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Melatonin secretion and the incidence of type 2 diabetes

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Abstract

Importance—Loss-of-function mutations in the melatonin receptor are associated with insulin resistance and type 2 diabetes. Additionally, lower nocturnal melatonin secretion is associated with increased insulin resistance in a cross-sectional analysis of non-diabetics.

Objective—We aimed 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 non-diabetic participants 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—Median urinary 6-sulfatoxymelatonin levels were 28.5ng/mg creatinine (5-95%, 5.5 to 84.2) among cases and 36.3ng/mg (5-95%, 6.9 to 110.8) among controls. Women with lower 6-

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sulfatoxymelatonin measurements had an increased OR of diabetes with multivariate OR 1.48 (95% CI, 1.11 to 1.98) per unit decrease in $\log_e$ aMT6s/creatinine ratio. Compared with women in the highest 6-sulfatoxymelatonin category, those in the lowest 6-sulfatoxymelatonin category had a multivariable odds ratio 2.17, 95% CI 1.18 to 3.98 of developing type 2 diabetes. Women in the highest 6-sulfatoxymelatonin category had an estimated incident rate of diabetes 427 cases per 100,000 person years compared to 927 cases per 100,000 patient 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.

Introduction

Melatonin is a pineal hormone under the control of the biologic clock, which is located in the hypothalamus and regulated by light exposure.[1] Secretion of melatonin follows a diurnal pattern, typically peaking 3-5 hours after sleep onset when it is dark, with almost no production ocurring 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 triglyceride levels, relative to controls.[6-8] In several large genome-wide association studies, single nucleotide polymorphisms (SNPs) in the type B melatonin receptor (MTNR1B) were associated with higher fasting glucose levels, higher hemoglobin A1c (HbA1c) and increased incidence of gestational and type 2 diabetes.[9-11] Among these SNPs, those which 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 association 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 (NHS).[12] The NHS began in 1976 when 121,701 registered nurses aged 30-55 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, where upon they 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 (urine creatinine >30mg/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. From power calculations we estimated that 370 matched case-control pair would provide >80% power to detect an odds ratio of at least 1.6 between the lowest and highest tertiles of melatonin with a two sided significance threshold of 0.05. Accordingly, we selected 370 participants who developed type 2 diabetes between 2000 and 2012. Controls were selected by risk-set sampling from participants free from type 2 diabetes at the time of each case diagnosis. Controls were matched one to one with cases within one year of age, within one 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 sugars and use of hypoglycemic medications. Details from the supplementary questionnaire were reviewed to ensure that the diagnosis of diabetes satisfied the criteria specified from 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 (98.4%) of cases.[14]

Nocturnal Melatonin Secretion

Melatonin secretion was estimated by measuring the concentration of its major metabolite, 6-sulfatoxymelatonin (aMT6s), in a first morning void urine specimen, and normalized to urine creatinine. The first morning void concentration of aMT6s normalized to urine creatinine (the urine aMT6s/creatinine ratio) has been used extensively to estimate overnight melatonin secretion. For example, two studies in populations similar to the current study have shown that the urinary aMT6s/creatinine ratio in a first morning void closely correlates with nocturnal plasma melatonin secretion (Spearman correlation coefficients 0.76). [15,16]

Urine concentrations of aMT6s were measured at Brigham and Women's Hospital Diagnostic Laboratory Facility using an enzyme-linked immunosorbant assay (ALPCO, Windham, NH); the inter-assay coefficient of variation (CV) was 10%. Urine creatinine was measured in the same laboratory by a modified Jaffe method (inter-assay CV = 5 %). To determine whether concentrations of aMT6s and creatinine remain stable after participants ship them back to our laboratory prior to aliquoting and freezing in liquid nitrogen, we performed a pilot study whereby aMT6s and creatinine were measured immediately after void, and then after 24 and 72 hours with a cold pack; the coefficients of variation for aMT6s and creatinine were 3% and 5%, respectively. In addition, aMT6s remains stable in frozen storage, as previously shown[17]. The 3-year stability of melatonin secretion, assessed by the aMT6s/creatinine ratio, was previously evaluated in a pilot study within the NHS; the intraclass correlation coefficient was 0.72[18].

Covariate data collection

Age, self-reported race, and menopausal status, as well as body mass index (BMI) 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 metabolic equivalent task scores (METs) 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 >90% of intake of most nutrients and was used to compute nutrient intakes of alcohol, cereal fiber, trans-saturated, polyunsaturated and saturated fats. The validity and reliability of this FFQ has been extensively studied.[20] Data from the FFQ was also used to compute both the alternative healthy eating index (AHEI), 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 carbohydrate 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 beta-blockers) were reported on the 2000 questionnaire, as was the current use of nonsteroidal antiinflammatory drugs (NSAIDs). Sleep duration and frequency of snoring were reported by women on the biennial questionnaire returned in 2000. Participants were categorized into one of four geographical regions in the U.S. based upon their home state at the time of urine and blood collection, as described in Table1.

All plasma biomarkers were measured in the research laboratory of Dr Nader Rifai (Children's Hospital, Boston). High sensitivity C-reactive protein (hs-CRP) was measured by a highly sensitive immunoturbidimetric assay with reagents and calibrators from Denka Seiken (Niigata, Japan). Interleukin 6 (IL-6), intercellular adhesion molecule 1(ICAM-1) and E-selectin were measured using commercial enzyme-linked immunosorbent assays (R & D Systems, Minneapolis, MN, USA). The inter-assay CVs were 1% for hs-CRP, 11% for IL-6, 5% for ICAM-1 and 9% for E-selectin. Insulin and triglyceride (TAG) levels were measured using radio-immunoassay and standard enzymatic methods, respectively. The CVs were 2% for insulin and 9% for triglycerides. The insulin sensitivity index (glucose disposal rate [M] corrected for free-fat mass; ie MFFM), or ISI, was calculated using the McAuley formula shown below.[23]

$$MFFM \Big/ \mathrm{I} = e^{[2.63 - 0.28(insulin) - 0.31\ ln(TAG)]}$$

All covariates were included in the analysis as continuous variables with the exception of treatment for hypertension (yes; no), snoring frequency (most nights; occasionally; almost never), sleep duration (less than five hours; five to six hours; seven to eight hours; nine to ten hours; greater than ten hours), and geographical region (north; south; midwest; west).

Statistical analyses

Levels of 6-sulfatoxymelatonin (aMT6s) were normalized to the creatinine level of the sample to account for differences arising from variations in urine concentrations (aMT6s/creatinine ratio, expressed as ng of aMT6s per mg of creatinine). The aMT6s/creatinine ratio was analyzed in two ways. First, we divided aMT6s/creatinine ratios into three categories; the cut-points for these categories were derived from tertiles of the aMT6s/creatinine ratios among the control participants. Second, we analyzed the aMT6s/creatinine ratio as a continuous variable, which was log-transformed due to substantial right skew in the distribution.

At baseline, the median and 5^{th} to 95^{th} percentiles of the aMT6s/creatinine ratio and all covariates were calculated for each participant. To determine the crude association of these covariates with diabetes, we compared the distributions of these covariates by case-control status using Wilcoxon signed rank test and Chi-squared test, as appropriate. We also compared the distribution of baseline covariates across the three categories of the urinary aMT6s/creatinine ratio using the Cochran-Armitage trend test for categorical covariates and

median regression for continuous covariates (with the category of the aMT6s/creatinine ratio evaluated as a continuous variable).

We used conditional logistic regression models, adjusting for medications that affect melatonin metabolism, namely beta-blockers and NSAIDs, 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 aMT6s/creatinine ratios (reported as odds ratios [ORs] with 95% confidence intervals [CIs]). A variety of sensitivity analyses were performed. First, we analyzed the possibility of a non-linear association between melatonin secretion and risk for diabetes by fitting restricted cubic splines; tests for non-linearity used the likelihood ratio test, comparing the model with only the linear term to the model with both linear and cubic spline terms. Second, we assessed for potential interactions between BMI, sleep duration, and snoring and the association between melatonin secretion and incident type 2 diabetes; interactions were evaluated using the -2 log-likelihood ratio test. Third, we analyzed the association of aMT6s/creatinine ratio and type 2 diabetes using unconditional logistic regression and stratification by BMI quintile; in these models, with 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 aMT6s/creatinine ratio and type 2 diabetes after adjustment for the insulin sensitivity index (using the McAuley formula[23]) to examine insulin resistance as a potential causal intermediate.

Since the controls were selected using risk set sampling, the OR from conditional logisitc regression are equivalent to the relative rates between categories.[24,25]. The incidence rate of diabetes in the source population is low, 6.3 per 1000 person-years, and so the distribution of melatonin secretion in the control group and source population was assumed to be equivalent. Therefore we were able to estimate the absolute incidence rate difference between categories of melatonin secretion using to an approach similar to previously described methods.[26]

All p-values were two tailed with 0.05 used as a significance threshold. All statistical analyses were performed with SAS, version 9.2 (SAS Institute, Inc., Cary, NC).

Results

Characteristics of the study Population

Baseline characteristics according to case-control status are shown in Table 1. The median urine aMT6s/creatinine ratio was significantly higher among controls (36.3 ng/mg; 5 to 95%, 6.9 to 110.8 ng/mg) than among cases (28.2 ng/mg; 5 to 95%, 5.5 to 84.2 ng/mg). However, urine creatinine was similar between controls and cases, 65.3 and 69.6mg/dL respectively (p-value = 0.20), suggesting that differences in melatonin secretion accounted for this difference in aMT6s/creatinine ratios. As expected in this matched sample, the median age (64 years; 5 to 95%, 56 to 75) was similar among controls and cases. Compared with controls, cases had a significantly higher BMI, were less physically active, consumed less alcohol and cereal fiber and more trans fat, and had lower overall diet quality scores based upon the aHEI. In addition, cases slept fewer hours per night, and were more likely to snore regularly, to use beta-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 (hs-CRP and IL-6) and of endothelial dysfunction (ICAM-1 and E-selectin) were higher among individuals who developed type 2 diabetes, compared with controls.

Baseline characteristics according to category of aMT6s/creatinine ratio are shown in Table 2. The median urine aMT6s/creatinine ratio in the lowest category was 14.4 ng/mg (5 to 95%, 4.2 to 24.8 ng/mg) and in the highest category was 67.0 ng/mg (5 to 95%, 50.2 to 177.5 ng/mg). As noted in the table, the urine creatinine concentration was virtually identical in all three categories, and therefore the categories of urine aMT6s/creatinine ratio were principally influenced by melatonin secretion. Women in the highest as compared with the lowest category of aMT6s/creatinine ratio 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 beta-blockers. In addition, insulin sensitivity calculated by the McAuley formula was higher among women with higher urine aMT6s/creatinine ratios with median values of 6.9, 7.4 and 7.5 accross categories of increasing urine aMT6s/ceatinine ratio (p-value for trend = 0.007), indicating that women with higher melatonin secretion had less insulin resistance. Other covariates did not significantly differ according to the urine aMT6s/creatinine ratio.

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 odds ratio (OR) comparing the lowest with highest category of aMT6s/creatinine ratio was 2.03 (95% CI, 1.38 to 3.01). A similar inverse association between melatonin secretion and incident type 2 diabetes was observed when the urine aMT6s/creatinine ratio was analyzed as a continuous variable (OR = 1.36 per unit decrease in $\log_e$ aMT6s/creatinine ratio; 95% CI, 1.14 to 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 beta-blockers or NSAIDs, region of the US and plasma biomarkers of diabetes risk, the OR comparing the lowest with highest category of urine aMT6s/creatinine ratio was 2.17 (95% CI, 1.18 to 3.98). The fully adjusted OR per unit decrease in $\log_e$ aMT6s/creatinine ratio was 1.48 (95% CI, 1.11 to 1.98).

In our non-parametric analysis, the association of the aMT6s/creatinine ratio with diabetes risk was linear. Interactions between melatonin secretion and BMI, sleep duration, and snoring history were not significant (p-values of 0.97, 0.76, and 0.32 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 to highest category of aMT6s/creatinine ratio was 1.91 (95% CI, 1.21 to 3.01); using the $\log_e$ aMT6s/creatinine ratio as a continuous variable, the OR per unit decrease in the ratio was 1.30 (95% CI, 1.06 to 1.61). Inclusion of insulin sensitivity (using the McAuley formula) in the final model did not alter the association between melatonin secretion and incident diabetes. The OR per unit decrease in $\log_e$ aMT6s/creatinine ratio was 1.70 (95% CI, 1.22 to 2.38) in fully adjusted models that also included insulin sensitivity.

The absolute incidence rate of incident diabetes following 2000 in the source population of the NHS is 631cases per 100,000 person years. From this the absolute incidence rate of diabetes in the highest category of melatonin secretion is estimated to be 427 cases per 100,000 person years and 927 cases per 100,000 person years in the lowest category of melatonin secretion.

Discussion

We found a and 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 aMT6s/creatinine ratio was 67.0ng/mg in highest category as

compared with 14.4ng/mg in the lowest. Participants in the lowest category of melatonin secretion had an Odds Ratio of 2.17 of developing type 2 diabetes compared with participants in the highest category of melatonin secretion, even after controlling for multiple potential confounders. This increased odds ratio equates to an absolute rate difference in the incidence of diabetes of 500 cases per 100,000 person years between the lowest and highest category of melatonin secretion.

Experimental studies suggest that melatonin has beneficial effects on glucose metabolism. Oral consumption of melatonin protected diabetic prone rats on a high calorie diet from developing hyperlipidemia, hyperglycemia and hyperleptinemia [6], while melatonin administration to insulin resistant mice reversed insulin resistance and improve 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 PI3K/AKT and MEK/ERK survival and growth pathways of *in-vitro* islet cells, potentially explaining the low density of pancreatic islets 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. found that serial nocturnal plasma melatonin levels were significantly lower in 6 diabetic patients compared with 5 controls ($p<0.01$).[35] Similarly, Hikichi et al. found in a study of 56 subjects that nocturnal plasma melatonin was significantly lower among subjects with proliferative diabetic retinopathy (N=14) compared with healthy subjects (N=26) (10.9 pg/ml versus 37.5 pg/ml, $p <0.01$), but not between diabetics without proliferative retinopathy (N=16) and healthy subjects (31.1 pg/ml versus 37.5 pg/ml).[36] The authors therefore suggested that the association between melatonin and diabetes was possibly mediated by dysfunctional retinal light perception and, consequently, reduced melatonin secretion. In a third 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]

Prospective evidence for a potential role for melatonin in glucose metabolism in humans comes from several large population genetic studies, including individuals of varied racial backgrounds.[9-11,39-42] Certain single nucleotide polymorphisms (SNPs) in the melatonin receptor MTNR1B are associated with fasting glucose levels, HbA1c, and the incidence of both gestational diabetes and type 2 diabetes. Among SNPs in MTNR1B associated with diabetes, those variants that lead to partial or complete loss of function of the MTNR1B receptor were 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 disease, including cancer [43,44]. Sleep disruption may also be associated with diabetes. As an example, men who reported sleeping <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 regular snoring were 2.2 times more likely to develop type 2 diabetes than women who did not snore[46], even after adjustment for adiposity. A recent study found that subjects 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 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 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 as evaluated by the questionnaire. Alternatively, sleep duration and snoring 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. Interestingly, 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. Interestingly, 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 measured by the McAuley index does not fully capture the effects of melatonin secretion on glucose homeostasis and that 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 are 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; as an 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 is 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 aMT6s 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, and were therefore limited in 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 and therefore we could not adjust for shift work in our multivariable models. However, four years earlier (in 1996), only 28 women (4% of the study population) was still working rotating night shifts, reflecting the age and seniority of the study population at that time. By the year 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 individual who were nurses at the time of study initiation and have continued to participate in an epidemiological study for 24 years. However the incidence rates of diabetes in this study population is similar to that seen in other population wide cohorts.[51] Finally, our study population was limited to woman, 97% of whom were white. Thus, it is unknown whether our findings can be applied to men or to other racial groups.

In conclusion, we found a and 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 indicated to determine whether or not 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.

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Table 1

Baseline characteristics of women who developed type 2 diabetes and their matched controls.

Covariates		Control (N=370)	Case (N=370)	p-value*
		Median (5 - 95% range)		
Urine aMT6s/creatinine (ng/mg)#		36.3 (6.9 – 110.8)	28.2 (5.5 – 84.2)	<0.001
Urine aMT6s (ng/dL) #		2410 (430 – 9920)	1910 (340 – 7980)	0.002
Urine creatinine (mg/dL) #		65.3 (33.7 – 160.4)	69.6 (35.2 – 162.0)	0.20
Age (years)		64.2 (55.8 – 75.2)	64.4 (55.5 – 75.7)	0.56†
BMI (kg/m^2)		25.3 (19.9 – 35.5)	29.3 (21.7 – 40.4)	<0.001
Physical activity, (METs/week)‡		13.3 (0.7 – 63.3)	10.2 (0.2 – 49.1)	0.003
AHEI score[i]		46.4 (26.7 – 65.4)	41.4 (24.9 – 61.2)	<0.001
Glycemic index		53.4 (48.0 – 58.2)	53.4 (47.4 – 58.9)	0.15
Polyunsaturated/saturated fat		0.59 (0.35 – 1.10)	0.59 (0.35 – 0.98)	0.46
%energy trans fat		1.47 (0.69 – 2.62)	1.61 (0.86 – 2.66)	<0.001
Cereal fiber in diet (gm)		6.2 (3.0 – 12.1)	5.4 (2.6 – 10.4)	<0.001
Alcohol consumption (gm/day)		1.5 (0.0 – 19.7)	0.9 (0.0 – 27.4)	0.007
E-selectin (ng/mL)		33.4 (15.8 – 56.8)	41.3 (18.0 – 74.4)	<0.001
hsCRP (mg/L)		2.0 (0.31 – 9.9)	3.7 (0.7 – 22.5)	<0.001
ICAM-1 (ng/mL)		258.1 (175.1 – 367.9)	281.4 (188.2 – 466.0)	<0.001
IL-6 (pg/mL)		1.2 (0.50 – 4.05)	1.8 (0.7 – 7.1)	<0.001
Insulin (uU/mL)		5.2 (1.8 – 13.3)	8.9 (2.6 – 31.1)	<0.001
Triglyceride(mg/dL)		116 (55 – 238)	164.0 (71.0 – 348.0)	<0.001
Insulin sensitivity (McAuley index)		8.1 (5.5 – 12.4)	6.2 (4.1 – 9.6)	<0.001
		N (%)		
White race		355 (96)	353 (95)	0.72
Smoking history	Current	20 (5.4)	26 (7.0)	0.36
	Past	166 (44.9)	172 (46.5)	0.66
Family history of diabetes		69 (18.7)	131 (35.4)	<0.001
History of HTN		146 (39.5)	230 (62.2)	<0.001
Treatment for HTN		116 (31.4)	210 (56.8)	<0.001
Snoring	Most nights	74 (20.0)	103 (27.8)	0.01
	Occasionally	24 (6.5)	27 (7.3)	0.66
	Almost never	260 (70.3)	232 (62.7)	0.03
Sleep duration (hours per night)	≤ 5	9 (2.4)	24 (6.5)	0.008
	6	69 (18.7)	78 (21.1)	0.41
	7-8	263 (71.1)	237 (64.1)	0.04
	9	17 (4.6)	23 (6.2)	0.33
	≥10	6 (1.6)	4 (1.1)	0.52
Region of the US[!]	North	175 (47)	204 (55)	0.03
	South	62 (17)	57 (15)	0.62
	Mid-west	80 (22)	97 (26)	0.14

Covariates		Control (N=370)	Case (N=370)	p-value*
	West	53 (14)	12 (3)	< 0.001
NSAID use		137(37)	156(42)	0.15
Beta-blocker use		57 (15)	80 (22)	0.03
Post-menopausal		362 (98)	366 (99)	0.24

*P-values for continuous variables calculated using Wilcoxon signed rank test and categorical variables using Chi-Square test.

#Urinary melatonin levels were assessed by measuring the urinary appearance of its primary metabolite (6-sulfatoxymelatonin [aMT6s]). aMT6s, aMT6s/creatinine and creatinine are displayed without log-transformation. In later analyses, aMT6s/creatinine is log-transformed when used as a continuous variable.

†Matching criteria in case-control selection.

‡Physical activity (METs/week), sum of the average time/week spent in each activity × MET value of each activity. METs measure the ratio of the work metabolic rate to the resting metabolic rate.

iAHEI is a diet-quality score that reflects a common dietary pattern associated with a lower risk of type 2 diabetes.

!!North region includes PA, NY, NJ, CT, RI, MA, NH, VT, and ME. South region includes OK, TX, LA, AR, MS, AL, FL, GA, SC, NC, VA, TN, KY, WV, DC, MD, and DE. Midwest region includes ND, SD, NE, KS, MN, IA, MO, WI, IL, MI, IN and OH. West region includes WA, OR MT, ID, WY, CA, NV, UT, CO, AZ, and NM.

Abbreviations: AHEI, alternative healthy eating index; hsCRP, high sensitivity c-reactive protein; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin 6; HTN, hypertension; METs, metabolic equivalent of task.

Table 2

Baseline characteristics of participants according to category of melatonin secretion

Covariates		Category of melatonin secretion†			p-value* (trend)
		Lowest (N=292)	Intermediate (N=246)	Highest (N=202)	
			Median (5-95% range)		
Urine aMT6s/creatinine (ng/mg)#		14.4 (4.2 – 24.8)	35.4 (27.0 – 47.6)	67.0 (50.2 – 177.5)	
Urine aMT6s (ng/dL)#		930 (240 – 2760)	2340 (1150 – 5730)	5075 (2080 – 17900)	<0.0001
Urine creatinine (mg/dL) #		69.1 (34.7 – 156.1)	64.3 (34.3 – 162.5)	68.9 (35.6 – 160.4)	0.75
Age (years)		65.1 (55.7 – 75.7)	64.3 (56.0 – 74.1)	63.7 (55.4 – 75.1)	0.07
BMI (kg/m^2)		27.8 (20.0 – 39.5)	27.4 (20.8 – 38.4)	26.1 (20.5 – 36.0)	0.003
Physical activity, (METs/week) ‡		12.3 (0.2 – 55.9)	12.2 (0.3 – 54.9)	10.6 (0.4 – 57.4)	0.18
AHEI Score i		43.1 (24.5 – 64.0)	44.3 (26.5 – 62.5)	44.1 (26.7 – 63.3)	0.35
Glycemic index		53.5 (48.3 – 58.9)	53.9 (47.3 – 58.5)	53.5 (46.8 – 58.1)	0.80
Polyunsaturated/saturated fat		0.58 (0.34 – 1.10)	0.61 (0.35 – 1.02)	0.58 (0.35 – 1.00)	0.96
%energy trans fat		1.54 (0.72 – 2.55)	1.51 (0.80 – 2.60)	1.62 (0.85 – 2.77)	0.56
Cereal fiber in diet (gm)		5.6 (2.7 – 10.8)	6.0 (2.7 – 11.4)	5.9 (2.8 – 11.8)	0.33
Alcohol consumption (gm/day)		0.9 (0.0 – 28.6)	1.5 (0.0 – 26.7)	0.9 (0.0 – 13.6)	1.00
E-selectin (ng/mL)		38.6 (17.6 – 68.3)	36.3 (17.8 – 70.7)	35.9 (16.3 – 60.7)	0.13
HsCRP (mg/L)		3.1 (0.6 – 21.2)	2.8 (0.5 – 19.6)	2.5 (0.4 – 18.0)	0.14
ICAM-1 (ng/mL)		271.0 (179.6 – 454.9)	267.7 (184.8 – 414.4)	263.8 (178.0 – 374.5)	0.25
IL-6 (pg/mL)		1.5 (0.7 – 5.6)	1.4 (0.5 – 6.6)	1.3 (0.5 – 4.4)	0.005
Insulin (uU/mL)		7.2 (2.4 – 28.0)	6.6 (1.8 – 22.9)	6.1 (1.9 – 20.9)	0.009
Triglyceride (mg/dL)		146 (71 – 316)	142 (56 – 273)	121 (57 – 313)	0.001
Insulin sensitivity (McAuley index)		6.9 (4.4 – 11.2)	7.4 (4.6 – 11.6)	7.5 (4.3 – 12.4)	0.007
			N(%)		
Control subjects		123(33)	124(34)	123(33)	
Case Subjects		169(45)	122(33)	79(21)	<0.0001
White race		281 (96)	231 (94)	196 (97)	0.22
Smoking history	Current	11 (4)	25 (10)	10 (5)	0.39
	Past	137 (47)	109 (44)	92 (46)	0.72

Covariates		Category of melatonin secretion†			p-value* (trend)
		Lowest (N=292)	Intermediate (N=246)	Highest (N=202)	
Family history of diabetes		77 (26)	65 (26)	58 (29)	0.59
History of HTN		165 (56)	119 (48)	92 (46)	0.01
Treatment for HTN		145 (50)	108 (44)	73 (36)	0.003
Snoring	**Most nights**	73 (25)	61 (25)	43 (21)	0.37
	Occasionally	18 (6)	18 (7)	15 (7)	0.57
	Almost never	194 (66)	160 (65)	138 (68)	0.71
Sleep duration (hrs per night)	**≤ 5**	16 (5)	10 (4)	7 (3)	0.27
	6	60 (21)	47 (19)	40 (20)	0.81
	7-8	192 (66)	163 (66)	145 (72)	0.18
	9	14 (5)	20 (8)	6(3)	0.52
	≥10	6(2)	2 (1)	1 (1)	0.14
Region of the US‼	**North**	148 (51)	128 (52)	103 (51)	0.92
	South	49 (17)	39 (16)	31 (15)	0.66
	Mid-west	74 (25)	56 (23)	47 (23)	0.56
	West	21 (7)	23 (9)	21 (10)	0.20
NSAID use		84(29)	73 (30)	64 (32)	0.78
Beta-blocker use		67 (23)	47 (19)	23 (11)	0.005
Post-menopausal		288 (99)	242 (98)	198 (98)	0.87

†Melatonin secretion was assessed by measuring the urinary appearance of its primary metabolite (6-sulfatoxymelatonin [aMT6s]) in the first morning urine with normalization using urinary creatinine.

*p-values for trend across categories of melatonin secretion were calculated using univariate median regression for continuous variables and the Cochran-Armitage trend test for categorical values.

#aMT6s, aMT6s/creatinine and creatinine are displayed without log-transformation. In later analyses, aMT6s/creatinine is log-transformed when used as a continuous variable.

ǂPhysical activity (METs/week) sum of the average time/week spent in each activity × MET value of each activity. METs measure the ratio of the work metabolic rate to the resting metabolic rate.

*i*AHEI is a diet-quality score that reflects a common dietary pattern associated with a lower risk of type 2 diabetes.

‼North region includes PA, NY, NJ, CT, RI, MA, NH, VT, and ME. South region includes OK, TX, LA, AR, MS, AL, FL, GA, SC, NC, VA, TN, KY, WV, DC, MD, and DE. Midwest region includes ND, SD, NE, KS, MN, IA, MO, WI, IL, MI, IN and OH. West region includes WA, OR MT, ID, WY, CA, NV, UT, CO, AZ, and NM.

Abbreviations: AHEI, alternative healthy eating index; hsCRP, high sensitivity c-reactive protein; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin 6; HTN, hypertension; METs, metabolic equivalent of task.

Table 3

Odds ratios for type 2 diabetes according to melatonin secretion.

	Odds Ratio of incident diabetes			
	Continuous (per unit decrease in log aMT6s/creatinine ratio)*	Decreasing category of aMT6s*		
		≥49.1 ng/mg	26.2-49.0 ng/mg	≤26.1 ng/mg
Number of cases	370	79	122	169
Number of controls	370	123	124	123
Model 1	1.36 (1.14 - 1.61)	Ref	1.54 (1.03 – 2.30)	2.03 (1.38 – 3.01)
Model 2	1.34 (1.11 – 1.61)	Ref	1.45 (0.92 – 2.28)	1.94 (1.26 – 2.99)
Model 3	1.47 (1.12 – 1.92)	Ref	1.33 (0.75 – 2.36)	2.31 (1.32 – 4.03)
Model 4	1.48 (1.11 – 1.98)	Ref	1.26 (0.66 – 2.39)	2.17 (1.18 – 3.98)

Model 1: Matched for Age and Race.

Model 2: Same as model 1 with adjustment for BMI.

Model 3: Same as model 2 with adjustment for physical activity, smoking, family history of diabetes, dietary factors (AHEI score, glycemic index, polyunsaturated/saturated fats, % energy from trans fats, cereal fiber intake, and alcohol intake), history or treatment of hypertension, sleep duration, history for snoring, use of beta-blockers, use of NSAIDs, menopausal status, and region of US.

Model 4: Same as model 3 with adjustment for E-selectin, HsCRP, ICAM-1, and IL-6.

*Melatonin secretion was assessed by measuring the urinary appearance of its primary metabolite (6-sulfatoxymelatonin [aMT6s]) in the first morning urine with normalization using urinary creatinine.