to creatinine in a first morning void closely correlates with nocturnal plasma melatonin secretion (Spearman rank correlation coefficient of 0.76).

Urinary concentrations of 6-sulfatoxymelatonin were measured at Brigham and Women's Hospital Diagnostic Laboratory Facility using an enzyme-linked immunosorbent assay (ALPCO); the interassay coefficient of variation was 10%. Urinary creatinine was measured in the same laboratory using a modified Jaffe method; the interassay coefficient of variation was 5%. To determine whether concentrations of 6-sulfatoxymelatonin and creatinine remain stable after participants ship them back to our laboratory prior to being aliquoted and frozen in liquid nitrogen, we performed a pilot study whereby 6-sulfatoxymelatonin and creatinine were measured immediately after void, and then after 24 and 72 hours with a cold pack; the coefficients of variation for 6-sulfatoxymelatonin and creatinine were 3% and 5%, respectively.

In addition, 6-sulfatoxymelatonin remains stable in frozen storage, as previously shown.[17] The 3-year stability of melatonin secretion (assessed by the ratio of 6-sulfatoxymelatonin to creatinine) was previously evaluated in a pilot study within the Nurses' Health Study; the intraclass correlation coefficient was 0.72.[18]

Covariate Data Collection

Age, self-reported race, menopausal status, body mass index (BMI; calculated as weight in kilograms divided by height in meters squared), 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 the biological sample collection, and expressed as the metabolic equivalent of tasks performed per week. These questionnaire-derived data on physical activity are highly correlated with activity diaries (r=0.79).[19]

Information about macro- and micronutrient intake was obtained from the semiquantitative food frequency questionnaire (FFQ) returned prior to submission of the urine specimen. The FFQ accounts for more than 90% of intake of most nutrients and was used to compute nutrient intakes of alcohol, cereal fiber, and *trans*-saturated, polyunsaturated, and saturated fats. The validity and reliability of this FFQ has been extensively studied.[20] Data from the FFQ also were used to compute both the alternative healthy eating in-

Table 1. Baseline Characteristics of Women Who Developed Type 2 Diabetes and Matched Controls

	Median (5%-95% Range)		
	Cases (n = 370)	Controls (n = 370)	*P* Value[a]
Urinary melatonin[b]			
Ratio of 6-sulfatoxymelatonin to creatinine, ng/mg	28.2 (5.5-84.2)	36.3 (6.9-110.8)	<.001
6-Sulfatoxymelatonin, ng/dL	1910 (340-7980)	2410 (430-9920)	.002
Urinary creatinine, mg/dL[b]	69.6 (35.2-162.0)	65.3 (33.7-160.4)	.20
Age, mean (SD), y	64.7 (6.3)	64.4 (6.1)	.56[c]
Body mass index[d]	29.3 (21.7-40.4)	25.3 (19.9-35.5)	<.001
Physical activity, METs/wk[e]	10.2 (0.2-49.1)	13.3 (0.7-63.3)	.003
Alternative healthy eating index score[f]	41.4 (24.9-61.2)	46.4 (26.7-65.4)	<.001
Glycemic index	53.4 (47.4-58.9)	53.4 (48.0-58.2)	.15
Ratio of polyunsaturated to saturated fat	0.59 (0.35-0.98)	0.59 (0.35-1.10)	.46
Energy from *trans* fat, %	1.61 (0.86-2.66)	1.47 (0.69-2.62)	<.001
Cereal fiber in diet, g	5.4 (2.6-10.4)	6.2 (3.0-12.1)	<.001
Alcohol consumption, g/d	0.9 (0-27.4)	1.5 (0-19.7)	.007
E-selectin, ng/mL	41.3 (18.0-74.4)	33.4 (15.8-56.8)	<.001
High-sensitivity C-reactive protein, mg/L	3.7 (0.7-22.5)	2.0 (0.3-9.9)	<.001
Intercellular adhesion molecule 1, ng/mL	281.4 (188.2-466.0)	258.1 (175.1-367.9)	<.001
Interleukin 6, pg/mL	1.80 (0.70-7.10)	1.20 (0.50-4.05)	<.001
Insulin, μIU/mL	8.9 (2.6-31.1)	5.2 (1.8-13.3)	<.001
Triglycerides, mg/dL	164.0 (71.0-348.0)	116 (55-238)	<.001
Insulin sensitivity index[23]	6.2 (4.1-9.6)	8.1 (5.5-12.4)	<.001
	No. (%)		
White race	353 (95.4)	355 (95.9)	.72
Smoking history			
Current	26 (7.0)	20 (5.4)	.36
Past	172 (46.5)	166 (44.9)	.66
Family history of diabetes	131 (35.4)	69 (18.6)	<.001
Hypertension			
History	230 (62.2)	146 (39.5)	<.001
Treatment	210 (56.8)	116 (31.4)	<.001
Snoring			
Most nights	103 (27.8)	74 (20.0)	.01
Occasionally	27 (7.3)	24 (6.5)	.66
Almost never	232 (62.7)	260 (70.3)	.03
Sleep duration per night, h			
≤5	24 (6.5)	9 (2.4)	.008
6	78 (21.1)	69 (18.6)	.41
7-8	237 (64.1)	263 (71.1)	.04
9	23 (6.2)	17 (4.6)	.33
≥10	4 (1.1)	6 (1.6)	.52
US region			
North[g]	204 (55.1)	175 (47.3)	.03
South[h]	57 (15.4)	62 (16.8)	.62
Midwest[i]	97 (26.2)	80 (21.6)	.14
West[j]	12 (3.2)	53 (14.3)	<.001
Use of NSAIDs	156 (42.1)	137 (37.0)	.15
β-Blocker use	80 (21.6)	57 (15.4)	.03
Postmenopausal	366 (99.9)	362 (97.8)	.24

Abbreviations: METs, metabolic equivalent of tasks; NSAIDs, nonsteroidal anti-inflammatory drugs.
SI conversion factors: To convert creatinine to μmol/L, multiply by 88.4; insulin to pmol/L, multiply by 6.945; triglycerides to mmol/L, multiply by 0.0113.
[a] Calculated using the Wilcoxon signed rank test for continuous variables and the χ^2 test for categorical variables.
[b] Assessed by measuring the urinary appearance of its primary metabolite (6-sulfatoxymelatonin). These levels are displayed without log transformation. In later analyses, the ratio of 6-sulfatoxymelatonin to creatinine was log transformed when used as a continuous variable.
[c] Matches criteria in case-control selection.
[d] Calculated as weight in kilograms divided by height in meters squared.
[e] Calculated as the sum of the average time per week spent in each activity multiplied by the MET value of each activity. METs measure the ratio of the work metabolic rate to the resting metabolic rate.
[f] Reflects a common dietary pattern associated with a lower risk for type 2 diabetes.
[g] Includes Pennsylvania, New York, New Jersey, Connecticut, Rhode Island, Massachusetts, New Hampshire, Vermont, and Maine.
[h] Includes Oklahoma, Texas, Louisiana, Arkansas, Mississippi, Alabama, Florida, Georgia, South Carolina, North Carolina, Virginia, Tennessee, Kentucky, West Virginia, District of Columbia, Maryland, and Delaware.
[i] Includes North Dakota, South Dakota, Nebraska, Kansas, Minnesota, Iowa, Missouri, Wisconsin, Illinois, Michigan, Indiana, and Ohio.
[j] Includes Washington, Oregon, Montana, Idaho, Wyoming, California, Nevada, Utah, Colorado, Arizona, and New Mexico.

dex (a predictor of type 2 diabetes that has been validated in similar cohorts[21]) and the dietary glycemic index.

The dietary glycemic index score for each participant was computed as the weighted average (weighted by the amount of carbohydrates consumed) of the individual glycemic indices of each food item.[22] Family history of diabetes was ascertained from the 1992 questionnaire. History of hypertension and treatment with antihypertensive medications (including β-blockers) were reported on the 2000 questionnaire, as was the current use of nonsteroidal anti-inflammatory drugs. Sleep duration and frequency of snoring were reported by women on the biennial questionnaire returned in 2000. Participants were categorized into 1 of 4 geographical regions in the United States based on their home state at the time of urine and blood collection (**TABLE 1**).

All plasma biomarkers were measured in the research laboratory of Nader Rifai, PhD (Boston Children's Hospital). High-sensitivity C-reactive protein was measured using a highly sensitive immunoturbidimetric assay with reagents and calibrators from Denka Seiken. Interleukin 6 , intercellular adhesion molecule 1, and E-selectin were measured using commercial enzyme-linked immunosorbent assays (R & D Systems).

The interassay coefficients of variation were 1% for high-sensitivity C-reactive protein, 11% for interleukin 6, 5% for intercellular adhesion molecule 1, and 9% for E-selectin. Levels of insulin and triglycerides were measured using radioimmunoassay and standard enzymatic methods, respectively. The coefficients of variation were 2% for insulin and 9% for triglycerides. The insulin sensitivity index (glucose disposal rate corrected for free-fat mass [MFFM]) was calculated using the formula by McAuley et al[23]:

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

All covariates were included in the analysis as continuous variables with

the exception of treatment for hypertension (yes or no), snoring frequency (most nights, occasionally, almost never), sleep duration (<5 hours, 5-6 hours, 7-8 hours, 9-10 hours, >10 hours), and US geographical region (North, South, Midwest, West).

Statistical Analyses

Levels of 6-sulfatoxymelatonin were normalized to the creatinine level of the sample to account for differences arising from variations in urinary concentrations (ratio of 6-sulfatoxymelatonin to creatinine, which was expressed as nanogram of 6-sulfatoxymelatonin per milligram of creatinine). The ratio of 6-sulfatoxymelatonin to creatinine was analyzed in 2 ways. First, we divided the ratio of 6-sulfatoxymelatonin to creatinine into 3 categories; the cut points for these categories were derived from tertiles of the ratio of 6-sulfatoxymelatonin to creatinine among the control participants. Second, we analyzed the ratio of 6-sulfatoxymelatonin to creatinine as a continuous variable, which was log transformed due to substantial right skew in the distribution.

At baseline, the median and 5th to 95th percentiles of the ratio of 6-sulfatoxymelatonin to creatinine and all covariates were calculated using data from all participants. To determine the crude association of these covariates with diabetes, we compared the distributions of these covariates by case-control status using the Wilcoxon signed rank test and the χ^2 test, as appropriate. We also compared the distribution of baseline covariates across the 3 categories of the urinary ratio of 6-sulfatoxymelatonin to creatinine using the Cochran-Armitage trend test for categorical covariates and median regression for continuous covariates (with the category of the ratio of 6-sulfatoxymelatonin to creatinine evaluated as a continuous variable).

We used conditional logistic regression models, adjusting for medications that affect melatonin metabolism, namely β-blockers and nonsteroidal anti-inflammatory drugs, as well as established risk factors for type 2 diabetes, including biomarkers of inflammation and endothelial dysfunction, to estimate the relative risk of type 2 diabetes according to ratio of 6-sulfatoxymelatonin to creatinine (reported as ORs with 95% confidence intervals). A variety of sensitivity analyses were performed.

First, we analyzed the possibility of a nonlinear association between melatonin secretion and risk for diabetes by fitting restricted cubic splines; tests for nonlinearity used the likelihood ratio test, comparing the model with only the linear term with the model with both linear and cubic spline terms. Second, we assessed for potential interactions between BMI, sleep duration, and snoring frequency and the association between melatonin secretion and incident type 2 diabetes; interactions were evaluated using the log-likelihood ratio test.

Third, we analyzed the association of the ratio of 6-sulfatoxymelatonin to creatinine and type 2 diabetes using unconditional logistic regression and stratification by BMI quintile; in these models, there was adjustment for the same covariates as in the primary models (including BMI as a continuous variable to further control for possible confounding of the association by BMI). Fourth, we analyzed the association between the ratio of 6-sulfatoxymelatonin to creatinine and type 2 diabetes after adjustment for the insulin sensitivity index (using the formula by McAuley et al[23]) to examine insulin resistance as a potential causal intermediate.

Because the controls were matched to cases using risk-set sampling, the OR from conditional logistic regression provides estimates of the relative rates.[24,25] The incidence rate of diabetes in the source population was low (6.3/1000 person-years); therefore, we assumed that the distributions of melatonin secretion in our control group closely represented the distribution of melatonin secretion in the source population. Thus, we were able to estimate the absolute incidence rate difference between the highest and lowest categories of melatonin secretion using a previously described approach,[26] in which the incidence rate in the reference category can be expressed as:

$$\frac{\text{Incidence rate of diabetes in reference category} - 3 \times \text{Expected incidence rate of diabetes in population}}{(1 + OR_2 + OR_3)}$$

For which OR_2 and OR_3 are the ORs of incident diabetes in categories 2 and 3 relative to the reference category and the expected incidence rate of diabetes is calculated from the source population.

All *P* values were 2-tailed with .05 used as a significance threshold. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc).

RESULTS

Characteristics of the Study Population

Baseline characteristics according to case-control status appear in Table 1. The median urinary ratio of 6-sulfatoxymelatonin to creatinine was significantly higher among controls (36.3 ng/mg; 5%-95% range, 6.9-110.8 ng/mg) than among cases (28.2 ng/mg; 5%-95% range, 5.5-84.2 ng/mg). However, urinary creatinine level was similar between controls and cases, 65.3 and 69.6 mg/dL, respectively (*P*=.20), suggesting that differences in melatonin secretion accounted for this difference in ratio of 6-sulfatoxymelatonin to creatinine. As expected in this matched sample, the median age (64 years; 5%-95% range, 56-75 years) was similar among controls and cases.

Compared with controls, cases had significantly higher BMIs, were less physically active, consumed less alcohol and cereal fiber and more *trans*-saturated fat, and had lower overall diet quality scores based on the alternative healthy eating index. In addition, cases slept fewer hours per night, and were more likely to snore regularly, to use β-blockers, and to have a personal history of hypertension and a family history of diabetes. Each of these associations has previously been reported in

this and other cohorts.[21,27-31] Similarly, biomarkers of inflammation (high-sensitivity C-reactive protein and interleukin 6) and of endothelial dysfunction (intercellular adhesion molecule 1 and E-selectin) were higher among individuals who developed type 2 diabetes compared with controls.

Baseline characteristics according to category of ratio of 6-sulfatoxymelatonin to creatinine appear in TABLE 2. The median urinary ratio of 6-sulfatoxymelatonin to creatinine in the lowest category was 14.4 ng/mg (5%-95% range, 4.2-24.8 ng/mg) and in the highest category was 67.0 ng/mg (5%-95% range, 50.2-177.5 ng/mg). The urinary creatinine concentration was virtually identical in all 3 categories; therefore, the categories of urinary ratio of 6-sulfatoxymelatonin to creatinine were principally influenced by melatonin secretion.

Women in the highest compared with the lowest category of ratio of 6-sulfatoxymelatonin to creatinine had significantly lower BMI values, lower plasma concentrations of each biomarker of inflammation and endothelial function, and were less likely to have a history of hypertension or use β-blockers. In addition, insulin sensitivity (calculated using the formula by McAuley et al[23]) was higher among women with higher urinary ratios of 6-sulfatoxymelatonin to creatinine with median values across the 3 categories of 6.9 (lowest), 7.4 (intermediate), and 7.5 (highest) (P=.007 for trend; Table 2), indicating that women with higher melatonin secretion had less insulin resistance. Other covariates did not significantly differ according to the urinary ratio of 6-sulfatoxymelatonin to creatinine.

Melatonin Secretion and Incident Type 2 Diabetes

Participants with lower melatonin secretion had a significantly higher incidence of type 2 diabetes (TABLE 3). After conditioning on matching factors, the OR comparing the lowest with the highest category for ratio of 6-sulfatoxymelatonin to creatinine was 2.03 (95% CI, 1.38-3.01). A similar inverse association between melatonin secretion and incident type 2 diabetes was observed when the ratio of urinary 6-sulfatoxymelatonin to creatinine was analyzed as a continuous variable (OR, 1.36 per unit decrease in estimated log ratio of 6-sulfatoxymelatonin to creatinine; 95% CI, 1.14-1.61). This association was not confounded by established diabetes risk factors.

After controlling for BMI and other lifestyle factors, menopausal status, family history of diabetes, history of hypertension, use of β-blockers or nonsteroidal anti-inflammatory drugs, region of the United States, and plasma biomarkers of diabetes risk, the OR comparing the lowest with the highest category of urinary ratio of 6-sulfatoxymelatonin to creatinine was 2.17 (95% CI, 1.18-3.98). The fully adjusted OR per unit decrease in estimated log ratio of 6-sulfatoxymelatonin to creatinine was 1.48 (95% CI, 1.11-1.98).

In our nonparametric analysis, the association of the ratio of 6-sulfatoxymelatonin to creatinine with diabetes risk was linear. Interactions between melatonin secretion and BMI, sleep duration, and snoring frequency were not significant (P values of .97, .76, and .32, respectively). Unconditional logistic regression models with stratification by BMI quintile yielded similar results. Specifically, the fully adjusted (including adjustment for BMI as a continuous variable in addition to stratification on BMI) OR comparing the lowest with the highest category of urinary ratio of 6-sulfatoxymelatonin to creatinine was 1.91 (95% CI, 1.21-3.01); using the calculated log ratio of 6-sulfatoxymelatonin to creatinine as a continuous variable, the OR per unit decrease in the ratio was 1.30 (95% CI, 1.06-1.61).

Inclusion of insulin sensitivity (calculated using the formula by McAuley et al[23]) in the final model did not alter the association between melatonin secretion and incident diabetes. The OR per unit decrease in the calculated log ratio of 6-sulfatoxymelatonin to creatinine was 1.70 (95% CI, 1.22-2.38) in fully adjusted models that also included insulin sensitivity.

The absolute incidence rate of diabetes in the source population (participants without diabetes from the Nurses' Health Study in 2000) was 6.31 cases/1000 person-years. Assuming that the distribution of melatonin secretion in the source population is similar to that in our control participants, the incidence rate of diabetes was estimated to be 4.27 cases/1000 person-years in the highest category of melatonin secretion and 9.27 cases/1000 person-years in the lowest category of melatonin secretion (absolute rate difference of 5 cases/1000 person-years).

COMMENT

We found an independent association between melatonin secretion and the subsequent development of type 2 diabetes. Secretion of melatonin varied widely among participants in our study; the median ratio of 6-sulfatoxymelatonin to creatinine was 67.0 ng/mg in the highest category compared with 14.4 ng/mg in the lowest category. Participants in the lowest category of melatonin secretion had an OR of 2.17 for developing type 2 diabetes compared with participants in the highest category of melatonin secretion, even after controlling for multiple potential confounders. This OR translated to an absolute rate difference in the incidence of diabetes of 5 cases/1000 person-years between the lowest and highest categories of melatonin secretion.

Experimental studies suggest that melatonin has beneficial effects on glucose metabolism. Oral consumption of melatonin-protected rats prone to diabetes from developing hyperlipidemia, hyperglycemia, and hyperleptinemia while receiving a high-calorie diet,[6] whereas melatonin administration to insulin-resistant mice reversed insulin resistance and improved glucose metabolism.[32] In vitro studies with human pancreatic islet cells demonstrated that prolonged exposure of islet cells to melatonin improved glucose sensitivity.[33,34] Melatonin exposure activates the phosphatidylinositol-3-kinase/protein kinase B survival pathway and the mitogen-activated protein

Table 2. Baseline Characteristics of Participants According to Melatonin Secretion Category

	Melatonin Secretion Category, Median (5%-95% Range)[a]			
	Lowest (n = 292)	Intermediate (n = 246)	Highest (n = 202)	*P* Value for Trend[b]
Urinary melatonin[c]				
Ratio of 6-sulfatoxymelatonin to creatinine, ng/mg	14.4 (4.2-24.8)	35.4 (27.0-47.6)	67.0 (50.2-177.5)	
6-Sulfatoxymelatonin, ng/dL	930 (240-2760)	2340 (1150-5730)	5075 (2080-17 900)	<.001
Urinary creatinine, mg/dL[c]	69.1 (34.7-156.1)	64.3 (34.3-162.5)	68.9 (35.6-160.4)	.75
Age, mean (SD), y	65.2 (6.6)	64.2 (5.6)	64.0 (6.4)	.07
Body mass index[d]	27.8 (20.0-39.5)	27.4 (20.8-38.4)	26.1 (20.5-36.0)	.003
Physical activity, METs/wk[e]	12.3 (0.2-55.9)	12.2 (0.3-54.9)	10.6 (0.4-57.4)	.18
Alternative healthy eating index score[f]	43.1 (24.5-64.0)	44.3 (26.5-62.5)	44.1 (26.7-63.3)	.35
Glycemic index	53.5 (48.3-58.9)	53.9 (47.3-58.5)	53.5 (46.8-58.1)	.80
Ratio of polyunsaturated to saturated fat	0.58 (0.34-1.10)	0.61 (0.35-1.02)	0.58 (0.35-1.00)	.96
Energy from *trans* fat, %	1.54 (0.72-2.55)	1.51 (0.80-2.60)	1.62 (0.85-2.77)	.56
Cereal fiber in diet, g	5.6 (2.7-10.8)	6.0 (2.7-11.4)	5.9 (2.8-11.8)	.33
Alcohol consumption, g/d	0.9 (0-28.6)	1.5 (0-26.7)	0.9 (0-13.6)	>.99
E-selectin, ng/mL	38.6 (17.6-68.3)	36.3 (17.8-70.7)	35.9 (16.3-60.7)	.13
High-sensitivity C-reactive protein, mg/L	3.1 (0.6-21.2)	2.8 (0.5-19.6)	2.5 (0.4-18.0)	.14
Intercellular adhesion molecule 1, ng/mL	271.0 (179.6-454.9)	267.7 (184.8-414.4)	263.8 (178.0-374.5)	.25
Interleukin 6, pg/mL	1.5 (0.7-5.6)	1.4 (0.5-6.6)	1.3 (0.5-4.4)	.005
Insulin, μIU/mL	7.2 (2.4-28.0)	6.6 (1.8-22.9)	6.1 (1.9-20.9)	.009
Triglycerides, mg/dL	146 (71-316)	142 (56-273)	121 (57-313)	.001
Insulin sensitivity index[23]	6.9 (4.4-11.2)	7.4 (4.6-11.6)	7.5 (4.3-12.4)	.007
	No. (%)			
White race	281 (96)	231 (94)	196 (97)	.22
Smoking history				
Current	11 (4)	25 (10)	10 (5)	.39
Past	137 (47)	109 (44)	92 (46)	.72
Family history of diabetes	77 (26)	65 (26)	58 (29)	.59
Hypertension				
History	165 (56)	119 (48)	92 (46)	.01
Treatment	145 (50)	108 (44)	73 (36)	.003
Snoring				
Most nights	73 (25)	61 (25)	43 (21)	.37
Occasionally	18 (6)	18 (7)	15 (7)	.57
Almost never	194 (66)	160 (65)	138 (68)	.71
Sleep duration per night, h				
≤5	16 (5)	10 (4)	7 (3)	.27
6	60 (21)	47 (19)	40 (20)	.81
7-8	192 (66)	163 (66)	145 (72)	.18
9	14 (5)	20 (8)	6 (3)	.52
≥10	6 (2)	2 (1)	1 (1)	.14
US region[g]				
North	148 (51)	128 (52)	103 (51)	.92
South	49 (17)	39 (16)	31 (15)	.66
Midwest	74 (25)	56 (23)	47 (23)	.56
West	21 (7)	23 (9)	21 (10)	.20
Use of NSAIDs	84 (29)	73 (30)	64 (32)	.78
β-Blocker use	67 (23)	47 (19)	23 (11)	.005
Postmenopausal	288 (99)	242 (98)	198 (98)	.87

Abbreviations: METs, metabolic equivalent of tasks; NSAIDs, nonsteroidal anti-inflammatory drugs.
SI conversion factors: To convert creatinine to μmol/L, multiply by 88.4; insulin to pmol/L, multiply by 6.945; triglycerides to mmol/L, multiply by 0.0113.
[a] Assessed by measuring the urinary appearance of its primary metabolite (6-sulfatoxymelatonin) during the first morning urine with normalization using urinary creatinine.
[b] Calculated using univariate median regression for continuous variables and the Cochran-Armitage trend test for categorical values.
[c] These levels are displayed without log transformation. In later analyses, the ratio of 6-sulfatoxymelatonin to creatinine was log transformed when used as a continuous variable.
[d] Calculated as weight in kilograms divided by height in meters squared.
[e] Calculated as the sum of the average time per week spent in each activity multiplied by the MET value of each activity. METs measure the ratio of the work metabolic rate to the resting metabolic rate.
[f] Reflects a common dietary pattern associated with a lower risk for type 2 diabetes.
[g] The states are listed by region in Table 1 footnotes g-j.

Table 3. Type 2 Diabetes According to Melatonin Secretion[a]

	Incident Diabetes, Odds Ratio (95% CI)[b]			
		Decreasing Category of Ratio of 6-Sulfatoxymelatonin to Creatinine[c]		
	Continuous[c]	≥49.1 ng/mg	26.2-49.0 ng/mg	≤26.1 ng/mg
No. of cases	370	79	122	169
No. of controls	370	123	124	123
Model				
1: Matched for age and race	1.36 (1.14-1.61)	1 [Reference]	1.54 (1.03-2.30)	2.03 (1.38-3.01)
2: Same as model 1 plus adjustment for body mass index	1.34 (1.11-1.61)	1 [Reference]	1.45 (0.92-2.28)	1.94 (1.26-2.99)
3: Same as model 2 plus adjustment for physical activity, smoking, family history of diabetes, dietary factors,[d] history or treatment of hypertension, sleep duration, history for snoring, use of β-blockers, use of NSAIDs, menopausal status, and US region	1.47 (1.12-1.92)	1 [Reference]	1.33 (0.75-2.36)	2.31 (1.32-4.03)
4: Same as model 3 plus adjustment for E-selectin, high-sensitivity CRP, ICAM-1, and IL-6	1.48 (1.11-1.98)	1 [Reference]	1.26 (0.66-2.39)	2.17 (1.18-3.98)

Abbreviations: CRP, C-reactive protein; ICAM-1, intercellular adhesion molecule 1; IL-6, interleukin 6; NSAIDs, nonsteroidal anti-inflammatory drugs.
[a] Assessed by measuring the urinary appearance of 6-sulfatoxymelatonin in the first morning urine with normalization using urinary creatinine.
[b] Unless otherwise indicated.
[c] Per unit decrease in log ratio of 6-sulfatoxymelatonin to creatinine.
[d] Include alternative healthy eating index score, glycemic index, ratio of polyunsaturated to saturated fats, percentage of energy from *trans* fats, cereal fiber intake, and alcohol intake.

kinase/extracellular signal-regulated kinase growth pathway of in vitro islet cells, potentially explaining the low density of pancreatic islet cells observed in rats following pinealectomy.[4]

A protective effect of melatonin regarding diabetes development is also supported by cross-sectional studies in humans, most of which were small. Peschke et al[35] found that serial nocturnal plasma melatonin levels were significantly lower in 6 patients with diabetes compared with 5 controls ($P<.01$). Similarly, Hikichi et al[36] found in a study of 56 participants that nocturnal plasma melatonin was significantly lower among those with proliferative diabetic retinopathy (n=14) compared with healthy participants (n=26) (10.9 vs 37.5 pg/mL, respectively, $P<.01$), but not among participants with diabetes but without proliferative retinopathy (n=16) and healthy participants (n=26) (31.1 vs 37.5 pg/mL, respectively). The authors suggested that the association between melatonin and diabetes was possibly mediated by dysfunctional retinal light perception and, consequently, reduced melatonin secretion.

In a small, cross-sectional analysis of 21 patients with metabolic syndrome and 19 healthy controls, nocturnal plasma melatonin and insulin levels were positively correlated among those with metabolic syndrome ($r=0.64$) but not among controls.[37,38] In a larger cross-sectional analysis of 1075 women without diabetes, the OR for insulin resistance was 0.45 (95% CI, 0.28-0.74) in women who were in the highest compared with lowest quartile of nocturnal melatonin secretion (C. McMullan, MD, unpublished data, March 2013).

Prospective evidence of a potential role for melatonin in glucose metabolism in humans comes from several large-population genetic studies that included individuals of varied racial backgrounds.[9-11,39-42] Certain single nucleotide polymorphisms in the melatonin receptor MTNR1B are associated with fasting glucose levels, hemoglobin A_{1c}, and the incidence of both gestational diabetes and type 2 diabetes. Among single nucleotide polymorphisms in MTNR1B associated with diabetes, those variants that lead to partial or complete loss of function of the MTNR1B receptor are associated with the highest incidence of type 2 diabetes.[5]

Prior studies have suggested that exposure to light at night, as with rotating night shift work or sleep restriction, is associated with various diseases, including cancer.[43,44] Sleep disruption may also be associated with diabetes. For example, 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.[27,45] Similarly, women who reported snoring on a regular basis were 2.2 times more likely to develop type 2 diabetes than women who did not ever snore,[46] even after adjustment for adiposity. A recent study found that participants who were exposed to prolonged sleep restriction had impaired glucose tolerance due to inadequate pancreatic insulin secretion.[47]

Consistent with these prior studies, both short sleep duration and snoring frequency were associated with incident type 2 diabetes in our case-control study. Because sleep disruption is also associated with decreased melatonin secretion,[48,49] it is possible that sleep disruption, if related to diabetes via a mechanism other than melatonin, could confound the association seen between melatonin and diabetes. However, adjustment for sleep duration and snoring frequency in our multivariable analysis did not significantly alter the association of melatonin secretion with incident type 2 diabetes. One possibility for this lack of confounding may be due to reporting error in sleep duration and snoring frequency on the questionnaire.

Alternatively, sleep duration and snoring frequency may not fully capture all aspects of sleep disruption that

can impair melatonin secretion. In addition, variation in melatonin secretion is affected by factors other than sleep disruption, some of which may be unknown and may explain the large distribution of melatonin secretion observed in this cohort. These factors may also explain how melatonin has beneficial effects in rodents, who are nocturnally active during the period of elevated melatonin yet derive metabolic benefit from exogenous melatonin ingestion.

Given the previously described association between lower melatonin secretion and increased insulin resistance, it is possible that insulin resistance is an intermediate step in the causal pathway between melatonin secretion and type 2 diabetes. Adjusting for insulin sensitivity did not alter the association between melatonin secretion and incident diabetes in our study. The reasons for this are unclear; however, it is possible that insulin sensitivity as calculated using the formula by McAuley et al[23] does not fully capture the effects of melatonin secretion on glucose homeostasis and more direct physiological measurements of glucose homeostasis, either by hyperglycemic or hyperinsulinemic clamp, may better determine the effects of melatonin on pancreatic insulin secretion and peripheral insulin resistance.

Our study has limitations. First, because it was an observational study, we were unable to draw conclusions about causality. It is possible that, in someone with insulin resistance, hyperinsulinemia may activate the insulin receptors located on the pineal gland, thereby suppressing melatonin secretion. However, genetic studies suggest that alterations in melatonin signaling produce insulin resistance, rather than the other direction.

Second, observational studies are subject to potential confounding; for example, our use of snoring status as a proxy for sleep-disordered breathing is imperfect and therefore residual confounding from sleep apnea may exist. However, we had reliable information about and controlled for multiple known risk factors for diabetes, including diet, lifestyle, personal and family history of disease, and circulating markers of inflammation and endothelial dysfunction.

Third, diabetes was self-reported in our analyses. However, ascertainment of diabetes in this cohort was confirmed by collecting supplementary information that has proven highly reliable.[14] Fourth, we were not able to sample multiple overnight plasma levels of melatonin. However, first morning void urine measurements of 6-sulfatoxymelatonin normalized to creatinine have been shown to provide reliable estimates of overnight melatonin production.[15,16,50]

Fifth, we did not have genetic data on the women in our study, which limits our ability to comment on interactions between melatonin secretion and variants in the melatonin receptor. Sixth, information about rotating night shift work was not collected at baseline when women returned urine and blood samples; therefore, we could not adjust for shift work in our multivariable models. However, 4 years earlier (in 1996), only 28 women (4% of the study population) were still working rotating night shifts, reflecting the age and seniority of the study population at that time. By 2000 (the baseline year in the current study), it is likely that even fewer women were performing rotating night shift work. Thus, it is highly unlikely that our results were confounded by rotating night shift work.

Seventh, the study population is limited to nurses at the time of study initiation who have continued to participate in an epidemiological study for 24 years. However, the incidence rates of diabetes in this study population were similar to that seen in other population-wide cohorts.[51] In addition, our study population was limited to women, 97% of whom were white. Thus, it is unknown whether our findings can be applied to men or to other racial groups.

CONCLUSION

We found an independent association between decreased melatonin secretion and an increased risk for the development of type 2 diabetes. It is interesting to postulate from these data, in combination with prior literature, whether there is a causal role for reduced melatonin secretion in diabetes risk. Further studies are needed to determine whether increasing melatonin levels (endogenously via prolonged nighttime dark exposure or exogenously via supplementation) can increase insulin sensitivity and decrease the incidence of type 2 diabetes.

Author Contributions: Dr McMullan had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: McMullan, Schernhammer, Hu, Forman.
Acquisition of data: Schernhammer, Hu, Forman.
Analysis and interpretation of data: All authors.
Drafting of the manuscript: McMullan.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: McMullan, Forman.
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Administrative, technical, or material support: McMullan, Hu, Forman.
Study supervision: Schernhammer, Forman.
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