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# *Higher 25-hydroxyvitamin D3 Levels Increase Optimal Daily Sleep Duration*

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***Abstract.*** Sleep disorders have risen to prominence as a major priority in the context of global public health. As the academic community has deepened its understanding of the possible impact of 25-hydroxyvitamin D3 (25OHD3) on sleep behavior, it has also attracted much attention in the field of sleep medicine. The study wanted to see if there was a relationship among circulating 25OHD3 levels and optimal sleep duration in the human body. We conducted a cross-sectional study using 2021 - 2023 National Health and Nutrition Examination Survey (NHANES) data. We drew a flow chart to screen the final study population, used a weighted linear regression model for analysis, and also performed a sensitivity analysis to see if the model was stable. In accordance with NHANES requirements, we used sample weights to ensure that the data was representative of the entire United States. After controlling for race, sex at assigned birth, age and degree of staying in the shadows in overall samples, we found that higher serum 25OHD3 concentrations were associated with longer sleep periods. Group analysis showed that this association was most evident among adults aged 20-35. Among individuals aged 20 to 35 years, a positive linear correlation was identified between serum 25-hydroxyvitamin D3 (25OHD3) concentration and sleep duration.

***Keywords:*** serum 25-hydroxyvitamin D3 (25OHD3), sleep duration, NHANES (National Health and Nutrition Examination Survey)

## 1. Introduction

Sufficient, good-quality sleep is beneficial to the body; it also helps enhance our thinking abilities and regulate emotions [1-4]. Studies have found that sleeping 6-8 hours is the most appropriate, and sleeping too little or too much may make people more likely to get sick or even die [5-6]. However, if you don't sleep well for a long time, it may disrupt normal sleep patterns, cause body inflammation, and increase the risk of problems such as obesity, mental illness and heart diseases [1-3, 7].

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Sleep problems are a major global public health challenge. A study on global sleep conditions showed that more than 850,000,000 adults aged 20 and older (16.2% of the global population) have clinically significant sleep disorders, and nearly half of them have severe symptoms [8]. In the United States alone, more than 50,000,000 people suffer from sleep problems; roughly one-third of adults report symptoms of insomnia and 10%-15% suffer from impaired daytime function [9].

Sleep time is related to many dietary factors, of which 25-hydroxyvitamin D3 (25OHD3) is particularly important because it plays many key roles in the body and can also regulate various physiological functions [10-12]. To assess whether an individual has adequate vitamin D levels, doctors rely on 25OHD3, which serves as the primary form of vitamin D circulating in the body [13]. If the 25OHD3 in the blood is less than 50 nmol/L, it means that vitamin D is deficient; if it is less than 525 nmol/L, it means that vitamin D is deficient [14]. In addition to being important for bone growth, too low 25OHD3 is also related to many problems, such as depression, cancers, immune system problems, respiratory diseases, and type 1 diabetes [15-21].

Previous studies have found that there is a relationship between serum 25OHD3 concentration and sleep-the higher the 25OHD3 level, the better the objective sleep quality and the longer sleep time [22-24]. But these studies have several obvious problems. First, they usually only studied specific groups of people, such as children, the elderly, or residents of a community [12, 22 -24]. Our study used a larger, more representative sample and randomly selected people aged 20 and older across the United States, making the results more reliable. In addition, past studies have not clarified standards for healthy sleep duration and have ignored the possible health risks of sleeping too long (>8 hours) [12, 22]. This time we define healthy sleep as 6-8 hours, so that the association linking serum 25OHD3 to healthy sleep patterns can be analyzed with greater accuracy through our approach.

For this research, we wanted to see if there was a correlation between blood levels of 25OHD3 and healthy weekday sleep. We hypothesized that if serum 25OHD3 levels were higher, then it would be more likely to sleep within the recommended 6 to 8 hours per day.

## 2. Methods

### 2.1. Study design

The study used data from the 2021 - 2023 National Health and Nutrition Examination Survey (NHANES) for a cross-sectional evaluation. To estimate the health and nutritional profiles of non-hospitalized U.S. residents, NHANES employs a probability sampling approach that operates in multiple stages. This analysis extracted sleep time data on weekdays and weekends, and also measured serum 25-hydroxyvitamin D3 (25OHD3) concentrations.

### 2.2. Participants selection

Initially, 11,933 people participated (5,575 men and 6,358 women), all aged 20 to 59. Later, some people were removed because they lacked important information, such as sleep duration, serum 25OHD3 levels, degree of staying in the shadow, marital status, and income-to-poverty ratio. After removing these people, 2,625 people were left for analysis. Details regarding the selection of participants are outlined in Figure 1.

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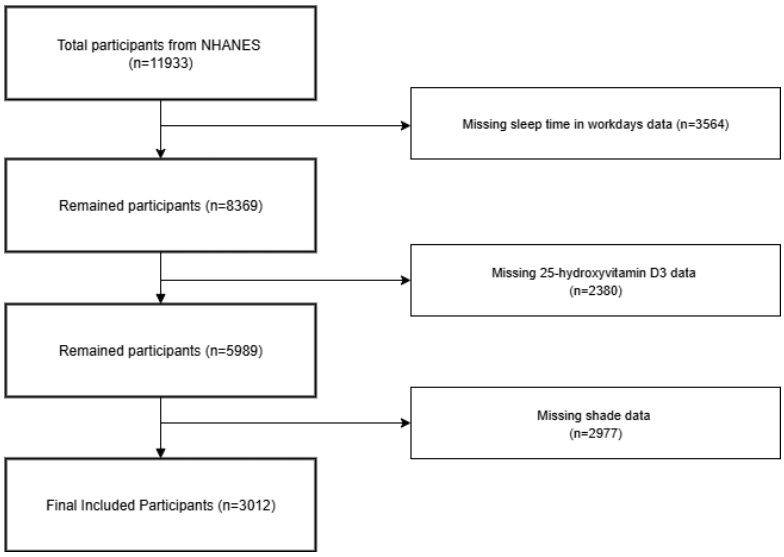



Figure 1. A flow diagram illustrating how participants were selected and included in the study

## 2.3. Serum 25-hydroxyvitamin D3 levels (Exposure)

NHANES uses high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) to measure the total 25OHD3 concentration in serum, in nanomoles per liter (nmol/L). Using this method, NHANES measured reliable serum values of 25OHD3 in 7,308 study participants.

## 2.4. Sleep duration (outcome)

Trained interviewers used the Computer-Assisted Personal Interviewing (CAPI) platform to collect information on everyone's sleep time during face-to-face home visits. Respondents provided their usual total sleep time on weekdays and weekends. We obtained total weekday sleep data for 8,318 participants. In this analysis, sleeping 6-8 hours a day was classified as the optimal sleep time.

## 2.5. Covariates (other factors)

We collected basic data through unified household surveys, including sex assigned at birth, age, race, household income-to-poverty ratio, and education level. The age grouping is coding like this: people under 35 years old are labeled as 1, and people 35 years old and above are labeled as 0. There are three levels of education: a high school diploma or lower, some college or associate's degree, and bachelor's degree or higher. Ethnic groups are divided into Mexican American, white, black and others. Age, sex at assigned birth assigned at birth, and race were used as covariates that may affect the exposure: serum 25OHD3 levels. In addition, we also consider shade exposure as a possible confounding factor. Participants were asked how often they stayed in the shade when spending more than one hour outside on a very sunny day. The responses were divided into "always", "sometimes" or "never" in the shade to measure how much they avoided the sun.

## 2.6. Statistical analysis

In accordance with NHANES regulations, we use different sample weights to ensure that the data is representative of the national situation. The Phone Follow-Up Subsample 2-Year Weight

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(WTPH2YR) was used to measure serum 25OHD3 concentrations, while the Interview Subsample 2-Year Weight (WTINT2YR) was used for sleep duration and other data in the questionnaire. In order to investigate the link among serum 25OHD3 and daily sleep time, we performed a weighted linear regression analysis. First, we made the simplest model without taking into account other confounders, then added age and sex at assigned birth, and finally added race. Before doing the regression, we took the logarithm of the value of serum 25OHD3 levels, which makes the data more concentrated. To see if the model is stable, we also did sensitivity analysis, removing the extreme values of sleep time and serum 25OHD3 levels to see if the results changed. All statistical tests were two-sided, with $p < 0.05$ deemed statistically significant. Where multiple testing was involved in the analyses, Bonferroni's adjustment was implemented to establish the appropriate p-value threshold for determining significance, as required. The entire analytical process was conducted utilizing R software, specifically version 4.4.0.

## 3. Results

### 3.1. Baseline characteristics

The study's population profile (N = 3,012) showed that among the average non-institutional adult in the United States, the sex at assigned birth was roughly equal. Table 1 shows that the average age of the participants was 39.9 ± 11.5 years. Most people are married (62.2%) and consider themselves White (56.6%). The average concentration of 25OHD3 in serum was 68.4 ± 32.1 nmol/L.

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Table 1. Demographic characteristics[1] of participants

| Characteristic | Total |
|---|---|
| N | 3,012 |
| Age (mean (SD[2])) | 39.9 (11.5) |
| sex at assigned birth, n (%) | |
| Male | 1,476 (49.0) |
| Female | 1,536 (51.0) |
| Marital Status, n (%) | |
| Married | 1,873 (62.2) |
| Never married | 759 (25.2) |
| Widowed/Divorced/Separated | 380 (12.6) |
| Race, n (%) | |
| White | 1,705 (56.6) |
| Black | 313 (10.4) |
| Mexican | 262 (8.7) |
| Other | 732 (24.3) |
| Education, n (%) | |
| High school diploma/GED or below | 903 (30.0) |
| Some college or AA degree | 1,159 (38.5) |
| College graduate or above | 949 (31.5) |
| Degree of staying in the shadow[3] (%) | |
| Many | 1,036 (34.4) |
| Moderate | 1,292 (42.9) |
| Few | 684 (22.7) |
| 25OHD3 (mean (SD)) | 68.4 (32.1) |

[1] All variables adjusted for 2020-2023 NHANES complex survey design weights to be representative of the United States; missing data exist for education variables wherein categories do not add up to the column total.

[2] SD, standard deviation

[3] Defined by NHANES questionnaire question: “When you go outside on a very sunny day, for more than one hour, how often do you stay in the shade?”

### 3.2. The association among 25OHD3 levels and sleep duration

The original study analyzed the relationship between serum 25OHD3 concentrations and sleep time in Table 2. In Model A without adjusting for other factors, the effect estimate is β=0.123 (95% CI: −0.007 to 0.252), which means that for every additional unit of log conversion of 25OHD3, sleep time will increase by 0.123 hours. When age and sex at birth (Model B) were taken into account, the estimate became β=0.193 (95% CI: 0.060 to 0.325), which was 56.9% higher than Model A, indicating that age and sex at assigned birth affected the initial results. In Model C, which adjusted for more factors for additional covariates, the estimate was β=0.171 (95% CI: 0.025 to 0.317), which was 11.4% lower than Model B, but the relationship was still statistically significant.

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Table 2. Weighted linear regression results for sleep duration in workdays (hrs.)

| Characteristic | Model A | Model B | Model C |
|---|---|---|---|
| log of 25OHD3 | 0.123 (-0.007, 0.252) 0.061* | 0.193 (0.060, 0.325) 0.008* | 0.171 (0.025, 0.317) 0.026* |

*Values represent regression coefficients (β) with 95% confidence intervals in parentheses and p-values.

Model A: No covariates were adjusted

Model B: Adjusted for age and sex at assigned birth

Model C: Adjusted for age, sex at assigned birth, race, and degree of staying in the shadows

### 3.3. The relationship between 25OHD3 levels and sleep duration

Stratified analysis found that the relationship between serum 25OHD3 concentration and people's sleep time varied greatly among different groups of people. In Table 3, when grouped by sex at assigned birth, there was a significant positive correlation for men (β = 0.29, 95% CI: 0.11 - 0.47) and no significant correlation for women (β = 0.07, 95% CI: −0.09 - 0.23). When grouped by ethnicity, Black participants had the strongest association (β = 0.33, 95% CI: 0.11 - 0.54), while trends were not evident for White (β = 0.15, 95% CI: −0.01 - 0.32), Mexican (β = 0.16, 95% CI: −0.13 - 0.46), and other races (β = 0.14, 95% CI: −0.13 - 0.41). In Figure 2, the association was greatest among young people aged 20-35 years (β = 0.46, 95% CI: 0.22 - 0.71), and the older they were, the weaker the effect (35-45 years: β = 0.08, 95% CI: −0.15 - 0.30;45-59 years: β = −0.06, 95% CI: −0.27 - 0.14).

To formally evaluate effect corrections, we added interaction terms to the regression analysis. The interaction between 25OHD3 and age significantly improved the model fit (ANOVA p = 0.013), demonstrating that age does affect the results. On the other hand, when the interaction terms of gender (p = 0.100) or ethnicity at birth (p = 0.673) were added, the model's fitness did not improve significantly compared to Model C. Then in the new model with the interaction terms, as shown in Figure 3, <35 years group (blue circle line): As Vitamin D concentration increases, sleep duration increases significantly, showing a clear upward trend. And for other lines, the corresponding trends are stable, indicating that the effect of Vitamin D on sleep duration could be tiny.

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Table 3. Association between 25OHD3 and sleep duration in different demographic subgroups

| Group | N | β (95% CI[1]) |
|---|---|---|
| Stratified by sex at assigned birth | | |
| Male | 1,318 | 0.29 (0.11, 0.47) |
| Female | 1,694 | 0.07 (-0.09, 0.23) |
| Stratified by race | | |
| White | 1,581 | 0.15 (-0.01, 0.32) |
| Black | 391 | 0.33 (0.11, 0.54) |
| Mexican | 274 | 0.16 (-0.13, 0.46) |
| Other | 766 | 0.14 (-0.13, 0.41) |
| Stratified by age (years) | | |
| 20-35 | 1,017 | 0.46 (0.22, 0.71) |
| 35-45 | 861 | 0.08 (-0.15, 0.30) |
| 45-59 | 1,134 | -0.06 (-0.27, 0.14) |

[1] CI, confidence interval

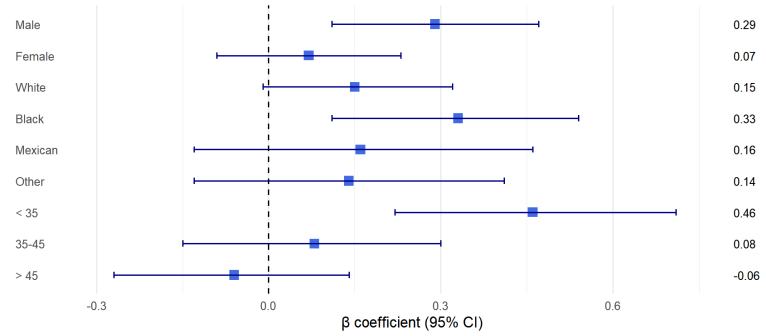



Figure 2. Table 3 presents a forest plot that includes effect size estimates for reaction outcomes, along with their corresponding 95% confidence intervals.




Figure 3. The role of age as a moderator in the dose-response relationship linking 25OHD3 levels to sleep duration

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## 4. Discussion

The study found that only 61.8% of people met the recommended 6-8 hours of sleep, indicating that sleep deprivation is common among adults. This association is particularly evident in people under the age of 35, and serum 25OHD3 concentrations increase steadily with increasing sleep time over the 6-8 hour range. These results suggest that public health needs to pay more attention to sleep-too little or too much sleep can affect health, which may explain broader health inequalities.

People with higher blood 25OHD3 levels experienced better sleep quality longer, which is consistent with previous studies [12, 22, 24, 25]. Why can higher 25OHD3 levels prolong healthy sleep? There may be several reasons [26-31]. For example, vitamin D receptors (VDRs) are ubiquitous in the suprachiasmatic nucleus (SCN) of the hypothalamus, which controls our biological clock [27-28]. Therefore, changes in 25OHD3 can affect the signals emitted by VDR, triggering a series of sleep-related reactions that ultimately change sleep time [26, 29]. Another possibility is that too low 25OHD3 will cause the brain to produce less serotonin, which may cause sleep breathing problems and undermine healthy sleep [30-31].

Findings from a number of prior studies align with our results, as they too identified a positive association between circulating 25OHD3 levels and sleep duration across various age groups. For example, a study of primary school students in China showed that there was a significant link among low 25OHD3 levels and short sleep time, which is consistent with our findings after adjusting for other influencing factors-the higher the 25OHD3 level, the longer the sleep time [32]. In the children group, studies also found that children with 25OHD3 deficiency had shorter sleep times and lower sleep efficiency, which further supports a possible biological link between vitamin D and sleep regulation [12]. In addition, a randomized controlled trial of vitamin D supplementation to adults aged 20-50 showed improvements in multiple sleep indicators, including sleep time, which also confirmed our observation that increased 25OHD3 levels and extended sleep time There is a linear relationship, especially in those with lower 25OHD3 levels [33]. Overall, these studies support the view that 25OHD3 levels may affect sleep time and quality.

However, other studies have not found a fixed link between 25OHD3 levels and total sleep time, which is different from our results. For example, the German community sample study said that overall 25OHD3 levels have little to do with how long people sleep, and they focus more on specific sleep indicators such as midsleep time [22]. The Japanese DOSANCO Health Study found that serum 25OHD3 levels was associated with subjective sleep quality and sleeping pill use, rather than objective sleep duration. After adjusting for depression and overall health factors, these associations weakened, suggesting that other conditions may have influenced outcomes. Different studies used different variables and population characteristics, which may explain the differences in results [34].

Further subgroup analysis showed that people with higher serum 25OHD3 concentrations tended to sleep longer. This relationship is most evident in men, probably because men have a stronger 25OHD3 receptor response and are more sensitive to changes in circadian rhythm [35]. Strangely enough, this connection was not found in women aged 35-59, which may be related to the reduction of melatonin caused by aging, the effects of steroid hormones in vivo and in vitro, and gender-related hormone differences at birth [36, 37]. The study also found a clear association between Black race and lower 25OHD3 levels, which is consistent with previous studies-people with darker skin had lower overall 25OHD3 levels than people with lighter skin; racially related socioeconomic factors may further reduce 25OHD3 concentrations [38, 39].The strength of this study is its inclusion of the general adult population, unlike prior research that has typically limited its focus to either older adults or younger individuals. This wider coverage allows for a more thorough understanding of how 25OHD3 levels are associated with sleep duration in adults. Furthermore, the

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study employed stratified analyses to examine this relationship both within the overall sample and across various subgroups, a method that is rarely employed in existing research.

However, this study has several limitations. First, because it is a cross-sectional study, it cannot determine causality; a longitudinal cohort study is needed to further explore these associations. In addition, ecological studies can assess 25OHD3 concentration levels in people in different regions. Another limitation is that the research relies on self-reported data, which introduces the possibility of errors and information-related biases. Finally, possible distractions such as chronic diseases (such as diabetes and depression) and dietary factors were not taken into account and could affect the results of the study.

## 5. Conclusion

In this study of adults aged 20-59 in the United States, we found that the relationship between 25OHD3 and sleep time was only true in people under the age of 35, and age influenced this effect. What is particularly important is that for young people, increasing the 25OHD3 level from 30 nmol/L to 110 nmol/L can increase sleep time by about 0.4 hours. Future research should explore the relationship between age and 25OHD3 status through stratified analysis. Long-term follow-up research can also help us confirm these findings and understand the possible reasons behind them.

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