Effects of Melatonin Supplementation on Renal Physiology in a Habitual Sleep Restricted Population.

NCT02631148

02/06/2018

Background and Significance

In the developed world changes to lifestyle and work practices have diminished the amount of nightly sleep many people obtain and altered the times workers are required to be awake.[1,2] Both phenomena act to desynchronize an individual's metabolic and physical demands against the internal physiological circadian rhythms controlled by external circadian cues and the central and peripheral circadian pacemakers.[3,4] Evidence that this is detrimental comes from epidemiological studies demonstrating short sleep duration, fragmented sleep, and shift work are all associated with increased incidence of diabetes, cardiovascular disease and overall mortality, probably **via as yet undetermined derangements in circadian regulated physiology**.[5-9] Many of the kidney's physiological functions follow diurnal periodicity with 13% of the protein coding genes expressed in the kidney following a circadian pattern.[4,10-16] Coordination of the periodicity of these processes in the kidney allows anticipation of the metabolic and physiological workload expected of the kidney throughout a 24-hour cycle.

Sleep measurements and renal function: Preliminary evidence suggests that disruption of the sleep-wake cycle promotes the development of four major chronic kidney disease (CKD) risk factors: **hypertension, diabetes, obesity and increased** renin–angiotensin–aldosterone system **activation (RAAS),** thereby leading to a more rapid decline in glomerular filtration rate (GFR) and increased proteinuria (**Figure 1)**. Individuals who report sleep duration 5 hours or less per night are 20% more likely to develop hypertension than those sleeping 7 hours per night.[17] Similarly, in detailed polysomnography (PSG) studies, elderly men with reduced duration of "restorative" or "deep" slow wave sleep (SWS or stage N3 sleep) had an increased risk of incident hypertension[18]. In a study of 6,834 adults, those who slept 6 or fewer hours per night were 1.7 times more likely to develop proteinuria than individuals who slept for 7 hours per night[19] and in a small cross-sectional study of industrial employees, rotating shift workers had a higher prevalence of proteinuria relative to daytime only workers[20]. We have preliminary data showing that short self reported sleep duration is associated with a more rapid decline in renal function independent of blood pressure and other factors (see, Preliminary Studies**)**.

Sleep disruption and mechanisms for CKD: Decreased sleep duration, de-synchronization of sleep time, or fragmentation of sleep may cause renal damage through increased blood pressure (BP) and activation of RAAS,[10,11,21-23] impaired glucose homeostasis,[6,24] and endothelial dysfunction,[25,26] all established risk factors for development of CKD.[27] Several studies have now demonstrated that acute (1-3 weeks) sleep deprivation increases insulin resistance, decreases pancreatic β islet cell function, and chronic sleep deprivation is a risk factor for development of diabetes.[6,24,28] Similarly, short self-reported sleep duration and reduced measured SWS are associated with an increased risk of hypertension.[17,18] Meanwhile, extension of sleep duration by 1 hour per night for 6 weeks has been found in small studies to decreased fasting insulin levels and brachial BP, suggesting sleep duration is a modifiable risk factor for CKD.[29,30] However it was not clear if decreased fasting insulin levels were due to pancreatic β islet function or improved insulin sensitivity.

Changes in sleep patterns are expected to change the 24 hour periodicity of the RAAS.[10,11,22,29,31] Of particular interest is the effect on 24-hour aldosterone secretion. Beyond the physiological control of sodium retention, aldosterone has detrimental effects on kidney function, stimulating expression of PAI-1

and TGF-β, which promote renal fibrosis, endothelial dysfunction, and altered response of vascular smooth muscle cells to catecholamines.[32-36] However, data on the effects of circadian disruption on aldosterone are conflicting. In a small short-term study involving subjects on continuous enteral feeding, one night of sleep deprivation decreased serum aldosterone over 24 hours.[22] In contrast, a long-term study found that subjects on a fixed sodium diet for up to 205 days had increased (as opposed to decreased) 24-hour urinary aldosterone excretion on days of shift work with associated increase sodium retention and blood pressure.[31] These discrepant results may have been due to difference in potassium intake, which was higher during days of shift work in the long-term study, and could have affected aldosterone production.[5,31]

Preliminary Work**:** Disruption of the diurnal regulation of several physiological processes, including, BP, activity of the RAAS, and glycemic control, may lead to increased risk for CKD. For the past 3 years I have investigated the association of circadian rhythms with cardiometabolic risk factors and disease in a series of epidemiological analysis (described below). I have also gained extensive experience in the performance of inpatient physiological studies in the context of randomized control trials which have involved measurement of renal specific RAAS activity using para-aminohippurate (PAH), blood pressure measured with 24-hour ambulatory monitoring, pulse wave analysis, and glucose regulation with glycemic clamps. During my fellowship I have demonstrated competence in the statistical and clinical study methods required for successful completion of the proposed study.

While others have shown that exposure to light at night during shift work depresses nocturnal melatonin secretion (the chief hormonal output from the central circadian pacemaker),[37,38] we have found that individuals with decreased nocturnal melatonin secretion at baseline have a significantly increased risk of later developing type 2 diabetes[39,40] and cardiovascular disease (unpublished work), both important risk factors for the development of CKD (**Table 1**).

Table 1: **Odds Ratio for incident type 2 diabetes and myocardial infarction according to nocturnal melatonin secretion**

	Odds Ratio (per decrease in log [urine melatonin/creatinine])	
	Type 2 diabetes	Myocardial infarction
Crude	1.36 (1.14-1.61)	1.51 (1.16-1.96)
Adjusted	1.48 (1.11-1.98)	1.69 (1.14-2.51)

Less nocturnal BP dipping is related to poor sleep efficiency[41] and short sleep duration.[42] We therefore analyzed the association of decreased nocturnal dipping with incident CKD among individuals recruited from the Jackson Heart Study and found that decreased dipping was associated with an increased risk of incident CKD (**Table 2**)[43].

Table 2: Odds Ratio of incident CKD according to nocturnal dipping

	Nocturnal dipping (per 10% of awake SBP)
Unadjusted (N = 603)	0.58 (0.42 – 0.79)
Adjusted (N = 408)	0.55 (0.32 – 0.96)

These results, in addition to work by others (see, Background and Significance) suggest that changes in an individual's sleep-wake cycle lead to disruption of normal diurnal regulation of physiological processes, potentially promoting the development of CKD. Thus, we analyzed the association of short self-reported sleep duration with longitudinal changes in eGFR among women enrolled in the Nurses' Health Study and demonstrated that individuals who had lower sleep duration had faster declines in eGFR and higher odds of albuminuria (unpublished results, **Tables 3&4**), further supporting the hypothesis that circadian disruption is detrimental to renal function.

Table 3: Odds ratio for eGFR decrease of ≥25% over 10 years by self-reported sleep duration

Sleep duration	Unadjusted	Adjusted
≤5 hours	1.64 (1.14-2.37)	1.54 (1.05-2.27)
6 hours	1.23 (1.03-1.46)	1.17 (0.97-1.41)
7-8 hours (ref)	1.0 (ref)	1.0 (ref)
9 hours	0.89 (0.59-1.34)	0.73 (0.47-1.14)
≥10 hours	1.38 (0.45-4.25)	1.00 (0.31-3.19)

Table 4: Odds ratio of albuminuria (UACR>30mg/g) by self-reported sleep duration

Sleep duration	Unadjusted	Adjusted
≤5 hours	2.31 (1.42-3.79)	2.12 (1.26-3.55)
6 hours	1.36 (1.02-1.81)	1.30 (0.96-1.76)
7-8 hours (ref)	1.0 (ref)	1.0 (ref)
9 hours	1.00 (0.51-1.93)	0.86 (0.42-1.76)
≥10 hours	2.15 (0.48-9.59)	1.33 (0.26-6.74)

Together, these studies suggest that sleep disruption and reduced nocturnal melatonin secretion are risk factors for incident CKD. While animal studies have demonstrated the reno-protective effect of sleep regulation and supplemental melatonin in preventing renal fibrosis, this has not been studied in humans. However, two small studies have demonstrated that increasing sleep duration among individuals with reduced sleep duration has beneficial effects on some CKD risk factors. In a study of 16 healthy individuals with habitual sleep < 7hours per night, for example, participants were asked to extend their sleep by one hour per night for 6 weeks.[30] Achieved sleep duration increased by 49 minutes/night and participants had decreased insulin levels and increased insulin sensitivity indicating a beneficial metabolic effect from sleep extension. Similarly, a small open label study of 36 individuals with diabetes with insomnia, found that controlled release melatonin improved sleep efficiency (time spent in bed asleep), sleep latency (time taken to fall asleep) and reduced HBA1c from 9.1 to 8.5% over 5 months.[44]

This preliminary evidence suggests the importance of both duration and neurophysiological characteristics of sleep and sleep timing on renal function similar to what has been found for a range of metabolic functions. It also motivates us to perform carefully controlled experiments to investigate the mechanisms by which sleep-wake cycle disruption may lead to a more rapid decline in renal function and if correction of commonly occurring sleep disorders, such as chronic sleep restriction, is beneficial in ameliorating CKD risk factors.

Specific Aims

In health, many of the kidney's physiological functions follow diurnal periodicity synchronized to the sleep-wake cycle.[4,10-15] This periodicity affects transcription of solute transporters,[4] excretion of sodium and calcium,[12,13] blood pressure, renal blood flow, and glomerular filtration rate (GFR), which are all increased during the day.[15] In contrast, the activity of the renin-angiotensin-aldosterone system (RAAS) is increased during the night. [10,11] Coordination of the periodicity of these processes in the kidney allows anticipation of the metabolic and physiological workload expected of the kidney throughout a 24-hour cycle.

Preliminary evidence suggests that disruption of the sleep-wake cycle is a potential risk factor for the development of chronic kidney disease (CKD). Animals with genetic disruption of circadian rhythms develop renal fibrosis, preventable by strictly controlling light-dark periodicity to restore circadian rhythm.[45] Human studies also suggest that disruption of the sleep wake cycle, whether by sleep restriction, or circadian de-synchronization (from shift work), may produce renal damage. In a study of 6,834 adults, those who slept 6 or fewer hours per night were 1.7 times more likely to develop proteinuria than individuals who slept for 7 hours per night.[19] In a small cross-sectional study of industrial employees, rotating shift workers had a higher prevalence of proteinuria relative to daytime only workers.[20] We have preliminary evidence that decreased sleep duration is associated with a more rapid decline in renal function. Decreased sleep duration, de-synchronization of sleep time, or fragmentation of sleep may cause renal damage through increased RAAS activation, increased nocturnal blood pressure,[10,11,21-23] impaired glucose homeostasis,[6,24] increased sympathetic outflow,[46,47] systemic inflammation,[48,49] and endothelial dysfunction[25,26] all established risk factors for development of CKD.[27]

Melatonin, a hormone secreted by the pineal gland mainly during the night, may be a critical mediator between circadian disruption and renal injury. Specifically, perturbations of the sleep-wake cycle lead to reduced nocturnal melatonin secretion,[50-54] which in animals studies is reversibly linked to renal fibrosis[27] and in humans with important risk factors for kidney disease including increased risk for incident hypertension,[55] type 2 diabetes,[39] and cardiovascular disease.[27,56] Supplemental melatonin prevents kidney injury in animal models of acute and chronic kidney disease.[57-59] Systemically, melatonin reduces sympathetic outflow to the cardiovascular system while regulating aldosterone production.[60-63]

We hypothesize that disruption of the sleep-wake cycle by sleep restriction increases risk for CKD, potentially via an increase in aldosterone secretion and reduction of nocturnal melatonin secretion with its pleiotropic effects on blood pressure, glucose metabolism, and endothelial function. We also hypothesize that correction of circadian disruption in high-risk, habitually sleep-restricted individuals by sleep extension or by melatonin supplementation using a controlled-release formulation administered at bedtime can reduce overall risk for CKD. These hypotheses give rise to the following specific aim:

1. **Sleep extension and melatonin supplementation and CKD risk factors**
   In a 6 week pilot study, 20 individuals with habitual sleep restriction will all be asked to extend their nightly sleep by 1 hour, and will then be randomized 1:1 to nightly controlled-release oral melatonin (2mg) or placebo. We will assess whether sleep extension and nightly melatonin supplementation in the community is a feasible intervention with a beneficial effect on the following CKD risk factors: systemic and renal specific RAAS activation (systemic plasma renin activity, plasma angiotensin II

levels, 24-hour urine aldosterone excretion, and renal plasma flow response to captopril); nocturnal blood pressure measured by 24-hour ambulatory blood pressure monitor; central blood pressure measured by pulse wave analysis; and glucose metabolism measured by Minimal Model assessment of insulin resistance and β-cell response to a mixed meal protocol.

Subject Selection

An obese, pre-diabetic, pre-hypertensive population with habitual sleep restriction is ideally suited for a study of the effects of sleep extension and melatonin on CKD risk factors. We have shown that obesity and insulin resistance are associated with reduced endogenous melatonin secretion. [39,64] Obesity predisposes individuals to aldosterone mediated inflammation and endothelial dysfunction. Thus, measures that reduce aldosterone secretion in these individuals may be particularly beneficial.[10,23,65]

We will recruit individuals from the Boston area who meet the inclusion and exclusion criteria listed in **Table 5**. Recruitment will take place through several venues. First, we will recruit from those who have already completed our MODERATE randomized trial (N>200 who have completed so far). The vast majority these individuals still reside in Boston. MODERATE participants have provided consent to be re-contacted for future studies. We will use the MODERATE database to identify individuals who may be eligible for our study, and contact them directly to invite them to participate.

Second, we will simultaneously attempt to enroll study participants who fulfill the age and BMI requirements using routine media outlets in the local community and also online advertising. These include: (1) billboards placed in Boston mass-transit buses and subways; (2) advertisements in local print media, such as the Boston Metro and the Boston Globe; (3) online advertisement with websites such as Craig's List and Oodle (these sites have been productive sources for recruitment in the past).

Third, we will use recruitment opportunities provided through Partners, such as Partners RSVP, and ClinicalTrials@Partners.

We will include both men and women and all races and ethnicities. The only exception will be women who are pregnant because certain medications used during the inpatient evaluations (e.g., nitroglycerine), and certain study medications (i.e., captopril) are contraindicated during pregnancy.

Table 5. Eligibility Criteria

Inclusion Criteria

- BMI ≥ 30 kg/m^2
- Age ≥18 years
- HbA1c 5.7-6.4%
- Self reported sleep <7 hours per night

Exclusion Criteria

- Current or prior history of diabetes mellitus
- Use of hypoglycemic meds or random serum glucose ≥ 200 mg/dL
- Estimated GFR <60 mL/min/1.73m^2
- Inability to extend sleep by 1 hour per night
- Pregnant or breast-feeding
- Preexisting lung disease requiring oxygen

- Preexisting CVD
- Active or uncontrolled psychotic disorder
- Active or uncontrolled bipolar illness
- Active malignancy within the prior 5 years
- Use of antihypertensive meds
- Hepatic impairment
- Job requires rotating night shifts
- Bariatric surgery within the prior 12 months
- Use of hypnotic meds
- Hematocrit <36%(women) or <41% (men)
- Use of natural sleep aids
- Known allergy/intolerance of ACE inhibitors
- History of stroke
- Recent (within 2 weeks of study) or upcoming travel across time zones
- Known obstructive sleep apnea or use of continuous positive airway pressure
- Insomnia
- Known allergy to melatonin
- Hypertension Type 1 or 2 (BP >150/90)
- Lactose Intolerance
- Autoimmune Disease

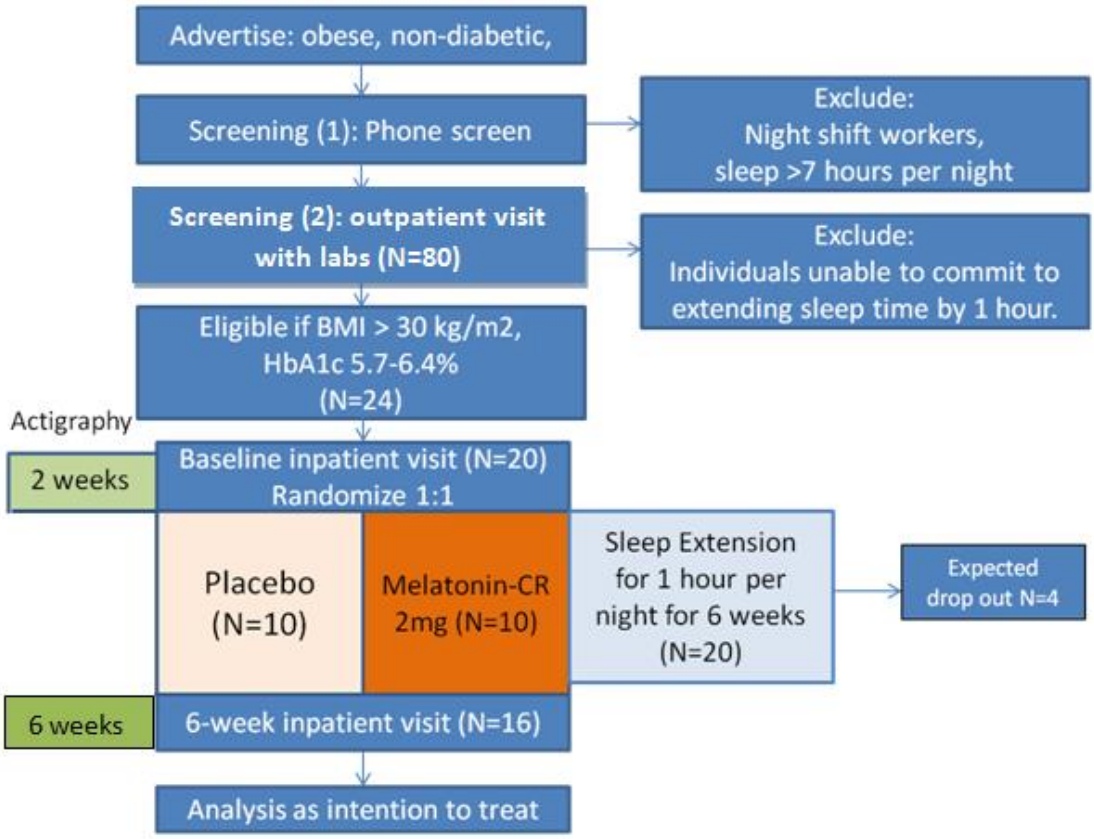
Subject Enrollment

Responders to advertisements will be pre-screened by telephone to obtain a BMI estimate and a medical history to gauge potential eligibility (**Table 5**). For those who may be eligible following this phone query, the basic study schema (**Figure 1**) will be described to identify those who remain interested in participation after learning the duration, inpatient requirements, and potential medication use. These individuals will then be invited to attend an outpatient screening visit.

We anticipate that the sex and race/ethnicity breakdown of subjects enrolled into EMC-PILOT will be similar to that which we have experienced in the MODERATE trial. Thus far in the MODERATE trial, enrolled subjects are 63% female, 28% black, 7% more than one race, and 13% Hispanic.

The pilot study is a double-blind, placebo-controlled randomized trial, in which 16 subjects with obesity (BMI≥30 kg/m^2), pre-diabetes (HbA1C, 5.7-6.4%), and self-reported short sleep duration (<7 hours/night) are randomly assigned 1:1 to receive either placebo or 2 mg of controlled-release melatonin, taken orally every evening 1 hour before bed for 6 weeks. **All subjects will be asked to extend their sleep time by one hour** during the 6 week period between inpatient visits. Endpoints will be measured at both the baseline and 6-week inpatient visits.

Figure 1: Schema for pilot study with sleep extension and nightly melatonin as treatments.



Screening Visit: Screening visits will take place at the Brigham and Women's Outpatient Center for Clinical Investigation (CCI; 221 Longwood Avenue). During the outpatient screen, Dr. McMullan will explain the study in detail, review the consent form, and answer questions. After obtaining written informed consent for participation, a history and physical examination will be performed in order initially judge eligibility based upon the criteria set out in **Table 5**. Those individuals deemed potentially eligible based upon the screening history and exam will undergo phlebotomy and analysis for HbA1c, serum chemistries and glucose, and CBC to confirm eligibility using the criteria presented in the table.

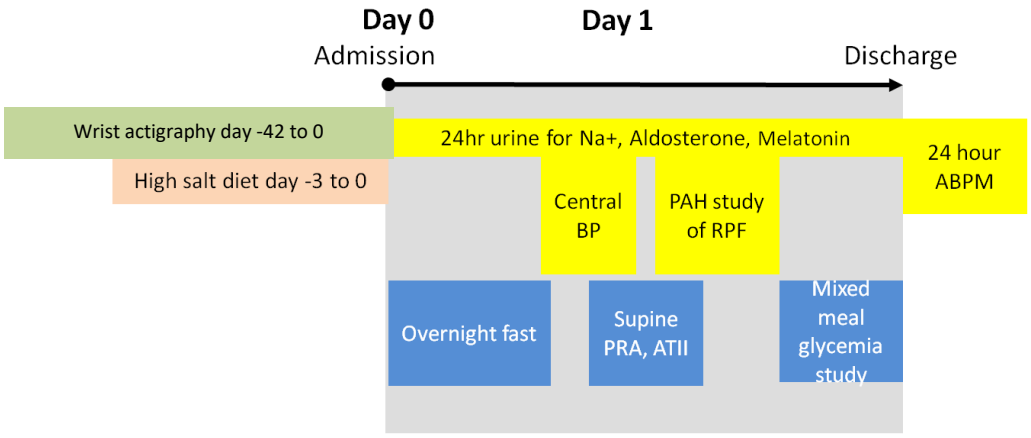
Study Procedures

Pre-admission: All participants will wear a wrist activity monitor for a total of 8 weeks; 2 weeks prior to the baseline inpatient visit and for the 6 weeks between inpatient visits, **Figure 2**. They will complete a daily sleep log during the 2 weeks preceding the baseline visit and 6 weeks preceding second visit. Three days before admission, participants will be placed on a high salt diet by supplementing their usual diet with 150 mmol sodium/day for 3 days using dry bouillon added to food. All participants will also be asked to do a 24 hour diet recall for the day before admission.

Treatment assignments: Prior to admission for the baseline visit, **subjects will be randomized (1:1)** to receive study medication (melatonin 2mg of controlled release formulation or placebo). **All subjects will be asked to extend their sleep time by one hour** during the 6 week period between inpatient visits.

Baseline and Follow-up inpatient visit: Baseline and 6-week inpatient visits will be virtually identical. Each inpatient visit will begin at 4:00PM on Day 0 and end at 4:00PM on Day 1, **Figure 2**.

Figure 2: Schema for inpatient visit studies at 6-week visit.



Day 0 — We will complete two 12-hour urine collections for each subject. All subjects will begin the first 12-hour urine collection at their home at 7:00AM on day 0 of admission to the Brigham and Women's Hospital Center for Clinical Investigation (CCI). The first 12-hour urine collection will be ended at 7:00PM that evening. The second 12-hour collection will begin and continued overnight until 7:00AM on day 1. The nurses will keep the daytime (7AM to 7PM) and nighttime (7PM to 7AM) urine separate to permit analysis of diurnal variation in urinary analytes. Subjects will fast after 9 pm to ensure a 12-hour fast by the next morning.

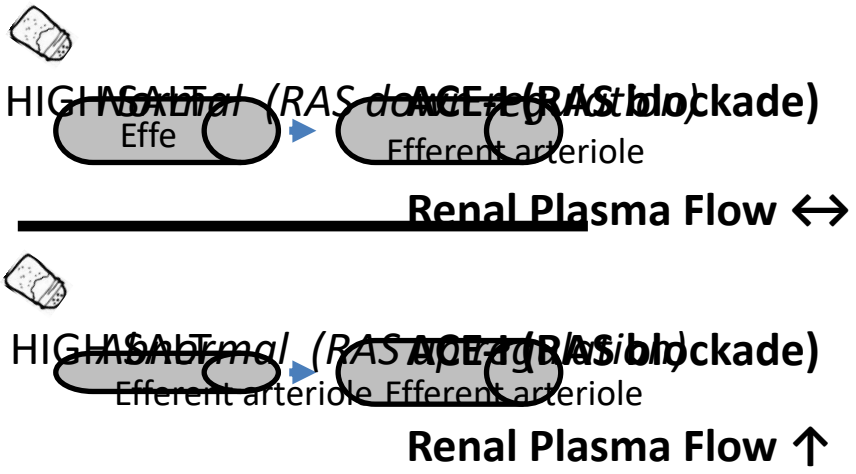
Day 1 — The second 12-hour urine collection will conclude at 7:00AM on day 1 and will be used to measure excretion of sodium and aldosterone. At 8:00AM, central blood pressure and vascular compliance will be assessed, followed at approximately 9:00AM by measurement of renal specific RAAS activity via para-aminohippurate (PAH) renal plasma flow testing with captopril, and systemic RAAS will be measured by plasma renin activity (PRA), angiotensin II (Ang II) and urine aldosterone. Next the subject will receive a mixed meal (10kcal/kg, 45% carbohydrate, 15% protein, 40% fat, 1g/kg glucose) to be consumed in 15 minutes with blood draws at 0, 10, 20, 30, 60, 90 and 120 minutes from the time the meal had begun to be consumed. The mixed meal will achieve glucose of 1g/kg by using specific amounts of a Trutol drink; the amounts are based on the subject's weight, **Table 6**. Plasma glucose, insulin and c-peptide will be measured in all 7 samples of blood. Finally the participant will be fitted with a Spacelabs 90207 ambulatory BP monitor prior to discharge to be worn for 24-hours.

Table 6. Sample Mixed Meal based on 180 lb participant							
Food Description	**Amount**	**Measure**	**Energy**	**Glucose (g)**	**Protein (%)**	**Fat (%)**	**Carbohydrate (%)**
Whole milk, Hood	70	gram	44.49		21.05	47.37	31.58
Egg, whole	117	gram	174.33		34.45	62.18	3.37
Butter	7	gram	50.19		0.46	99.5	0.03
SF peanuts, dry roasted	30	gram	175.5		15.09	71.2	13.71
Turkey sausage	30	gram	46.68		42.56	53.85	3.59
Trutol, glucose drink	323	gram	327.52	81.72	0	0	100
Total			818.71	81.72	14.18	40.78	45.04

Follow-up — During the study, subjects will be phoned weekly by a study coordinator to assess for compliance and potential side effects. Dr McMullan will call all subjects every 2 weeks to discuss difficulties encountered with their new sleep schedule, to recommend improvements and encourage compliance with the protocol.

Parameters Measured: At the inpatient visits, the following measurements will be made:

Renal Specific RAAS — The activity of the local kidney RAAS can be measured by examining the change in effective renal plasma flow (RPF) after oral captopril while in high sodium balance. Effective RPF is measured as clearance of para-aminohippuric acid, because PAH is cleared solely and nearly entirely by the kidney. The protocol to measure PAH clearance is performed with the participant supine, **Figure 5**.



Systemic RAAS — PRA and ang II levels will be measured on the first morning blood draw.

Central Blood Pressure — Will be measured using pulse wave analysis using the SphygmoCor *system (AtCor Medical***,** West Ryde, NSW, Australia), which measures central blood pressure and arterial stiffness.

Glucose metabolism — Will be evaluated by applying the Oral Minimal Model method to the results of a 2 hour 7- measurement, mixed meal tolerance test.[2] Subject will be given breakfast in the form of a mixed meal (10kcal/kg, 45% carbohydrate, 15% protein, 40% fat, 1g/kg glucose) which must be consumed within 15 minutes with glucose, insulin and c-peptide measured from arterialized blood drawn at 0, 10, 20, 30, 60, 90 and 120 minutes from start of meal. Glucose will be measured using a YSI glucose analyzer (CV <2%) for rapid measurement (YSI, Inc, Yellow Springs, OH), insulin will be measured with a radioimmunoassay (CV=10%) and c-peptide will be measured using an electrochemiluminescence

immunoassay(CV=12%). From the glucose, insulin and c-peptide measurements calculation of insulin sensitivity (reciprocal of insulin resistance) and β-cell responsitivity will be calculated.

Drugs to be used:

Captopril - The 25mg oral dose of captopril used during the renal specific RAAS measurement. Severe hypotensive reactions to a single dose of captopril are very uncommon in our experience among those we've studied who were free from pre-existing HTN, particularly in high sodium balance; nevertheless, participants will undergo constant monitoring during the RPF study, and the study will be stopped if the BP following captopril falls below 90 mmHg systolic or 50 mmHg diastolic, at which point the participant will receive medical treatment from the attending physician. Captopril may also be associated with angioedema, but typically occurs after repeated doses and even so the risk is <1%.

Controlled release melatonin (Circadin®) - The 2 mg controlled-release formulation of melatonin has been studied previously in diabetic subjects and improves sleep duration[44]. Pharmacokinetic data suggests that this dose of melatonin achieves nocturnal serum melatonin levels at or above physiological concentrations among healthy volunteers and is therefore adequate to replete nocturnal melatonin in individuals who have relatively low endogenous secretion [67]. The tolerability of controlled-release melatonin has been widely studied[68-71]. In the largest series, 791 subjects were evaluated for 3 weeks in a randomized placebo-controlled trial; adverse event rates were 0.8% with melatonin and 0.6% with placebo. After 3 weeks, 711 subjects were re-randomized for an additional 26 weeks; during this prolonged period, the incidence of adverse events was 0.6% and 0.7% in melatonin and placebo arms, respectively[69]. In a separate, smaller open label study of controlled-release melatonin, the most common adverse events attributed to melatonin were dizziness and headache[70]. Controlled-release melatonin was found to have no effect on psychomotor function or memory in adults relative to placebo[71].

Devices to be used:

Central Aortic Blood Pressure Measurement –Studies of central aortic blood pressure will be performed while the subject is supine and fasting, prior to blood draw. The SphygmoCor XCEL PWA device is a portable machine that will be used to measure central aortic blood pressure. A thin pillow is placed under the arm to align the arm with the heart. A blood pressure cuff is placed on the patient's arm, over the brachial artery. The SphygmoCor XCEL system takes the subject's brachial systolic and diastolic pressure, and then captures the subject's brachial waveform. The brachial waveform is then analyzed by the SphygmoCor Brachial GTF to provide a Central aortic waveform and Central blood pressure measurements. The entire procedure will take approximately 10 minutes to perform. Coded data is automatically stored via the SphygmoCor EXCEL System and will be downloaded onto a secure research computer for storage and analysis.

Ambulatory blood pressure monitor – The device, the SPACELABS Inc. 90207, consists of a 4.5" x 3.4" x 1.1" box weighing 12 oz. that is usually worn on the belt or pant/skirt-line, and a chord that connects the box underneath the clothing to the cuff which is worn on the upper arm. Multiple cuffs of various sizes (small 17-26 cm, standard 24-32 cm, large 32-42 cm, and extra large 38-50 cm) will be available and the appropriate cuff for each participant will be utilized. Once affixed, the device is switched on and the study coordinator will manually initiate two or three readings so that the participant may become accustomed to how the monitor works/feels before he/she leaves the CCI. The monitor will be pre-

programmed to record the BP every 30 minutes during the day and every 60 minutes at night. Instructions to participants will include removal of the device before bathing/showering to keep the device dry. At the end of the 24hr monitoring period, the participant will switch off the device and mail it back to the study coordinator in a pre-paid mailer (which will be provided prior to CCI discharge). Once received, the study coordinator will download the data using SPACELABS Inc. 92506 Report Management System (a PC-compatible Interface).

Data Collection:

This study will collect demographic information (age, self-described race and ethnicity, sex), and physical data (height, weight, screening blood pressure). Blood and urine samples will be analyzed for various biomarkers to collect the data necessary to carry out the specific aims, as described in the section above *Parameters Measured*. All of these data will be recorded in association with a study ID number, and kept as secure excel files by the statistician and programmer. The only individually identifiable private data that will be collected are names, addresses, and contact phone numbers so that participants can be screened with follow-up phone calls, and they will be admitted to the inpatient CCI under their name. This personal information, however, will not be associated with the results obtained from the various tests performed, as the latter will be associated with a study ID number. The file linking the study ID number and the personally identifiable information will be kept by the PI in a secure location and will not be violated unless it becomes medically necessary to contact a participant on the basis of one of their lab screening tests (e.g., elevated liver function tests).

Biostatistical analysis

The primary purpose of the trial is to generate pilot data for a larger randomized control trial. Therefore we aim to calculate means and standard errors for the parameters as well as estimates of effects of sleep extension and melatonin supplementation which will allow accurate power calculations and facilitate the design of a larger study. While we suspect we will be underpowered in this study to find significant differences in endpoints due to sleep extension or melatonin supplementation we will perform statistical analysis on the outcomes as follows. For the main outcomes (renal specific RAAS, BP, glycemic control [insulin sensitivity and β-islet cell function]), we can assume a normal approximation for the changes in log transformed endpoints and so will use an ANCOVA analysis to compare the change in outcome from baseline to 6 week follow-up between melatonin (N=8) and placebo groups (N=8) of the study. Finally we will test the interaction of sleep extension and melatonin supplementation using multivariable linear regression of the parameter change with predictors which will include treatment assignments (sleep extension and melatonin supplementation) and the interaction term **sleep extension *melatonin supplementation** in an analysis of all study participants (N=16).

For the purposes of this grant we have performed power calculations for sleep extension based on either pilot data from obese individuals from our own studies or published results.

Renal specific RAAS Activity – Based upon published data, we assume that change in renal specific RAAS activity ($\Delta RPF_{captopril}$) is 46ml/min/1.73m^2 in obese individuals, SD 12[72], and From these values, we estimate that we will have 80% power to detect a change in $\Delta RPF_{captopril}$ with sleep extension of 15 ml/min/1.73m^2 (33% from baseline) with type I error of 0.05 (two-tailed), **Table 7**.

Central Blood Pressure – Based on pilot data, we assume a mean central systolic BP of 106 mmHg and SD 10 mmHg with a between measurement correlation coefficient 0.89. Thus, for ANCOVA analysis the SD is 6.0 for change in BP. From these values, we estimate we will have 80% power to detect a change in BP of 8 mmHg due to sleep extension with type I error of 0.05 (two-tailed), **Table 7**.
24 hour Ambulatory Blood Pressure – Based on pilot data, we assume a mean 24-hour systolic BP of 122.8 mmHg and SD of 10.4mmHg with a between-measurement correlation coefficient 0.74. Thus for ANCOVA analysis the SD is 5.0 for change in BP. From these values, we estimate that we will have 80% power to detect a 6 mmHg change in 24-hour mean SBP due to sleep extension with a type I error rate of 0.05 (two-tailed).
Insulin sensitivity –Based on published data, we assume a mean insulin sensitivity of 19.03 dl/kg/min/pmol/l and SD of 1.38 dl/kg/min/pmol/l and a between-measurement correlation coefficient of 0.7.[2,73] Thus for ANCOVA analysis the SD for change in insulin sensitivity is 1.1 dl/kg/min/pmol/l. From these values we estimate that we will have 80% power to detect a change in insulin sensitivity of 1.4 dl/kg/min/pmol/l due to sleep extension with type I error of 0.05 (two-tailed), **Table 7**
β-islet cell responsitivity – Based on published data, we assume a mean β islet cell response (dynamic response,ϕ_d) of 494.88 and SD of 25.16 and a assume a measurement correlation coefficient of 0.7.[2,73] . Thus for ANCOVA analysis the SD for change in ϕ_d is 17.68. From these values we estimate that we will have 80% power to detect a change in β islet cell response of 24 due to sleep extension with type I error of 0.05 (two-tailed), **Table 7**. All power calculation performed with PS power and sample size calculator, version 3.0.34.

Table 7: Comparison of the change in outcome we are powered to detect compared with measured changes in outcomes detected in other studies with lifestyle interventions or BMI comparisons.

Outcome	Intervention/Comparison	Δ Measured	Detectable change in this study
Renal Specific RAAS[72]	Obese vs overweight	9ml/min/1.73m^2	15ml/min/1.73m^2
Central systolic BP[46]	Diet and exercise	12 mmHg	8mmHg
24hour ABPM[47]	Difference in BMI of 5kg/m^2	3 mmHg	6mmHg
Insulin Sensitivity[22]	Diet and exercise	19% change	7% change
β-islet cell function[40]	Diet	20% change	5% change

Risks and Discomforts

Potential complications to participants include: (i) the discomfort from having phlebotomy; (ii) the discomfort from having intravenous peripheral catheters placed during CCI admissions; (iii) potential side effects from being on a high salt diet for 3 days, such as elevation of BP or hypokalemia; (iv) potential side effects from medications administered during the inpatient CCI stays, including drop in BP from oral captopril consumption (for the renal plasma flow study (RPF)); (v) side effects from melatonin such as allergic reaction, medication interactions, and elevation of liver function tests; and finally (vi) the chance

of a confidentiality breach such that personal information becomes de-identified and linked to the results of the various studies and blood tests.

i. Phlebotomy: This risk will occur by study design but is minimal.

ii. Peripheral intravenous catheter placement: This risk will occur by study design but is minimal.

iii. High salt diet: A high salt diet is common to the US population, and enrolled participants will not have pre-existing HTN or hypokalemia; thus, a high salt diet for 3 days is unlikely to cause harm.

iv. Inpatient procedures: Severe hypotensive reactions to the oral captopril are very uncommon in our experience among those we've studied who were free from pre-existing HTN; nevertheless, participants will undergo constant monitoring during the RPF study, and captopril will not be given if the BP prior to scheduled captopril administration is less than 100 mmHg systolic or 60 mmHg diastolic.

v. Side effects from Melatonin: The tolerability of controlled-release melatonin has been widely studied. In the largest series, 791 subjects were evaluated for 3 weeks in a randomized placebo-controlled trial; adverse event rates were 0.8% with melatonin and 0.6% with placebo. After 3 weeks, 711 subjects were re-randomized for an additional 26 weeks; during this prolonged period, the incidence of adverse events was 0.6% and 0.7% in melatonin and placebo arms, respectively. In a separate, smaller open label study of controlled-release melatonin, the most common adverse events attributed to melatonin were dizziness and headache. Controlled-release melatonin was found to have no effect on psychomotor function or memory in adults relative to placebo.

vi. Breach of confidentiality: This poses a psychological/financial risk, should information about medical history, laboratory information, or test results be breached. To guard against this, all recorded data, identified by study number only, will be housed separately from the name-address-phone number file of participants in the study. Only the principal investigator (or designated assistant) will have need (and necessary access) to cross-reference the files, and this will be done only when it is necessary to contact a participant for medical reasons, or if the participant is seeking additional information.

If subjects experience study related adverse events, they will be followed up by the study until the symptoms resolve. Depending on severity, this can range from daily to weekly phone calls or possibly study visits.

None of the inpatient studies use radiation and so participants will not be at additional radiation risk from enrollment in the study.

Adequacy of Protection Against Risks

Overall, as described above, the risks associated with this proposal are either minimal, or else unlikely to occur. Informed consent will be obtained from all participants, and all possible protections against risk will be employed.

a. Informed Consent. Our plans for recruitment were outlined in the Research Strategy. Essentially, potential participants will be identified though public advertisements and interested individuals will initially be screened by telephone to judge potential eligibility. At the screening visit, informed consent will be obtained by the PI (Dr. McMullan), or occasionally, by the Co-investigator (Dr. Forman). All consent forms will have been pre-approved by the institutional review board at Brigham and Women's Hospital. The process of informed consent will provide potential participants with the following information:

i. Purpose of the study

ii. Drugs to be administered and the possible use of placebo

iii. A description of randomization and blinding

iv. Reasons why the participant may eligible for the study

v. The number of participants expected to be enrolled

vi. The funding source for the study

vii. Duration of the study

viii. A detailed description of all outpatient and inpatient study protocols and tests to be performed

ix. Storage of blood samples for possible future use

x. Circumstances under which participation may be terminated by the investigator

xi. Procedures for safe and orderly termination if the participant wishes to withdraw

xii. A detailed explanation of all potential risks, including unforeseen risks, and potential benefits

xiii. Remuneration

b. Protections Against Risk. As described above, we will exclude from enrollment individuals who are at increased risk for adverse events resulting from study medications. We will screen for side effects of study medications weekly, and monitor for biochemical side effects through laboratory screening. Individuals who have serious adverse reactions will have study participation terminated. Risk associated with the outpatient screening and checkup visits (phlebotomy) is minimal. Risk associated with inpatient procedures (the EDD and RPF studies) will be minimized by continuous monitoring by a physician (the PI or Co-investigator) and nurse, and – as noted – participants will already be hospital inpatients at that time so any serious adverse events can quickly be addressed. No vulnerable populations will be enrolled.

Potential Benefits

Potential benefit to society: As described in detail in the **Study Procedure** section, we will perform a small pilot study of obese individuals. We plan to enroll a total of 20 individuals into a randomized controlled trial. All participants will be adults (age≥18 years), and will be recruited from the Boston area. Volunteers will be recruited and screened for inclusion based upon having a BMI≥30 kg/m2, and having HbA1C between 5.7 and 6.4%. Other than having these "conditions", potential participants will be expected to otherwise be in general good health, without severe co-morbid conditions as described in the **Subject Enrollment** section. There will be no involvement of special vulnerable populations. After randomization, participants will be retained by weekly phone calls; additionally, the duration of this trial (6 weeks) is relatively short. Study group assignment will be random, of the 20 participants, 10 will receive placebo, and 10 will receive controlled release melatonin (2.0mg of Circadin® nightly for 6 weeks). The 2 mg controlled-release formulation of melatonin has been studied previously in diabetic subjects and improves sleep duration. Pharmacokinetic data suggests that this dose of melatonin achieves nocturnal serum melatonin levels at or above physiological concentrations among healthy volunteers and is therefore adequate to replete nocturnal melatonin in individuals who have relatively low endogenous secretion.

The key potential benefit is the knowledge that is gained on potentially modifiable mediators of important pathophysiologic mechanisms of disease. The segment of the US population that would meet our study criteria is large; specifically, over half of the US adult population is obese, and nearly a quarter of these individuals have pre-diabetes. Thus, the results of this study could have profound public health implications.

Potential benefit to participants: It is possible that participants will gain direct benefit from these studies. First of all, it is possible that individuals who are not randomized to placebo will benefit from treatment. On the other hand, because this study is only 12-weeks in duration, these benefits (if present) will likely be short-lived.

Monitoring and quality assurance

We will submit our data safety monitoring plan (DSMP) to the Brigham and Women's Hospital Institutional Review Board for approval. It is based upon the guidelines set forth by the Brigham and Women's IRB that we have formulated our Safety Monitoring Plan.

a. Type of DSMP. We plan to implement a fully independent data safety monitoring board (DSMB), which will meet every 6 months. The DSMB will have 2 members independent of Drs. McMullan and Forman; a statistician and a clinical investigator. The first meeting will be prior to subject enrollment to allow agreement on expectations of investigator and DSMB, with review of the study protocol approved by IRB. Safety data regarding adverse effects will be presented at each DSMB meeting as will unblinded outcome data. At each meeting the DSMB will decide on the continuation of the trial after considering the study risk based on the accumulation of adverse events and the futility of the trial based on the outcomes data. In addition to the DSMB a committee of investigators, including Drs. McMullan and Forman, as well as a research pharmacist will provide safety monitoring on a weekly basis allowing "on-the-spot" monitoring for abnormal safety labs or protocol breaches as well as providing decisions regarding termination of an individual's participation. This will allow greater flexibility and speed to react to safety issues should they arise.

b. Data to be reviewed. The safety committee made of investigators will review data pertaining to side effects and adverse events.

ClinicalTrials.gov Requirements

The principal investigator of this proposal, as the responsible party, will comply with Public Law 110-85 (FDAAA), and will register this clinical trial on ClinicalTrials.gov.

References:

1. EU. *Fifth European Working Conditions Survey.* Luxembourg: Publications Office of the European Union; 2012 2012.

2. Tsc SL, Wang F, Zhang L, et al. 0174 Preliminary results of shift work and cardiovascular risk factors: analysing baseline data of a prospective night shift worker cohort in Shenzhen, China. *Occupational and environmental medicine.* Jun 2014;71 Suppl 1:A81-82.

3. Bixler E. Sleep and society: an epidemiological perspective. *Sleep Med.* Sep 2009;10 Suppl 1:S3-6.

4. Fine DM, Wasser WG, Estrella MM, et al. APOL1 risk variants predict histopathology and progression to ESRD in HIV-related kidney disease. *J Am Soc Nephrol.* Feb 2012;23(2):343-350.

5. Cai H, Shu XO, Xiang YB, et al. Sleep Duration and Mortality: A Prospective Study of 113 138 Middle-Aged and Elderly Chinese Men and Women. *Sleep.* Oct 28 2014.

6. Buxton OM, Cain SW, O'Connor SP, et al. Adverse metabolic consequences in humans of prolonged sleep restriction combined with circadian disruption. *Sci Transl Med.* Apr 2012;4(129):129ra143.

7. Pan A, Schernhammer ES, Sun Q, Hu FB. Rotating night shift work and risk of type 2 diabetes: two prospective cohort studies in women. *PLoS Med.* Dec 2011;8(12):e1001141.

8. Ford ES. Habitual sleep duration and predicted 10-year cardiovascular risk using the pooled cohort risk equations among US adults. *Journal of the American Heart Association.* Dec 2014;3(6):e001454.

9. Hermansson J, Gillander Gadin K, Karlsson B, Reuterwall C, Hallqvist J, Knutsson A. Case fatality of myocardial infarction among shift workers. *International archives of occupational and environmental health.* Sep 27 2014.

10. Plantinga L, Lee K, Inker LA, et al. Association of sleep-related problems with CKD in the United States, 2005-2008. *Am J Kidney Dis.* Oct 2011;58(4):554-564.

11. Agarwal R, Light RP. Sleep and activity in chronic kidney disease: a longitudinal study. *Clin J Am Soc Nephrol.* Jun 2011;6(6):1258-1265.

12. Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science (New York, N.Y.).* Aug 13 2010;329(5993):841-845.

13. Nikolaeva S, Pradervand S, Centeno G, et al. The circadian clock modulates renal sodium handling. *J Am Soc Nephrol.* Jun 2012;23(6):1019-1026.

14. Kopp JB, Nelson GW, Sampath K, et al. APOL1 genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol.* Nov 2011;22(11):2129-2137.

15. Foster MC, Coresh J, Fornage M, et al. APOL1 variants associate with increased risk of CKD among African Americans. *J Am Soc Nephrol.* Sep 2013;24(9):1484-1491.

16. Zhang R, Lahens NF, Ballance HI, Hughes ME, Hogenesch JB. A circadian gene expression atlas in mammals: implications for biology and medicine. *Proc Natl Acad Sci U S A.* Nov 11 2014;111(45):16219-16224.

17. Gangwisch JE, Feskanich D, Malaspina D, Shen S, Forman JP. Sleep Duration and Risk for Hypertension in Women: Results from The Nurses' Health Study. *Am J Hypertens.* Apr 2013.

18. Fung MM, Peters K, Redline S, et al. Decreased slow wave sleep increases risk of developing hypertension in elderly men. *Hypertension.* Oct 2011;58(4):596-603.

19. Herzog N, Jauch-Chara K, Hyzy F, et al. Selective slow wave sleep but not rapid eye movement sleep suppression impairs morning glucose tolerance in healthy men. *Psychoneuroendocrinology.* Oct 2013;38(10):2075-2082.

20. Madhavan SM, O'Toole JF, Konieczkowski M, Ganesan S, Bruggeman LA, Sedor JR. APOL1 localization in normal kidney and nondiabetic kidney disease. *J Am Soc Nephrol.* Nov 2011;22(11):2119-2128.

21. Ito K, Bick AG, Flannick J, et al. Increased burden of cardiovascular disease in carriers of APOL1 genetic variants. *Circulation research.* Feb 28 2014;114(5):845-850.

22. Warram JH, Gearin G, Laffel L, Krolewski AS. Effect of duration of type I diabetes on the prevalence of stages of diabetic nephropathy defined by urinary albumin/creatinine ratio. *J Am Soc Nephrol.* Jun 1996;7(6):930-937.

23. Mitsiades CS, McMillin D, Kotoula V, et al. Antitumor effects of the proteasome inhibitor bortezomib in medullary and anaplastic thyroid carcinoma cells in vitro. *J Clin Endocrinol Metab.* Oct 2006;91(10):4013-4021.

24. Yaggi HK, Araujo AB, McKinlay JB. Sleep duration as a risk factor for the development of type 2 diabetes. *Diabetes Care.* Mar 2006;29(3):657-661.

25. Neilan TG, Mongeon FP, Shah RV, et al. Myocardial extracellular volume expansion and the risk of recurrent atrial fibrillation after pulmonary vein isolation. *JACC. Cardiovascular imaging.* Jan 2014;7(1):1-11.

26. Neilan TG, Shah RV, Abbasi SA, et al. The incidence, pattern, and prognostic value of left ventricular myocardial scar by late gadolinium enhancement in patients with atrial fibrillation. *J Am Coll Cardiol.* Dec 10 2013;62(23):2205-2214.

27. Taal MW, Brenner BM. Renal risk scores: progress and prospects. *Kidney Int.* Jun 2008;73(11):1216-1219.

28. Buxton OM, Pavlova M, Reid EW, Wang W, Simonson DC, Adler GK. Sleep restriction for 1 week reduces insulin sensitivity in healthy men. *Diabetes.* Sep 2010;59(9):2126-2133.

29. Haack M, Serrador J, Cohen D, Simpson N, Meier-Ewert H, Mullington JM. Increasing sleep duration to lower beat-to-beat blood pressure: a pilot study. *Journal of sleep research.* Jun 2013;22(3):295-304.

30. Leproult R, Deliens G, Gilson M, Peigneux P. Beneficial Impact of Sleep Extension on Fasting Insulin Sensitivity in Adults with Habitual Sleep Restriction. *Sleep.* Oct 28 2014.

31. Rakova N, Jüttner K, Dahlmann A, et al. Long-Term Space Flight Simulation Reveals Infradian Rhythmicity in Human Na+ Balance. *Cell metabolism.* 2013;17(1):125-131.

32. Brown NJ, Nakamura S, Ma L, et al. Aldosterone modulates plasminogen activator inhibitor-1 and glomerulosclerosis in vivo. *Kidney Int.* Sep 2000;58(3):1219-1227.

33. Juknevicius I, Segal Y, Kren S, Lee R, Hostetter TH. Effect of aldosterone on renal transforming growth factor-beta. *American journal of physiology. Renal physiology.* Jun 2004;286(6):F1059-1062.

34. Ikeda U, Kanbe T, Nakayama I, Kawahara Y, Yokoyama M, Shimada K. Aldosterone inhibits nitric oxide synthesis in rat vascular smooth muscle cells induced by interleukin-1 beta. *Eur J Pharmacol.* Jul 18 1995;290(2):69-73.

35. Kornel L, Smoszna-Konaszewska B. Aldosterone (ALDO) increases transmembrane influx of Na+ in vascular smooth muscle (VSM) cells through increased synthesis of Na+ channels. *Steroids.* Jan 1995;60(1):114-119.

36. Oberleithner H. Aldosterone makes human endothelium stiff and vulnerable. *Kidney Int.* May 2005;67(5):1680-1682.

37. Bonmati-Carrion MA, Arguelles-Prieto R, Martinez-Madrid MJ, et al. Protecting the Melatonin Rhythm through Circadian Healthy Light Exposure. *International journal of molecular sciences.* 2014;15(12):23448-23500.

38. Claustrat B, Brun J, Chazot G. The basic physiology and pathophysiology of melatonin. *Sleep Med Rev.* Feb 2005;9(1):11-24.

39. McMullan CJ, Schernhammer ES, Rimm EB, Hu FB, Forman JP. Melatonin secretion and the incidence of type 2 diabetes. *JAMA.* Apr 2013;309(13):1388-1396.

40. McMullan CJ, Curhan GC, Schernhammer ES, Forman JP. Association of nocturnal melatonin secretion with insulin resistance in nondiabetic young women. *Am J Epidemiol.* Jul 15 2013;178(2):231-238.

41. Ross AJ, Yang H, Larson RA, Carter JR. Sleep efficiency and nocturnal hemodynamic dipping in young, normotensive adults. *Am J Physiol Regul Integr Comp Physiol.* Oct 1 2014;307(7):R888-892.

42. Borel AL, Benhamou PY, Baguet JP, et al. Short sleep duration is associated with a blood pressure nondipping pattern in type 1 diabetes: the DIAPASOM study. *Diabetes Care.* Sep 2009;32(9):1713-1715.

43. McMullan CJ, Hickson DA, Taylor HA, Forman JP. Prospective analysis of the association of ambulatory blood pressure characteristics with incident chronic kidney disease. *J Hypertens.* Sep 2015;33(9):1939-1946.

44. Garfinkel D, Zorin M, Wainstein J, Matas Z, Laudon M, Zisapel N. Efficacy and safety of prolonged-release melatonin in insomnia patients with diabetes: a randomized, double-blind, crossover study. *Diabetes Metab Syndr Obes.* 2011;4:307-313.

45. Martino TA, Oudit GY, Herzenberg AM, et al. Circadian rhythm disorganization produces profound cardiovascular and renal disease in hamsters. *Am J Physiol Regul Integr Comp Physiol.* May 2008;294(5):R1675-1683.

46. Taylor J. 2013 ESH/ESC guidelines for the management of arterial hypertension. *Eur Heart J.* Jul 2013;34(28):2108-2109.

47. Mattix HJ, Hsu CY, Shaykevich S, Curhan G. Use of the albumin/creatinine ratio to detect microalbuminuria: implications of sex and race. *J Am Soc Nephrol.* Apr 2002;13(4):1034-1039.

48. Connell SJ, Hollis S, Tieszen KL, McMurray JR, Dornan TL. Gender and the clinical usefulness of the albumin: creatinine ratio. *Diabet Med.* Jan-Feb 1994;11(1):32-36.

49. Boudjeltia KZ, Faraut B, Esposito MJ, et al. Temporal dissociation between myeloperoxidase (MPO)-modified LDL and MPO elevations during chronic sleep restriction and recovery in healthy young men. *PLoS One.* 2011;6(11):e28230.

50. McMullan CJ, Schernhammer ES, Forman JP. Melatonin level and risk for type 2 diabetes--in reply. *JAMA.* Aug 7 2013;310(5):537.

51. Freedman BI, Kopp JB, Langefeld CD, et al. The apolipoprotein L1 (APOL1) gene and nondiabetic nephropathy in African Americans. *J Am Soc Nephrol.* Sep 2010;21(9):1422-1426.

52. Tzur S, Rosset S, Shemer R, et al. Missense mutations in the APOL1 gene are highly associated with end stage kidney disease risk previously attributed to the MYH9 gene. *Human genetics.* Sep 2010;128(3):345-350.

53. Hernández C, Abreu J, Abreu P, Castro A, Jiménez A. Nocturnal melatonin plasma levels in patients with OSAS: the effect of CPAP. *Eur Respir J.* Sep 2007;30(3):496-500.

54. Ulasi, II, Tzur S, Wasser WG, et al. High population frequencies of APOL1 risk variants are associated with increased prevalence of non-diabetic chronic kidney disease in the Igbo people from south-eastern Nigeria. *Nephron. Clinical practice.* 2013;123(1-2):123-128.

55. Forman JP, Curhan GC, Schernhammer ES. Urinary melatonin and risk of incident hypertension among young women. *J Hypertens.* Mar 2010;28(3):446-451.

56. Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia-Gonzalez M, Reiter RJ. Prognostic value of nocturnal melatonin levels as a novel marker in patients with ST-segment elevation myocardial infarction. *Am J Cardiol.* Apr 2006;97(8):1162-1164.

57. Ayas NT, White DP, Manson JE, et al. A prospective study of sleep duration and coronary heart disease in women. *Arch Intern Med.* Jan 27 2003;163(2):205-209.

58. Lettieri CJ, Eliasson AH, Andrada T, Khramtsov A, Raphaelson M, Kristo DA. Obstructive sleep apnea syndrome: are we missing an at-risk population? *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine.* Oct 15 2005;1(4):381-385.

59. Leong WB, Arora T, Jenkinson D, et al. The prevalence and severity of obstructive sleep apnea in severe obesity: the impact of ethnicity. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine.* Sep 2013;9(9):853-858.

60. McMullan CJ. HD, Taylor HA, Forman JP. Prospective analysis of the association of ambulatory blood pressure characteristics with incident chronic kidney disease. . *Journal of Hypertension.* 2015;in press.

61. Ray CA. Melatonin attenuates the sympathetic nerve responses to orthostatic stress in humans. *J Physiol.* Sep 2003;551(Pt 3):1043-1048.

62. Arangino S, Cagnacci A, Angiolucci M, et al. Effects of melatonin on vascular reactivity, catecholamine levels, and blood pressure in healthy men. *Am J Cardiol.* May 1999;83(9):1417-1419.

63. Cagnacci A, Arangino S, Angiolucci M, Maschio E, Melis GB. Influences of melatonin administration on the circulation of women. *Am J Physiol.* Feb 1998;274(2 Pt 2):R335-338.

64. Matsushita K, Selvin E, Bash LD, Franceschini N, Astor BC, Coresh J. Change in estimated GFR associates with coronary heart disease and mortality. *J Am Soc Nephrol.* Dec 2009;20(12):2617-2624.

65. Feraco A, Armani A, Mammi C, Fabbri A, Rosano GM, Caprio M. Role of mineralocorticoid receptor and renin-angiotensin-aldosterone system in adipocyte dysfunction and obesity. *The Journal of steroid biochemistry and molecular biology.* Sep 2013;137:99-106.

66. Assessment report for Circadin to the European Medicines Agency: European Medicines Agency.

67. Aldhous M, Franey C, Wright J, Arendt J. Plasma concentrations of melatonin in man following oral absorption of different preparations. *Br J Clin Pharmacol.* Apr 1985;19(4):517-521.

68. Lemoine P, Wade AG, Katz A, Nir T, Zisapel N. Efficacy and safety of prolonged-release melatonin for insomnia in middle-aged and elderly patients with hypertension: a combined analysis of controlled clinical trials. *Integr Blood Press Control.* 2012;5:9-17.

69. Wade AG, Crawford G, Ford I, et al. Prolonged release melatonin in the treatment of primary insomnia: evaluation of the age cut-off for short- and long-term response. *Curr Med Res Opin.* Jan 2011;27(1):87-98.

70. Lemoine P, Garfinkel D, Laudon M, Nir T, Zisapel N. Prolonged-release melatonin for insomnia - an open-label long-term study of efficacy, safety, and withdrawal. *Ther Clin Risk Manag.* 2011;7:301-311.

71. Lyseng-Williamson KA. Melatonin prolonged release: in the treatment of insomnia in patients aged ≥55 years. *Drugs Aging.* Nov 2012;29(11):911-923.

72. Feldman HI, Appel LJ, Chertow GM, et al. The Chronic Renal Insufficiency Cohort (CRIC) Study: Design and Methods. *J Am Soc Nephrol.* Jul 2003;14(7 Suppl 2):S148-153.

73. McMullan CJ, Yano Y, Bakris GL, Kario K, Phillips RA, Forman JP. Racial impact of diurnal variations in blood pressure on cardiovascular events in chronic kidney disease. *J Am Soc Hypertens.* Apr 2015;9(4):299-306.