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Is Insomnia a Risk Factor for Decreased Influenza Vaccine Response?

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Abstract

Healthy young adult college students ($N = 133$) with Insomnia ($n = 65$) or No Insomnia ($n = 68$) were compared on influenza serum antibody levels pre- and four weeks postvaccination. Volunteers underwent structured clinical interviews for sleep disorders to ensure insomnia diagnoses, as well as psychiatric interviews, physical examinations, and drug testing to ensure comorbid health problems were not potential confounds. There were significant time (both groups had increases in antibody levels pre- to postvaccination) and group (Insomnia group had lower HI antibody levels overall) main effects, but the time $\times$ group interaction was nonsignificant. Exploratory analyses did find significant PSQI $\times$ Time ($p < .001$) and Insomnia Status $\times$ Time ($p = .002$) interaction effects. Results indicate insomnia may be a risk factor for lowered immunity to the influenza virus.

Influenza virus infection is a serious public health concern, resulting in up to 28,000 deaths each year in the United States (Centers for Disease Control & Prevention, 2010). The influenza vaccine is recommended for everyone (World Health Organization, 2005), and about 35% of the United States population is vaccinated with trivalent, inactivated flu vaccine, a number that increased to 42% in 2011–2012 (Centers for Disease Control and Prevention, 2013; Park, 2013). A recent meta-analysis demonstrated that vaccination is ineffective in a significant percentage (i.e., 33–49%) of cases (Osterholm, Kelley, Sommer, & Belongia, 2012). Inadequate vaccination response can occur for a variety of reasons, one of which might be health problems (Ader, 2007; Burns et al., 2003; Miller et al., 2001; Moynihan et al., 2004; Segerstrom & Miller, 2004). Chronic insomnia is a highly prevalent health problem (approximately 15% of the United States population) and may be one cause of attenuated influenza vaccine responses. To the best of our knowledge, no study has evaluated the relationship between chronic insomnia and immune function in vaccination response (e.g., influenza vaccine).

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Several previous studies have shown lowered immune functioning in individuals who reported poor sleep, sleep deprivation, or insomnia (Burgos et al., 2005; Cover & Irwin, 1994; Hall et al., 1998; Irwin, 2002; Irwin, Clark, Kennedy, Christian Gillin, & Ziegler, 2003; Irwin et al., 1994; Irwin et al., 1996; Miller et al., 2004; Prather et al., 2012). Studies to date, however, have fallen into one of two categories: (a) the measurement of immune cell numbers or cytokines (e.g., Cover & Irwin, 1994; Hall et al., 1998; Savard et al., 2003) in persons with insomnia, or (b) examination of vaccine responses (e.g., Miller et al., 2004; Prather et al., 2012) in relation to poor sleep or reduced sleep duration (Frey, Fleshner, & Wright, 2007; Lange et al., 2003; Spiegel et al., 2002).

Studies have shown that insomnia is related to suppression of natural killer (NK) cell activity (Cover & Irwin, 1994; Hall et al., 1998), lymphocyte counts (Savard, Laroche, Simard, Ivers, & Morin, 2003), as well as chronic increases in certain proinflammatory cytokines (e.g., IL-6; Burgos et al., 2005; Vgontzas et al., 2002). However, it is unclear how these cross-sectional insomnia-immune relationships will translate to vaccination response (e.g., influenza vaccine).

Sleep deprivation studies have also found a link between immune function and sleep. For instance, Lange, Perras, Fehm, and Born (2003) found that healthy adults allowed to sleep the night after a hepatitis A vaccination had a nearly twofold greater antibody titer four weeks later relative to subjects staying awake throughout that night. In a study by Spiegel, Sheridan, and Van Cauter, young men with their normal sleep time (e.g., 7.5–8 hr) restricted to 4 hr for six nights had a significantly weaker antibody response to the influenza vaccine than those allowed to maintain their normal sleep schedule during this time (Spiegel, Sheridan, & Van Cauter, 2002). Conversely, studies have found that shorter sleep durations (measured naturalistically) were related to reduced influenza (Miller et al., 2004) and hepatitis B (Prather et al., 2012) vaccine response in healthy young and middle-aged adults, respectively, without sleep problems.

Similarly, Cohen and colleagues (Cohen, Doyle, Alper, Janicki-Deverts, & Turner, 2009) found that individuals with self-reported sleep durations < 7 hr or sleep efficiencies < 92% were more likely to develop a cold after exposure to the virus than those sleeping > 8 hr or > 98%. These results were confirmed when sleep duration was measured behaviorally using actigraphy watches (Prather, Janicki-Deverts, Hall, & Cohen, 2015). Participants sleeping fewer than 6 hr per night were at an increased risk of developing a clinical cold following virus exposure.

Unfortunately, sleep deprivation and naturalistic short sleep duration are not the same as chronic insomnia. For instance, several studies have shown that people with insomnia do not demonstrate excessive daytime sleepiness (objectively or subjectively) indicative of sleep deprivation, but in fact look more like people *without* insomnia who have had a full night's sleep (Riedel & Lichstein, 2000; Stepanski, Zorick, Roehrs, & Young, 1988). There are many potential causes of naturalistic short sleep durations such as shift work, other circadian rhythm disorders, or insufficient opportunity for sleep due to work or social factors (for a review see Taylor, Lichstein, & Durrence, 2003). It is important to study the relationship of individuals with rigorously defined chronic insomnia, excluding those with circadian rhythm

disorders and other issues reducing their sleep duration, so that a better understanding can be gained of the relationship between *insomnia* and *vaccine response*.

The current study was a repeated measures cohort design (healthy young adults with vs. without chronic insomnia) assessing in vivo (e.g., influenza vaccine) immune responses pre- and postvaccination. The primary endpoint was the hemagglutination inhibition (HI) assay, which is the clinical outcome of flu-specific antibody levels in blood serum, assessing how well antibodies recognize and bind to an influenza virus. The primary hypothesis was that the Insomnia group would have lower levels of influenza antibodies at four weeks postvaccination than the No Insomnia group. Exploratory analyses examined other variables previously linked with reduced influenza vaccine response such as gender (Furman et al., 2014), perceived stress (Kiecolt-Glaser, Glaser, Gravenstein, Malarkey, & Sheridan, 1996; Miller et al., 2004), smoking (Cruijff et al., 1999), and obesity (Sheridan et al., 2012) to determine if they might be significant confounders of the relationship between insomnia and influenza antibody levels.

METHOD

Participants

A total of 133 (Insomnia = 65, No Insomnia = 68) healthy college students were recruited through announcements in class and flyers around campus, and they successfully completed all phases of the study over two years (2011: Insomnia = 31 and No Insomnia = 28; 2012: Insomnia = 34 and No Insomnia = 40). To be included in the study, participants had to be 18–29 years old, enrolled at the University of North Texas, not received the influenza vaccine the previous year, not have another sleep disorder, Axis I or II psychiatric disorder, or serious chronic medical condition (e.g., cancer, HIV, pain, immune, Guillain-Barre syndrome), not be taking any medications that could alter immune responses (e.g., steroids, opioids), not be pregnant or nursing, and not have egg or mercury allergies. The sample was 60% female, with a mean age of 20.24 ($SD = 2.60$). Ethnic categories were 69% non-Hispanic or Latino, 29% Hispanic or Latino, and 2% of unknown ethnicity. Racial categories were 64% white, 11% Black or African American, 13% multiracial, 4% Asian, and 8% unknown or not reported. Within the insomnia group, 67.7% had sleep onset insomnia, 24.6% had maintenance insomnia, and 20% had terminal insomnia at least three nights per week. The study was approved by the university's institutional review board for human subjects.

Power analysis—There were no previous data on which to derive an effect size, but Miller et al. (2004) did find that sleep duration was moderately correlated with antibody response ($r = -.20$; $d = .41$) in a healthy young adult population. We decided to use a more conservative, yet clinically significant effect of $d = .25$ at $\alpha < .05$, with an expected moderate repeated measures correlation ($\rho = 0.40$). Given these assumptions, a total $n = 62$ (i.e., $n = 31$ per group per year) was needed to find statistical significance for any given strain. It was decided a priori to collect data over two influenza seasons to amass a final sample size of 32 per group for each of 2 years (i.e., $N = 128$) to ensure we were adequately powered to find statistical significance by aggregating across years if difficulties in

recruitment arose. As can be seen above, we were able to recruit 5 participants more than were needed for our primary hypothesis.

Measures

The majority of assessments in this study have well-documented psychometric profiles and are those deemed essential in the recommendations of the consensus conference on assessment of insomnia in research (Buysse, Ancoli-Israel, Edinger, Lichstein, & Morin, 2006). Descriptions of the measures are provided below, followed by procedures used.

General Health Questionnaire (GHQ)—The GHQ was the primary measure for collecting self-report data on age, race and ethnicity, height, weight, and other health behaviors (i.e., medication use [prescription, over-the-counter, dietary supplements], substance use [nicotine, caffeine, alcohol, illicit drugs], treatment of psychiatric disorders [current and past], family history of psychiatric disorders and treatment). Self-report information was obtained using a series of checklists and yes, no, and open-ended questions. To date, no reliability or validity data exist for this measure, but the GHQ is optimal for the measurement of certain variables based on the limited availability of established measures. Because alcohol and nicotine use were both significantly positively skewed, those data were dichotomized into users and nonusers.

Insomnia Severity Index (ISI)—The ISI is a 7-item self-report measure designed to assess perceived severity of insomnia (Morin, 1993). Each item uses a 5-point Likert scale from 0 (not at all satisfied) to 4 (very much satisfied). The items sum to produce a total score, ranging from 0 to 28, with higher scores representing greater insomnia severity, and scores ≥ 15 representing clinically significant insomnia. The ISI has shown excellent reliability in college student samples with an internal consistency α coefficient of 0.86 in a previous study (Taylor, Bramoweth, Grieser, Tatum, & Roane, 2013) and 0.94 in the current sample.

Epworth Sleepiness Scale (ESS)—The ESS is an 8-item self-report measure designed to assess the overall level of daytime sleepiness. Each item uses a 4-point Likert scale to assess the chance of dozing (0 = *would never* to 3 = *high chance*) in a variety of situations. The item's scores sum to produce a total score (range 0–24). Scores > 10 are considered to be suggestive of significant daytime sleepiness; scores > 15 have been associated with pathological sleepiness that may be due to conditions such as obstructive sleep apnea or narcolepsy. The ESS has shown good reliability in college student samples with an internal consistency α coefficient of 0.68 in a previous study (Taylor et al., 2013) and 0.83 in the current sample.

Morningness/Eveningness Questionnaire (MEQ)—The MEQ is a 19-item measure intended to classify people along a dimension of morningness-eveningness in circadian rhythms (Horne & Ostberg, 1976). A higher score indicates a greater degree of morningness and a lower score indicates a greater degree of eveningness, with scores < 31 representing definite evening types in a young adults sample (Horne & Ostberg, 1976). The MEQ has

shown excellent reliability with an internal consistency α coefficient of 0.83 in previous studies (Anderson, Petros, Beckwith, & Mitchell, 1991) and 0.82 in the current sample.

Pittsburgh Sleep Quality Index (PSQI)—The PSQI is a 19-item self-rated questionnaire composed of 15 multiple-choice items and 4 write-in items that cover the domains of subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medications, and daytime dysfunction (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). The PSQI generates seven scores that correspond to the seven domains previously mentioned. The domain scores range from 0 (no difficulty) to 3 (severe difficulty) and when combined produce a global score ranging from 0 to 21, with higher scores indicating worse sleep quality. The PSQI has shown good reliability in college student samples with an internal consistency α coefficient of 0.83 in a previous study (Taylor et al., 2013) and 0.84 in the current sample.

Perceived Stress Scale (PSS)—The PSS is a 14-item self-report questionnaire used to assess the stress domains of unpredictability, lack of control, burden overload, and stressful life circumstances. Respondents specify the frequency of feelings, thoughts, or circumstances described on a 5-point Likert scale (0 = never to 4 = very often), with a total score with a possible range of 0–56 (higher scores reflect greater perceived stress). The PSS has shown good reliability in college student samples with an internal consistency α coefficient of 0.88 in a previous study (Taylor et al., 2013) and 0.89 in the current sample.

Structured Clinical Interview for the DSM-IV (Axis I & II)—All subjects who advanced to the diagnostic evaluation phase of the study were interviewed by advanced clinical health psychology doctoral students with the SCID-I and II to assess for current and past psychiatric disorders (First, Spitzer, Gibbon, & Williams, 1996, 1997). Caseness was performed as needed with a licensed clinical psychologist (DJT) who had extensive experience using the SCID to diagnose psychiatric disorders in clinical trials. The SCID-I and SCID-II incorporate DSM-IV diagnostic criteria in a structured, standardized interview that yields formal psychiatric diagnoses. The SCID-I generally produces interrater reliability scores (Kappa) in the 0.6 range, with scores in the 0.8 range for major depression and generalized anxiety disorders (Segal, Hersen, & Van Hasselt, 1994).

Structured Clinical Interview Schedule for DSM-5 sleep disorders (SCISD)—All subjects who proceeded to the diagnostic evaluation phase of the study were interviewed by trained interviewers with the SCISD to assess for current sleep disorders as defined by the *Diagnostic and Statistical Manual of Mental Disorders*, fifth edition (DSM-5; American Psychiatric Association, 2013). This measure was specifically designed to allow an independent evaluator with a master's degree or higher, and minimal training in sleep disorders and administration, to obtain reliable sleep disorder diagnoses in adults. As with other sleep interviews, this one has yet to be validated, but this process is ongoing. Caseness was performed as needed with the first author (DJT), who is a licensed clinical psychologist, board-certified in both sleep medicine and behavioral sleep medicine.

Sleep diary—Sleep diaries were used to measure subjective sleep patterns and determine insomnia status (i.e., Insomnia vs. Normal sleepers). Participants were asked to make daily

diary entries for seven days with an estimate of their sleep the night before (e.g., bedtime, sleep onset). To ensure diaries were completed nightly, subjects were asked to call in each morning to an answering machine and give their sleep diary data from the night before. Sleep diaries are significantly correlated with PSG on wake time after sleep onset, total sleep time, and sleep efficiency ($r = .46-.59$; Lichstein et al., 2006) in people with insomnia. Diaries are also better than single-point retrospective estimates of sleep (Coursey, Frankel, Gaarder, & Mott, 1980). Use of the sleep diary allowed the researchers to assess the quantitative components (e.g., sleep onset latency > 30 min) of the criteria required for a diagnosis of insomnia.

Hemagglutination Inhibition (HI) assay—The HI assay is a standard clinical laboratory technique used to detect the presence of anti-influenza (all class) antibodies in serum. The assay was performed using a standard microtiter procedure (Kohut et al., 2004). The influenza viruses used in the assay were the specific viruses chosen for use in the 2011–2012 and 2012–2013 influenza vaccines obtained from the Center for Disease Control Center for Influenza, Atlanta, GA. All influenza viruses were propagated in 9- or 10-day-old embryonated chicken eggs by inoculation via allantoic-amnionic route. Because HI titers are positively skewed, those data were log transformed (log2).

Procedures

All 133 participants underwent vigorous screening to verify the diagnosis of insomnia, or lack thereof. They next underwent a physical examination and drug testing, and had blood drawn for influenza antibody assessment prior to receiving the vaccine. Blood was redrawn four weeks later. All procedures occurred between September and the first week of November.

Consent and diagnostic evaluation—The study was described upon arrival for diagnostic evaluation. Each participant was asked to provide informed consent, and then began a thorough screening process to ensure he or she met the inclusion and exclusion criteria. They first completed the ISI, ESS, PSQI, and MEQ. Each volunteer then met with a research assistant to clarify responses and verify the absence of exclusionary medical disorders as well as make sure participants in the No Insomnia group did not score in the clinically significant range on sleep disturbance questionnaires (i.e., PSQI ≥ 5 ; MEQ 31–69; ISI < 15 , ESS < 15). Volunteers then met with a trained doctoral-level graduate student who administered the structured clinical interviews to verify the absence of any exclusionary mental or sleep disorders. Finally, volunteers met with a physician or nurse practitioner at the university Health and Wellness Center, who was blind to group membership, and underwent a history and physical examination to ensure no underlying conditions or substances were present that may have affected sleep or vaccine response. Bloodwork was performed to ensure measurements within normal ranges on a number of immune and endocrine indices. A urine drug test was performed to verify the absence of any unreported medication or illicit drugs.

Blood draw followed by influenza vaccination—The laboratory staff at the Health and Wellness Center were responsible for all blood draws and vaccinations and were blind to

group membership. Once participants were cleared by the study physician, each had blood drawn using red-topped tubes for serum. There does not appear to be a consistent diurnal variation in the response to the influenza vaccine (Langlois, Smolensky, Glezen, & Keitel, 1995), but circadian variability was still controlled for by drawing blood between 12:00 p.m. and 2:00 p.m. After blood was collected, it sat at room temperature for approximately 60 min to allow for clotting, and was subsequently centrifuged at 2,500 rpm for 20 min, and then frozen in aliquots at -80 Celsius. Immediately following the baseline blood draw, each participant received the Novartis Fluvirin preparation of the influenza vaccine through intramuscular injection. In 2011–2012, the trivalent vaccine contained A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2), and B/Brisbane/60/2008 viruses. In 2012–2013, the trivalent vaccine again contained A/California/7/09 (H1N1), as well as the new A/Victoria/361/2011 (H3N2), and B/Wisconsin/1/2010 viruses.

Four-week post vaccination blood draws—Participants returned four weeks postvaccination for a follow-up blood draw. After all blood samples were drawn each year, they were express shipped to the Iowa State University (ISU) laboratory, where assays were performed. Participating ISU lab members were blind to group membership.

Remuneration—To enhance retention, the following financial incentives were offered for completion of each stage of the study: completion of all screening (\$25), initial blood draw and vaccination (\$40 + free influenza vaccination), four-week follow-up blood draw (\$40).

Statistical Analyses

All statistical analyses were performed using SPSS version 20.0. Prior to analyses, data were screened for normality, missing data, and outliers (Tabacnick & Fidell, 2007). Baseline comparisons were performed comparing groups on demographics (i.e., age, gender, race, and ethnicity), sleep questionnaires (ISI, PSQI, ESS, MEQ), health variables (i.e., nicotine use, alcohol use, body mass index, continuous and categorized), and perceived stress. To test the primary hypothesis that the Insomnia group would have a poorer response to the vaccine at four week follow-up, a multivariate analysis of variance (MANOVA) was completed with group (Insomnia vs. No Insomnia) as the independent variable, time (baseline vs. four weeks postvaccination) as the repeated measure, and log2 transformed HI antibody levels of each viral strain in both years as the dependent variables. Finally, an exploratory repeated measures MANOVA was performed with variables previously linked with reduced influenza vaccine response (i.e., age, gender, BMI, nicotine use, and stress), sleep diary total sleep time (TST), sleep questionnaires (ISI, PSQI, ESS, MEQ), and other health variables (i.e., alcohol use) entered in as independent variables, time (baseline vs. four weeks postvaccination) as the repeated measure, and log2 transformed HI antibody levels of each viral strain in both years as the dependent variables.

RESULTS

Recruitment

A total of 1,088 participants underwent phone screening to determine preliminary eligibility (e.g., did not participate in previous year, 18–29 years old, currently enrolled at the

University of North Texas (UNT), usual bedtime 10:00 p.m.–1:00 a.m. or rise time 4:00 a.m.–10:00 a.m.; if Insomnia group, difficulty falling or stay asleep 3 nights/week, for 3 months, with daytime complaints; if No Insomnia group, average sleep duration > 7.5 hr). A total of 331 (30%) volunteers passed screening, were sent the packet of questionnaires, and were scheduled for in-person meetings for consenting and diagnostic interviews. A total of 171 (52%) participants passed this stage and were scheduled for a history and physical and laboratory assessments to ensure no underlying conditions or substances were present that may have affected sleep or vaccine response. Of those, 149 (87%) participants passed and had their blood drawn followed by an influenza vaccine, and 139 (93%) participants completed follow-up blood draws four weeks postvaccination. There were no pre- to postvaccination attrition differences between groups (No Insomnia = 7 [8.8%], Insomnia = 3 [4.3%]; $p = .284$).

Baseline Comparisons

As can be seen in Table 1, there were no group differences on demographic variables (i.e., gender, ethnicity, race, or age). With respect to health variables (i.e., BMI, nicotine use, alcohol use), significantly more of the Insomnia group (17.2%) used nicotine than the No Insomnia group (4.4%). Finally, as expected, individuals with Insomnia reported higher scores on the ISI, PSQI, ESS, and PSS, and lower scores on the MEQ (i.e., eveningness) than the No Insomnia group.

As expected, the majority of participants had serum HI antibody titers at baseline (i.e., before vaccination) indicative of previous exposure (i.e., HI > 40; Cox et al., 2002) to the influenza strains included in the vaccine (H3N2 = 99.2%; B = 97%; H1N1 = 95.5%), or to closely related strains, even though none had received the vaccine the previous year. Chi-square tests of independence demonstrated no baseline differences in rates of previous exposure between the groups on any of the three strains: H3N2 (Insomnia = 98.5% vs. No insomnia = 100%, $p = .982$), B (Insomnia = 95.4% vs. No insomnia = 98.5%, $p = .580$), H1N1 (Insomnia = 92.3% vs. No insomnia = 98.6%, $p = .190$).

Insomnia and Hemagglutination Inhibition (HI) Antibodies

The repeated measures MANOVA demonstrated significant time, Wilks's $\Lambda = .252$, $F(3, 129) = 127.44$, $p < .001$, partial eta-squared (η_p^2) = .75, and between group main effects, Wilks's $\Lambda = .889$, $F(3, 129) = 5.39$, $p = .002$, $\eta_p^2 = .11$, but not a Group x Time interaction, Wilks's $\Lambda = .976$, $F(3, 129) = 1.06$, $p = .369$, $\eta_p^2 = .02$. Follow-up univariate comparisons were then performed with the same group and time variables as above, but with individual virus strains as the independent variable. As can be seen in Table 2, there were significant time effects (i.e., response to vaccination) across all assays, strains, and years. As can be seen in Table 2 and Figure 1, a significant between-groups main effect was found for H3N2 in both years ($p = .041$, $\eta_p^2 = .08$ and $p = .008$, $\eta_p^2 = .09$ respectively), with the Insomnia group having lower antibody amounts at baseline and continuing to be relatively lower at four weeks postvaccine. As can be seen in Table 2 and Figure 1, significant between-groups main effects were also found for B, but only in 2012–2013 ($p = .009$, $\eta_p^2 = .09$). H1N1 data was aggregated across years because the same strain was used each year, which allowed for greater power on this particular strain. As can be seen in Table 2, a similar but nonsignificant

trend was seen for the between-subjects effects ($p=.098$, $\eta_p^2=.02$). Pairwise comparisons of groups at baseline and postvaccination, separated by strain and year, as well as Group x Time interactions, were provided for additional information and for future comparisons.

Exploratory Analyses

As can be seen in Table 3, the repeated measures MANCOVA, with potentially confounding variables entered as covariates, did not find any significant between-subjects effects. This model did, however, find significant PSQI x Time ($p<.001$) and Insomnia Status x Time ($p=.002$) interaction effects.

DISCUSSION

This was the first study to examine the relationship between insomnia and influenza vaccine response. As expected, most of these healthy young participants were already protected (H3N2 = 99.2%; B = 97%; H1N1 = 95.5%), due to previous exposures to the influenza strains included in the vaccine, or to closely related strains. In addition, there was a significant time effect (all $ps<.001$), demonstrating a robust antibody responses to the vaccine. Although the raw data showed no Group x Time interactions, the Insomnia group did have lower overall HI antibody amounts ($p=.002$, $\eta_p^2=.11$) than the No Insomnia group when averaging pre- and postvaccination responses over time and across viral strain. Further examination of the individual strains indicated the Insomnia group had lower H3N2 antibodies in both years and B antibodies in 2012 (all $ps<.05$, all $\eta_p^2>.08$) and a trend for H1N1 antibodies ($p<.10$, $\eta_p^2=.02$), when averaging over time. Exploratory analyses controlling for potential confounds such as demographic (i.e., age, gender), health (i.e., BMI, nicotine use, alcohol use), stress, and other continuous sleep variables (i.e., TST, ISI, PSQI, ESS, MEQ), did show that both sleep quality (i.e., PSQI; $p<.001$) and insomnia status ($p=.002$) were predictive of influenza antibody responses. These results indicate insomnia and sleep quality may be a risk factor for lowered immunity to the influenza virus. Perhaps most intriguing was that these results were found in healthy young adults, who presumably have stronger immune systems than young children, older adults, and other immune-compromised individuals.

The above results were largely in agreement with those found in previous studies showing lowered immunity in individuals who reported poor sleep, sleep deprivation, or insomnia (Burgos et al., 2005; Cover & Irwin, 1994; Hall et al., 1998; Irwin, 2002; Irwin et al., 2003; Irwin et al., 1994; Irwin et al., 1996; Miller et al., 2004; Prather et al., 2015; Prather et al., 2012). Compromised immunity has long been hypothesized as a mediator for the relationship between insomnia and poor health (e.g., Taylor et al., 2003; Taylor et al., 2007). The pattern of raw results, however, was somewhat surprising, as it was hypothesized that the groups would start out equivalent on antibody levels and that the insomnia group would show decreased response to the vaccine, similar to what was found for short sleep duration and influenza (Miller et al., 2004) and hepatitis B (Prather et al., 2012) vaccine response. It is of note, that the H3N2 results were replicated over two years with two different samples.

In hindsight, it should not have been a surprise that there were group differences at baseline. Our hypothesis was based on the idea that people with insomnia would have worse overall

immune function, and thus a poorer vaccine-induced antibody response. Immune dysregulation has been shown in previous cross-sectional analyses of immune cell numbers or cytokines (e.g., Cover & Irwin, 1994; Hall et al., 1998; Savard et al., 2003) in individuals with insomnia. Additionally, extensive research by Vgontzas and colleagues (Vgontzas et al., 2002; Vgontzas et al., 2001) indicates that the pathophysiology of insomnia is one of hyperarousal with 24-hr increases in ACTH and cortisol secretion, and the effects of cortisol on immune suppression are well established (e.g., Ader, 2007). Participants in our study may have had altered HPA axes that adversely affected responses. As a consequence, it is possible that, similar to the results in the current study (e.g., H3N2 and B), the Insomnia group developed fewer antibody titers after their *original* exposure to the vaccine strains, or closely related strains, which decreased at similar rates over time, resulting in baseline differences in the current study. Conversely, it is possible that the two groups generated similar levels of antibodies during their *original* exposure, but that the Insomnia group had a more rapid reduction in titers over time, resulting in baseline differences in the current study. Finally, it is possible that the *original* exposure occurred longer in the past for the Insomnia group than the No Insomnia group, which allowed for more time for the titers to decrease in the Insomnia group, resulting in baseline differences in the current study.

Unfortunately, given the cross-sectional nature of the current study, it is not possible or appropriate to attempt to answer the above questions with the current data set. For one, it is not possible to know when the participants were previously exposed to the viral strains, or closely related viral strains. Therefore it is not possible to determine the effect of time on baseline levels of antibody titers. In addition, although it is possible to statistically control for baseline differences on antibody titers, this is considered a misuse of these statistical procedures in this type of cohort design (Miller & Chapman, 2001), as these group differences are likely meaningful and may be a function of having chronic insomnia. Future studies are needed that follow the same participants over multiple years of vaccination to determine if chronic insomnia affects the rate of antibody titer decay over the course of multiple months and to parse out the relationship between chronic insomnia and antibody response using more sophisticated multilevel statistical procedures.

The current study was unable to replicate previous studies that found gender (Furman et al., 2014), smoking (Cruijff et al., 1999), obesity (Sheridan et al., 2012), and perceived stress (Kiecolt-Glaser et al., 1996; Miller et al., 2004) predicted poorer influenza antibody response. The most likely reasons for the null findings in the current study were our sample being healthy young adults and restriction of range. For instance, in our sample the oldest participant was 29 years old, the median number of cigarettes smoked was zero, and the mean was 1.53 *per week*. Conversely, Cruijff et al. (1999) had a sample of participants who were over 60 years old, and smokers who averaged 1–9, 10–19, and > 20 cigarettes *per day*. Similarly, the Sheridan et al. (2012) sample averaged 45.6–59.7 years of age, depending on the group, and had 164 obese participants compared to only 16 obese participants in the current study. It should be noted that in the present study our measure of stress was subjective (the PSS). We did not assess any physiological correlates of stress (e.g., cortisol, cytokines), and, as noted, Vgontzas and colleagues (Vgontzas et al., 2002; Vgontzas et al., 2001) have reported 24-hr increases in ACTH and cortisol secretion as well as cytokine shifts that may account for daytime fatigue (Vgontzas et al., 2002; Vgontzas et al., 1999).

Thus, the lack of any physiological stress measures may have weakened any effects reported, as we were limited to a single (subjective) indicator. Given this, and the type of statistical analyses (cross-sectional) it is difficult to make any strong inferences concerning these results (Miller et al., 2004).

Another factor that may be a limitation was the use of college students. Typically influenza is a problem for the very young and old, because they are more immunocompromised or vulnerable to influenza. Indeed, our healthy sample were largely protected at baseline, leaving less room for a vaccine response. However, both groups showed both clinical and statistical increases in HI antibodies pre- to postvaccine, and earlier research showed that the variability of HI antibody responses to the influenza vaccine is similar between young (college-age) and older adults (Kohut et al., 2005). Therefore, it is not clear how much of a limitation it was that this sample was not immunocompromised, and indications are that healthy young adults were an appropriate population to examine. In addition, the CDC found that during the 2009 H1N1 pandemic, those aged 15–29 years had 4.8 times the rate of infection as those 60 years old (Centers for Disease Control and Prevention, 2009). Finally, in order to determine if *insomnia alone* was a risk factor for decreased influenza vaccine response, it was necessary to test this hypothesis in otherwise healthy participants without confounding medical disorders or medications, which allowed for greater internal validity (i.e., clearer interpretation of significant results). In addition, studying older adults without confounding disorders or medications would have poor external validity because such individuals are not representative of that population.

In summary, this study suggests that otherwise healthy individuals suffering from insomnia may have compromised immune function, potentially leaving them more vulnerable to infection. This may be especially important in at-risk populations, as insomnia might further impair immune responses in individuals who are already immune-compromised. The current study adds to the literature examining sleep and immunity in several ways. It is the first study to examine influenza vaccine responses in a clinically diagnosed insomnia population. It was also important in that the sample was a very well characterized healthy, medication-free sample, which allows for a cleaner interpretation of the results. Future research is needed to determine the long-term effects of insomnia on influenza antibody titers, and these results need to be replicated in other populations (e.g., immune-compromised individuals) as well as with other vaccinations (e.g., hepatitis B, meningitis). In addition, future research is needed to determine if insomnia interventions might improve influenza vaccination response.

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FIGURE 1.

Mean and standard error of log₂ transformed hemagglutination inhibition (HI) levels of the Insomnia and No Insomnia groups before and after vaccinations for H3N2 anti-influenza antibodies in 2011–2012 and 2012–2013 and B H3N2 anti-influenza antibodies in 2012–2013.

TABLE 1

Baseline Sample Characteristics by Group

	Insomnia (n = 65)		No Insomnia (n = 68)		χ^2	p	OR (95% CI)
	% within group		% within group				
Sex, female	60.0%		60.3%		.001	.972	.99 (.49–1.98)
Ethnicity, Hispanic	27.7%		30.9%		2.21	.332	1.12 (.53–2.37)
Race					2.78	.595	N/A
White	63.1%		64.7%				
Black/African American	12.3%		10.3%				
Multiracial	12.3%		13.2%				
Asian	1.5%		5.9%				
Unknown	10.8%		5.9%				
BMI					1.85	.605	N/A
Underweight	6.6%		10.4%				
Normal weight	52.5%		53.7%				
Overweight	31.1%		22.4%				
Obese	9.8%		13.4%				
Nicotine users	17.2%		4.4%		5.68	.017	4.50 (1.19–16.96)
Alcohol users	32.3%		25.0%		.870	.351	1.43 (.67–3.05)

	<i>M</i> (<i>SD</i>)	<i>M</i> (<i>SD</i>)	<i>t</i>	<i>p</i>	<i>Cohen's d</i>
Age, y	20.35 (2.8)	19.94 (2.2)	-.94	.348	0.16
ISI	16.17 (4.03)	2.65 (2.32)	-23.86	<.001	4.16
PSQI	10.72 (2.84)	2.72 (1.43)	-20.62	<.001	3.60
ESS	9.86 (4.28)	4.68 (2.99)	-8.13	<.001	1.42
MEQ	43.98 (8.96)	49.49 (8.65)	3.60	<.001	0.63
PSS	19.45 (5.93)	12.40 (5.14)	-7.34	<.001	1.27
BMI	24.95 (5.44)	24.27 (6.19)	-.67	.505	0.12

Note. *M* = Mean, *SD* = Standard Deviation, BMI = Body Mass Index, ISI = Insomnia Severity Index, PSQI = Pittsburgh Sleep Quality Index, ESS = Epworth Sleepiness Score, MEQ = Morningness-Eveningness Questionnaire, PSS = Perceived Stress Score.

TABLE 2
Pairwise and Repeated Measures Analyses of No Insomnia Versus Insomnia Groups at Pre- Versus Four Weeks Postvaccination

	Prevaccine				Four weeks postvaccine				Pre- to postvaccine			
	No Insomnia		Insomnia		No Insomnia		Insomnia		Time		Group	
	M (SD)	M (SD)	t	p	M (SD)	M (SD)	t	p	F	p	F	p
H3N2												
2011–2012	8.71 (0.99)	8.13 (1.49)	1.76	.084	10.25 (1.02)	9.52 (1.80)	1.91	.062	55.83	<.001	4.75	.033
2012–2013	7.85 (1.15)	7.03 (1.53)	2.62	.011	11.00 (1.38)	10.35 (1.55)	1.89	.062	300.49	<.001	7.45	.008
B												
2011–2012	7.82 (1.29)	7.26 (1.36)	1.63	.109	9.00 (1.22)	9.03 (1.55)	−0.09	.933	66.76	<.001	0.75	.389
2012–2013	7.50 (1.39)	6.97 (1.57)	1.53	.130	10.22 (1.28)	9.35 (1.27)	2.93	.004	187.59	<.001	7.11	.009
H1N1												
2011–2013	8.26 (1.88)	7.54 (1.97)	2.18	.031	10.95 (1.91)	10.72 (2.11)	0.70	.506	256.36	<.001	2.78	.098

Note. Analyses are on Log₂ transformed HI antibodies for H3N2 and B across 2011–2012 (No Insomnia = 28; Insomnia = 31) and 2012–2013 (No Insomnia = 40; Insomnia = 34) vaccinations, and H1N1 combined across years (No Insomnia = 68; Insomnia = 65) because same strain was used in virus both years. *M* = Mean, *SD* = Standard Deviation; HI = Hemagglutination Inhibition assay; IgG = Immunoglobulin G assay; IgG = IgG 1 subclass antibody.

TABLE 3

Exploratory Repeated Measures Multivariate Analysis of Variance (MANOVA) of Demographic, Health, Sleep, and Stress Variables

Effect	Wilks' s Λ	F	P	Partial η^2
Between subjects				
Intercept	.64	22.75	< 0.001	.36
Age	.98	.62	.603	.02
Gender	.96	1.76	.158	.04
BMI	.96	1.73	.164	.04
Nicotine use	.95	2.12	.101	.05
Alcohol use	1.00	.03	.992	< .01
TST	.99	.26	.857	.01
PSQI	.98	.90	.441	.02
ESS	.97	1.11	.346	.03
MEQ	.99	.45	.718	.01
PSS	.96	1.66	.178	.04
Insomnia	.97	1.15	.330	.03
Within subjects				
Time	.95	2.71	.048	.06
Time x age	.98	.74	.532	.02
Time x gender	.97	1.22	.306	.03
Time x BMI	.98	.25	.860	.01
Time x nicotine use	.98	.93	.431	.02
Time x alcohol use	.99	.50	.686	.01
Time x TST	.99	.40	.756	.01
Time x PSQI	.86	6.45	< .001	.14
Time x ESS	.99	.36	.781	.01
Time x MEQ	.98	.66	.580	.02
Time x PSS	.99	.60	.619	.02
Time x insomnia	.88	5.25	.002	.12

Note. Variables entered as independent variables, time (baseline vs. four weeks postvaccination) as the repeated measure, and log2 transformed HI antibody levels of each viral strain in both years as the dependent variables. Hypothesis $df=3$, Error $df=117$, BMI = Body Mass Index, TST = total sleep time, PSQI = Pittsburgh Sleep Quality Index, ESS = Epworth Sleepiness Score, MEQ = Morningness-Eveningness Questionnaire, PSS = Perceived Stress Score.