

# Sleep and Antibody Response to Hepatitis B Vaccination

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**Study Objectives:** Experimental evidence links poor sleep with susceptibility to infectious illness; however, it remains to be determined if naturally occurring sleep is associated with immune responses known to play a role in protection against infection. The aim of this study was to determine whether sleep duration, sleep efficiency, and sleep quality, assessed in the natural environment, predict magnitude of antibody responses to a novel antigen among community volunteers in midlife.

**Design:** Observational.

**Measurements and Results:** Healthy midlife adults ( $n = 125$ ; 70 female; age 40-60 yr) received the standard 3-dose hepatitis B vaccination series. Actigraphy and electronic sleep diaries were used to assess sleep duration, sleep efficiency, and subjective sleep quality. Viral-specific antibody titers were obtained prior to the 2nd and 3rd vaccination to assess primary and secondary antibody responses. Clinical protection status (anti-hepatitis B surface antigen immunoglobulin G  $\geq 10$  mIU/ml) was assessed 6 mo after the final immunization. Regression analyses revealed that shorter actigraphy-based sleep duration was associated with a lower secondary antibody response independent of age, sex, body mass index, and response to the initial immunization. Shorter sleep duration, measured by actigraphy and sleep diary, also predicted a decreased likelihood of being clinically protected from hepatitis B at the conclusion of the vaccination series. Neither sleep efficiency nor subjective sleep quality were significant predictors of antibody response.

**Conclusions:** Short sleep duration in the natural environment may negatively affect *in vivo* antibody responses to novel antigens, providing a possible explanation for observed associations of poor sleep with increased susceptibility to infectious disease.

**Keywords:** Sleep, antibody, vaccination, immune function

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## INTRODUCTION

Short sleep duration (< 6 or 7 hr/night) and poor sleep efficiency predict increased risk for several chronic diseases,<sup>1-5</sup> susceptibility to acute infectious illness,<sup>6,7</sup> and all-cause mortality.<sup>8-10</sup> Experimental sleep deprivation studies support the immune system as a potential mechanism linking sleep disturbance and disease risk. Indeed, acute and chronic sleep loss interferes with immune processes integral to host resistance,<sup>11</sup> including reductions in natural killer cell activity,<sup>12,13</sup> T-cell function,<sup>14,15</sup> shifts in helper T-cell cytokine production,<sup>16,17</sup> and increases in levels of proinflammatory cytokines.<sup>18-26</sup>

Much of the research linking sleep and immunity has been limited to *in vitro* measures of immunocompetence that are often of unknown clinical significance and provide a poor estimate of the body's host defense against disease. Consequently, researchers have turned to *in vivo* measures of immunity, including antibody response to vaccination. Preliminary experimental evidence demonstrates that young, healthy par-

ticipants deprived of sleep in the laboratory produce fewer antibodies to hepatitis A,<sup>27,28</sup> hepatitis B,<sup>28</sup> and influenza vaccines.<sup>29,30</sup> It remains unclear, however, whether these findings generalize beyond young adults or accurately reflect natural variation in sleep observed outside the laboratory. Recent prospective evidence shows that adults who sleep for shorter periods of time or with lower efficiency are more likely to develop a biologically verified upper respiratory infection after exposure to a rhinovirus than those who sleep better.<sup>6</sup> These findings raise the possibility that sleep patterns may affect immune processes relevant for protection against infection. To explore this possibility, the following study examined whether sleep duration, efficiency, and subjective quality predicted antibody response to hepatitis B vaccination among healthy, midlife community volunteers who were naïve to the hepatitis B antigen. Antibody response to this vaccination provides an *in vivo* measure of the competence of the immune system to respond when exposed to a novel antigen.

## METHODS

### Participants

Data for the current study were derived from the Vaccination Immunity Project, a longitudinal study examining associations of psychosocial, physiologic, and behavioral factors with antibody response to hepatitis B vaccination. Participants were 70 women and 55 men recruited via mass mail solicitation in Western Pennsylvania (primarily Allegheny County). Eligible participants were nonsmokers, in good gen-

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eral health (including no history or symptoms of myocardial infarction, asthma, cancer treatment in the past year, current or past psychiatric illness, or other systemic disease known to affect the immune system), and free from medications known to affect the nervous, endocrine, or immune systems in the past 3 mo (not including oral contraceptives). Women who were pregnant or lactating were ineligible to participate. In addition, participants more than 30% overweight, as estimated by sex-specific height-weight tables,<sup>31</sup> were excluded. Prior to full enrollment, blood samples were drawn from otherwise eligible participants to assess levels of hepatitis B surface antigen (HBsAg) and antibodies to hepatitis B core and surface antigens (anti-HBc and anti-HBs), indicating current or past exposure or prior vaccination, respectively. Individuals who showed any serologic evidence of prior exposure to the antigen were excluded.

### Procedures

All participants were administered the standard 3 20- $\mu$ g doses of recombinant hepatitis B vaccine (Engerix-B, Glaxo SmithKline, Research Triangle Park, NC) administered into the deltoid muscle. The 1st and 2nd doses were administered 1 mo apart, followed by a booster dose at 6 mo. Because all participants were naïve to the hepatitis B antigen at baseline, antibodies produced in response to the initial immunization constituted a primary antibody response, whereas responses recorded after doses 2 and 3 constituted secondary antibody responses. For the 7 days surrounding each of the 3 vaccinations (3 days before, the day of, and 3 days afterward), participants completed electronic diaries assessing bedtime, wake time, and subjective sleep quality. A subgroup of participants ( $n = 104$ ) also wore actigraph watches over the same 7-day period surrounding the 1st immunization. Blood was drawn immediately before administration of the 2nd and 3rd dose of the vaccine to assess primary and secondary antibody responses, whereas the blood drawn 6 months after the final vaccination assessed clinical protective status (anti-HBs immunoglobulin G (IgG)  $\geq 10$  mIU/ml). Participants were paid \$230 for participating in the study. Informed consent was obtained from all participants in accordance with the University of Pittsburgh Institutional Review Board.

### Hepatitis B Antibody Levels

Blood samples for the determination of hepatitis B antibodies were allowed to coagulate and were centrifuged, and the serum was removed and frozen at  $-80^{\circ}\text{C}$  until analysis. Frozen samples were sent to Central Laboratory Services (University of Pittsburgh Medical Center) for the determination of antibody titers by enzyme-linked immunoassay using commercially available kits (Abbott Laboratories, Abbott Park, IL). Antisera with known titers were used to determine the international units (IU)/ml of antibody in each sample. If antibody levels were greater than 1,000 mIU/ml, the highest levels detectable by the enzyme-linked immunoassay methods, they were sent to a commercial laboratory (Arup Laboratories, Salt Lake City, UT) where they were diluted and re-run. This permitted the quantification of high antibody levels. Excellent reliability was observed between the two laboratories ( $r = 0.998$ ).

## Sleep Measures

### Actigraphy

Actigraphy was used to obtain an objective measure of sleep duration and efficiency collected continuously on 6 consecutive nights (3 nights before and 3 nights after the initial immunization). Participants wore an actigraph (Actiwatch-64; Respironics, Bend, OR) on their nondominant wrist to assess rest and activity; these findings provide behavioral data to infer sleep/wake patterns. Data were collected in 1-min epochs and validated manufacture-supplied software (Actiware 5.02, Minimitter, Inc., Bend, OR) was used to estimate sleep patterns, which were averaged over the 6 nights of collection. Actigraphy has been shown to be a reliable and valid measure of sleep behavior in healthy community samples.<sup>32,33</sup> Because actigraphy was added after initiation of this study, only a subset of participants ( $n = 104$ ) wore wrist activity monitors. There were no significant sociodemographic differences between participants who did and did not complete the actigraphy protocol.

Actigraphy data was lost on 11 participants due either to hardware malfunction or user error, yielding 93 participants available for data editing. To obtain a stable average of actigraphy-derived sleep duration and efficiency at least 4 days of complete data were required, which further reduced the sample to 88 participants.

### Sleep diaries

All participants ( $n = 125$ ) completed electronic diaries (Palm Zire21, Sunnyvale, CA) each morning of the 7-day period surrounding each of the three vaccinations to assess sleep times. In the case of the first vaccination period, the sleep times were used to set rest intervals for actigraphy editing. In addition, self-reported bedtimes, minutes until sleep onset, and wake times were also used to calculate subjective sleep duration. Participants also rated the quality of the past night's sleep (from 1 = very poor to 4 = very good), providing a measure of subjective sleep quality. Sleep diary measures were averaged over the 7 days surrounding the first immunization to coincide with actigraphy measurement as well as over the 21 days of collection in this study.

### Other Variables

A number of sociodemographic variables previously shown to be related to antibody response to hepatitis B immunization<sup>34-37</sup> were assessed and treated as covariates in analyses, including age, sex, and body mass index (BMI;  $\text{kg}/\text{m}^2$ ). Because previous studies support an inverse association between psychological stress and antibody response to vaccination,<sup>38-40</sup> participants also completed the 4-item Perceived Stress Scale (PSS)<sup>41</sup> 5 times per day via electronic daily diary to obtain an average level of daily stress over the 7 days surrounding each of the three vaccination periods. The PSS is a well-validated measure for assessing stress perception; psychometrics of this measure are good with internal consistency reported to be above 0.84 and test-retest reliability above 0.85.<sup>41</sup> Similar to the sleep diary data, the diary stress measures were averaged over the 7 days surrounding the first immunization to coincide with actigraphy measurement as well as over the 21 days of diary collection.

## Statistical Analyses

All analyses were performed using Predictive Analytics Software (PASW), Version 18.0 (SPSS: An IBM Company, Armonk, NY). Viral-specific antibody titers were measured after all 3 vaccinations of the series. Consistent with existing literature,<sup>42</sup> only 25% of participants (31 of 125) mounted detectable levels of antibody after the first vaccination. Thus, individuals were categorized as early responders or early nonresponders. In contrast, 89% of the 123 individuals on whom we had antibody levels after the second vaccination had detectable levels. One participant was a clear outlier (> 9 standard deviations above the mean) and was dropped from analyses, leaving 122 participants with secondary antibody data. Antibody levels to the second immunization were natural log-transformed before analysis. This transformation was successful in yielding a normal distribution of the data. Finally, antibody levels measured 6 mo after the final vaccination were used to assess whether participants had mounted a clinically protective response to the vaccination series. Here, 18 of 123 participants failed to meet the accepted clinical threshold for protection (anti-HBs IgG  $\geq$  10 mIU/ml).<sup>43</sup> Consequently, a dichotomous variable was created (protected versus unprotected) to examine associations between sleep and clinical protection status.

Unadjusted and adjusted binary logistic and linear regression analyses were computed to predict primary and secondary antibody responses and clinical protection status to the vaccination. In adjusted analyses, sociodemographic covariates, including age, sex, and BMI, were entered in the first step of the model, with the sleep measure entered in the second step. In separate models, sociodemographic covariates were entered in the first step followed by the measure of psychological stress in the second step, with the sleep measure entered in the final step. Preliminary analyses found that responders to the first immunization mounted higher antibody levels to the second injection ( $r = 0.22$ ,  $P < 0.05$ ). Thus, in models predicting antibody levels to the second vaccination, responder status (responder versus nonresponder) to the first immunization was also entered as a covariate in the first step. Because secondary antibody responses were natural log-transformed, predicted geometric means were calculated to aid in interpreting magnitude of effect. Additionally, to better clarify associations of sleep with hepatitis B clinical protection status and provide clearer estimates of effect sizes, we fit regression equations using categorical measures of sleep (approximate tertiles) and reported odd ratios (ORs) and 95% confidence intervals (CIs).

## RESULTS

### Sample Characteristics

The sample included 125 relatively healthy participants (56% female, 91.2% Caucasian) age 40 to 60 yr (mean = 50.1  $\pm$  5.4 yr). Sociodemographic characteristics, sleep averages, and antibody levels are provided in Table 1. Antibody levels were largely unrelated to sociodemographic characteristics, with the exception of sex. In this regard, women displayed higher secondary antibody levels ( $t(120) = 3.83$ ,  $P < 0.001$ ) and were more likely to be clinically protected at the conclusion of the vaccination series ( $X^2(1) = 6.87$ ,  $P < 0.01$ ) than men. Antibody levels were unrelated to diary measures of perceived stress ( $P > 0.35$ ).

**Table 1**—Sociodemographic characteristics, sleep averages, and anti-HBs IgG antibody levels for the study sample

Variable	Mean (SD) or Percentage
Gender (% Female)	56%
Age (years)	50.1 (5.4)
Race (% Caucasian)	91.2%
Body Mass Index (kg/m <sup>2</sup> )	25.2 (3.3)
Education: Some college (%)	92.5%
Employment Status (% Full time)	64.8%
Family Income (%)	
< \$50,000	31.2%
\$50,000-\$74,999	20.0%
\$75,000-\$99,999	13.6%
$\geq$ \$100,000	24.8%
No response	10.4%
Diary-based perceived psychological stress score <sup>a</sup>	5.1 (2.8); 4.9 (2.8)
Actigraphy-based sleep duration (hrs)	6.7 (0.8)
Actigraphy-based sleep efficiency (%)	80.6 (7.1)
Diary-based sleep duration (hrs) <sup>a</sup>	7.1 (0.7); 7.0 (0.8)
Diary-based subjective sleep quality (very poor [0-4] very good) <sup>a</sup>	3.2 (0.4); 3.2 (0.4)
Primary antibody levels (mIU/ml)	4.76 (26.5) median = 0.0
Secondary antibody levels (mIU/ml)	96.0 (217.4) median = 23.1
% clinically protected ( $\geq$ 10 mIU/ml)	84.8%

<sup>a</sup>Values of diary measures averaged over the 21 days of collection and the 7 days surrounding the first immunization.

Older adults displayed more efficient sleep ( $r = 0.23$ ,  $P < 0.05$ ) and reported better sleep quality ( $r = 0.18$ ,  $P < 0.05$ ). Actigraphy-derived sleep duration was positively correlated with diary-based sleep duration averaged over the three collection periods ( $r = 0.75$ ,  $P < 0.001$ ) and when constrained to diary-based sleep duration measured over the 7 days surrounding the first vaccination period ( $r = 0.86$ ,  $P < 0.001$ ). Actigraphy-derived sleep duration was also associated with actigraphy-derived sleep efficiency ( $r = 0.26$ ,  $P = 0.01$ ). Conversely, subjective sleep quality was unrelated to actigraphy-derived measures of efficiency, duration, and diary-based sleep duration ( $P > 0.20$ ).

### Sleep and Antibody Response

Initial bivariate analyses showed no associations of actigraphy-derived sleep efficiency, diary-based sleep duration, or subjective sleep quality with magnitude of secondary antibody response. In contrast, shorter sleep duration, as measured by actigraphy, was associated with lower secondary antibody levels ( $r = 0.23$ ,  $P = 0.04$ ). Linear regression analyses, adjusting for age, sex, BMI, and response to the initial dose of vaccine, continued to support this association;  $F(5, 79) = 5.35$ ,  $P < 0.001$ ;  $\Delta R^2 = 0.04$ ;  $b = 0.44$ ,  $SE = 0.22$ ,  $P < 0.05$  (Table 2). To illustrate this relationship (Figure 1), sleep duration was categorized into approximate tertiles (< 6 hr per night,  $n = 19$ ; 6-7 hr per night,  $n = 37$ ; > 7 hr per night,  $n = 29$ ). Estimated from

predicted geometric means (model adjusted for age [mean = 50.4 yr], sex [female], BMI [mean = 25.3 kg/m<sup>2</sup>], and responder status [nonresponder]), each additional hr of sleep was associated with a 56% increase in secondary antibody levels. Shorter sleep duration remained an independent predictor of secondary antibody response after further adjustment for diary-based perceived psychological stress averaged over 21 days of daily stress assessment (b = 0.47, SE = 0.22, P = 0.04); however, this association was slightly attenuated when adjusting for diary-based perceived psychological stress averaged over the 7 days of the first vaccination (b = 0.42, SE = 0.22, P = 0.065). Sleep measures were unrelated to likelihood of a detectable primary antibody response, with the exception of actigraphy-derived

sleep efficiency (r = -0.25, P = 0.02); however, this association did not withstand covariate adjustment (OR, 0.94; 95% CI, 0.87-1.01, P = 0.09).

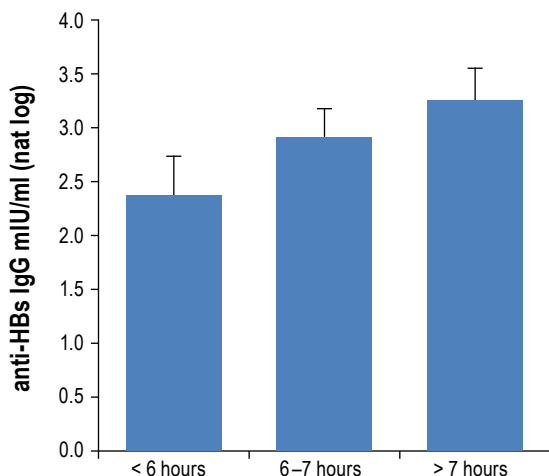
### Sleep and Clinical Protection

In the current study, 14.6% of participants failed to meet the threshold for clinical protection (anti-HBs IgG ≥ 10 mIU/ml), as assessed 6 mo after the final dose of vaccine. Logistic regression analyses demonstrated that shorter actigraphy-derived sleep duration was associated with decreased likelihood of clinical protection, which remained significant after adjustment for age, sex, and BMI (OR, 3.53; 95% CI, 1.22-10.27, P = 0.02). This graded relationship is shown in Figure 2. Furthermore, using approximate tertiles of sleep duration to predict protection status, sleeping fewer than 6 hr conferred a significant risk of being unprotected as compared with sleeping more than 7 hr per night (for < 6 hr/night, OR, 0.09; 95% CI, 0.01-0.94; for 6-7 hr/night, OR, 0.36; 95% CI, 0.04-3.67; > 7 hr/night [reference]). Further, this association remained significant after adjustment for diary-based psychological stress, averaged over 21 days of collection (OR, 3.77; 95% CI, 1.30-10.90, P = 0.01) and 7 days surrounding the first vaccination (OR, 3.70; 95% CI, 1.24-11.06, P = 0.02). Shorter diary-based sleep duration, averaged over the 7 days surrounding the first vaccination, was also associated with a decreased likelihood of clinical protection, and withstood adjustment for sociodemographic factors and psychological stress (OR, 2.73; 95% CI, 1.20-6.23, P = 0.02) (findings were similar when analyses were restricted to participants for whom actigraphy assessment was available). Likelihood of clinical protection was unrelated to actigraphy-derived sleep efficiency, diary-based sleep

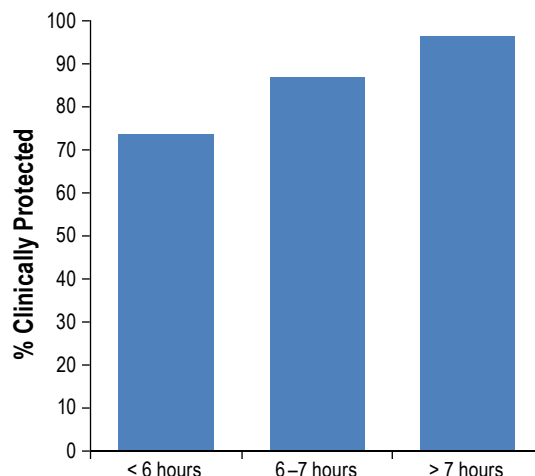
**Table 2**—Unadjusted and adjusted (age, gender, BMI, responder status) linear regression analyses examining whether average sleep duration, efficiency, and quality predicts secondary antibody levels to the hepatitis B immunization

Dependent Variable: anti-HBs IgG levels	b	SE	P-value	R <sup>2</sup>	ΔR <sup>2</sup>
<b>Sleep Duration</b>					
Actigraphy-based sleep duration	0.50	0.23	0.04	0.04	—
After adjustment for covariates	0.44	0.22	0.045	0.21	0.04
Diary-based sleep duration <sup>1</sup>	0.19	0.25	0.77	0.00	—
After adjustment for covariates	0.06	0.23	0.79	0.17	0.00
Diary-based sleep duration <sup>2</sup>	0.26	0.24	0.28	0.01	—
After adjustment for covariates	0.18	0.22	0.41	0.20	0.01
<b>Sleep Efficiency</b>					
Actigraphy-based sleep efficiency	0.02	0.03	0.55	-0.01	—
After adjustment for covariates	0.03	0.03	0.34	0.17	0.00
<b>Sleep Quality</b>					
Diary-based sleep quality <sup>1</sup>	-0.06	0.45	0.90	-0.01	—
After adjustment for covariates	-0.13	0.42	0.75	0.17	0.00
Diary-based sleep quality <sup>2</sup>	-0.01	0.42	0.98	0.00	—
After adjustment for covariates	-0.01	0.38	0.98	0.19	0.00

<sup>1</sup>Averaged over 21 days of diary collection; <sup>2</sup>averaged over 7 days of diary collection around the first immunization.



**Figure 1**—Average actigraphy-based sleep duration predicts anti-HBs IgG levels in response to the second hepatitis B immunization after adjustment for age, gender, BMI, and responder status.



**Figure 2**—Average actigraphy-based sleep duration predicts likelihood of being clinically protected (i.e. anti-HBs IgG ≥ 10 mIU/ml) 6-months following the third hepatitis B immunization.

duration, when averaged over the 21 days of collection, and subjective sleep quality.

## DISCUSSION

Shorter sleep duration, assessed objectively using actigraphy and averaged over 6 consecutive days of collection, was associated with lower secondary antibody response to hepatitis B antigen and decreased likelihood of mounting a clinically protective response to the hepatitis B vaccination series. The latter finding was also predicted by self-reported sleep duration measured via electronic sleep diary. These findings were independent of several factors known to be associated with magnitude of vaccination response, including age, sex, and BMI.<sup>34-37</sup> Conversely, measures of sleep efficiency and sleep quality were largely unrelated to magnitude antibody response to the hepatitis B vaccine.

Existing evidence linking sleep and antibody response comes from controlled laboratory studies using total or partial sleep deprivation. These studies show that sleep restriction for several consecutive nights prior to<sup>29</sup> and immediately after immunization<sup>27,28</sup> are associated with reduced antibody responses when compared with responses among nonrestricted sleepers. The current study extends this laboratory evidence by showing that shorter sleep duration, assessed in the natural environment, is associated with lower antibody response as well as decreased likelihood of clinical protection. Moreover, our data are consistent with recent evidence that short sleep duration predicts increased susceptibility to infectious disease.<sup>6</sup> These findings provide initial evidence that natural variation in sleep may contribute to clinically relevant differences in the magnitude of immune responses to vaccination, possibly providing a physiologic basis for observed differences in susceptibility to infection. Indeed, the magnitude of the sleep effect observed in this study is comparable to other known risk factors (age, sex, BMI, smoking status) for clinical nonprotection after the hepatitis B vaccination series.<sup>34,36,44</sup>

The literature supports attenuated vaccination responses in individuals experiencing elevated levels of psychological stress.<sup>38-40</sup> In this study, however, daily psychological stress, measured via electronic diaries, was unrelated to antibody levels and likelihood of clinical protection 6 mo after concluding the vaccination series. Adjustment for psychological stress, averaged over 7 days of measurement, in statistical models predicting secondary antibody responses, reduced the amount of variance accounted for by sleep duration from 4.0% to 3.5%, representing a 12.5% decrease. This suggests that although psychological stress plays some role in the sleep-vaccination response link in this sample, its effect is modest. Indeed, actigraphy and diary-based sleep duration were significant predictors of likelihood of clinical protection, independent of sociodemographic covariates and psychological stress, providing further evidence for the unique influence of sleep duration on vaccination response.

What mechanisms might link sleep and magnitude of antibody response to vaccination? Sleep loss is associated with fluctuations in several immune processes integral to antigen presentation and antibody production following immunization.<sup>11</sup> Indeed, prolonged sleep deprivation is associated with shifts in numbers of T and B cells in peripheral circulation,<sup>14,45,46</sup> dimin-

ished antigen-specific T-helper cell response,<sup>28</sup> declines in the production of interleukin-12<sup>47</sup> (a cytokine critical in orchestrating T-helper cell maturation), and increased levels of systemic inflammation.<sup>18,21-23</sup> Sleep-related alterations in neuroendocrine outputs also likely play a role. Indeed, elevations in sympathetic activation and cortisol output have been observed sleep-deprived participants.<sup>48,49</sup> Moreover, administration of exogenous epinephrine and cortisol at physiologic levels modulate several lymphocyte subsets, including natural killer cells and naïve and T-helper cells.<sup>50</sup> Notably, the effects of catecholamines on T and B cells are somewhat inconsistent.<sup>51</sup> Indeed, other biochemical mediators, including growth hormone and prolactin, have been shown to have stronger influences on antigen-specific T-helper cell activity than epinephrine and norepinephrine.<sup>28</sup> Further research exploring these sleep-immune pathways in the context of vaccination and antibody production is warranted.

Strengths of this study include its prospective design, the use of actigraphy as a more objective method for assessing sleep in the natural environment, and use of a clinically relevant *in vivo* model of immunocompetence. However, there are a number of limitations that must be considered when interpreting the current findings. For instance, actigraphy was only assessed around the time of the 1st vaccination, which limited our ability to illuminate possible “critical periods” when short sleep duration contributes to antibody response. Prior studies demonstrate that the timing of psychological and behavioral factors during vaccination influence subsequent antibody production.<sup>28,52,53</sup> For example, Miller and colleagues<sup>53</sup> found that higher daily stress experienced 8 days after immunization uniquely predicted fewer circulating antibodies to the influenza vaccine among healthy college students. Data from the current study failed to reveal any individual nights of sleep as uniquely predictive of antibody response or likelihood of clinical protection (data not shown); however, further research using continuous actigraphy measurements across the vaccination series could potentially identify periods during antibody production that are sensitive to changes in sleep.

It is important to note that the first immunization occurred in the middle of the actigraphy assessment. Evidence shows that peripheral immune activation can result in sleep disturbance; thus, it is possible that the immune response to the antigen affected subsequent sleep.<sup>54</sup> An examination of sleep parameters across the assessment days, however, did not reveal any significant changes in sleep on the days after vaccination, making it unlikely that the vaccination contributed to shortened sleep duration. Moreover, this finding is consistent with prior research.<sup>28</sup> It is recognized that actigraphy assesses activity levels, not sleep specifically. As such, a confirmatory study using home-based polysomnography is warranted. This is particularly relevant given recent laboratory evidence showing that higher levels of slow wave activity during non-rapid eye movement sleep on the nights after hepatitis A vaccination were associated with higher percentages of hepatitis A-specific T-helper cells 18-20 wk and 1 yr later.<sup>28</sup> Finally, there are several considerations with respect to the study sample that should be noted. First, the menstrual phase of female participants was not controlled in this study, a limitation given that fluctuations in sex steroids have been shown to affect immune function.<sup>55,56</sup> Second, better and more efficient sleep was positively correlated with age, which

is inconsistent with literature showing that quality of sleep declines with increasing age.<sup>57,58</sup> This contradictory finding may be explained by the fact that there was limited variance in the age of the sample, which was restricted to participants between 40 and 60 yr old. Further, enrollment into this study required participants to meet strict health criteria, including being free for 3 months from immune-related conditions and medications known to affect immune, endocrine, and nervous system function. As such, the participants may not accurately reflect midlife adults in the general population where underlying illness is more common. Third, this study did not formally assess for clinical sleep impairment or use a diary assessment of sleep efficiency, limiting our ability to generalize these findings to clinical populations. Relatedly, the presence of clinical sleep disorders, such as obstructive sleep apnea (OSA), was not assessed in the current sample. However, associations of sleep duration with antibody response were independent of BMI, a strong correlate of OSA.<sup>59</sup> Moreover, a recent study found no differences in antibody responses to influenza vaccination between individuals with OSA and normal sleepers.<sup>60</sup> Investigation into how sleep affects immune function among individuals with diagnosed clinical sleep impairments is a fertile area for future research.

Observed associations of shorter sleep duration with lower antibody responses to vaccination and decreased likelihood of mounting a clinically protective response raises the possibility that interventions designed to promote sleep may be of health benefit. However, given the preliminary nature of this work, further exploration of sleep-immune relationships is needed because it may assist in the development of novel therapeutic strategies, including potential sleep interventions to modify infectious disease risk.

In summary, this study provides initial evidence that natural variation sleep duration is positively associated with secondary antibody response to hepatitis B vaccination and likelihood of clinical protection. Further investigation into the influence sleep has on clinically relevant measures of immunity is warranted.

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## DISCLOSURE STATEMENT

This was not an industry supported study. The authors have indicated no financial conflicts of interest.

## REFERENCES

1. Ayas NT, White DP, Al-Delaimy WK, et al. A prospective study of self-reported sleep duration and incident diabetes in women. *Diabetes Care* 2003;26:380-4.
2. Ayas NT, White DP, Manson JE, et al. A prospective study of sleep duration and coronary heart disease in women. *Arch Intern Med* 2003;163:205-9.
3. Gangwisch JE, Heymsfield SB, Boden-Albala B, et al. Sleep duration as a risk factor for diabetes incidence in a large U.S. sample. *Sleep* 2007;30:1667-73.

4. Mallon L, Broman JE, Hetta J. Sleep complaints predict coronary artery disease mortality in males: a 12-year follow-up study of a middle-aged Swedish population. *J Intern Med* 2002;251:207-16.
5. Meisinger C, Heier M, Lowel H, Schneider A, Doring A. Sleep duration and sleep complaints and risk of myocardial infarction in middle-aged men and women from the general population: the MONICA/KORA Augsburg cohort study. *Sleep* 2007;30:1121-7.
6. Cohen S, Doyle WJ, Alper CM, Janicki-Deverts D, Turner RB. Sleep habits and susceptibility to the common cold. *Arch Intern Med* 2009;169:62-7.
7. Patel SR, Malhotra A, Gao X, Hu FB, Neuman MI, Fawzi WW. A prospective study of sleep duration and pneumonia risk in women. *Sleep* 2012;35:97-101.
8. Cappuccio FP, D'Elia L, Strazzullo P, Miller MA. Sleep duration and all-cause mortality: a systematic review and meta-analysis of prospective studies. *Sleep* 2010;33:585-92.
9. Heslop P, Smith GD, Metcalfe C, Macleod J, Hart C. Sleep duration and mortality: The effect of short or long sleep duration on cardiovascular and all-cause mortality in working men and women. *Sleep Med* 2002;3:305-14.
10. Kripke DF, Garfinkel L, Wingard DL, Klauber MR, Marler MR. Mortality associated with sleep duration and insomnia. *Arch Gen Psychiatry* 2002;59:131-6.
11. Lange T, Dimitrov S, Born J. Effects of sleep and circadian rhythm on the human immune system. *Ann N Y Acad Sci* 2010;1193:48-59.
12. Irwin M, Mascofich A, Gillin JC, Willoughby R, Pike J, Smith TL. Partial sleep deprivation reduces natural killer cell activity in humans. *Psychosom Med* 1994;56:493-8.
13. Irwin M, McClintick J, Costlow C, Fortner M, White J, Gillin JC. Partial night sleep deprivation reduces natural killer and cellular immune responses in humans. *FASEB J* 1996;10:643-53.
14. Bollinger T, Bollinger A, Skrum L, Dimitrov S, Lange T, Solbach W. Sleep-dependent activity of T cells and regulatory T cells. *Clin Exp Immunol* 2009;155:231-8.
15. Cardinali DP, Esquifino AI, Srinivasan V, Pandi-Perumal SR. Melatonin and the immune system in aging. *Neuroimmunomodulation* 2008;15:272-8.
16. Dimitrov S, Lange T, Tieken S, Fehm HL, Born J. Sleep associated regulation of T helper 2 cytokine balance in humans. *Brain Behav Immun* 2004;18:341-8.
17. Sakami S, Ishikawa T, Kawakami N, et al. Coemergence of insomnia and a shift in the Th1/Th2 balance toward Th2 dominance. *Neuroimmunomodulation* 2002;10:337-3.
18. Meier-Ewert HK, Ridker PM, Rifai N, et al. Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. *J Am Coll Cardiol* 2004;43:678-83.
19. Moldofsky H, Lue FA, Davidson JR, Gorczyński R. Effects of sleep deprivation on human immune functions. *FASEB J* 1989;3:1972-7.
20. Vgontzas AN, Zoumakis E, Bixler EO, et al. Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. *J Clin Endocrinol Metab* 2004;89:2119-26.
21. Chennaoui M, Sauvet F, Drogou C, et al. Effect of one night of sleep loss on changes in tumor necrosis factor alpha (TNF-alpha) levels in healthy men. *Cytokine* 2011;56:318-24.
22. Frey DJ, Fleschner M, Wright KP Jr. The effects of 40 hours of total sleep deprivation on inflammatory markers in healthy young adults. *Brain Behav Immun* 2007;21:1050-7.
23. Haack M, Sanchez E, Mullington JM. Elevated inflammatory markers in response to prolonged sleep restriction are associated with increased pain experience in healthy volunteers. *Sleep* 2007;30:1145-52.
24. Irwin M, Wang M, Campomayor CO, Collado-Hidalgo A, Cole S. Sleep deprivation and activation of morning levels of cellular and genomic markers of inflammation. *Arch Intern Med* 2006;166:1756-62.
25. Mullington JM, Simpson NS, Meier-Ewert HK, Haack M. Sleep loss and inflammation. *Best Pract Res Clin Endocrinol Metab* 2010;24:775-84.
26. Miller MA, Kandala NB, Kivimaki M, et al. Gender differences in the cross-sectional relationships between sleep duration and markers of inflammation: Whitehall II study. *Sleep* 2009;32:857-64.
27. Lange T, Perras B, Fehm HL, Born J. Sleep enhances the human antibody response to hepatitis A vaccination. *Psychosom Med* 2003;65:831-35.
28. Lange T, Dimitrov S, Bollinger T, Diekelmann S, Born J. Sleep after vaccination boosts immunological memory. *J Immunology* 2011;187:283-90.
29. Spiegel K, Sheridan JF, Van Cauter E. Effect of sleep deprivation on response to immunization. *JAMA* 2002;288:1471-2.

30. Benedict C, Brytting M, Markstrom A, Broman JE, Schioth HB. Acute sleep deprivation has no lasting effects on the human antibody titer response following a novel influenza A H1N1 virus vaccination. *BMC Immunol* 2012;13:1.
31. Metropolitan height and weight tables. *Stat Bull Metropol Life Insur Co* 1983;64:2-9.
32. Ancoli-Israel S, Cole R, Alessi C, Chambers M, Moorcroft W, Pollak CP. The role of actigraphy in the study of sleep and circadian rhythms. *Sleep* 2003;26:342-92.
33. Littner M, Kushida CA, Anderson WM, et al. Practice parameters for the role of actigraphy in the study of sleep and circadian rhythms: an update for 2002. *Sleep* 2003;26:337-41.
34. Averbhoff F, Mahoney F, Coleman P, Schatz G, Hurwitz E, Margolis H. Immunogenicity of hepatitis B vaccines. Implications for persons at occupational risk of hepatitis B virus infection. *Am J Prev Med* 1998;15:1-8.
35. Bock HL, Kruppenbacher J, Sanger R, Hobel W, Clemens R, Jilg W. Immunogenicity of a recombinant hepatitis B vaccine in adults. *Arch Intern Med* 1996;156:2226-31.
36. Wood RC, MacDonald KL, White KE, Hedberg CW, Hanson M, Osterholm MT. Risk factors for lack of detectable antibody following hepatitis B vaccination of Minnesota health care workers. *JAMA* 1993;270:2935-39.
37. Zuckerman JN. Protective efficacy, immunotherapeutic potential, and safety of hepatitis B vaccines. *J Med Virol* 2006;78:169-77.
38. Cohen S, Miller GE, Rabin BS. Psychological stress and antibody response to immunization: a critical review of the human literature. *Psychosom Med* 2001;63:7-18.
39. Burns VE, Carroll D, Ring C, Drayson M. Antibody response to vaccination and psychosocial stress in humans: relationships and mechanisms. *Vaccine* 2003;21:2523-34.
40. Pedersen AF, Zachariae R, Bovbjerg DH. Psychological stress and antibody response to influenza vaccination: A meta-analysis. *Brain Behav Immun* 2009;23:427-33.
41. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav* 1983;24:385-96.
42. Glaser R, Kiecolt-Glaser JK, Bonneau RH, Malarkey W, Kennedy S, Hughes J. Stress-induced modulation of the immune response to recombinant hepatitis B vaccine. *Psychosom Med* 1992;54:22-9.
43. Leads from the MMWR. Recommendations of the immunization practices advisory committee update on hepatitis B prevention. *JAMA* 1987;258:437, 444-5, 449.
44. Roome AJ, Walsh SJ, Cartter ML, Hadler JL. Hepatitis B vaccine responsiveness in Connecticut public safety personnel. *JAMA* 1993;270:2931-4.
45. Born J, Lange T, Hansen K, Mølle M, Fehm HL. Effects of sleep and circadian rhythm on human circulating immune cells. *J Immunol* 1997;158:4454-64.
46. Born J, Uthgenannt D, Dodt C, et al. Cytokine production and lymphocyte subpopulations in aged humans. An assessment during nocturnal sleep. *Mech Ageing Dev* 1995;84:113-26.
47. Dimitrov S, Lange T, Nohroudi K, Born J. Number and function of circulating human antigen presenting cells regulated by sleep. *Sleep* 2007;30:401-11.
48. Balbo M, Leproult R, Van Cauter E. Impact of sleep and its disturbances on hypothalamo-pituitary-adrenal axis activity. *Int J Endocrinol* 2010;2010:759234.
49. Meerlo P, Sgoifo A, Suchecki D. Restricted and disrupted sleep: effects on autonomic function, neuroendocrine stress systems and stress reactivity. *Sleep Med Rev* 2008;12:197-210.
50. Dimitrov S, Benedict C, Heutling D, Westermann J, Born J, Lange T. Cortisol and epinephrine control opposing circadian rhythms in T cell subsets. *Blood* 2009;113:5134-43.
51. Kin NW, Sanders VM. It takes nerve to tell T and B cells what to do. *J Leukoc Biol* 2006;79:1093-1104.
52. Edwards KM, Burns VE, Reynolds T, Carroll D, Drayson M, Ring C. Acute stress exposure prior to influenza vaccination enhances antibody response in women. *Brain Behav Immun* 2006;20:159-68.
53. Miller GE, Cohen S, Pressman S, Barkin A, Rabin BS, Treanor JJ. Psychological stress and antibody response to influenza vaccination: when is the critical period for stress, and how does it get inside the body? *Psychosom Med* 2004;66:215-23.
54. Bryant PA, Trinder J, Curtis N. Sick and tired: Does sleep have a vital role in the immune system? *Nat Rev Immunol* 2004;4:457-67.
55. Grossman CJ. Regulation of the immune system by sex steroids. *Endocr Rev* 1984;5:435-55.
56. Gameiro C, Romao F. Changes in the immune system during menopause and aging. *Front Biosci (Elite Ed)* 2010;2:1299-1303.
57. Unruh ML, Redline S, An MW, et al. Subjective and objective sleep quality and aging in the sleep heart health study. *J Am Geriatr Soc* 2008;56:1218-27.
58. Ohayon MM, Carskadon MA, Guilleminault C, Vitiello MV. Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: developing normative sleep values across the human lifespan. *Sleep* 2004;27:1255-73.
59. Patil SP, Schneider H, Schwartz AR, Smith PL. Adult obstructive sleep apnea: pathophysiology and diagnosis. *Chest* 2007;132:325-37.
60. Dopp JM, Wiegert NA, Moran JJ, Muller D, Weber S, Hayney MS. Humoral immune responses to influenza vaccination in patients with obstructive sleep apnea. *Pharmacotherapy* 2007;27:1483-89.