

# Association between Night-shift Work and Serum 25-hydroxyvitamin D Levels in Japanese Male Indoor Workers: A Cross-sectional Study

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**Abstract:** Although low vitamin D status resulting from night work is a suspected cause of various health disorders, few studies have investigated the association between night-shift work and vitamin D status. Here, we examined serum 25-hydroxyvitamin D (25OHD) levels in 19 Japanese indoor workers, including night-shift workers, in blood samples collected at the annual medical checkup (late July) in a metal tool factory. Analyses were finally restricted to 14 male workers (33–59 yr) in 3 groups: fixed daytime work (n=6), and rotating shift work with (n=4) and without (n=4) night shifts. No significant differences in serum 25OHD levels were observed among the three groups ( $p=0.98$ , Kruskal-Wallis test). One to two participants in each group had 25OHD levels lower than the 20 ng/ml reference value for vitamin D deficiency even in summer. These results clearly indicate the need for large-scale studies to test the hypothesis that night-shift work is associated with lower 25OHD levels.

**Key words:** Work schedule, Night work, Shift work, Work hours, Vitamin D, Biomarker

## Introduction

Approximately 20% of the working population in developed countries works on shifts<sup>1</sup>. Night-shift work in particular has been shown to increase the risks for various health disorders, including cardiovascular diseases and endocrine cancers<sup>2–4</sup>. For example, a prospective study of nurses conducted in the U.S. revealed that night-shift work for  $\geq 20$  yr was associated with an elevated risk of hip and wrist fractures<sup>2</sup>. The increased risk of developing these disorders is also linked with

low vitamin D status<sup>5, 6</sup>, as individuals who work at night may be insufficiently exposed to sunlight which is related to the development of vitamin D deficiency<sup>7</sup>. Vitamin D-related problems have likely been exacerbated by the emergence of a 24-h society, in which increasing demands result in work hours that are spread across both day and night.

25-Hydroxyvitamin D (25OHD) is the primary circulating vitamin D metabolite, which possess a much longer biological half-life in human blood than other vitamin D metabolites and vitamin D itself, and is commonly used as an indicator of individuals' vitamin D status<sup>5</sup>. Exposure to solar ultraviolet B radiation changes 7-dehydrocholesterol to previtamin D by pho-

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tosynthesis in the skin<sup>5</sup>). Previtamin D is completely converted to vitamin D through thermal isomerization at body temperature<sup>5</sup>). Once formed, vitamin D is transported in the blood bound to vitamin D-binding protein, and converted into 25OHD in the liver<sup>5</sup>). Although low vitamin D status resulting from night work is a suspected cause of night-shift work-related health problems<sup>2</sup>), few studies have investigated the association between night-shift work and circulating 25OHD levels. To date, the findings related to 25OHD levels have presented mixed results<sup>8, 9</sup>). Clarification of the effect of night work on an individual's vitamin D status would allow the development of effective countermeasures for protecting night-shift workers from bone fracture and other hypovitaminosis D-related health problems.

In this cross-sectional study, we investigated the association between serum 25OHD levels and night-shift work in Japanese male workers.

## Methods

### *Subjects*

A cross-sectional survey was carried out in late July, 2009. Subjects were 42 annual medical checkup examinees who worked at a metal tool factory located in Osaka Prefecture, Japan (34.5°N), an area of high annual sunlight. In all, 25 workers aged 22–62 yr agreed to participate in the study. Of these, 19 workers provided fasting blood at their annual medical check-up examination conducted in late July. Participants completed self-administered questionnaires concerning job history, including work schedule and job category, and lifestyles, including sleeping habits and frequency of going outside during daylight hours (only during a week of night shifts). The examination also provided individual anthropometric, demographic, and health information, including age, gender, height, and body weight. Each participant provided signed informed consent to participate in the study, and the protocol was approved by the Institutional Review Board of the National Institute of Occupational Safety and Health, Japan.

Subjects were categorized into three groups based on their current work schedules as follows: fixed daytime workers (mainly 8:30–17:00), rotating shift workers without night shifts (mainly 8:30–17:00 and 13:10–21:35; otherwise, 8:30–17:00 and 10:30–19:00), and rotating shift workers with night shifts. The night-shift worker group consisted of three teams that rotated shifts weekly as follows: day shift (8:30–17:00), afternoon shift (13:10–21:35), and night shift (21:30–6:00). Since the factory operated on a five-day week, the individual number of night shifts was five per three-week period.

### *Laboratory assay for 25OHD*

Following blood collection, serum was separated by centrifugation, frozen on site, shipped on dry ice to the laboratory, and then stored at  $-80^{\circ}\text{C}$  until analysis. Samples were thawed only once, immediately prior to analysis. After the extraction of serum 25OHD with acetonitrile and centrifugation using standard procedures, 25OHD in each sample was measured using a radioimmunoassay (25-Hydroxyvitamin D  $^{125}\text{I}$  RIA Kit, DiaSorin, Inc., Stillwater, MN, USA) at a commercial clinical laboratory (SRL, Tokyo, Japan). All samples were measured on the same day in a single batch to exclude the confounding effects caused by between-day variation and to ensure the comparability of measured values. Laboratory analysts were not informed of the work schedules of the subjects. Five blinded quality-control samples were included in the batch analysis; the coefficient of variation for 25OHD was 33.3%. The coefficients of intra- and inter-day variation in routine quality-control data for 25OHD reported from the commercial laboratory ranged between 4.3–7.0% (at mean levels of 8.50, 14.10, and 50.92 ng/ml).

### *Statistical analyses*

Subjects were categorized into three groups by their work schedule, as described above. Initially, 19 participants donated their blood specimen. Women ( $n=4$ ) were excluded from the analyses because all night-shift workers were men. One male worker was also excluded from the analyses due to insufficient information concerning his work schedule. Ultimately, analysis was restricted to the remaining 14 male workers (aged 33–59 yr): 6 fixed daytime workers, 4 rotating shift workers without night shifts, and 4 rotating shift workers with night shifts.

The Kruskal-Wallis test was used to compare the basic characteristics and serum 25OHD levels among the three groups. Analysis of covariance (ANCOVA) was also performed with age (continuous) as a covariate. All analyses were two-sided and the statistical significance level was chosen as  $p<0.05$ . Statistical analyses were performed using the statistical analysis platform R, version 2.10.1 (R Development Core Team (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

## Results

Subject characteristics are summarized in Table 1. No statistically significant differences were observed among the three work schedule groups, with the exception of age (fixed daytime workers were older;  $p=0.02$ ).

**Table 1. Subject characteristics by work schedule**

Characteristic	Fixed daytime workers	Rotating shift workers without night shift	Rotating shift workers with night shift	<i>p</i> -value*
No. of men who provided blood specimens	6	4	4	—
Age [yr]	51.8 ± 6.1	43.5 ± 4.8	39.5 ± 4.7	0.02
Body mass index [kg/m <sup>2</sup> ]	23.6 ± 1.5	23.6 ± 1.5	22.6 ± 1.1	0.44
Working time per month [h] <sup>†, ‡</sup>	160 ± 8.2	141 ± 24.6	135 ± 15	0.21
Sleeping time [h] <sup>†</sup>	6.00 ± 0.63	6.00 ± 0.81	6.88 ± 0.63	0.14
Commuting time [min] <sup>†</sup>	51.7 ± 35.9	56.3 ± 20.6	41.3 ± 22.5	0.68
No. of subjects with 25OHD levels lower than 20 ng/ml	2	1	1	—

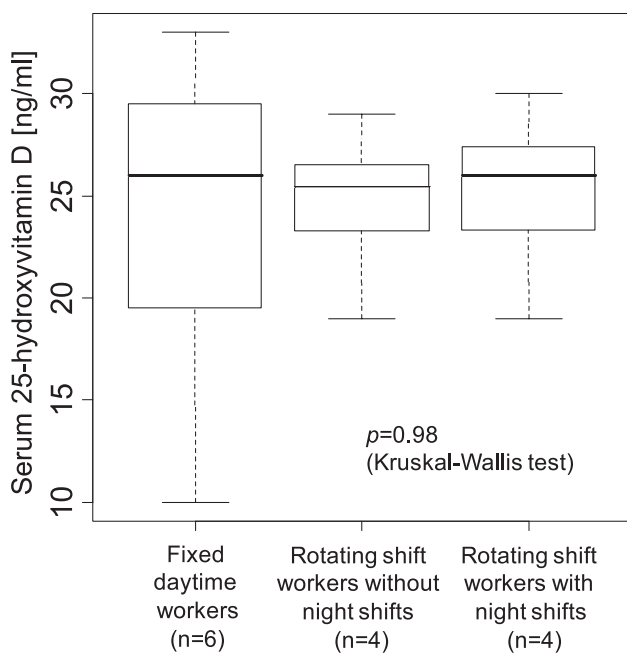
Data are presented as the mean ± SD, with the exception of the subject number.

\*Kruskal-Wallis test.

<sup>†</sup>Self-reported.

<sup>‡</sup>Sample size varies due to missing information (n=4, 4, and 3, respectively).

25OHD, 25-hydroxyvitamin D.



**Fig. 1. Serum 25-hydroxyvitamin D levels according to work schedule among Japanese male indoor workers.**

Bold horizontal lines represent the median of the distribution; values above and below these lines are divided into quartiles by horizontal lines (hinges of the box) represent the two quartiles nearest the midpoint. The whiskers extend to the most extreme data point, which is no more than 1.5 times the box length away from the box.

Although night-shift workers reported shorter working and commuting times, and longer sleeping hours than the other two groups, the differences were not statistically significant. Participants did not work overtime or outdoors, except for one fixed daytime worker who reported working both indoors (85%) and outdoors (15%).

The serum 25OHD concentrations of all collected blood samples were determined and compared (Fig. 1). Although serum 25OHD was detected in all samples, no significant differences were detected in its levels among

the three work schedule groups ( $p=0.98$ ). Medians and interquartile ranges in fixed daytime workers, rotating shift workers without night shifts, and rotating shift workers with night shifts were 26.0 (19.5–29.5), 25.5 (23.5–26.8), and 26.0 (23.5–27.8) ng/ml, respectively. Even after adjustment for age by ANCOVA, no significant differences were detected ( $p=0.87$ ). However, a few 25OHD levels ( $n=1$  or  $2$ ) were below the proposed threshold value for vitamin D deficiency of 20 ng/ml<sup>5, 10</sup>.

## Discussion

In this cross-sectional study, we investigated circulating 25OHD levels in Japanese indoor workers with one of three work schedules: fixed daytime, and rotating shift work with and without night shifts. Among these groups, serum 25OHD levels were not significantly different, contrary to our prediction of lower 25OHD levels in night shift workers based on expected insufficiencies in their sunlight exposure. Even though the study was conducted during a sunny time of year, a few participants in all groups had 25OHD levels lower than the threshold value for vitamin D deficiency of 20 ng/ml. This point should be emphasized. It is recommended that every indoor worker should be exposed to more ultraviolet radiation irrespective of his work schedule.

The apparent lack of association between night-shift work and 25OHD is consistent with a previous study that demonstrated serum 25OHD concentrations during summer did not significantly differ among physicians working at least one night shift a week, medical students, or indoor workers<sup>8</sup>). In addition, a comparison of female nursing-home workers with or without a single weekly night shift found no significant association between night-shift work and serum 25OHD concentrations measured in winter after controlling for relevant factors, such as age and sunshine duration<sup>9</sup>). As our

study was conducted in summer (July), the critical question of whether or not the 25OHD levels in mid-winter would differ among the three groups of workers is raised. Another study showed that most 25OHD levels in indoor daytime male workers were lower than 20 ng/ml in winter (February)<sup>11</sup>. The present findings are apparently not compatible with the case report of markedly low 25OHD (<12 nmol/l) in a 46-yr-old man who worked night shifts for 10 yr with minimal exposure to sunlight<sup>7</sup>.

In the present study, the mean age of fixed daytime workers was significantly higher than the other two groups, which was not unexpected as older workers are less likely to perform shift work. However, according to the ANCOVA that accounted for the effects of age, this factor did not influence the between-group differences in 25OHD concentrations. Although an inverse association between age and epidermal concentrations of 7-dehydrocholesterol, the precursor of vitamin D<sub>3</sub>, in the skin of Caucasian patients has been reported<sup>12</sup>, the association between age and 25OHD is not clear as conflicting results have been reported. According to multiple linear regression analyses, positive associations in Emirati and Norwegian women<sup>13, 14</sup>, and an inverse and no association in American elderly women and men, respectively, aged 67–95 yr<sup>15</sup> have been reported for 25OHD with age. After seasonal stratification, the direction and significance of the associations are inconsistent among seasons and studies<sup>13, 15</sup>.

Sunlight exposure during the night-shift week may have attenuated the association between night-shift work and 25OHD levels. Indeed, three of four participants in the rotating shift with night shift group occasionally went outside during the day according to their self-report. If the fixed daytime workers spent nearly all working time indoors, the night-shift workers may have received higher sunlight exposure than their daytime counterparts. Regrettably, there is no literature comparing sunlight exposure levels between fixed daytime workers and night-shift workers. Future studies which measure the difference in sunlight exposure levels between fixed daytime workers and night-shift workers by a method other than self-report may provide very important and meaningful results. A few of the serum samples from all the groups of indoor workers contained 25OHD levels that were lower than 20 ng/ml among the present indoor workers. The 25OHD levels of indoor workers are generally lower than those of outdoor workers<sup>16</sup>.

The present findings imply that factors other than low vitamin D may exist which increase the risk of bone fracture in night-shift workers. One possible candidate may be melatonin<sup>2</sup>, as this molecule is closely associat-

ed with bone health<sup>17</sup>. However, it may be difficult to determine its role independent of vitamin D, given that both biomarkers are affected by exposure to sunlight.

Several limitations of the study warrant mention. First, the small number of subjects limited the statistical power to detect differences among the worker groups. Although the present data should be considered as preliminary, the findings are of value, given the little evidence that exists concerning the association between night-shift work and 25OHD<sup>8, 9</sup>. Second, it is not clear how our results could be generalized to other shift-working men or women, even though it is known that 25OHD levels in men would be higher than those in women *a priori*<sup>6</sup>. Other limitations of this study include imprecision in the quantification of 25OHD, and the measurement of 25OHD based on one blood sample that was collected at a single time point, similar to the approach of past studies<sup>8, 9</sup>. A few studies have demonstrated that the radioimmunoassay for 25OHD is more reliable than competitive protein-binding assays<sup>18, 19</sup>, which is an important consideration as assay variation and standardization of 25OHD measurements are an important issue in this field of research<sup>19–22</sup>. Non-differential measurement error and subsequent misclassification would lead to a null association. Although the measurement of 25OHD using high performance liquid chromatography isotope-dilution tandem mass spectrometry is considered to be the most reliable approach<sup>21</sup>, the high cost of this equipment and the reagents limit their use.

In conclusion, the present results did not support the hypothesis that night-shift work is associated with lower vitamin D levels. Additional studies with larger numbers of subjects including women and various types of night shifts are required to confirm the association between night-shift work and vitamin D status.

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