Urinary melatonin and risk of incident hypertension among young women

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Objective Administered in supraphysiologic doses, the hormone melatonin may reduce blood pressure, particularly nocturnal blood pressure. However, whether lower physiologic levels of melatonin are an independent risk factor for the development of hypertension has never been reported.

Methods We examined the association between first morning urine melatonin levels and the risk of developing hypertension among 554 young women without baseline hypertension who were followed for 8 years. Cox proportional hazards models were adjusted for age, BMI, physical activity, alcohol intake, smoking status, urinary creatinine, and family history of hypertension.

Results During 8 years of follow-up, a total of 125 women developed hypertension. The relative risk for incident hypertension among women in the highest quartile of urinary melatonin (>27.0 ng/mg creatinine) as compared with the lowest quartile (<10.1 ng/mg creatinine) was 0.49 (95% confidence interval 0.28–0.85, $P<0.001$).

Conclusion First morning melatonin levels are independently and inversely associated with incident hypertension; low melatonin production may be a pathophysiologic factor in the development of hypertension. *J Hypertens* 28:446–451 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Introduction

Many biologic functions in humans follow 24-h circadian patterns, including blood pressure (BP), which is normally lower during the night and increases in the morning [1–3]. Secretion of melatonin by the pineal gland also follows a circadian rhythm; it is secreted exclusively during the dark phase of a light–dark cycle [4]. In animals, melatonin receptors are found in the central nervous system and on endothelial cells [5]. In these animals, melatonin leads to relaxation of the aorta and pulmonary circulation, a decrease in sympathetic outflow, and an increase in nitric oxide production [5–7]. In humans, short-term physiologic studies [8,9] demonstrated relaxation of carotid and axillary arteries, as well as reductions in circulating norepinephrine after administration of oral melatonin; furthermore, some short-term interventional studies [8–12] comparing supraphysiologic doses of melatonin to placebo demonstrated reductions in either nocturnal or 24-h BP.

Whether physiologic differences in melatonin levels can predict long-term risk of developing hypertension among nonhypertensive individuals, however, has never been examined. Nocturnal plasma melatonin levels are accurately reflected by first morning urinary levels of 6-sulphatoxymelatonin (aMT6s) [13,14]. In order to determine the independent association between urinary
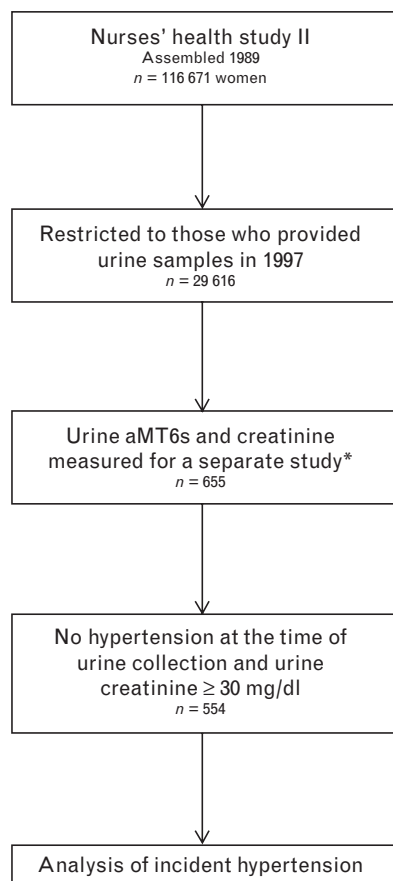
aMT6s levels and the risk of incident hypertension, we conducted a prospective cohort study of 554 premenopausal women from second Nurses' Health Study (NHS II).

Methods
Study population

The derivation of the study population for the current analysis is summarized in Fig. 1. The NHS II is a prospective cohort of 116 671 female registered nurses who were 25–42 years of age when they returned an initial questionnaire in 1989. Subsequent questionnaires have been mailed every 2 years to update information on health-related behaviors and medical events. From 1997 through 1999, 29 616 women agreed to submit urine samples, which were returned by courier and stored in liquid nitrogen freezers. The women who provided urine specimens were similar to those who did not except that they were somewhat less likely to be current smokers. Of the women who provided first morning urine specimens, 576 were selected for a previous case–control study [15] of urinary melatonin and breast cancer risk (192 breast cancer patients and 384 controls). In addition to this breast cancer case–control study, 79 women who were part of a study of hormone stability also had urine melatonin measurements and were included. The urine specimens from these women were assayed for

Fig. 1

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┌─────────────────────────────┐
│     Nurses' health study II  │
│        Assembled 1989        │
│       n = 116 671 women      │
└─────────────────────────────┘
               │
               ▼
┌─────────────────────────────┐
│  Restricted to those who     │
│  provided urine samples in   │
│            1997              │
│        n = 29 616            │
└─────────────────────────────┘
               │
               ▼
┌─────────────────────────────┐
│   Urine aMT6s and creatinine │
│   measured for a separate    │
│          study*              │
│          n = 655            │
└─────────────────────────────┘
               │
               ▼
┌─────────────────────────────┐
│  No hypertension at the time │
│  of urine collection and urine│
│     creatinine ≥ 30 mg/dl    │
│          n = 554            │
└─────────────────────────────┘
               │
               ▼
┌─────────────────────────────┐
│  Analysis of incident        │
│       hypertension           │
└─────────────────────────────┘
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Assembly of the study population. *The 655 woman who already had aMT6s and creatinine measurements included 576 women from a nested case–control study of breast cancer (192 women who ultimately developed breast cancer and 384 who did not), and 79 women who were included in a study of hormone stability. aMT6s, 6-sulphatoxymelatonin.

creatinine and for aMT6s, the major metabolite of melatonin, which is highly correlated with blood and saliva melatonin levels [13,16–21].

Our study population for this particular analysis of urinary melatonin and hypertension risk included the subset of these 655 women who had available urinary aMT6s and creatinine levels, and who did not already have hypertension when they submitted their urine specimens. In addition, we further excluded women with very low urinary creatinine concentrations (<30 mg/dl), leaving 554 women in the analysis [22]. All 554 women were premenopausal at the time of urine collection. A spot urine with less than 30 mg/dl of creatinine is currently defined by the WHO as too dilute for adequate analysis [22]. The institutional review board at Brigham and Women's Hospital reviewed and approved this study.

Ascertainment of normalized urinary 6-sulphatoxymelatonin

Urine aMT6s was assayed in the Endocrine Core Laboratory of Dr M. Wilson (Emory University, Atlanta, Georgia, USA) using an ELISA (ALPCO, Windham, New Hampshire, USA); the coefficient of variation for this assay using blinded quality control samples was 13.9%. Urine creatinine (coefficient of variation = 9.2%) was assayed in the same laboratory using a modified Jaffe method. For each participant, urine aMT6s was divided by urine creatinine to obtain a normalized urine aMT6s (expressed as ng/mg).

Ascertainment of hypertension

The baseline and biennial follow-up questionnaires inquired about physician-diagnosed hypertension and the year of diagnosis. Self-reported hypertension was found to be highly reliable in the NHS [23]. In a subset of women who reported hypertension, medical record review confirmed a documented SBP and DBP, respectively, higher than 140 and 90 mmHg in 100% and higher than 160 and 95 mmHg in 77% of participants [23]. Women were considered to have prevalent hypertension at the time of urine collection if they reported a diagnosis of hypertension on any previous biennial questionnaire or reported use of antihypertensive medications on the questionnaire that preceded urine collection. To analyze incident hypertension, women with prevalent hypertension were excluded. Among those without prevalent hypertension at baseline, women were considered to have incident hypertension if they reported, after the date of urine collection, an initial diagnosis of hypertension or new use of antihypertensive medications.

Ascertainment of other factors

Age, BMI (kg/m^2), and smoking status were obtained from a supplemental questionnaire that accompanied the urine collection. Physical activity [metabolic equivalent task scores (METS)] and alcohol intake (g/day) were self-reported on the main biennial questionnaire just preceding urine collection. Women reported the amount of time they spent doing various light to vigorous physical activities (in minutes per week), including walking, jogging, running, swimming, racquet sports, bicycling, or other aerobic activity; questionnaire derived information about these activities has been validated in comparison with physical activity diaries ($r = 0.79$) [24]. Alcohol intake was computed from a validated food frequency questionnaire; information about alcohol intake on this questionnaire also compares favorably to alcohol intake recorded in dietary diaries ($r = 0.90$) [25]. Information about intakes of sodium, potassium, calcium, and magnesium were also available from the food frequency questionnaire. Information on history of hypertension in a first-degree relative was available on the 1989 questionnaire.

Statistical analyses

Because they were not normally distributed, urinary aMT6s/creatinine ratios were examined in quartiles, using the lowest quartile as the reference group. Person-time was counted from the date of urine collection to the date the last biennial questionnaire was returned (2005), and allocated according to exposure status. Person-time was truncated when an event occurred. Participants were censored at the date of death, or, if they did not return a subsequent questionnaire, they were censored at the date the subsequent questionnaire was mailed. Associations between quartiles of normalized urinary aMT6s and incident hypertension were analyzed using Cox proportional hazards regression. We computed hazard ratios [reported as relative risks (RRs)] for age-adjusted models, as well as multivariable-adjusted models; all multivariable RRs reported were adjusted for established hypertension risk factors, including age (continuous) [26], BMI (continuous) [27], the square of BMI [$(BMI)^2$, continuous], physical activity (quintiles) [27], smoking (never, past, or current), urinary creatinine (continuous), family history of hypertension (yes/no), and alcohol intake (seven categories) [27]. Tests for linear trend were assessed using log-transformed normalized aMT6s levels as a continuous variable.

In the principal analysis, all 554 participants (including both breast cancer patients and controls, as well as women from the hormone stability study) were included. Because breast cancer diagnosis may in some way have impacted hypertension risk, we performed a secondary analysis after excluding women who ultimately developed breast cancer ($n = 401$). Other secondary analyses included analysis of nonnormalized aMT6s levels (without dividing by creatinine), analysis of log-transformed aMT6s/creatinine ratio as a continuous variable, adjustment for night shift work, adjustment for baseline BP, and exclusion of current smokers from the analyses.

In order to provide an effect size estimate demonstrating the potential clinical relevance of melatonin in the development of hypertension, we calculated a hypothetical attributable fraction using the highest three quartiles of urine aMT6s/creatinine ratios as the 'unexposed' group and the lowest quartile as the 'exposed' group. The adjusted RR for the exposed group was used in the following equation to calculate an attributable fraction, where P_e is equal to 25% (the prevalence of the exposed):

$$\text{Attributable fraction} = [(RR - P1) \times P_e] \div \{[(RR - 1) \times P_e] + 1\}$$

The interpretation of the hypothetical attributable fraction is the % of all cases of incident hypertension that conceivably could have been prevented if all women in the population had aMT6s/creatinine ratios that fell in the 'unexposed' range.

For all RRs, we calculated 95% confidence intervals (95% CIs). All P values are two-tailed. Statistical tests were performed using SAS statistical software, version 9 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Baseline characteristics

The median aMT6s/creatinine ratio was 17.0 ng/mg [interquartile range (IQR) 10.1–27.0]. The median age was 44 years (IQR 41–48) and the median BMI was 23.6 kg/m^2 (IQR 21.8–26.6). Baseline characteristics are shown in Table 1 stratified by quartile of aMT6s/creatinine ratio. With increasing quartile, median values for age, BMI, and current smoking all were lower. Alcohol intake, physical activity, urinary creatinine, and family history of hypertension did not appear to differ consistently with differences in the aMT6s/creatinine ratio.

Urinary 6-sulphatoxymelatonin and risk of hypertension

Among 554 women without prevalent hypertension at the time of urine collection, there were 125 individuals with incident hypertension identified through 8 years of follow-up. Compared with women whose urine aMT6s/creatinine ratio was in the lowest quartile (<10.1 ng/mg), the multivariable RR (adjusting for age, BMI, BMI^2, physical activity, smoking status, urinary creatinine, family history of hypertension, and alcohol intake) for incident hypertension among those in the highest quartile (>27.0 ng/mg) was 0.49 (95% CI 0.28–0.85, $P < 0.001$; Table 2).

We also performed various secondary analyses. First, we adjusted for the number of night shifts worked in the 2 weeks prior to submitting the urine sample in addition to our full multivariable model; the RR for the highest quartile as compared with lowest quartile of aMT6s/creatinine ratio was 0.51 (95% CI 0.29–0.89). Addition of dietary intakes of sodium, potassium, calcium, and magnesium to the multivariable models likewise had little impact on the results; the RR for the highest aMT6s/creatinine ratio was 0.48 (95% CI 0.27–0.83). Exclusion of current smokers from the analysis did not substantially impact the results (multivariable RR = 0.54 comparing the highest with lowest quartile, 95% CI 0.31–0.97, $P = 0.001$). Urine aMT6s/creatinine ratios were inversely related to both baseline SBP (correlation coefficient $r = -0.12$, $P = 0.005$) and baseline DBP ($r = -0.12$, $P = 0.004$). Even though BP is presumably on the causal pathway, we analyzed the association between melatonin and incident hypertension after also controlling for baseline BP. The RR comparing the highest with lowest quartile was 0.57 (95% CI 0.32–1.01).

We also analyzed our data after excluding women who were selected based upon developing breast cancer ($n = 401$ with 89 hypertension cases). Although with less statistical power in this analysis, the CIs were

Table 1 Baseline characteristics

Characteristic	Quartiles of aMT6s/creatinine ratio (ng/mg) [median, range]			
	5.5 [<10.1]	13.8 [10.1−16.9]	21.0 [17.0−27.0]	36.9 [>27.0]
No. of participants	138	139	139	138
		Median (IQR)		
Age (years)	46 (42−49)	45 (42−48)	44 (40−47)	44 (41−47)
BMI (kg/m^2)	25.5 (22.3−29.0)	23.8 (22.1−26.6)	23.4 (21.4−26.5)	22.6 (20.8−24.6)
Physical activity (METS)	11.9 (4.6−30.8)	15.1 (5.5−26.1)	11.5 (5.4−23.5)	15.6 (5.7−29.9)
Alcohol intake (g/day)	1.8 (0−6.0)	1.3 (0−5.7)	0.9 (0−4.1)	1.8 (0−3.7)
Urine creatinine (mg/dl)	97 (63−142)	102 (70−155)	100 (71−150)	88 (58−126)
		Percentage		
Current smoking	11.6	7.9	4.3	6.5
Past smoking	29.7	21.6	18.7	25.4
Family history of hypertension	46.4	50.4	50.4	42.8

aMT6s, 6-sulphatoxymelatonin; IQR, interquartile range; METS, metabolic equivalent task scores.

wider, the multivariable RR for the highest as compared with lowest quartile of urine aMT6s/creatinine was not different from the analysis that included breast cancer patients and was still significant (RR = 0.49, 95% CI 0.26–0.93, P = 0.01).

In addition, we analyzed quartiles of urinary aMT6s that were not normalized to the urine creatinine concentration. In this analysis, women in the highest as compared with lowest quartile of nonnormalized aMT6s had a multivariable RR for incident hypertension of 0.40 (95% CI 0.20–0.78). Furthermore, we analyzed the urinary aMT6s/creatinine ratio as a continuous variable after log-transformation (log-transformation resulted in a distribution resembling normal). The RR for a one unit higher log-transformed aMT6s/creatinine ratio was 0.68 (95% CI 0.55–0.84).

Finally, we repeated our analyses after stratifying by BMI (<25 vs. ≥25 kg/m^2). The multivariable RR for incident hypertension comparing the highest with lowest quartile of urine aMT6s−creatinine was 0.39 (95% CI 0.18–0.85) among those women whose BMI was less than 25 kg/m^2 and was 0.45 (95% CI 0.18–1.11) among women whose BMI was more than 25 kg/m^2 (P value for interaction = 0.20).

To estimate a hypothetical attributable fraction, we defined the lowest aMT6s/creatinine quartile (<10.1 ng/mg)

as the 'exposed' group and the highest three quartiles (≥10.1 ng/mg) as the 'unexposed' group. The adjusted RR comparing the exposed with unexposed group was 1.88 (95% CI 1.27–2.77). Using the aforementioned equation, we calculated the hypothetical attributable fraction (the percentage of new cases of hypertension that could conceivably have been avoided if all women had aMT6s/creatinine ratios ≥ 10.1 ng/mg) as 18% (95% CI 5–31).

Discussion

Among 554 nonhypertensive young women, higher morning urinary aMT6s levels, and by implication, higher nocturnal plasma melatonin levels were independently associated with a decreased risk of developing hypertension. To our knowledge, this is the first prospective study to demonstrate an association between melatonin levels with long-term hypertension risk.

The existing literature dealing with melatonin and hypertension in humans consists of eight small, short-term (1–4-week duration) interventional studies [8–12,28–30] of supraphysiologic melatonin doses (ranging from 1 to 10 mg per dose). Although the mean physiologic nocturnal melatonin concentrations range from 7.8 to 158 pg/ml (depending on study and hour of measurement) [31–34], a single 1-mg dose of melatonin can lead to a 570-fold increase in plasma melatonin levels within 90 min [9]. Of these eight studies, two [28,29] were performed in

Table 2 Normalized urinary melatonin and risk of incident hypertension

	Quartiles of aMT6s/creatinine ratio (ng/mg) [median, range]				
	5.5 [<10.1]	13.8 [10.1−16.9]	21.0 [17.0−27.0]	36.9 [>27.0]	P
No. of participants	138	139	139	138	
No. of cases	49	33	22	21	
Age-adjusted RR (95% CI)	1.0 (ref)	0.63 (0.40−0.98)	0.42 (0.25−0.70)	0.38 (0.23−0.65)	<0.001
Multivariable RR (95% CI)	1.0 (ref)	0.66 (0.42−1.05)	0.42 (0.25−0.72)	0.49 (0.28−0.85)	<0.001

Multivariable models adjusted for age, BMI, (BMI)^2, physical activity, alcohol intake, smoking, status, urine creatinine, and family history of hypertension. aMT6s, 6-sulphatoxymelatonin; CI, confidence interval; RR, relative risk.

adolescent type 1 diabetic patients and demonstrated no significant effect on BP. A third study [30] randomized 40 treated hypertensive patients in a crossover design to 4 weeks of melatonin (5 mg nightly) or placebo and observed a 6.5/4.9-mmHg increase in average 24-h BP among treated individuals. The remaining five studies demonstrated significant reductions in at least one BP outcome. Two studies [10,12] of hypertensive individuals documented decreases of 6/4 and 6/3 mmHg in nocturnal BP, without any change in daytime BP. Another randomized crossover study [11] among normotensive individuals noted a 6.4-mmHg decrease in 24-h average SBP. The remaining two studies [8,9] conducted in young healthy men and women measured only acute changes in BP 90 min after dosing and found reductions of 9.5/7.5 and 9/4 mmHg.

In addition to melatonin, rotating shift work has been demonstrated in some studies to impact BP. Yamasaki *et al.* [35] demonstrated that women working in evening and night shifts were six times less likely to appropriately 'dip' their BP during the subsequent sleep period as compared with women working in a day shift. In addition, women working in night or evening shifts had a paradoxical increase in urine catecholamines during sleep [35]. Similar findings were reported by Kitamura *et al.* [36] and Lo *et al.* [37] who observed a switch from dipping to nondipping status following a night shift, although 4 days of continuous night shift work appeared to restore the normal dipping pattern, suggesting that cycle disruption (rather than night work *per se*) is a key to disruption of normal BP cycles [36]. In our study, however, controlling for the number of night shifts worked in 2 weeks prior to urine collection did not substantially attenuate the association; thus, rotating night work cannot fully explain the association between melatonin and hypertension.

The potential mechanisms mediating melatonin's impact on BP have been studied extensively in laboratory experiments. In cultured aortic rings, melatonin increases nitric oxide levels and decreases levels of reactive oxygen species (ROS) [38]; additionally, the vasoactive effects of norepinephrine and phenylephrine are blunted by melatonin [38,39]. Treatment of spontaneously hypertensive rats with melatonin decreases BP while increasing nitric oxide activity, decreasing the levels of ROS and catecholamines, and causing relaxation of vascular smooth muscle cells [6,39–41]. By comparison, few mechanistic studies have been performed in humans. In two studies [8,9] of healthy young women and men, treatment with melatonin led to relaxation of carotid and axillary arteries and decreased circulating levels of norepinephrine.

Alternative explanations exist for the inverse association between melatonin and hypertension risk. It is well established that sleep-disordered breathing is a risk factor for the development of hypertension [42], presumably through activation of the sympathetic nervous system [43,44]. Moreover, there is some evidence that sleep-disordered breathing leads to abnormal patterns in melatonin secretion [33,45], and individuals with obstructive sleep apnea have lower overall nocturnal plasma melatonin levels [33]. Therefore, lower melatonin levels may simply be one mechanism linking sleep-disordered breathing with hypertension. On the contrary, rats are nocturnal animals with peak melatonin levels occurring during periods of wakefulness; therefore, the BP-lowering effect of melatonin (at least in rats) cannot be explained as being mediated by disrupted sleep. Another possible explanation for the inverse association we observed is 25-hydroxyvitamin D [25(OH)D], which has emerged as an independent risk factor for hypertension [46]. It is conceivable that individuals with lower nocturnal melatonin production, potentially because of more nocturnal wakefulness, happen to also have less daytime sun exposure and lower 25(OH)D levels.

Our study has limitations that deserve mention. First, each participant submitted a single morning urine specimen rather than multiple specimens or an overnight collection. Because of potential night-to-night within-person variation in melatonin production, some participants in our study likely had their melatonin status misclassified. However, the correlation between two urinary aMT6s measurements collected 3 years apart among 79 participants of this cohort was 0.72, suggesting that profound misclassification was unlikely. Furthermore, this type of misclassification, if it occurred, would likely be random and, therefore, tend to bias the results toward an underestimation of the true association. Second, we did not directly measure our participants' BP. Nevertheless, all participants are trained health professionals, and self-reporting of hypertension has been validated in this cohort. Third, we lacked information about sleep-disordered breathing in these participants and also lacked plasma 25(OH)D measurements; therefore, we could not analyze these factors as either potential mechanisms or confounders. Fourth, the prospective association between urine melatonin and incident hypertension fell just below the significance threshold when we adjusted for baseline BP, so it is conceivable through some unrecognized mechanism that higher BPs suppress melatonin production or that a third, yet unknown, factor is present that influences both melatonin and BP. Finally, our study population is predominately white and entirely female, so the results may not be generalizable to other racial groups or to men.

In conclusion, higher melatonin excretion is associated with a decreased risk of developing hypertension. Low melatonin levels may be a pathophysiologic factor in the development of hypertension. Experimental studies are required to further explore potential mechanisms, and

additional clinical studies are needed to confirm this association. Melatonin may hold promise as a novel risk factor for developing hypertension, paving the way for long-term interventions to prevent high BP.

Acknowledgements

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