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# Gender differences in the relationships between chronic kidney disease, asymmetric dimethylarginine, and sleep quality: The HEIJO-KYO cohort

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ABSTRACT

The association between chronic kidney disease (CKD), serum levels of asymmetric dimethylarginine (ADMA), and sleep quality has not been studied in large populations. In this cross-sectional study of 1115 elderly individuals (mean age, 71.9 years), we measured serum levels of ADMA, and objective and subjective sleep quality using actigraphy and a standardized self-reported questionnaire, respectively. Multivariable analysis adjusted for potential confounders revealed that in females, compared with the non-CKD/low-ADMA group ($n = 312$), sleep efficiency was significantly lower in the CKD/high-ADMA group ($n = 52$) by 3.5% for objective sleep quality [95% confidence interval (CI), 1.1–5.9] and by 4.2% (95% CI, 0.3–8.0) for subjective sleep quality but not in the non-CKD/high-ADMA ($n = 179$) and CKD/low-ADMA ($n = 36$) groups. In males, no significant associations between CKD, ADMA levels, and sleep quality were observed. Wake time after sleep onset was significantly longer by 11.3 min (95% CI, 3.0–19.6) for objective sleep quality and by 25.9 min (95% CI, 4.9–46.9) for subjective sleep quality in the CKD/high-ADMA group than in the non-CKD/low-ADMA group in females but not in males. Mediation analysis revealed a significant effect of serum ADMA levels on the association between renal function and parameters of sleep quality among females. In conclusions, both objective and subjective sleep quality were poorer in elderly females with CKD/high-ADMA than in those with non-CKD/low-ADMA, but not in males. Association between CKD and sleep disturbances might be mediated by ADMA levels.

## 1. Introduction

Sleep disturbances increase with age and are more common in females (1). Previous epidemiological studies have suggested that the sleep disturbance prevalence was up to 40% in Western and Japanese elderly populations [1–5] and that sleep disturbances are associated with increased risks of psychiatric and neurodegenerative disorders, cardiovascular diseases, and early mortality [6–9]. Recently, several studies have provided evidence suggesting that sleep disturbances are highly prevalent in chronic kidney disease (CKD) patients even without hemodialysis, ranging to up to 60%–85% [10]. However, in most of the studies, sleep quality was evaluated using questionnaires, and few studies have documented the association between CKD and sleep quality using objective methods in large populations. Furthermore, mechanisms underlying the association between CKD and sleep disturbances remain largely unknown.

Nitric oxide (NO) is a biological neurotransmitter synthesized by NO synthases (NOS), located in the nervous system as well as other organs. It is well known that NO is involved in a variety of neural functions, such as neurosecretion, thermoregulation, and sleep homeostasis [11]. Previous evidence in aged rats includes that administering an NOS inhibitor significantly reduced slow-wave sleep (SWS) and rapid eye movement (REM) sleep; decreased SWS and REM sleep recovered by NO donor administration [12].

Asymmetric dimethylarginine (ADMA), a major endogenous competitive NOS inhibitor partly controlling the bioavailability of all NOS isoforms in humans, is partly cleared by renal excretion, resulting in elevated serum ADMA levels in CKD patients that can inhibit NOS activity [13,14]. Further, serum ADMA levels are inversely associated with endogenous NO levels [13]. Therefore, elevated ADMA levels in CKD patients may be a mediator of sleep disturbances; however, the association between CKD, ADMA levels, and sleep quality has not been explored in humans. The purpose of the present study was to evaluate the relationship between CKD, serum levels of ADMA, and sleep quality in a large population.

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## 2. Methods

### 2.1. Participants

We analyzed baseline data from a study entitled "Housing Environments and Health Investigation among Japanese Older People in Nara, Kansai Region: a prospective community-based cohort (HEIJO-KYO) study", including 1115 community-dwelling elderly individuals aged ≥60 years who had undergone measurements of serum ADMA levels and objective or subjective sleep quality [15]. All participants provided written informed consent; the Nara Medical University Ethics Committee approved the study protocol.

### 2.2. Measurements of objective and subjective sleep quality

An actigraph (Actiwatch 2; Respironics Inc., PA, USA), worn on the non-dominant wrist, was used to measure objective sleep quality at 1-min intervals on two consecutive nights. The sleep statuses at each epoch, sleep onset, and sleep termination were automatically determined by Actiware version 5.5 (Respironics Inc.) using the default algorithm [16]. Epochs with higher activity counts than a moderate threshold (40 counts/min) were treated as awake. Sleep onset was defined as the first minute followed by a 10-min immobility period comprising ≥4 counts/min, and sleep termination was the last minute following a 10-min immobility period. Five actigraphic sleep parameters were determined using the following objective data (sleep status and sleep onset and termination) and self-reported data (bedtime and rising time): sleep efficiency (SE) [percentage calculated from time spent sleeping (below the activity threshold of 40 counts/min) between sleep onset and sleep offset divided by the time between bedtime and rising time]; wake time after sleep onset (WASO) [time spent awake (above the activity threshold of 40 counts/min) between sleep onset and rising time]; sleep onset latency (SOL) [time between bedtime and sleep onset]; total sleep time (TST) [sleep duration (time between bedtime and rising time) multiplied by SE]; and fragmentation index (FI) [100 × number of 1-min immobile epochs divided by the total number of immobile epochs between bedtime and rising time]. Further, potential sleep-disordered breathing was defined as SE < 70% and/or TST < 5 h because the odds ratio for sleep-disordered breathing is significantly higher in elderly populations [17].

Four subjective sleep parameters were determined using the Pittsburgh Sleep Quality Index questionnaire [18], which sleep parameters over the previous month are asked: SE (percentage calculated from the time spent sleeping divided by the time in bed), WASO (time in bed minus the time spent sleeping), SOL (time between bedtime and sleep onset), and TST (time spent sleeping).

### 2.3. Measurement of serum ADMA levels and renal function

After overnight fasting, venous blood was collected between 10:00 a.m. and 11:00 a.m. Serum samples were obtained by centrifugation and stored at −80 °C. A commercial laboratory (SRL Co. Inc., Tokyo, Japan) measured serum ADMA levels using high-performance liquid chromatography with a lower ADMA detection limit of 0.1 μmol/L. No sample had ADMA levels below the detection limit. The intra- and inter-assay coefficients of variation were 5.2% and 5.5%, respectively [19].

The estimated glomerular filtration rate (eGFR) was calculated using the formula from the Japanese Society of Nephrology-Chronic Kidney Disease Practice Guide [20]: eGFR (mL/min/1.73 $m^2$) = 194 × [serum creatinine (mg/dL)]$^{-1.094}$ × [age (years)]$^{-0.287}$ × 0.739 (if female). CKD was defined as eGFR < 60 mL/min/1.73 $m^2$. In an additional analysis, the formula from the CKD-Epidemiology Collaboration (EPI) definition for the Japanese population was also used: eGFR = 0.813 × 141 × min.(serum creatinine/κ, 1)$^{\alpha}$ × max.(serum creatinine/κ, 1)$^{-1.209}$ × 0.993$^{age}$ × 1.018 (if female), where κ is 0.7 for females and 0.9 for males, α is −0.329 for females and −0.411 for males, min. indicates the minimum serum creatinine/κ value or 1, and max. indicates the maximum serum creatinine/κ value or 1 [21].

### 2.4. Other measurements

Body mass index (BMI) was calculated as weight (kg) per [height (m)] [2]. Current smoking status and information on medication use were evaluated using a questionnaire. Hypertension was defined based on the medical history and current anti-hypertensive therapy. Medical history, current anti-diabetic therapy, and blood glucose levels (fasting plasma glucose levels ≥7.0 mmol/L and glycated hemoglobin levels ≥6.5% of the National Glycohemoglobin Standardization Program value) were used to define diabetes mellitus. Daytime physical activity was calculated as the average number of physical activity counts from getting out of bed in the morning to bedtime in the evening and was measured at 1-min intervals for 2 days using an actigraph (Actiwatch 2) worn on the non-dominant wrist.

### 2.5. Statistical analyses

Normally distributed variables are reported as mean ± standard deviation. Means and proportions between genders were compared using the unpaired *t*-test and chi-square test, respectively. Participants were divided into four groups according to renal function (CKD or non-CKD) and serum ADMA levels (above or below the median value); *P*-values for differences in objective and subjective sleep parameters between the reference group (non-CKD/low-ADMA) and the other groups were calculated using linear regression analysis with dummy variables. Multivariable linear regression models of associations were adjusted for potential confounders, such as age (≥70 vs. < 70 years), BMI (≥25 vs. < 25 kg/$m^2$), smoking status (yes vs. no), hypertension (yes vs. no), diabetes (yes vs. no), and daytime physical activity (100 counts/min). Statistical analyses were performed using SPSS version 19.0 for Windows (IBM SPSS Inc., Chicago, IL, USA). A two-sided *P*-value < 0.05 was considered statistically significant. Mediation analysis was performed using linear regression analysis and Sobel's test in PROCESS (using model 4) for SPSS ver. 2.16.3 [22]. Analysis included eGFR defined by the CKD-EPI as an independent variable, objective and subjective sleep parameters as dependent variables, and serum ADMA levels as the mediation variable.

## 3. Results

Mean age of the study participants ($n$ = 1115) was 71.9 ± 7.1 years and 588 (52.7%) were female. Significantly lower prevalence of diabetes mellitus and CKD were observed in females, and serum ADMA levels were significantly lower in females (Table 1). Compared with males, females had significantly better objective sleep quality, such as higher SE, shorter WASO, and lower FI, but subjective sleep quality such as SE and WASO were comparable.

Participants were divided into four groups according to renal function (CKD or non-CKD) and serum ADMA levels (above or below the median value); i.e. non-CKD/low-ADMA ($n$ = 315 for females, 213 for males), non-CKD/high-ADMA ($n$ = 182 for females, 198 for males), CKD/low-ADMA ($n$ = 37 for females, 53 for males), and CKD/high-ADMA ($n$ = 54 for females, 63 for males). The CKD/high-ADMA group exhibited significantly older age, higher BMI, higher prevalence of hypertension, and lower physical activity compared with those in the non-CKD/low-ADMA group (Supplemental Table 2). Among females, compared with the non-CKD/low-ADMA group, the group with CKD/high-ADMA demonstrated significantly worse objective sleep parameters, such as lower SE (86.1 vs. 81.8%), longer WASO (42.0 vs. 57.7 min), longer SOL (17.5 vs. 23.0 min), and higher FI (1.8 vs. 2.5). TST was comparatively longer in the two CKD groups than the non-CKD/low-ADMA group (Table 2). Regarding subjective sleep