Stress, Influenza, and Health Behavior: Implications for Pandemic Influenza and Infection Control

by

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Dedication

To my parents.

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Abstract

The impact of 2009 pandemic influenza A H1N1 on morbidity and mortality among university-aged adults, and the noted disparities in attack rates in different populations, point to the need for research to identify novel risk factors for explaining variability in susceptibility and disease status in affected populations. Exposure to psychological stress may constitute one such novel risk factor for acquiring influenza infection that has yet to be examined and well understood, particularly in the university setting. Furthermore, simple health behaviors and practices that might be altered due to psychological stress have not been well studied in relation to influenza acquisition. This dissertation utilizes prospective data from the M-Flu study, a randomized intervention trial conducted among students living in residence halls at the University of Michigan during the 2007-2008 flu season, to demonstrate if increased exposure to psychological stress is significantly associated with increased rates of influenza-like illness (ILI), increased rates of naturally acquired influenza A infection, and higher influenza viral load, a potential biomarker of disease severity. This dissertation also explores the behavioral response to circulating seasonal influenza among participants living in this high-risk setting for transmission of infection. The main findings from this work indicate that (1) differential exposure to psychological stress significantly affects the rate of ILI and naturally acquired infection; (2) increased levels of perceived stress are significantly associated with increased levels of viral load among young adults with confirmed influenza; and (3) young adults in this environment seeking clinical verification of their

ILI along with laboratory testing report sub-optimal compliance with non-pharmaceutical recommendations for mitigating the spread of influenza. Implications of these findings for pandemic influenza and infection control within the university setting are discussed.

Chapter 1

Introduction

Disparities between human populations in attack rates of both seasonal and pandemic influenza have generated much discussion on plausible key factors responsible for this documented variation.[1-5] Psychological risk factors such as perceived stress in response to life events among individuals constitute one novel pathway by which disparities in susceptibility and disease severity may exist. Several studies have established a positive temporal association between psychological stressors and incident outcomes related to poor respiratory health.[6-14] In contrast, the available data on psychological stressors and perceived stress with respect to outcomes of influenza-like illness (ILI or flu-like illness), confirmed influenza infection, and/or biomarkers of disease severity among those infected have yielded inconsistent findings.[15-21] Research has also shown that social support networks act as a stress-buffering mechanism against self-reported or clinically verified (i.e. confirmed) respiratory infection in older adults and small children.[7, 8] However, there is no data in relation to the role of social support as a buffering mechanism for perceived stress on rates of flu-like illness or influenza among young adults.

Up to date, key epidemiologic questions regarding the control of influenza disease transmission have remained unanswered. Among these questions is if the early detection of flu circulating in the community affects public compliance with non-pharmaceutical

measures for influenza.[22] The threat of avian influenza and the emergence of the 2009 pandemic influenza A H1N1 have prompted researchers to examine the behavioral response to influenza epidemics since some responses during outbreaks are critical in containing and spreading disease.[23] Evidence of a change in self-reported anticipated and actual compliant behavior with recommended non-pharmaceutical measures for the current flu pandemic has been reported in some cross-sectional studies.[24-26] The long-term effect, however, of flu-like illness and influenza on compliant behavior with these measures in a high-risk community setting for transmission of disease is unknown.

The current research on the subject of perceived stress, influenza, and the behavioral response to an influenza outbreak in the community is limited. To date, studies have not examined the extent to which perceived stress is a risk factor for confirmed or survey reported ILI, naturally acquired seasonal influenza A infection, and influenza A viral load, a possible biomarker of disease severity, in understudied populations like young adults at high risk for pandemic flu. In addition, researchers have not yet examined these associations among young adults living within a university setting at high risk for transmission of infection. Moreover, no studies have examined the behavioral response to an influenza outbreak in a university community environment over an extended period of time. Taken together, the study of perceived stress, influenza, and the behavioral response to an influenza outbreak among young adults in the university setting has implications for susceptibility to pandemic variant strains and infection control in a crowded environment.

Using data from a cluster randomized intervention trial conducted among 1,111 young adults living within university residence halls during the 2007-2008 flu season, a

season noted to have a high influenza attack rate,[27] the objectives of this dissertation were (1) to examine the influence of perceived stress on rates of ILI and assess if social support networks modified this association; (2) to examine the influence of perceived stress on rates of naturally acquired influenza A infection and influenza A viral load; and (3) to examine if the onset of ILI influences participants' reduction in exposure to social contacts and adherence to hand hygiene measures that can ultimately affect the spread of influenza within the community.

1.1 Specific Aims and Hypotheses

Aim 1: Examine if increased levels of perceived stress are associated with increased rates of ILI among young adults.

Hypothesis 1a: Exposure to higher vs. lower levels of perceived stress at baseline will be associated with increased rates of clinically observed or survey reported ILI over the follow-up period.

Hypothesis 1b: The observed association between perceived stress and rates of ILI will be attenuated among participants with larger social support networks.

Aim 2: Examine if increased levels of perceived stress are associated with increased rates of influenza A infection and a higher influenza A viral load among young adults.

Hypothesis 2a: Exposure to higher vs. lower levels of perceived stress at baseline will be associated with an increased rate of naturally acquired influenza A infection over the follow-up period.

Hypothesis 2b: Among those with confirmed influenza A infection, exposure to higher vs. lower levels of perceived stress at baseline will be associated with a higher influenza A viral load.

Aim 3: Examine if the confirmation of ILI elicits compliance with recommended hand hygiene measures and the voluntary reduction in exposure to social contacts.

Hypothesis 3a: During the week of illness confirmation compared to the week prior, participants with clinically verified ILI and with a greater number of verified symptoms will report greater compliance with hand hygiene measures for mitigating disease spread and a reduction in their number of social contacts compared to (1) participants who reported ILI but were not clinically examined and (2) participants who did not report ILI either clinically or on any weekly survey. A sustained adherence with these non-pharmaceutical measures is hypothesized among confirmed ILI cases.

1.2 Background

Psychobiological models for stress, infectious disease susceptibility, and clinical characteristics of illness

In 1936, Dr. Hans Selye, a pioneer in the field of medicine who coined the term "stress", published on a set of symptoms observed among his laboratory rats in response to stressful stimuli (i.e. the *General Adaptation Syndrome*).[28, 29] As stated in his 1936 paper, "A Syndrome Produced by Diverse Nocuous Agents," Dr. Selye wrote:

"We consider the first stage to be the expression of a general alarm of the organism when suddenly confronted with a critical situation, and therefore term it the "general alarm reaction." Since the syndrome as a whole seems to represent a generalized effort of the organism to adapt itself to new conditions, it might be termed the "general adaptation syndrome." It might be compared to other general defense reactions such as inflammation or the formation of immune bodies... It seems to us that more or less pronounced forms of

this three-stage reaction represent the usual response of the organism to stimuli such as temperature changes, drugs, muscular exercise, etc., to which habituation or inurement can occur."[28, 29]

Selye provided the foundation for stress research in the latter-half of the 20th century onward, resulting in numerous experimental studies in relation to stress-induced hormonal responses and alterations in human immune function.[30] All definitions of stress have revolved around one pivotal point, namely, that stress is a process in which "environmental demands tax or exceed the adaptive capacity of an organism, resulting in psychological and biological changes that may place persons at risk for disease."[31]

Stress research encompasses three general traditions.[31] The first is the environmental tradition, in which environmental demands, stressors, or life events objectively influence susceptibility to disease. The second is the psychological tradition, namely, an individual's subjective assessment of a stressful situation (i.e. perceived stress). The third tradition is the human stress response, which is an individual's emotional, behavioral, and/or biological reaction to environmental and/or psychological stressors.[31]

Two psychobiological models on how stress influences the onset of infectious disease and the clinical course of illness have been proposed (see Figures 1.1 and 1.2).[32] Of note, these models do not suggest that stress is the sole etiologic factor in disease onset and its progression, but instead it is one of multiple factors that may explain variability in infectious disease susceptibility.[32] An overview of the immune-altering effects of stress and stress effects on health behaviors will now be addressed.

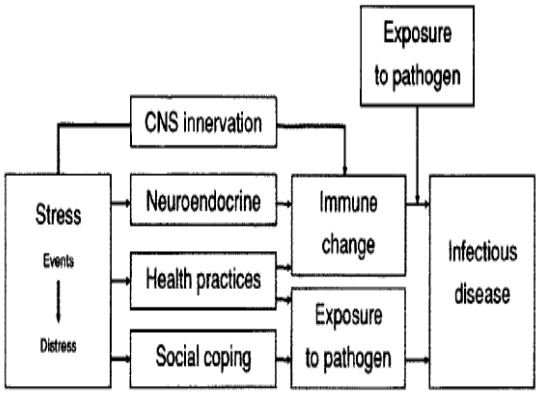

Figure 1.1 Pathways by which stress impacts the manifestation of infectious disease

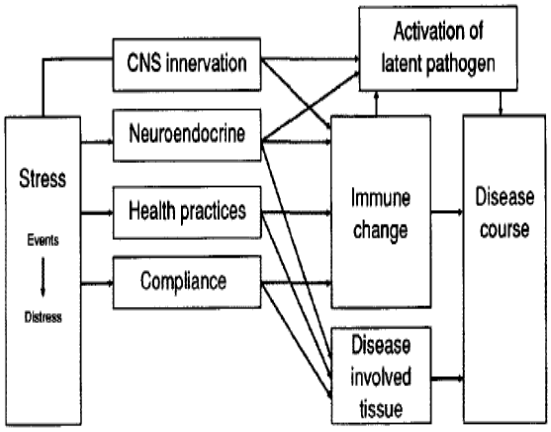

Figure 1.2 Pathways by which stress impacts disease course

The central nervous system (CNS)

The role of stress in immune function has been directly linked to effects on the CNS and hypothalamic-pituitary-adrenal (HPA) axis.[33, 34] Stressors can activate the stress response through corticotropin-releasing factor (CRF) from the hypothalamus.[34, 35] Shortly following the experience of an acute stressor, CRF is released, whereby the axons within the hypothalamus terminate and secretion of CRF into capillaries of the hypophysealportal venous plexus occurs, resulting in entry into the anterior pituitary gland.[33] CRF then causes the basophilic cells of the anterior pituitary gland to make proopiomelanocortin, which in turn stimulates hormones such as adrenocorticotropic hormone (ACTH) and β-endorphin. ACTH induces corticosteroids from the adrenal gland, one of the main classes of stress hormones.[33] CRF also results in the secretion of norepinephrine and epinephrine into the medulla of the adrenal gland, another major class of stress hormones, both of which are substantially elevated and present during the human stress response.[33]

Neuroendocrine hormones and the immune system

During the stress response, the adrenal medulla releases catecholamines (e.g. norepinephrine, epinephrine, and dopamine), which are synthesized in the brain, into the bloodstream. Catecholamines stimulate two subclasses of receptors, α- and β-adrenergic receptors, which result in various physiologic actions.[34] The physiologic effects of catecholamines affect a number or organs (e.g. brain, muscle, liver, skin) and systems (e.g. cardiovascular, pulmonary, and gastrointestinal systems).

At the same time, hypothalamic CRF activates the release of a number of hormones including ACTH, which stimulates the cortex of the adrenal gland to release cortisol and other glucocorticoid (steroid) hormones.[34] Glucocorticoids control a number of immune cell expression and functions including cortisol's role in carbohydrate, lipid, and protein metabolism and its anti-inflammatory and pro-inflammatory effects.[34] During stress, increased levels of cortisol are present, which decreases both T- and B-cell activity, thereby potentially preventing tissue damage by extended cell contact with increased levels of specific cytokines.[34] However, whether cortisol-induced physiologic effects are beneficial or harmful is dependent on the type of stressor, whether it is chronic or acute, and how one perceives the stressful situation or event and the following concentration and length of cortisol exposure.[34] Other hormones released during stress include endorphins, growth hormones, prolactin, oxytocin, and sex steroids such as testosterone.

Both direct and indirect changes in immune function can occur as a result of neuroendocrine hormone secretion during the stress response. Neuropeptides (e.g. hypothalamic and pituitary peptides) and neuroendocrine hormones may directly affect

immune change through manipulation of biochemical occurrences influencing cell function and cell proliferation and differentiation.[34] These hormones may also act indirectly on immune function by affecting cytokine production which then influences immune cell function.[34] For example, murine models have shown that among restraint-stressed mice infected with either herpes simplex virus (HSV) or influenza A/Puerto Rico/8/34 virus, catecholamines play a major role in suppressing virus-specific CD8+ cytotoxic T-lymphocytes which are critically involved in proper cellular immune system function.[36] This finding is important to note given that influenza-specific T-helper cells which stimulate the production of antibody responses to influenza hemagglutinin promote the generation of virus-specific CD8+ cytotoxic T-lymphocytes.[37] Hence, suppression of virus-specific CD8+ cytotoxic T-lymphocytes indicates an impaired immunity to influenza infection. Other studies[38-40] have shown that neuroendocrine activation during the stress response suppresses natural killer cell activity, which is the first line of defense against viral pathogens in humans, including influenza viruses. Two other studies[41, 42] provide further evidence for the role of the neuorendocrine hormone response in respiratory infection. One study[41] found positive correlations between greater severity of acute respiratory synctial virus (RSV) bronchiolitis and elevated levels of growth hormones and prolactin. The second study[42] showed a statistically significant association between increased levels of plasma cortisol and a decreased T-helper 1-type cytokine response in RSV infection, indicating interference of antibody production during viral infection. In summary, neuroendocrine hormone secretion during the stress response plays an integral role in immune function and susceptibility to respiratory viral infection.

Behavioral pathways

Behavioral pathways linking stress to infectious disease and disease course have been implicated as a result of one's psychological stress manifesting into behavioral changes.[34] Certain behavioral changes such as poor hygienic practices, changes in physical activity, and increased alcohol consumption may, for example, work in a manner that influence exposure to respiratory pathogens, such as influenza viruses, which then place individuals at greater risk for exposure to these agents.[32, 43-45] Some changes in health practices related to stress may directly affect host tissue and organs leading to greater susceptibility to respiratory infection. For example, smoking may be increased during stress, which in turn could irritate and cause damage to nasal and lung tissues thereby increasing the likelihood of viral infection.[32, 45] Alternatively, stress can be linked to behavioral changes that may reduce exposure to pathogenic agents. One example of this is social withdrawal, whereby individuals with increased levels of perceived stress may limit their social interaction with others through social withdrawal and therefore decrease their risk for acquiring an infectious illness.[32, 46] However, individuals who limit their social interactions due to stress, once exposed to a pathogen, may be more susceptible to illness as a result of stress-related down regulation of immune function.[34, 47]

Stress may also influence compliance with health recommendations such as hand hygiene and other health practices. For example, in a large hospital-wide survey conducted by the Centers for Disease Control and Prevention (CDC), poor compliance with hand washing was associated with greater levels of patient care intensity, which reflected heavier workloads among medical staff and understaffing.[44] Another study[48]

examined compliance with recommended dietary behavior changes among adults who were at increased risk for developing cardiovascular disease. The authors reported that 71% of the 334 patients in the study population viewed stress as one of the main barriers to adherence with a diet regimen to control blood sugar and blood pressure. A third study[49] examined the effects of exam stress on dental hygiene behavior among 24 medical students, with half the participants preparing for a major academic exam and the other half, the control group, not preparing. Deinzer et al.[49] found significantly higher rates of plaque and gingivitis following the exam period in students preparing for the exam compared to controls. Hence, the literature provides evidence of an association between stress and compliance with health recommendations, whereby higher levels of stress are associated with non-compliant behavior in both clinical and non-clinical study populations.

1.3 Psychological stress and respiratory illness in humans

Evidence of a temporal association between psychological stress and respiratory illness is strong.[6-14] However, there is only a handful of prospective studies examining stress and incident outcomes of influenza infection and ILI.[15-21] The following sections will first discuss differences and similarities between experimental (i.e. viral-challenge) and observational study designs for examining these temporal relationships and, second, will discuss study findings with respect to respiratory illness outcomes in the current human stress literature.

Experimental and observational study designs

Respiratory viral-challenge studies are studies in which healthy volunteers complete baseline assessments of environmental stressors and/or psychological stress

prior to inoculation with a respiratory virus. Subjects are then typically kept in isolation (i.e. quarantined) and have their symptoms recorded and/or clinically verified over the study period.[32] Observational study designs, in contrast, allow study participants to be observed in their natural environment. As with respiratory viral-challenge studies, baseline assessments of stress are collected. Subjects are then followed for a period of time where self-report and/or clinical examination of illness is recorded.

One advantage of an experimental study design is that it allows for control of viral dosage and biological verification of pathogens,[32] which are ideal for controlling exposure pathways (i.e. differential behaviors). For example, stress may influence one's behavioral and coping mechanisms, such as increased smoking, which work to influence susceptibility to respiratory infection. Experimental studies also typically measure and control for baseline levels of antibodies to the pathogen of interest which indicates prior exposure and a degree of immunity to the challenge pathogen. There are however several drawbacks to experimental studies, including selected, specialized populations that consist of volunteers who are in very good health, are willing to be inoculated with respiratory viruses, and willing to be quarantined. Volunteers who are taking medications, smoke, consume alcohol regularly, and who may have any underlying (chronic) illness are automatically excluded. These studies also consist primarily of homogeneous adult populations.[9, 10, 14, 32, 50] Feasibility and institutional review board ethical considerations also make experimental studies difficult to implement. In contrast, observational studies allow for examination of more heterogeneous populations and do not require that participants be healthy volunteers, but rather disease-free at the outset of the study. Therefore, an observational study can examine a cohort of individuals that is

more representative of a general population sample than an experimental study. Observational studies should, however, implement control for possible confounders that are usually controlled for by exclusion criteria often used in experimental studies. Evidence regarding the influence of perceived stress on respiratory illness in both experimental and observational studies will now be discussed.

Psychological stress and upper respiratory tract infection

Studies[8, 51, 52] have suggested that levels of perceived stress are positively associated with the incidence of upper respiratory tract infection (URTI). For example, Cobb et al.[8] showed that higher levels of perceived stress were associated with an increased incidence of clinically verified URTI independent of health practices such as smoking and physical activity. In another study,[52] participants with greater impairment of immune reactivity to stressors at baseline were more likely to report an episode of URTI following a week that was perceived to be highly stressful compared to a week with lower perceived stress. Edwards et al.[51] found that among an undergraduate study population, higher levels of perceived stress interacted with levels of cortisol secretion in predicting incidence of self-reported URTI over a 2-week period. These studies therefore show that increased levels of perceived stress may be temporally associated with an increased incidence of URTI.

Psychological stress and the common cold

Cohen et al.[10] found that baseline assessments of perceived stress were strongly associated with an increased risk of clinical colds among adults who had been inoculated with rhinovirus. This association was found to be primarily attributable to increased rates of biologically verified infection as confirmed via nasal wash samples of viral isolation

and levels of IgA and IgG antibodies. In another study,[50] healthy adult volunteers were either administered one of five common cold viruses or nasal saline drops for comparison. Cohen et al.[50] showed that clinically verified colds increased in a dose-response manner with higher levels of perceived stress among adults. In fact, increased rates of infection, rather than a greater frequency of reported cold symptoms following infection, were primarily responsible for the relation between perceived stress and infectious respiratory illness. In contrast, Stone et al.[14] observed no relation between perceived stress and development of common cold symptoms following experimental rhinovirus infection in a sample of 17 healthy undergraduates. Using an observational study design, Takkouche et al.[13] also showed that perceived stress was associated with the common cold among faculty and staff at a university over the course of one year (adjusted incidence rate ratio [aIRR] = 2.8, 95% CI: 1.7-4.6 for 4th (highest) quartile vs. 1st quartile (lowest) of perceived stress). Strong evidence for a positive association between increased levels of perceived stress and an increased susceptibility to the common cold has therefore been consistently established in adult populations. Interestingly, some of these findings suggest that perceived stress is more strongly related to clinically-verified cold infections rather than symptom-based reports of the common cold.[10, 50]

Psychological stress and respiratory disease

Only one study[11] examining the role of perceived stress in relation to chronic respiratory conditions was identified. Wright et al.[11] found that higher levels of baseline caregiver perceived stress when infants were between 2 and 3 months old predicted an increased risk of multiple wheeze episodes among children in their first 14 months

independent of race/ethnicity, birth weight, maternal smoking, and lower respiratory infection at baseline. Of note, respiratory disease such as wheezing in small children is considered a risk factor for heightened susceptibility to other respiratory infections such as influenza. Among adults psychological stress has also been identified as a potential risk factor for mortality from respiratory disease. Nielsen et al.[53] showed that psychological stress at baseline, as measured by two questions with respect to the intensity and frequency of perceived stress, was linked to greater rates of mortality from respiratory diseases in adult men with higher levels of stress compared to those with lower levels of stress over the course of 25 years (adjusted hazard ratio [aHR]: 1.79, 95% CI: 1.10, 2.91). No relation was found among adult women.[53] Hence, there is some evidence that perceived stress contributes to respiratory disease in both children and adults. Results from studies with longer follow-up periods can provide a broader picture of the long-term effects of these risk factors in children as they age. Similarly, the relation between perceived stress and mortality due to respiratory disease requires further investigation and better measurements of stress in the epidemiologic literature.

Psychological stress and the immune response to influenza vaccination

Several studies have identified stress-induced effects on the immune response to influenza vaccination.[54-64] Despite the limited sample size in most of these studies, the majority report an inverse relationship between psychological stress and the immune response to vaccination, whereby individuals with greater levels of stress produce a significantly lower number of antibodies to at least one viral strain, indicative of an altered protection to the virus.[54, 56-64] In fact, a recent meta-analysis revealed that psychological stress resulted in a significantly poorer antibody response to A/H1N1 and

B influenza viral strains compared to the A/H3N2 strain following vaccination.[65] In summary, the majority of vaccination studies have found a negative correlation between stress and the immune response to influenza vaccination.

1.4 Incident outcomes of influenza infection and flu-like illness

Since 1966, four observational and three experimental studies have published on the temporal association between stress and influenza infection and/or self-reported ILI.[15-21] Discussion of these studies is now presented.

Current and future research: the role of stress in ILI and influenza infection

Only one published study has examined exposure to perceived stress as a predictor of self-reported ILI. Smolderen et al.[17] prospectively examined the association between perceived stress using a validated scale[66] and self-reported ILI among adult volunteers (mean age: 46 years). ILI was defined as the sudden onset of fever >38°C plus headache or muscle pain and at least one of running nose, coughing, sore throat, or chest pain. The authors found stress to be moderately predictive of self-reported ILI (adjusted odds ratio [aOR] = 1.03, 95% confidence interval [CI]: 1.00-1.07).

In an earlier study by Mohren et al.,[20] the temporal association between the stress of job insecurity and common infections including self-reported ILI and other health complaints among adult employees (mean age: 41 years) in an occupational setting was examined. Job insecurity was measured dichotomously and based on a single question, "Do you fear losing your job on short notice?," hence, a validated measure for stress was not used. Data on common infections were based on the common cold, gastroenteritis, and ILI as defined by fever (characterized by a temperature of at least 38^{o}C) and at least four of the following six ILI symptoms: muscular pain, fatigue, sore throat, clogged or

runny nose, coughing, and headache. The results showed that job insecurity stress was more strongly predictive of self-reported ILI risk than the risk of reporting a common cold or gastroenteritis (ILI aOR: 1.39, 95% CI: 1.22 to 1.57; common cold aOR: 1.04, 95% CI: 0.95 to 1.13; gastroenteritis aOR: 1.20, 95% CI: 1.03 to 1.40).

Two studies[15, 21] examining rates of confirmed influenza infection reported no statistically significant associations with exposure to psychological risk factors and/or an increased number of reported stressors at baseline. For example, Cluff et al.[21] found that rates of confirmed infection during an influenza epidemic among male employees of a military research installation were not significantly higher among men who were classified as "psychologically vulnerable" at baseline compared to men who were psychologically non-vulnerable. Clover et al.[15] examined the effects of perceived family functioning and stressful life events on the development of confirmed influenza B infection in 66 families during a 1984 influenza epidemic and found no statistically significant association between a greater number of negative life events and presence of infection. Of note, neither study explicitly examined perceived stress as the risk factor of interest.

Three experimental studies[16, 18, 19] examining the impact of stressors and psychological stress on the manifestation of symptoms in response to an influenza viral challenge have been published. Among these, only one study[16] reported a statistically significant finding. Cohen et al.[16] inoculated 55 adult volunteers with influenza A/Kawasaki/86 H1N1 and found that increased perceived stress prior to viral challenge predicted higher overall illness symptom scores in the 7 days post-inoculation. Additional analyses showed that stress was most strongly associated with symptoms on days 2 and 3

following viral challenge, when symptom scores were dramatically increased, but not significantly associated with symptoms between days 4 and 7 when symptom scores began to approach pre-challenge symptom levels.[16]

Taken together, the stress literature pertaining to outcomes of influenza infection and flu-like illness provides mixed findings. None of the identified studies examined the role of perceived stress in predicting rates of clinically verified ILI or naturally acquired influenza A infection, specifically. Only two of the aforementioned studies[16, 17] examined perceived stress as a predictor of illness, but they were not conducted in the community setting. Single-item measures of stress such as in the Mohren et al.[20] study of job insecurity as a predictor of self-reported ILI can introduce bias from measurement error, thus making it difficult to properly interpret study findings. In addition, the two studies[15, 21] examining rates of confirmed influenza were published in 1966 and 1989 and utilized antiquated methodology for longitudinal data. Neither study examined exposure to perceived stress but rather exposure to psychological vulnerability[21] and stressful life events.[15] Therefore, well-controlled observational studies utilizing appropriate statistical methods for examining temporal relations between influenza A infection, a pandemic variant strain, and clinically verified ILI in response to perceived stress are needed. Such potential associations observed, particularly in understudied populations like young adults who are at high risk for pandemic flu, can have important implications for future study of stress-reduction interventions and their necessity in alleviating the burden of influenza within community settings.

1.5 Buffering the negative impact of stress: the role of social support

The concept of social support refers to a social network's ability to provide "psychological and material resources intended to benefit an individual's ability to cope with stress."[67] Several researchers have shown that social support via increased social integration and diverse social networks contributes positively to health and the well-being of an individual.[7, 8, 67-76] A person's social network provides three core resources, namely, instrumental support (i.e. material aid; e.g. financial aid), informational support (i.e. pertinent information which assists an individual in coping with life difficulties; e.g. advice), and emotional support (e.g. social networks that provide the opportunity for emotional expression, caring, trust, etc.). The resources provided by a social network can vary depending on the type of stressor(s) and/or by the personality of the individual facing a stressful situation.[67]

The notion of social support as a promoter of health and well-being can be explained by two models: the stress-buffering model and the main effects model.[67, 69, 72, 77-80] In the first model, the buffering hypothesis suggests that social support protects individuals from the harmful effects of stress by promoting less perceived stress and more successful ways for dealing with it. The key factor here is that stressed individuals will perceive others in their support system to have the ability to provide instrumental, informational, and/or emotional support.[67] On the other hand, the main effects model suggests that social support acts in a positive way to influence health independently of stress via promotion of positive psychological states. The main effects model supports the notion that social support is beneficial for all persons and not just highly stressed individuals. Therefore, diversity of one's social network(s) and their ability to socially

integrate within a network play a key role in this model.[67] Social support can be very important in an individual's ability to manage their stress. Consequently, given the evidence supporting a relationship between perceived stress and respiratory illness, improved coping techniques for stress and a reliance on one's social network may lead to a decreased vulnerability to influenza infection and/or ILI.

In line with the stress-buffering model, this dissertation aims to determine if psychological stress statistically interacts with perceived social support. There is in fact an evidence base for a statistical interaction between these two factors in predicting URTI.[7, 8] For example, Cobb et al.[8] conducted a prospective study among adults to examine the joint effects of life event stress and perceived social support on both self-reported and clinically verified URTI and found that high levels of social support were not protective among participants reporting high life event stress; however, high levels of perceived social support were found to be protective of URTI under low life event stress. A similar finding was reported in a second study[7] conducted among a group of children such that social support was only protective against incident URTI at low life event stress levels. The authors stated that "the interaction with life events is due to the fact that occurrence of upper respiratory infection increased with greater life event stress, but only among subjects with high social support ($p<0.025$). In the low support children, illness did not vary with life event experience."[7] Given the current literature, there are no studies to the best of my knowledge that have examined the stress-buffering hypothesis within the context of influenza, particularly in young adults. This dissertation will therefore be the first to examine the joint effects of psychological stress and perceived social support

on both self-reported and clinically verified ILI among young adults within a university setting.

1.6 Stress and influenza viral load

Currently only one study, of which I am aware, has examined the temporal association between exposure to psychological stress and a biomarker of influenza disease severity in persons with influenza infection.[16] This study[16] looked at mucus production among adult volunteers in response to perceived stress which was measured one day prior to an influenza A/Kawasaki/86 H1N1 viral challenge. Cohen et al.[16] report that mucus production peaked at days 2 and 3 following inoculation (indicating a peak in disease severity), but began to rapidly decrease throughout days 4 and 7. Baseline levels of stress were positively associated with increased mucus production within 2 to 4 days following infection, only.[16] The current research stands to benefit from analyzing other biomarkers of disease severity such as influenza viral load. Influenza viral load, a biological measure quantifying the level of virus represented by the interaction between viral replication and the host's ability to clear infection,[81] has never been examined in the literature as a primary outcome with respect to psychological risk factors, particularly in young adults.

There are data to support the hypothesis that higher viral load biologically correlates with greater disease severity in persons infected with influenza. For example, higher influenza viral loads have been linked to more severe pro-inflammatory responses and tissue injury in otherwise healthy adults infected with avian influenza A H5N1 compared to previously healthy adults infected with seasonal influenza A H1N1 or H3N2.[82] Boivin et al.[83] observed a substantially faster recovery from flu symptoms when Oseltamivir was administered within the first 24 hours of symptom onset, which

corresponded to a substantial decrease in viral load and thus a decrease in infectivity. In a separate study,[84] patients with severe 2009 pandemic H1N1 infection had substantially protracted declines in viral infectivity and increased cytokine reactions compared to persons with mild clinical disease. A mathematical modeling study[85] of the human immune response to seasonal influenza A infection showed that for low levels of initial viral load, the course of disease remained asymptomatic; for medium levels of viral load, the course of disease began to show constant duration and some severity of infection with various times of symptom onset; and for high levels of initial viral load the severity of illness greatly increased.

There are however some limitations in using viral load as a biomarker of influenza disease severity. Two of the main limitations are that the level of virus detected may be affected by the method of specimen collection[84, 86, 87] and that this measure may not represent the true concentration of influenza A RNA if semi-quantitative PCR methods are utilized. Despite these limitations, viral load still provides researchers with important data on virus-host interactions.[81, 84, 87]

Given the documented relationship between increased levels of perceived stress and greater mucus production in adults, and evidence that greater influenza viral load corresponds to more severe disease in humans, it may be important to examine if a temporal association between perceived stress and viral load of seasonal influenza A exists. Using viral load as a biomarker for disease severity among persons with confirmed infection may help to further develop the risk profile for influenza viral pathology, particularly among young adults at high risk for pandemic influenza. Therefore, this dissertation aims to elucidate, for the first time, if psychological stress is a novel risk

factor for viral load of seasonal influenza A in a young adult cohort. Examining viral load data in conjunction with clinically verified and/or self-reported ILI and laboratory-confirmed influenza infection from the same study will help uncover if psychological stress impacts not only subjective and objective measures of influenza illness but also pathological markers of influenza infection.

1.7 Confirmation of flu-like illness, influenza, and subsequent health behavior

The first two aims of this dissertation examine if psychological stress predicts rates of ILI, rates of naturally acquired influenza infection, and influenza viral load among young adults living in shared housing units within the university setting. The third aim now explores the health behaviors among individuals who become sick with influenza. Research in this area is critical for identifying how ill persons respond to their illness and behave in a manner that can positively or negatively impact the transmission of influenza within a community setting. Specifically, the third aim of this dissertation examines if the confirmation of flu-like illness influences every day behaviors that are known to play an important role in the spread of disease within this environment.

Several studies have reported on infection control measures with respect to prevention and control of outbreaks from influenza.[88-95] However, there have been very few epidemiological studies examining if an influenza outbreak in the community affects behavior with recommended measures for mitigating the spread of influenza.[22, 96] Such measures include proper hand hygiene, use of alcohol-based hand sanitizers to disinfect hands, and limiting social interactions/contact with others to minimize exposure and transmission of disease.[97] According to reports from the CDC, hand washing with soap and water is one of the most important measures for reducing the spread of pathogens

such as influenza viruses to others.[98] The CDC also recommends the use of alcohol-based hand sanitizers as an effective alternative for hand disinfection within the influenza context.[98] In addition, the CDC along with the World Health Organization (WHO) recommend that persons with ILI limit their contact with others during an influenza epidemic, ideally in isolation, when symptoms appear.[99] Limited contact with others when symptomatic is an effective approach in reducing secondary attack rates, particularly in university settings.[99] Given the 2009 influenza A H1N1 pandemic, examining the relation between illness confirmation and adherence to non-pharmaceutical measures for mitigating the spread of influenza, particularly among persons living in a high risk setting for transmission of infection, has important implications regarding transmission of disease within the community.

Recent population-based studies have begun to examine if influenza outbreaks impact public compliance with non-pharmaceutical measures in hopes of aiding in the development of a global pandemic preparedness agenda. For example, one population-based cross-sectional study[24] surveying adults between May 8 and May 12, 2009 in the United Kingdom found that in response to the 2009 H1N1 influenza pandemic, 38% of participants reported engaging in at least one of three recommended behavior changes, including increasing hand washing frequency, surface cleaning, and engaging in "avoidance behavior." Another population-based cross-sectional survey on the 2009 H1N1 pandemic conducted in April 2009 by the Harvard School of Public Health[23] following the WHO's declaration of a pandemic imminent reported that 59% of adult Americans in the US responded to the outbreak by washing their hands or using alcohol-based hand gels "more frequently". Hence, the urgency of pandemic preparedness today

has highlighted the need to quantify compliance with such non-pharmaceutical measures for influenza, particularly in community environments where influenza is prevalent.

This aspect of my dissertation will help elucidate if individuals who live in crowded community settings during a seasonal influenza outbreak adhere to health precautions intended to limit the spread of disease by quantifying compliance via participants' reported hand hygiene behavior and reduction in exposure to social contacts. Examining the behavioral response to an influenza outbreak and illness confirmation in an institutional setting over an extended time frame is another aspect of this study that will contribute greatly to the literature on compliance within a community where influenza is known to be circulating. Through comparisons between illness cases and disease-free control subjects, this dissertation will quantify for the first time trends in reported hand hygiene and other daily health behaviors in response to flu-like illness among university students. The findings from this research will have important implications for infection control within crowded community settings where influenza is present.

1.8 Public health significance

Although there is mounting evidence linking respiratory illness to psychological stress in children and adult populations, no research has prospectively examined the extent to which psychological stress influences ILI, seasonal influenza A infection, and influenza A viral load in an understudied population of healthy young adults at high risk for pandemic flu in the community setting. This dissertation will identify novel temporal associations between perceived stress and susceptibility to influenza infection and clinically verified ILI within this understudied population in a university setting. Given

the growing interest in individuals' behavioral response to influenza outbreaks, this dissertation will also provide invaluable information on everyday health behaviors that have major implications for the spread or containment of disease in a crowded community environment.

The public will benefit from these dissertation findings and their implications for future study of stress-reduction/management and behavioral interventions in alleviating the burden of influenza within community settings. Regarding the first two aims of this dissertation, examining the natural course of infection due to perceived stress in university settings, in particular, can have important consequences for respiratory health through educational outreach and stress interventions targeting at-risk students. Moreover, novel identifications of potential mechanisms such as social support networks will provide further intervention targets for reducing transmission of influenza in residence halls. On a larger scale, given the pandemic threat of swine A H1N1 and the potential economic and social disruption that may occur if this viral strain becomes more pathogenic, stress may play a key role in determining the extent of disease in healthy persons who fail to adhere to protective measures such as proper hand hygiene. Whether perceived stress plays a role in susceptibility to pandemic influenza and disease severity is currently unknown. Work examining these relationships using seasonal influenza A as an outcome may help provide insight regarding the impact of stress on current and future pandemic strains.

Regarding the third aim of this dissertation, exploration of young adults' adherence to recommended non-pharmaceutical measures for influenza such as hand hygiene will aid in the development of infection control protocols on university

campuses, specifically within residential hall units. By examining the sustained behavioral response to influenza among young adults, researchers can begin to understand trends in behavior and risk factors for compliance in this specific population, develop effective targeted interventions for behavioral change, and help mitigate the challenges university health care systems face. Hence, this work has ramifications regarding the circulation of influenza within the university setting and implications for multi-level behavioral interventions (i.e. institutional- and individual-level interventions) targeting those most likely to spread disease.

Chapter 2

Exposure to Psychological Stress is Associated with Incidence of Influenza-like Illness

2.1 Background

Although a strong and consistent relation between psychological stress and susceptibility to respiratory illness in humans has been established,[8-11, 13, 14, 50] there is a paucity of studies examining the role of psychological stress in influencing the incidence of ILI. The extent to which perceived stress and psychological stressors predict rates of ILI has varied between a moderately increased risk to a substantial risk in older adult populations.[17, 20] However, psychological risk factors for ILI among young adults in the community setting have not been well characterized or documented. The impact of 2009 pandemic influenza A H1N1 on morbidity and mortality among university-aged adults[100] and the noted disparities in attack rates among different racial/ethnic and socioeconomic groups[1, 2] necessitates epidemiologic research to identify novel risk factors contributing to the observed variability in susceptibility and disease status in these affected populations. As was established in an occupational setting,[20] the burden of increased exposure to psychological stressors may indeed constitute a unique risk factor for symptoms of ILI among young adults living in a university setting. Understanding this relationship in an understudied population of young adults at high risk for pandemic influenza may provide an avenue for devising effective stress-reduction and psychological interventions in university settings. Furthermore, developing a comprehensive risk profile for young

adults in this environment presents a novel opportunity for understanding the role of coping behaviors that may influence the impact of perceived stress on incidence of ILI.

Social support networks have been theorized to mechanistically affect psychological well-being, thus exerting influence on a diverse range of health outcomes including respiratory health, via two separate models: the stress-buffering and main effects models.[67, 69, 72, 77-80] In the stress-buffering model, perceived social support protects from the deleterious stress-effects on health via an individual's perception of their ability to depend on members of their social network to provide help when needed.[67] Individuals in one's social support network are, in this model, believed to aid in reducing the effect(s) of stressors on an individual, therefore allowing one to perceive the stressor as less threatening. This allows one to more effectively cope with one's perceived stress. In the main effects model, social support networks are hypothesized to influence health independently of stress via successful social integration into one's network and the promotion of positive psychological states that can elicit health-benefitting biological responses.[67] Studies have shown social support networks to be an independent risk factor for and act as a stress-buffering mechanism against self-reported or clinically verified respiratory infection in populations of older adults and small children.[7, 8, 101] There is, however, no evidence regarding if perceived social support buffers against the deleterious effects of high psychological stress on rates of ILI in young adult populations.

In this study, the influence of perceived stress on rates of ILI among young adults in a university setting during the 2007-2008 flu season was examined. Social support networks were also examined as an effect modifier of the stress-ILI association in this study population.

2.2 Methods

Study population and design

Data were obtained from the second year of a cluster randomized intervention trial known as M-Flu conducted during the 2007-2008 influenza season on the University of Michigan campus (Clinicaltrials.gov number, NCT00490633). Details of the original M-Flu study have been previously published.[102] The primary goal of M-Flu was to investigate the efficacy of face masks with and without the use of hand hygiene in comparison to no intervention on incidence of influenza among students living in residence halls at the University of Michigan. In total, 1,111 students living in five randomly selected residence halls at the University of Michigan during the 2007-2008 academic year were eligible for analysis.

A total of 15 residence halls are located throughout campus. Based on housing composition, size, and the demographic representativeness of the residence halls, five of the 15 residence halls were selected for inclusion into the study. Each of the selected residence halls consisted of residence houses (total N=37). Randomization was based on these 37 residence houses, whereby each house was randomly assigned to one of three study groups (Control, Face mask only, Face mask and Hand Hygiene). Participants in both intervention arms were asked to wear their provided face masks when in the residence hall for at least 6 hours per day during the intervention period. In addition, participants in the face mask and hand hygiene arm were asked to use the provided alcohol-based hand gel (Purell hand sanitizer with 62% ethyl alcohol, Pfizer Consumer Healthcare, Morris Plains, New Jersey). Hand sanitizer was given in a 2 oz. portable bottle and also in an 8 oz. pump. Participants in the control arm did not receive any

intervention materials, however all participants received education on proper hand hygiene practices and respiratory etiquette through an e-mail video link and the study website.

Baseline and weekly surveys

Distribution of surveys followed the first laboratory-confirmation of influenza on campus, which occurred during the week of 21 January 2008 as part of ongoing surveillance. The baseline survey was administered on-line to all participants prior to the intervention period beginning on 28 January 2008. Characteristics of the study population such as age, gender, self-reported race/ethnicity, alcohol consumption, sleep quality, physical activity, employment status, vaccination history, and prior diagnosis of either asthma or allergies were collected. The survey also asked questions regarding hand hygiene habits, respiratory illness symptoms in the previous week, and the number of roommates participants reportedly had. Perceived stress related to life events in the past month[66] and data on participants' social network(s)[103] were also collected.

Eight weekly surveys were distributed on-line to participants. Surveys collected data on reported presence or absence of respiratory illness symptoms experienced within the previous seven days.

Variables of interest

The main outcome was ILI, adapted from a case definition used in influenza surveillance with high sensitivity, specificity, and positive predictive value for laboratory confirmed influenza infection.[104] Presence of ILI was determined by clinical assessment and/or weekly surveys, both of which were defined by symptoms of persistent cough with at least one of fever/feverishness, body aches, and/or chills. Clinical verification of

reported ILI was performed by study-affiliated clinical assistants. For the purpose of this analysis, ILI status was determined based upon clinical assessment or, if no clinical report was available, survey report.

The main predictor variable was psychological stress as measured at baseline using the Perceived Stress Scale (PSS).[66] There are 14 items in which scores were ranked on a 0 to 4 scale ranging in responses of never to very often. Questions pertained to participants' feelings and thoughts during the previous month and indicated *how often* they felt or thought a specific way regarding a stressful life situation. To obtain an individual's stress score at baseline, values for all 14 items were summed across. Only respondents with data available for each item were included for analysis. A higher overall score indicated a greater level of perceived stress (range: 0-56).

Baseline covariates included: gender (male/female), race (white/other), previous diagnosis of allergies (yes/no) and asthma (yes/no), influenza vaccination status (yes/no), current alcohol consumption at least once a week (yes/no), current employment status (yes/no), and level of physical activity (high/low) as defined by the recommended amount of exercise for adults by the CDC (high level of physical activity defined by a very or extremely hard rate for 4 or more times per week for at least 20 minutes each time *or* an easy, medium, or hard rate for 5 or more times per week for at least 30 minutes each time);[105] sleep quality over the past month (fairly/very bad vs. fairly/very good); a composite variable for optimal hand washing (optimal/suboptimal) based on CDC recommendations of duration and the average number of handwashes (mean number = 5) among participants (i.e. optimal hand washing was defined by washing with soap and water at least 5 times on average per day for at least 20 seconds each time);[98, 106] a

composite variable for use of alcohol-based hand sanitizer (proper use/otherwise) defined by the use of at least a quarter-sized amount of hand gel (recommended by the CDC) twice daily (the average number of uses among study participants);[98, 106] and the number of roommates reported to be living with (0, 1, or 2 or more persons). Intervention group, that is the intervention or control group participants were randomized to at the outset of the study, was also considered a potential confounder.

Effect measure modifier

The Lubben Social Network Scale (LSNS-18),[103, 107] designed to quantify the size of one's current social network, perceived support network, and perceived network of individuals one can confide in, consisted of 18 items in which scores were ranked from 0 to 5. Baseline scores were calculated by summing across all items, whereby a higher score represented a larger social network (range: 0 to 90). Because the LSNS-18 is an expanded version of the original scale,[107] which was validated in elderly populations, the psychometric properties of the LSNS-18 with M-Flu data were examined using factor analysis and Cronbach's alpha for different subscales. Slight modification of the wording used in the original LSNS-18 was performed for this study population.

Analytic methods

The distribution of potential confounders and social support network size at baseline was examined among 860 ILI-free participants who completed the PSS in its entirety. Chi-squared tests for categorical data and ANOVA for continuous data adjusting for within-residence house correlation were examined.[108] Observed differences in possible confounders between students with low and high perceived stress based on the mean value cut-off were determined using $P < 0.05$.

Complementary log-log (Cloglog) models adjusting for correlation within residence houses were used to examine the relationship between perceived stress and ILI incidence among an ILI-free cohort at baseline (N=938). Unlike Cox proportional hazard regression, discrete-time survival models using the Cloglog link in SAS Proc Genmod (SAS V.9.1 Cary, NC) allow for the effect of time to be estimated in discrete intervals.[109, 110] This method is robust to non-proportionality and is appropriate for this study since time, although continuous, was grouped into weekly intervals. Only the first report of clinical ILI or survey ILI was used for analysis if no clinical assessment was available. In total, 59 participants reported ILI more than once in the follow-up period.

To determine which covariates to include in the multivariate analysis, univariate Cloglog models were used to examine the association between each potential confounder at baseline and ILI incidence ($P< 0.15$). Variables displaying a statistically significant difference between low and high stress groups were also included in the multivariate model ($P< 0.05$). To properly model the functional form of continuous independent variables (i.e. perceived stress and social support networks) in relation to ILI incidence, separate univariate Cloglog models examining a trend across quintile categorizations of stress and social support networks were examined. Based on a quintile examination of stress, a U-shaped trend was identified when predicting ILI incidence; therefore, to ease interpretation and given a large number of incident cases (N=277), stress scores were categorized based on the variable's functional form into three groups, namely, "low" (0-21), "moderate" (22-29), or "high" (30-56). Quintile examination of social support network scores showed a linear trend in predicting ILI incidence, therefore results are

reported for every interquartile-range (IQR=15.5) increase in perceived social support. Statistical significance was determined using $P< 0.05$ in adjusted-Cloglog models.

2.3 Results

Table 2.1 shows baseline characteristics of the 860 ILI-free participants with complete stress data who were eligible for analysis. More than half of the participants reported being female (55%), of non-Hispanic white race/ethnicity (63%), having sub-optimal hand hygiene (74%), and having never been vaccinated for influenza, including the 2007-2008 season (83%). Participants with exposure to high levels of perceived stress (a score greater than the mean=22.78; SD=7.72) had significantly lower perceived social support scores on average than participants with low perceived stress. In addition, the LSNS-18[103] showed excellent internal validity, with a Cronbach's alpha greater than 0.90 for all three subscales.

Table 2.1 Demographic characteristics among participants with reported stress data at baseline (N=860)[a]

	No. (%) of Participants								
Characteristics	Overall		ICC[b]	Low Stress		High Stress		P	
Total Number Of Participants	860			413		447			
Intervention Randomized To									
Mask/ Hygiene	264	(31)		126	(31)	138	(31)		
Mask Only	306	(36)		149	(36)	157	(35)		
Control	290	(34)		138	(33)	152	(34)		
Social Support Score, mean (SD)[c]	51.9	(11.3)	0.02	54.2	(10.8)	49.4	(11.6)	<.0001[h]	
Gender			0.19						

Female	469	(55)		208	(50)	261	(58)	0.20
Male	391	(45)		205	(50)	186	(42)	
Race/Ethnicity			0.03					
Non-Hispanic white	528	(63)		291	(72)	237	(54)	<.0001[h]
Other[d]	309	(37)		111	(28)	198	(46)	
Current Employment			0.03					
Yes	343	(40)		160	(39)	183	(42)	0.56
No	504	(60)		247	(61)	257	(58)	
Current Drinker			0.02					
Yes	270	(32)		140	(34)	130	(30)	0.21
No	574	(68)		267	(66)	307	(70)	
Exercise[e]			0.03					
Low Rate	644	(76)		300	(74)	344	(78)	0.21
High Rate	206	(24)		108	(26)	98	(22)	
Roommates In Room Assignment[f]			0.14					
0	186	(22)		81	(20)	105	(24)	0.41
1	581	(68)		285	(69)	296	(66)	
2 or more	92	(11)		47	(11)	45	(10)	
Recent Flu Vaccine[g]			0.03					
No	695	(83)		331	(82)	364	(83)	0.53
Yes	147	(17)		75	(18)	72	(17)	
Flu Vaccination Prior To 2008 Season			< 0					
No	523	(63)		246	(61)	277	(65)	0.26
Yes	304	(37)		155	(39)	149	(35)	
Optimal Hand Washer			0.02					

No	637	(74)	293	(71)	344	(77)	0.07
Yes	222	(26)	120	(29)	102	(23)	
Proper Use Of Hand Sanitizer		< 0					
No	453	(94)	211	(95)	242	(94)	0.70
Yes	28	(6)	12	(5)	16	(6)	
Sleep Quality		0.0001					
Very/Fairly Bad	181	(21)	52	(13)	129	(29)	<.0001[h]
Fairly/Very Good	678	(79)	361	(87)	317	(71)	
Prior Allergy Diagnosis		< 0					
No	613	(71)	292	(71)	321	(72)	0.72
Yes	247	(29)	121	(29)	126	(28)	
Prior Asthma Diagnosis		< 0					
No	729	(85)	351	(85)	378	(85)	0.86
Yes	131	(15)	62	(15)	69	(15)	

[a]Stress scores dichotomized among 860 participants with complete stress data based on the average score (22.78)

[b]ICC=Intracluster Correlation Coefficient

[c]N=788 participants completed the Lubben-18 scale among 860 participants with stress data; a higher score indicates a higher level of perceived social support

[d]Includes black or African American, Asian, Hispanic, Hawaiian or Pacific Islander, American Indian or Alaskan Native, Multi-Ethnic

[e]High rate was defined by a very or extremely hard rate for 4 or more times per week for at least 20 minutes each time or an easy, medium, or hard rate for 5 or more times per week for at least 30 minutes each time

[f]Categories compared for 0 vs. 1 or more roommates

[g]Recent flu vaccine defined as having a flu vaccine for the 2007-2008 flu season at baseline

[h]P-values computed using cluster-adjusted chi-square test for categorical characteristics and cluster-adjusted ANOVA for continuous characteristics; $P < 0.05$ considered statistically significant

Table 2.2 shows results from the univariate discrete-time survival models for rates of ILI. Based on the functional form of perceived stress in the univariate analysis, individuals with *moderate* perceived stress (score range: 22-29) had a 31% reduced incidence of ILI (hazard ratio [HR]=0.69, 95% confidence interval [CI]: 0.52, 0.91)

compared to participants with *low* stress (score range: 0-21); and, individuals with *high* perceived stress (score range: 30-56) had a 67% increased incidence of ILI compared to those with *moderate* stress (HR=1.67, 95% CI: 1.18, 2.37). In addition, for every IQR increase in social support network scores, the rate of ILI increased by 15% over the study period (95% CI: 0.97 to 1.37; see Table 2.2). Other factors associated with rates of ILI in univariate analyses included being in the control arm, of female gender, and having had a prior diagnosis of allergies (all $P< 0.15$; see Table 2.2).

Table 2.2 Univariate associations between each potential confounder at baseline and self-reported or clinically verified influenza-like illness over the follow-up period

Variable	n	ICC[a]	HR[b]	95% CI[c]	P
Perceived Stress[d]	860	< 0			
High vs. Low			1.15	(0.83 to 1.59)	0.40
Medium vs. Low			0.69	(0.52 to 0.91)	0.01[k]
High vs. Medium			1.67	(1.18 to 2.37)	0.004[k]
Intervention Randomized To	938	< 0			
Mask/ Hygiene			0.75	(0.55 to 1.01)	0.06[k]
Mask Only			1.00	(0.76 to 1.31)	0.99
Control			Ref		
Social Support Score[e]	808	< 0	1.15	(0.97 to 1.37)	0.11[k]
Gender	934	< 0			
Female			1.29	(1.02 to 1.65)	0.04[k]
Male			Ref		
Race/Ethnicity	908	< 0			
Non-Hispanic white			1.14	(0.88 to 1.47)	0.31
Other[f]			Ref		
Current Employment	919	< 0			
Yes			0.98	(0.77 to 1.25)	0.87

No			Ref		
Current Drinker	899	< 0			
Yes			1.12	(0.87 to 1.44)	0.40
No			Ref		
Exercise[g]	900	< 0			
High Rate			1.06	(0.80 to 1.39)	0.70
Low Rate			Ref		
Roommates in Room Assignment	933	< 0			
2 or more			0.88	(0.55 to 1.40)	0.59
1			1.02	(0.76 to 1.36)	0.91
0			Ref		
Recent Flu Vaccine[h]	899	< 0			
Yes			1.02	(0.75 to 1.39)	0.90
No			Ref		
Flu Vaccination Prior to 2008 Season	880	< 0			
Yes			1.13	(0.89 to 1.45)	0.32
No			Ref		
Optimal Hand Washer[i]	931	< 0			
Yes			1.00	(0.76 to 1.32)	0.97
No			Ref		
Proper Use of Hand Sanitizer[j]	530	< 0			
Yes			1.03	(0.54 to 1.96)	0.92
No			Ref		
Sleep Quality	914	< 0			
Fairly/Very Good			0.83	(0.63 to 1.11)	0.21
Very/Fairly Bad			Ref		

Prior Allergy Diagnosis	921	< 0			0.09[k]
Yes			1.40	(1.09 to 1.79)	
No			Ref		
Prior Asthma Diagnosis	921	< 0			
Yes			1.09	(0.78 to 1.50)	0.62
No			Ref		

[a]ICC=Intracluster Correlation Coefficient; clustering was not accounted for when ICC <0

[b]HR=Hazard Ratio

[c]CI=Confidence Interval

[d]Modeled based on a quintile assessment of the functional form of stress (range: 0 to 56); 20th percentile of stress score=16; 40th percentile=21; 60th percentile=25; 80th percentile=29; 100th percentile=56

[e]Social support modeled linearly based on a quintile assessment of the functional form (range: 10 to 89); 20th percentile=42; 40th percentile=49; 60th percentile=55; 80th percentile=62; 100th percentile=89; interpreted as an interquartile range (IQR); IQR=15.5

[f]Includes black or African American, Asian, Hispanic, Hawaiian or Pacific Islander, American Indian or Alaskan Native, Multi-Ethnic

[g]High rate was defined by a very or extremely hard rate for 4 or more times per week for at least 20 minutes each time or an easy, medium, or hard rate for 5 or more times per week for at least 30 minutes each time

[h]Recent flu vaccine was defined as having a flu vaccine for the 2007-2008 flu season reported at baseline

[i]Optimal hand washing defined by washing with soap and water at least 5 times on average per day for at least 20 seconds each time; based on CDC recommendation of duration and the average number of handwashes reported among participants at baseline

[j]Proper use of alcohol-based hand sanitizer defined by the use of at least a quarter-sized amount of hand gel (recommended by the CDC) twice daily (the average number of uses among study participants)

[k]Independent variables entered into the multivariate model based on P<0.15 for the Wald statistic

Multivariate analyses are presented in Table 2.3. Model 1, which excludes the main effect for social support networks, and model 2, which includes the main effect for social support networks, both show a statistically significant curvilinear relationship between perceived stress and rates of ILI. Controlling for intervention/control study group, gender, race/ethnicity, sleep quality, and prior allergy diagnosis in Model 1 (see Table 2.3), individuals with *moderate* perceived stress had a 31% lower incidence of ILI compared to participants with *low* stress (HR=0.69, 95% CI: 0.52, 0.92); and, individuals with *high* perceived stress had slightly increased rates of ILI compared to those with *low* stress (HR=1.02, 95% CI: 0.72, 1.46) and a significantly increased rate of ILI compared

to those with *moderate* stress (HR=1.47, 95% CI: 1.02, 2.13). Further adjusting Model 1 for social support networks (Model 2; see Table 2.3), individuals with *moderate* perceived stress had a 32% lower incidence of ILI compared to participants with *low* stress (HR=0.68, 95% CI: 0.50, 0.92); and, individuals with *high* perceived stress had a slight increased incidence of ILI compared to those with *low* stress (HR=1.04, 95% CI: 0.70, 1.52) and a significantly increased incidence of ILI compared to those with *moderate* stress (HR=1.52, 95% CI: 1.02, 2.26). No evidence of a statistical interaction between perceived stress and social support was found (Model 3; see Table 2.3).

Table 2.3 Multivariate survival analyses of self-reported or clinically verified influenza-like illness over the follow-up period[a]

	Model 1				Model 2				Model 3			
Variable	n	ICC[b]	HR[c] 95% CI[d]	P	n	ICC[b]	HR[c] 95% CI[d]	P	n	ICC[b]	HR[c] 95% CI[d]	P
Perceived Stress[e]	799	< 0			738	< 0			738	< 0		
High vs. Low			1.02 (0.72 to 1.46)	0.91			1.04 (0.70 to 1.52)	0.86			1.26 (0.24 to 6.65)	0.79
Medium vs. Low			0.69 (0.52 to 0.92)	0.01[i]			0.68 (0.50 to 0.92)	0.01[i]			0.59 (0.13 to 2.62)	0.49
High vs. Medium			1.47 (1.02 to 2.13)	0.04[i]			1.52 (1.02 to 2.26)	0.04[i]			2.06 (0.35 to 11.99)	0.42
Intervention Randomized to												
Mask/ hygiene			0.68 (0.49 to 0.94)	0.02[i]			0.68 (0.48 to 0.95)	0.03[i]			0.70 (0.50 to 0.97)	0.03[i]
Mask only			0.93 (0.71 to 1.24)	0.64			0.92 (0.68 to 1.23)	0.56			0.94 (0.70 to 1.27)	0.71
Control			Ref				Ref				Ref	
Gender												
Female			1.35 (1.04 to 1.75)	0.02[i]			1.38 (1.04 to 1.82)	0.02[i]			1.36 (1.03 to 1.79)	0.03[i]
Male			Ref				Ref				Ref	
Race/Ethnicity												
Non-Hispanic white			1.10 (0.84 to 1.44)	0.51			1.10 (0.82 to 1.46)	0.52			1.10 (0.83 to 1.46)	0.49
Other[f]			Ref				Ref				Ref	
Sleep Quality												
Fairly/Very Good			0.81	0.19			0.77	0.11			0.78	0.12

	(0.60 to 1.11)		(0.56 to 1.06)		(0.56 to 1.07)	
Very/Fairly Bad	Ref		Ref		Ref	
Prior Allergy Diagnosis						
Yes	1.43 (1.11 to 1.86)	0.01[i]	1.41 (1.07 to 1.85)	0.01[i]	1.39 (1.06 to 1.83)	0.02[i]
No	Ref		Ref		Ref	
Social Support Score[g]	NA[j]		1.09 (0.91 to 1.32)	0.35	1.09 (0.83 to 1.42)	0.54
Interaction Term[h]	NA[j]		NA[j]			
Stress High vs. Low X Support					1.28 (0.33 to 4.98)	0.71
Stress Med. vs. Low X Support					0.67 (0.19 to 2.38)	0.54
Stress High vs. Med. X Support					2.11 (0.49 to 9.17)	0.32

[a]Model 1 includes potential confounders associated with the outcome in univariate analyses; Model 2 includes perceived social support; Model 3 includes the statistical interaction between perceived stress and perceived social support

[b]ICC=Intracluster Correlation Coefficient

[c]HR=Hazard Ratio

[d]CI=Confidence Interval

[e]Categories based on a quintile assessment for functional form; low range: 0 to 21, moderate range: 22 to 29, high range: 30 to 56; mean value = 22.78

[f]Includes black or African American, Asian, Hispanic, Hawaiian or Pacific Islander, American Indian or Alaskan Native, Multi-Ethnic

[g]Social support entered linearly and interpreted as an interquartile range (IQR); IQR=15.5

[h]Interaction terms based on the IQR for perceived social support

[i]Variables considered statistically significant at the P<0.05 level

[j]NA=Not Applicable

2.4 Discussion

This study found that differential exposure to psychological stress greatly affected the rate of ILI among young adults in the university setting during the 2007-2008 influenza season. Contrary to the initial study hypothesis of a positive linear relation between increased stress and a higher rate of ILI, a statistically significant curvilinear (i.e. U-shaped) association was identified. Young adults with the highest and lowest burden of psychological stress were placed at a significantly greater risk of ILI compared to participants with moderate levels of stress. Participants with the highest levels of perceived stress had a 52% *greater* incidence of ILI compared to participants with moderate stress. In contrast, participants with moderate levels of perceived stress had a 32% *reduced* incidence of ILI compared to participants with low stress. The study findings also showed that social support did not modify the association between stress and incidence of ILI, suggesting that social support networks did not impact coping among participants exposed to psychological stressors.[67] Adjustment for gender, race/ethnicity, sleep quality, and prior allergy diagnosis did not explain the observed association between stress and rates of ILI, suggesting that other pathways, including stress-induced behavioral responses and biological changes in immune susceptibility to flu-like illness, may account for the distinct stress thresholds that were observed in this study. Additional research is needed to understand the mechanistic pathways by which varying thresholds of psychological stress lead to differential rates of ILI within young adult populations in the university setting.

This study is unique in that it provides prospective, epidemiologic evidence of a statistically significant association between psychological stress and incidence of ILI in a

young adult cohort during the 2007-2008 flu season within the natural environment. The statistically significant curvilinear association between perceived stress and incidence of ILI suggests that moderate levels of stress appeared protective against ILI among young adults and that a certain amount of perceived stress may actually benefit health. Perceived stress itself is a normal physiological reaction to either positive or negative life demands and the literature argues that it is one's management of stress that can subsequently influence the immune response and overall health.[30, 111, 112] In a recent review of studies regarding the psychoneuroimmunology of human stress,[30] individuals experiencing high psychological stress, particularly sustained stress, have continuously exhibited down-regulation in their immune function while other studies have indicated a positive immune reaction (e.g. higher number of antibodies, increased neutrophils, monocytes, CD8+, CD2+, and CD26+) in response to acute psychological stressors. Variability in upward or downward regulation of individuals' immune function in response to psychological stressors may be attributed in part to the "immunomodulating effect" of "perceived controllability."[30] Adults with less control over stressors have often shown negatively altered immune changes such as a decrease in T helper cells and those with high control have often shown positive immune changes such as an increase in their number of B cells.[30] These notable changes in immune parameters have implications for risk of infection, particularly infection caused by influenza viruses due to systemic, local antibody, and cytotoxic T lymphocyte responses.[37] Perceived controllability is indeed a plausible reason for the curvilinear association observed in this study since the Perceived Stress Scale[66] aims to capture the extent to which individuals view their lives as unmanageable and overwhelming. In fact, highly effective stress management skills,

optimism, and/or positive affective states in response to stress have been linked to health-promoting immune responses such as increased natural killer cell activity and poor coping behaviors and low levels of personal resources like social support have been linked to significant impairments in immunity.[30, 111, 112] Taken together, perceived stress may influence respiratory function through individuals' stress management. Those with moderate perceived stress may have simply dealt with stressful circumstances more effectively than those with very high stress in this study population. Among young adults in community settings, coping styles and perceived controllability in response to psychological stressors have not been examined for rates of ILI and influenza. Future research should replicate this study with a focus on rigorously measured and well-defined behavioral responses, coping mechanisms, and immune responses influencing the mechanistic pathway between psychological stress and self-reported or clinically verified ILI.

Reasons for participants with low perceived stress having significantly increased rates of ILI compared to those with moderate stress remain to be fully understood. However, the data suggests some possible explanations. First, social support networks were inversely related to levels of perceived stress. An increased rate of ILI among those with low stress compared to those with moderate stress may therefore be attributed to an increased risk of pathogen exposure due to larger social support networks.[113] Second, although baseline health behaviors (e.g. alcohol consumption and exercise) and hand hygiene did not confound the relationship between stress and ILI, it is possible that other health practices not measured in this study could have influenced the risk of ILI among these individuals. Third, other unmeasured individual level characteristics (e.g.

personality traits such as neuroticism) not examined here may play a role in determining differences in rates of ILI between participants with differential levels of perceived stress.

No studies have established if perceived stress is associated with rates of ILI and confirmed influenza infection among young adults living in the university setting. Among young adult populations specifically, Burns et al.[61] and Larson et al.[114] examined the effects of perceived stress on antibody response to influenza vaccination in healthy undergraduate populations. However, only one study has examined the relationship between perceived stress and rate of ILI.[17] Smolderen et al.[17] examined older adults from the general population and found that increased psychological stress was slightly predictive of an increase in self-reported ILI (aOR=1.03, 95% CI: 1.00, 1.07). Similar to this aim 1 study, they[17] utilized the Perceived Stress Scale,[66] but it was a shortened 10-item version of the 14-item scale used in this current study. In addition, the ILI case definitions varied between the aim 1 study and that of Smolderen et al..[17] ILI in their[17] research was defined as the sudden onset of fever >38°C plus headache or muscle pain and at least one of running nose, coughing, sore throat, or chest pain. Cough and fever/feverishness are commonly used symptoms defining ILI[104] and were the best predictors of laboratory-confirmed influenza infection in the M-Flu study, with cough showing a greater risk for infection than fever/feverishness. This dissertation also utilized a composite ILI endpoint obtained from clinically assessed ILI or survey-reported ILI if clinical assessment was unavailable. Hence, this composite endpoint and the close follow-up of ILI cases may have reduced measurement error, therefore allowing a statistically significant association between differential levels of stress and rates of ILI to be detected.

Given that the main findings from this research show a statistically significant curvilinear association between stress and ILI incidence, with participants reporting the highest burden of perceived stress at greatest risk for ILI, the moderate association identified in the study by Smolderen et al.[17] may be attributed to modeling stress continuously and therefore not fitting an appropriate model to the available data. For example, had stress been modeled continuously even though it was curvilinearly associated with ILI in aim 1, an interquartile range increase in stress (IQR = 10.5) would have produced an HR estimate of 1.00 (95% CI: 0.85 to 1.19). Thus, no association between perceived stress and rates of ILI would have been mistakenly concluded. Given the published data in the Smolderen et al.[17] study, it is difficult to say if there was in fact a non-linear association between stress and ILI. It is also possible that adults living in the community setting do not experience the same stressors or have the same coping mechanisms as students. As such, the aim 1 findings of a curvilinear relationship may only apply to a university student study population when influenza activity is high. Indeed, results from previous research using original M-Flu data collected during the 2006-2007 influenza season, a season with low influenza activity, found that increased levels of perceived stress were associated with a 25% increased rate of ILI in young adults (manuscript under peer-review at the *Journal of Epidemiology and Community Health*).

Although participants with lower psychological stress reported having larger social support networks, on average, than persons with higher stress at baseline, social support networks did not modify the association between stress and rates of ILI. The concept of social support as a buffering mechanism for stressed individuals refers to a

social network's ability to provide "psychological and material resources intended to benefit an individual's ability to cope with stress."[67, 69] Therefore, despite having a fairly large study population and making use of validated instruments for stress and social support, the non-statistical interaction between stress and social support suggests that study participants did not perceive members of their social support networks to be able or willing to provide the help needed for effective coping with psychological stressors.[67] Indeed, this study found that only among participants with high perceived stress was the risk for ILI substantially increased. Cobb et al.[8] found that a greater social support did not confer protection against URTI to participants reporting high life event stress. In fact, only participants with low life event stress were protected from illness conditional on having greater social support, assuming a positively linear relationship between stress and URTI.[8] A similar finding was reported in a second study[7] examining a group of children such that social support was only protective against incident URTI among children exposed to low life event stress. Given the curvilinear association between stress and ILI in this aim 1 study, and the fact that participants with moderate levels of stress were protected against ILI, it may be that very high perceived stress outweighs the benefits provided by social support networks. Hence, future work should examine the role of social support networks among highly stressed individuals in predicting confirmed respiratory infection, like influenza. Studies should ideally use validated instruments of social support and stress, a large sample size, and have variability in perceived social support and the respiratory outcome(s) of interest in the study population.

Although adjustment for demographics and reported health practices did not explain the stress-ILI association observed, there is a noteworthy impact of these factors

on ILI. Participants in the face mask and hand hygiene intervention group reportedly had a 31% reduced incidence of ILI during the study period in comparison to the control group. This association is supported by previous findings using an intention-to-treat approach to analyze the role of face mask and hand hygiene in reducing primary incidence of ILI during the 2006-2007 flu season.[102] Females were also more likely than males to report ILI, a finding that is consistent with the existing literature on reporting differences of illness symptoms between males and females.[115-117] Participants who reported having a prior allergy diagnosis were observed to have a 39% increased incidence of ILI compared to participants with no diagnosis of allergies. This finding is not surprising since allergies can compromise respiratory function and therefore increase susceptibility to respiratory infections such as influenza.[118]

There are several strengths and limitations to this study. First, the design and study population allowed for control of many factors that can influence susceptibility to influenza such as age and seasonal variation. In addition, influenza transmission was enhanced due to the shared living arrangement of participants. Second, the study's longitudinal component made it possible to examine risk factors for ILI in the natural environment. Third, this work utilized validated instruments from stress and social support that were internally consistent within the study population. Limitations include the reliance on self-report data. Measures of self-report are subject to recall bias and are ideally followed-up by objective measures. However, the evidence supports a strong positive correlation between self-reported illness and clinically-verified respiratory infection.[10, 50, 52] Since this study population consisted of young adults from a randomized intervention trial in a university setting, these results may not be applicable to non-

university settings and other study designs. However, these findings may be generalizable to similar demographic populations residing in shared living spaces. Finally, data on stress management and other coping mechanisms were not collected; hence this study was unable to examine coping mechanisms for stress as possible mediators of the observed association between stress and incidence of ILI.

2.5 Conclusion

Among young adults living in residence halls within a university setting during the 2007-2008 flu season, exposure to high levels compared to moderate levels of psychological stress were associated with an increased incidence of ILI, and, exposure to moderate levels of stress compared to low stress predicted a significant reduction in the incidence of ILI. Social support networks did not modify the association between stress and ILI rates. Given the high flu activity documented during the 2007-2008 flu season, the strength of the observed association is considered substantial. Epidemiologic studies should aim to identify validated measurements of emotional and behavioral responses, coping mechanisms, and properly measured immune responses that can mediate the association between stress and incidence of ILI in young adults within the community setting. Future research on the impact of stress-reduction interventions within the university setting will greatly aid in understanding the role of psychological stress in predicting rates of ILI among young adults. This is the first study to provide empirical evidence on the associations between psychological stress, social support networks, and incidence of ILI among young adults at high risk for pandemic influenza within the university setting. Further studies should establish if psychological stress is a key

mechanism in predicting confirmed influenza infection in young, healthy adults within university and other community settings.

Chapter 3

Exposure to Psychological Stress is Associated with Rates of Influenza A Infection and Viral Load

3.1 Background

Recent studies have established a temporal relation between psychological stress and rates of ILI in adult populations.[17, 20] This relation was also established in a young adult population in an earlier chapter of this dissertation. Data on the association between perceived stress and rates of naturally acquired influenza infection, however, is scant. Findings from two earlier studies[15, 21] on exposure to psychological stressors and rates of infection have failed to establish an association. Three influenza viral-challenge studies[16, 18, 19] examining the influence of psychological stressors and perceived stress on manifestation of illness symptoms have been published and only one study[16] reported an association between perceived stress and illness within 3 days of viral inoculation in healthy adult volunteers. With the emergence of the 2009 pandemic A H1N1 influenza virus, current and future research needs to focus on determining if psychological stress increases the risk of infection in populations at high risk for contracting pandemic flu viruses. One such understudied population is university-aged adults living in a university setting. Given the 2009 H1N1 pandemic and its impact on morbidity and mortality among university-aged adults,[100, 119] psychological stress may in fact be a novel risk factor even when statistically controlling for prior influenza vaccination.

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Identifying if psychological stress influences the viral pathology of influenza is also very important. Only one such experimental study[16] has examined the effects of stress on a biological marker of influenza severity, namely mucus production, among infected adults. However, the current literature provides no evidence on the association between psychological stress and biomarkers for disease severity in persons with naturally occurring influenza infection. Influenza viral load, a measure quantifying virus levels represented by the biological interaction between viral replication and the host's ability to clear infection,[81] has never been examined with respect to psychological risk factors in young adults. Some studies support the hypothesis that higher viral load (i.e. higher viral infectivity) is biologically correlated with greater disease severity in persons infected with influenza.[82-84] For example, To et al.[84] conducted a retrospective cohort study and showed that patients with severe 2009 pandemic H1N1 infection, including some who had either developed acute respiratory distress syndrome or had fatal disease, had significantly slower declines in viral load and increased cytokine responses compared to patients with mild clinical disease. Hence, developing a comprehensive risk profile for disease severity in a university-aged population at high risk for pandemic influenza requires identifying and understanding novel risk factors for viral replication and clearance. Establishing a relation between stress and viral load in this susceptible population would therefore aid in understanding critical predictors of a potentially important biomarker for disease severity and could have implications for infection control.

In this study, an investigation was undertaken to examine if increased psychological stress predicts an increased incidence of naturally acquired influenza A

infection and a higher influenza A/H3 viral load among young adults in a university setting during the 2007-2008 influenza season.

3.2 Methods

Study population and design

Data used came from the second year of a 2-year university-based cluster randomized intervention trial conducted during the 2007-2008 influenza season. Details of the original study have been published.[102] In total, 1,111 students living in five randomly selected university residence halls were eligible for analysis. Randomization was based on 37 residence houses within these selected residence halls, whereby each house was randomly assigned to one of three study groups (Control, Face mask only, Face mask and Hand Hygiene). Participants in the control arm did not receive any intervention materials, however all participants received education on proper hand hygiene and respiratory hygiene/cough etiquette through an e-mail video link and the study website.

Baseline and weekly surveys

Web-based surveys were distributed to all study participants following the detection of the first case of laboratory-confirmed influenza throughout the university, which took place during the week of 21 January 2008 as part of surveillance. The baseline survey was distributed before the intervention period commenced on 28 January 2008. Information on age, gender, self-reported race/ethnicity, alcohol intake, quality of sleep, physical activity,[105] employment, history of influenza vaccination, and previous diagnosis of either asthma or allergies was collected. Data regarding hand hygiene habits, systemic and respiratory illness symptoms in the previous week, and the number of roommates participants reportedly lived with were also collected. In addition, the level of

psychological stress experienced in the past month[66] and information on participants' social network(s)[103] were obtained.

Eight weekly on-line surveys were distributed to study participants throughout the intervention period. Data on the reported presence or absence of systemic and respiratory illness symptoms within the previous week were collected.

Clinical specimens

All students in the participating residence halls were invited to provide specimens for virus identification when they experienced symptoms (yes/no) of ILI (defined as illness with cough plus at least one or more of fever/feverishness, chills, or body aches) during the follow-up period. Each time a participant reported ILI on a survey, he/she would immediately be contacted to see study personnel for clinical specimen collection. Trained clinical staff made a diagnosis of ILI at one of the onsite health service centers located throughout campus. Throat swab samples were collected from study participants and tested for influenza by reverse transcriptase polymerase chain reaction (Rt-PCR). All positive A H3N2 samples were tested using semi-quantitative Rt-PCR with standard dilutions of H3N2 A/Anhui/1239/2005 and H3N2 A/Mexico/1842/2007. Influenza A/H3 viral load was measured among positive influenza A subtype H3 isolates. A monetary incentive of $25.00 was given to all study participants providing a throat swab specimen.

Variables of interest

Two primary outcomes were examined. The first outcome, presence or absence of influenza A infection, was determined using Rt-PCR on specimens collected from study participants who provided throat swab samples during their clinical exam. The second outcome, influenza A/H3 viral load, was examined among the 46 participants with

confirmed influenza A H3N2 using cycle threshold (Ct) values obtained from Rt-PCR and serial dilutions of provided influenza A H3N2 strains. Of note, Ct values are inversely related to influenza viral load.

The main predictor variable was psychological stress as measured at baseline using the Perceived Stress Scale (PSS).[66] The PSS is a 14-item, validated scale in which scores are ranked from 0 to 4, with responses ranging between never to very often. This scale[66] aims to capture *how often* participants felt or thought a specific way regarding a stressful life event. To calculate a participant's stress score at baseline, values for all items were summed across. Only respondents with complete stress data were included for analysis. A higher overall score indicated a greater level of perceived stress (range: 0-56).

Baseline covariates analyzed included self-reported gender (male/female), race (white/other), previous diagnosis of allergies (yes/no) and asthma (yes/no), receipt of influenza vaccination (yes/no), alcohol intake at least once a week (yes/no), employment status (yes/no), and physical activity (high/low) as defined by the recommended amount of exercise for adults by the CDC.[105] Data on sleep quality over the past month (fairly/very bad vs. fairly/very good), hand washing (optimal/suboptimal) based on CDC recommendations of duration and the average number of hand washes (mean number = 5) among participants,[98, 106] appropriate use of alcohol-based hand sanitizer (proper use/otherwise) defined by the use of at least a quarter-sized amount (recommended by the CDC) twice daily (the average number of uses among study participants),[98, 106] and the number of roommates reported to be living with (0, 1, or 2 or more persons) were also collected. Intervention group as determined by the intervention or control group students were allocated to at study outset was also considered a potential confounder. Among viral

load data, potential confounders also included ILI symptom severity as measured during clinical assessment (scored on a 0-3 scale, whereby 0 indicated no symptom present and 3 indicated a high severity of the symptom) and the number of days between illness symptom onset and specimen collection.

Analytic methods

The distribution of potential confounders measured at baseline was examined among 1,024 participants who completed the Perceived Stress Scale in its entirety. Chi-squared tests for categorical data and ANOVA for continuous data adjusting for within-residence house correlation were examined.[108] Observed differences in potential confounders between students with low and high perceived stress based on the mean value cut-off were determined using $P< 0.05$. The distribution of these potential confounders was also examined among participants who had tested positive for seasonal influenza A throughout the follow-up period (total N=46).

Cloglog models using generalized estimating equations (GEEs) adjusting for correlation within residence houses were used to examine the relationship between perceived stress and incidence of laboratory-confirmed influenza A among the entire study cohort (N=1,111). Linear regression analyses using GEEs adjusting for correlation within residence houses were performed to examine perceived stress as a predictor of viral load among participants who had tested positive for seasonal influenza A (N=46). To properly model the functional form of perceived stress, separate univariate regression models examining a potential linear trend across quintile categorizations of stress and each outcome were examined (quintiles were specific to the population being examined for flu infection and viral load). Given the small number of incident cases of influenza A

and an observed U-shaped trend between stress, rates of infection, and viral load, stress scores were centered around zero and modeled using second-ordered polynomials (i.e. a stress-by-stress interaction term was included in all regression models). Visual diagnostics were utilized to ease interpretation.

To determine which baseline covariates to include in the multivariate analysis for seasonal influenza A infection, univariate Cloglog models employing GEEs were used to examine the association between each potential confounder at baseline and incidence of influenza A infection ($P< 0.15$). In addition, baseline characteristics displaying a statistically significant difference ($P< 0.05$) between low and high stress groups were also included in the multivariate model. For multivariate analyses examining influenza A/H3 viral load, univariate linear regression models employing GEEs were used to examine the association between each baseline covariate and viral load ($P< 0.10$ due to the smaller sample size). Statistical significance was determined using $P< 0.05$ in all adjusted-regression models. All analyses were performed using SAS v. 9.1 (Cary, NC).

3.3 Results

Table 3.1 shows baseline characteristics of the 1,024 participants with complete stress data at baseline who were eligible for analysis. More than half of the participants reported being female (56%), of non-Hispanic white race/ethnicity (63%), having sub-optimal hand hygiene (75%), and having never been vaccinated for influenza, including the 2007-2008 season (83%). For Table 3.1, participants were categorized as having a high or low stress score based on a mean value cut-off of perceived stress (mean=23.14, SD=7.63). Statistically significant differences between race/ethnicity, sleep quality, and perceived

social support were identified between low and high stress groups (all P <0.0001; see Table 3.1).

Table 3.1 Demographic characteristics among participants with reported stress data at baseline (N=1,024)[a]

Characteristics	No. (%) of Participants									
	Overall		ICC[b]	Low Stress		High Stress		P		
Total number of participants	1,024			512		512				
Intervention										
Randomized to										
Mask/ hygiene	314	(31)		155	(30)	159	(31)			
Mask only	370	(36)		188	(37)	182	(36)			
Control	340	(33)		169	(33)	171	(33)			
Gender			0.20							
Female	572	(56)		271	(53)	301	(59)	0.35		
Male	452	(44)		241	(47)	211	(41)			
Race/Ethnicity			0.04							
Non-Hispanic white	626	(63)		357	(72)	269	(54)	<.0001[j]		
Other[c]	369	(37)		142	(28)	227	(46)			
Current Employment			0.05							
Yes	400	(40)		192	(38)	208	(41)	0.44		
No	605	(60)		310	(62)	295	(59)			
Current Drinker			0.04							
Yes	342	(34)		180	(36)	162	(32)	0.36		
No	660	(66)		322	(64)	338	(68)			
Exercise[d]			0.02							
Low Rate	763	(75)		367	(73)	396	(78)	0.06		
High Rate	250	(25)		139	(27)	111	(22)			

Roommates in Room Assignment[e]			0.13					
0	213	(21)		96	(19)	117	(23)	0.35
1	696	(68)		355	(69)	341	(67)	
2 or more	114	(11)		61	(12)	53	(10)	
Recent Flu Vaccine[f]			0.01					
No	826	(83)		411	(82)	415	(84)	0.53
Yes	173	(17)		91	(18)	82	(16)	
Flu Vaccination Prior to 2008 Season			< 0					
No	618	(63)		310	(62)	308	(64)	0.68
Yes	363	(37)		187	(38)	176	(36)	
Optimal Hand Washer[g]			0.03					
No	764	(75)		367	(72)	397	(78)	0.08
Yes	259	(25)		144	(28)	115	(22)	
Proper Use of Hand Sanitizer[h]			0.0086					
No	553	(95)		269	(95)	284	(95)	0.98
Yes	31	(5)		15	(5)	16	(5)	
Sleep Quality			0.0036					
Very/Fairly Bad	227	(22)		68	(13)	159	(31)	<.0001[j]
Fairly/Very Good	795	(78)		443	(87)	352	(69)	
Prior Allergy Diagnosis			0.007					
No	732	(71)		363	(71)	369	(72)	0.69
Yes	292	(29)		149	(29)	143	(28)	
Prior Asthma Diagnosis			< 0					
No	868	(85)		438	(86)	430	(84)	0.49

Yes	156	(15)		74	(14)	82	(16)	
Social Support, mean (SD)[i]	51.8	(11.3)	0.02	54.0	(10.5)	49.3	(11.6)	<.0001[j]

[a]Perceived stress was dichotomized among 1,024 participants with complete stress data based on the mean value (23.14)

[b]ICC=Intracluster Correlation Coefficient

[c]Includes black or African American, Asian, Hispanic, Hawaiian or Pacific Islander, American Indian or Alaskan Native, Multi-Ethnic

[d]High rate was defined by a very or extremely hard rate for 4 or more times per week for at least 20 minutes each time or an easy, medium, or hard rate for 5 or more times per week for at least 30 minutes each time

[e]Categories compared for 0 vs. 1 or more roommates

[f]Recent flu vaccine was defined as having a flu vaccine for the 2007-2008 flu season at baseline

[g]Optimal hand washing defined by washing with soap and water at least 5 times on average per day for at least 20 seconds each time; based on CDC recommendation of duration and the average number of handwashes reported among participants at baseline

[h]Proper use of alcohol-based hand sanitizer defined by the use of at least a quarter-sized amount of hand gel (recommended by the CDC) twice daily (the average number of uses among study participants)

[i]A total of n=941 participants completed the Lubben-18 scale among 1,024 participants with complete stress data; a higher score indicates a higher level of perceived social support

[j]P-values computed using cluster-adjusted chi-square test for categorical characteristics and cluster-adjusted ANOVA for continuous characteristics; statistically significant difference determined if $P < 0.05$

Table 3.2 shows the distribution of perceived stress, Ct values, and potential confounders among the 46 participants who tested positive for seasonal influenza A sub-type H3. The mean perceived stress score was 23.55 (SD=10.09; N=44). A total of 45 infected individuals (98%) met the clinical case definition of ILI used and 57% of all infected participants were deemed as having a high ILI symptom severity score based on a mean value of 5.98 during clinical examination. Participants presented themselves for specimen collection, on average, 2.5 days following symptom onset (SD=1.13).

Table 3.2 Demographic characteristics among participants with confirmed influenza A infection (N = 46)

Characteristics	No. (%) or Mean (SD)
Perceived Stress[a]	23.55 (10.09)

Intervention Randomized to		
Face mask/ hygiene	14	(30)
Face mask only	17	(37)
Control	15	(33)
Clinical ILI Symptom Severity[b]		
High	26	(57)
Low	20	(43)
Days Between Illness Onset and Viral Detection[c]	2.5	(1.13)
Cycle Threshold[d]	31.53	(4.42)
Gender		
Female	33	(73)
Male	12	(27)
Race/Ethnicity		
Non-Hispanic white	19	(43)
Other[e]	25	(57)
Current Employment		
No	22	(49)
Yes	23	(51)
Current Drinker		
No	29	(64)
Yes	16	(36)
Exercise[f]		
Low Rate	34	(76)
High Rate	11	(24)
Roommates in Room Assignment		
0	5	(11)

1	35	(78)
2 or more	5	(11)
Recent Flu Vaccine[g]		
No	42	(93)
Yes	3	(7)
Flu Vaccination Ever (prior to 2008 season)		
No	32	(76)
Yes	10	(24)
Optimal Hand Washer[h]		
No	34	(76)
Yes	11	(24)
Proper Use of Hand Sanitizer[i]		
No	26	(96)
Yes	1	(4)
Sleep Quality		
Very Bad/Fairly Bad	8	(18)
Fairly Good/Very Good	37	(82)
Prior Allergy Diagnosis		
No	30	(67)
Yes	15	(33)
Prior Asthma Diagnosis		
No	40	(89)
Yes	5	(11)
Social Support Score[j]	51.88	(10.74)

[a]A total of 44 participants had complete stress data at baseline, range: 5 to 56

[b]One person (1/46) did not meet the clinical case definition of ILI, however was categorized in the "low" category; Mean=5.98, SD=2.41, range: 1 to 12

[c]N=46, median=2.5, range: 0 to 7; Only one person had virus detected after 7 days from illness onset.

[d]Real-time PCR results expressed in cycle threshold values (a higher value correlates inversely with viral load); N=46, median=31.86, range: 23.33 to 40

[e]Includes black or African American, Asian, Hispanic, Hawaiian or Pacific Islander, American Indian or Alaskan Native, Multi-Ethnic

[f]High rate was defined by a very or extremely hard rate for 4 or more times per week for at least 20 minutes each time or an easy, medium, or hard rate for 5 or more times per week for at least 30 minutes each time

[g]Recent flu vaccine was defined as having a flu vaccine for the 2007-2008 flu season at baseline

[h]Optimal hand washing defined by washing with soap and water at least 5 times on average per day for at least 20 seconds each time; based on CDC recommendation of duration and the average number of handwashes reported among participants at baseline

[i]Proper use of alcohol-based hand sanitizer defined by the use of at least a quarter-sized amount of hand gel (recommended by the CDC) twice daily (the average number of uses among study participants)

[j]A total of n=41 participants completed the Lubben-18 scale; a higher score indicates a higher level of perceived social support

Figure 3.1 and table 3.3 show the results from univariate analyses for incidence of influenza A infection. Based on a quintile examination of perceived stress in univariate analyses, an upward U-shaped association was identified (see Figure 3.1); therefore, stress scores were centered around zero and both linear (i.e. stress main effect) and quadratic terms (i.e. stress-by-stress interaction) of perceived stress were analyzed.

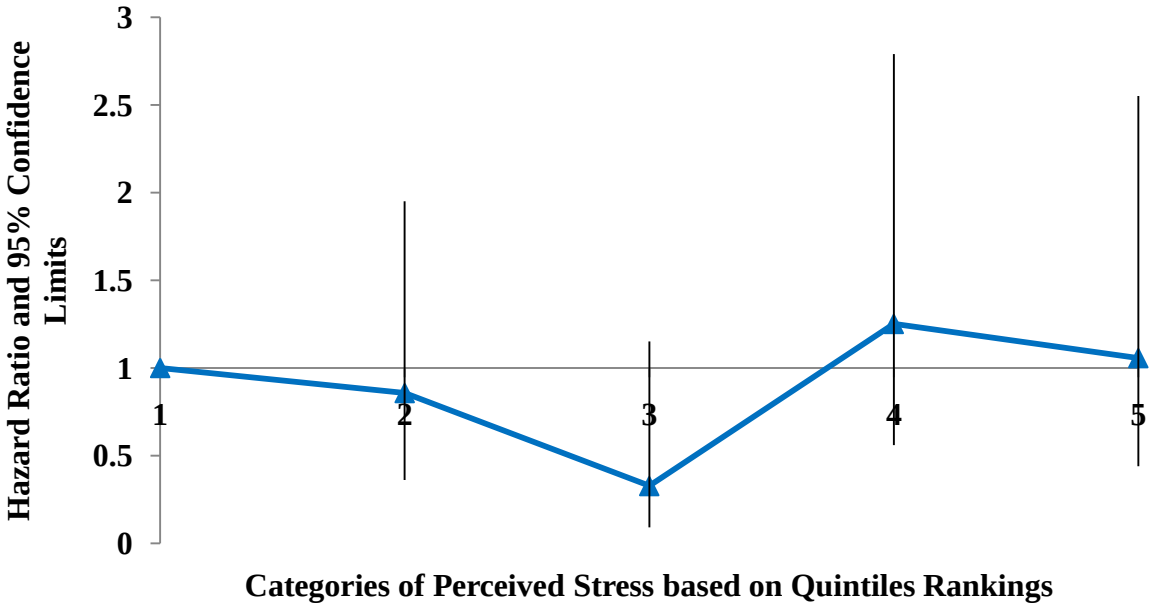


Figure 3.1 Quintile examination of the convex relation between perceived stress scores at baseline and rates of laboratory-confirmed influenza A infection throughout the follow-up period (N=1,024)

Note. Categories of perceived stress based on quintile rankings (from left to right): 20th percentile = stress score of 16; 40th percentile = stress score of 22; 60th percentile = stress score of 26; 80th percentile = stress score of 29; 100th percentile = stress score of 56; range of stress scores from 0 to 56; average stress score = 23.14, standard deviation = 7.63, interquartile range = 10.

Table 3.3 shows a statistically significant convex relation between stress and rates of infection (stress-by-stress interaction: HR=1.37, 95% CI 1.14, 1.66) for every interquartile range (IQR=10) increase in stress (N=1,024). This trend was visually displayed in Figure 3.1. Taken together, Figure 3.1 and Table 3.3 suggest that at the 60th percentile of stress scores in the study population, the protective stress-effects on respiratory function (as noted by an HR < 1 in Figure 3.1 and an HR < 1 for the linear stress term in Table 3.3) were canceled out by the deleterious effects of stress that were observed (as noted by an HR > 1 in Figure 3.1 and an HR > 1 for the quadratic stress term, indicating a convex relation, in Table 3.3). Using a $P< 0.15$ criterion for variable entry into a multivariate model, Table 3.3 shows that females were more likely to contract seasonal influenza A infection compared to males; participants who were employed compared to unemployed were more likely to be infected; and participants who had been vaccinated versus not had reduced rates of infection (influenza vaccination for 2008 season: HR=0.35, 95% CI: 0.11, 1.12; influenza vaccination "ever" vs. "never": HR=0.54, 95% CI: 0.27, 1.10) .

Table 3.3 Univariate complementary log-log models showing the relationship between stress and potential confounders at baseline with rates of influenza A infection over the follow-up period (N=1,111)

Variable	n	ICC[a]	HR[b]	95% CI[c]	P
Stress (linear term)[d]	1,024	< 0	0.99	(0.73 to 1.36)	0.97
Stress (quadratic term)[d]			1.37	(1.14 to 1.66)	0.001[k]
Intervention Randomized to	1,111	< 0			

Mask/ hygiene			0.99	(0.47 to 2.07)	0.97
Mask only			1.11	(0.56 to 2.23)	0.76
Control			Ref		
Gender	1,107	< 0			
Female			2.00	(1.05 to 3.82)	0.03[k]
Male			Ref		
Race/Ethnicity	1,074	< 0			
Non-Hispanic white			0.71	(0.39 to 1.30)	0.27
Other[e]			Ref		
Current Employment	1,086	< 0			
Yes			1.77	(0.98 to 3.20)	0.06[k]
No			Ref		
Current Drinker	1,066	< 0			
Yes			1.12	(0.61 to 2.07)	0.71
No			Ref		
Exercise[f]	1,072	< 0			
High Rate			1.01	(0.51 to 1.99)	0.98
Low Rate			Ref		
Roommates in Room Assignment	1,106	< 0			
2 or more			1.53	(0.47 to 5.01)	0.48
1			1.72	(0.72 to 4.10)	0.22
0			Ref		
Recent Flu Vaccination[g]	1,082	< 0			

Yes			0.35	(0.11 to 1.12)	0.08[k]
No			Ref		
Flu Vaccination Prior to 2008 Season	1,060	< 0			
Ever			0.54	(0.27 to 1.10)	0.09[k]
Never			Ref		
Optimal Hand Washer[h]	1,104	< 0			
Yes			0.99	(0.50 to 1.97)	0.98
No			Ref		
Proper Use of Hand Sanitizer[i]	638	NE[i]			
Yes			0.64	(0.09 to 4.76)	0.67
No			Ref		
Sleep Quality	1,086	0.0001			
Fairly/Very Good			1.38	(0.62 to 3.10)	0.43
Very/Fairly Bad			Ref		
Prior Allergy Diagnosis	1,094	< 0			
Yes			1.34	(0.72 to 2.50)	0.35
No			Ref		
Prior Asthma Diagnosis	1,094	< 0			
Yes			0.71	(0.28 to 1.81)	0.48
No			Ref		
Social Support Score[j]	965	< 0			
High (50-89)			0.90	(0.48 to 1.67)	0.73
Moderate (43-49)			0.30	(0.09 to 1.03)	0.06[k]

Low (0-42)	Ref

[a]ICC=Intracluster Correlation Coefficient; clustering was not accounted for when ICC <0

[b]HR=Hazard Ratio

[c]CI=Confidence Interval

[d]Modeled including a stress-by-stress interaction (i.e. quadratic term) based on a quintile assessment of the functional form of perceived stress

[e]Includes black or African American, Asian, Hispanic, Hawaiian or Pacific Islander, American Indian or Alaskan Native, Multi-Ethnic

[f]High rate was defined by a very or extremely hard rate for 4 or more times per week for at least 20 minutes each time or an easy, medium, or hard rate for 5 or more times per week for at least 30 minutes each time

[g]Recent flu vaccine was defined as having a flu vaccine for the 2007-2008 flu season at baseline

[h]Optimal hand washing defined by washing with soap and water at least 5 times on average per day for at least 20 seconds each time; based on CDC recommendation of duration and the average number of handwashes reported among participants at baseline

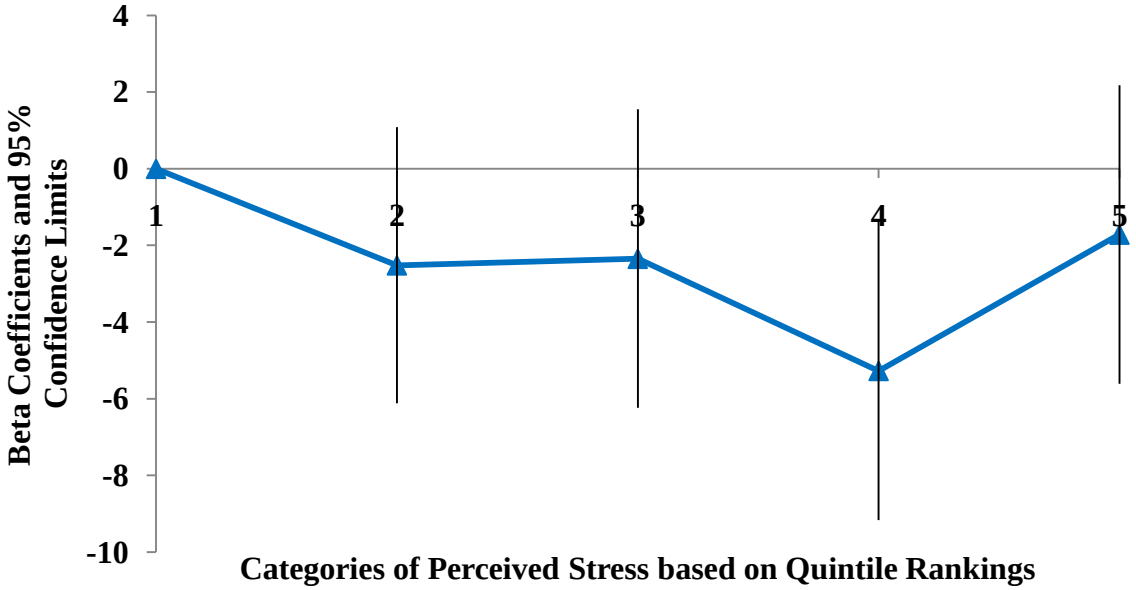
[i]Proper use of alcohol-based hand sanitizer defined by the use of at least a quarter-sized amount of hand gel (recommended by the CDC) twice daily (the average number of uses among study participants); NE= ICC not estimable due to missing data

[j]Catgories based on a quintile assessment of the functional form of social support (range: 0 to 89); 20th percentile=42; 40th percentile=49; 60th percentile=55; 80th percentile=61; 100th percentile=89

[k]Independent variables considered statistically significant based on P<0.15 for the Wald statistic

Based on a univariate analysis, Figure 3.2 and Table 3.4 also show a convex relation between stress and influenza viral load (stress-by-stress interaction: Beta coefficient [β]=0.006, 95% CI: -0.002, 0.01) for every 1-unit increase in stress (n=44). Taken together, Figure 3.2 and Table 3.4 suggest that as stress levels approached the 80th percentile of stress scores among those who were infected, the upward stress-effects on viral load were canceled out by the downward stress-effects that were observed (note that a negative β corresponds to a higher viral load). Using a $P< 0.10$ criterion for entry into a multivariate model, infected participants who had "ever" received influenza vaccination were more likely to have a lower viral load than those who had "never" been vaccinated; and, participants reporting "fairly good or very good" sleep quality at baseline compared to those reporting "fairly bad or very bad" sleep quality had a significantly higher viral load (see Table 3.4).

Figure 3.2 Quintile examination of the convex relation between perceived stress scores at baseline and levels of influenza A/H3 viral load among participants with confirmed infection (N=44)



Note. Negative regression coefficients for cycle threshold correspond to higher levels of viral load; categories of perceived stress based on quintile rankings (from left to right): 20th percentile = stress score of 13; 40th percentile = stress score of 21; 60th percentile = stress score of 27; 80th percentile = stress score of 30; 100th percentile = stress score of 56; range of stress scores from 5 to 56; average stress score = 23.55, standard deviation = 10.09, interquartile range = 12.5.

Table 3.4 Univariate linear associations between baseline characteristics and influenza A/H3 viral load over the follow-up period (N = 46)

Variable	n	ICC[a]	β[b]	95% CI[c]	P
Perceived Stress (linear term)[d]	44	0.10	-0.12	(-0.26 to 0.01)	0.08
Perceived Stress (quadratic term)[d]			0.006	(-0.002 to 0.01)	0.12
Intervention Randomized to	46	0.10			
Face mask/ hygiene			1.60	(-1.80 to 5.01)	0.36
Face mask only			2.55	(-0.72 to 5.82)	0.13
Control			Ref		
Gender	45	0.03	-2.19	(-5.05 to 0.67)	0.13
Female			Ref		
Male					

Race/Ethnicity	44	0.10			
Non-Hispanic white			-0.21	(-2.99 to 2.56)	0.88
Other[e]			Ref		
Current employment	45	0.11			
Yes			0.44	(-2.14 to 3.02)	0.74
No			Ref		
Current Drinker	45	0.11			
Yes			0.89	(-1.81 to 3.59)	0.52
No			Ref		
Exercise[f]	45	0.11			
High Rate			0.06	(-2.93 to 3.06)	0.97
Low Rate			Ref		
Roommates in Room Assignment	45	0.09			
2 or more			3.41	(-1.96 to 8.78)	0.21
1			2.01	(-2.11 to 6.12)	0.34
0			Ref		
Recent Flu Vaccination[g]	45	0.09			
Yes			0.99	(-4.19 to 6.16)	0.71
No			Ref		
Flu Vaccination Prior to 2008 Season	45	0.41			
Ever			3.66	(0.92 to 6.39)	0.01[k]
Never			Ref		
Optimal Hand Washer[h]	45	0.12			

Yes			-1.76	(-4.71 to 1.19)	0.24
No			Ref		
Proper Use of Hand Sanitizer[i]	27	-0.25			
Yes			3.14	(-5.49 to 11.77)	0.48
No			Ref		
Sleep Quality	45	0.21			
Fairly Good/Very Good			-3.53	(-6.74 to -0.31)	0.03[k]
Very Bad/Fairly Bad			Ref		
Prior Allergy Diagnosis	45	0.10			
Yes			0.87	(-1.82 to 3.57)	0.53
No			Ref		
Prior Asthma Diagnosis	45	0.09			
Yes			-0.97	(-5.09 to 3.15)	0.64
No			Ref		
Clinical ILI Symptom Severity	46	0.09			
High			-0.11	(-2.73 to 2.51)	0.94
Low			Ref		
Days Between Illness Onset and Viral Detection[j]	46	0.13			
>2.5 Days			0.98	(-1.56 to 3.52)	0.45
≤2.5 Days			Ref		
Social Support Score	41	0.15	0.02	(-0.11 to 0.15)	0.79

[a]ICC=Intracluster Correlation Coefficient; clustering was not accounted for when ICC <0

[b]β=The expected change in the outcome for a unit increase in the independent variable of interest; a negative regression coefficient corresponds to a higher viral load

[c]CI=Confidence Interval
[d]Modeled including a stress-by-stress interaction (i.e. quadratic term) based on a quintile assessment of the functional form of perceived stress
[e]Includes black or African American, Asian, Hispanic, Hawaiian or Pacific Islander, American Indian or Alaskan Native, Multi-Ethnic
[f]High rate was defined by a very or extremely hard rate for 4 or more times per week for at least 20 minutes each time or an easy, medium, or hard rate for 5 or more times per week for at least 30 minutes each time
[g]Recent flu vaccine was defined as having a flu vaccine for the 2007-2008 flu season
[h]Optimal hand washing defined by washing with soap and water at least 5 times on average per day for at least 20 seconds each time; based on CDC recommendation of duration and the average number of handwashes reported among participants at baseline
[i]Proper use of alcohol-based hand sanitizer defined by the use of at least a quarter-sized amount of hand gel (recommended by the CDC) twice daily (the average number of uses among study participants)
[j]Categorization based on mean value of the total number of days
[k]Independent variables entered into the multivariate model based on P<0.10 for the Wald statistic

Tables 3.5 and 3.6 show the multivariate models for rates of infection (N=931) and influenza A/H3 viral load (N=41), respectively. Adjusting perceived stress for covariates in the model predicting rates of influenza infection (see Table 3.5), a statistically significant convex relation was again found (stress-by-stress interaction: HR=1.44, 95% CI 1.19, 1.75) for every IQR-increase in stress. In the adjusted model for viral load (see Table 3.6), a statistically significant convex association was identified (stress-by-stress interaction: β=0.007, 95% CI: 0.001, 0.01) for every 1-unit increase in stress.

Table 3.5 Multivariate complementary log-log regression model for rates of influenza A infection over the follow-up period[a]

Variable	n	ICC[b]	HR[c]	95% CI[d]	P
Perceived Stress (linear term)[e]	931	< 0	0.92	(0.63 to 1.34)	0.66
Perceived Stress (quadratic term)[e]			1.44	(1.19 to 1.75)	0.0002[i]
Gender					
Female			2.27	(1.11 to 4.63)	0.02[i]
Male			Ref		

Race/Ethnicity			
Non-Hispanic white	0.66	(0.34 to 1.26)	0.21
Other[f]	Ref		
Social Support Score[g]			
High (50-89)	0.84	(0.41 to 1.75)	0.64
Moderate (43-49)	0.32	(0.09 to 1.14)	0.08
Low (0-42)	Ref		
Current Employment			
Yes	1.47	(0.77 to 2.81)	0.24
No	Ref		
Recent Flu Vaccination[h]			
Yes	0.43	(0.13 to 1.51)	0.19
No	Ref		
Flu Vaccination Prior to 2008 Season			
Ever	0.77	(0.36 to 1.65)	0.50
Never	Ref		
Sleep Quality			
Fairly/Very Good	2.00	(0.73 to 5.47)	0.17
Very/Fairly Bad	Ref		

[a]Multivariate analysis includes variables significantly different at baseline using P< 0.05 or that were predictive of infection rates using P < 0.15 in univariate analyses

[b]ICC=Intracluster Correlation Coefficient

[c]HR=Hazard Ratio

[d]CI=Confidence Interval

[e]Modeled including a stress-by-stress interaction (i.e. quadratic term) based on a quintile assessment of the functional form of perceived stress

[f]Includes black or African American, Asian, Hispanic, Hawaiian or Pacific Islander, American Indian or Alaskan Native, Multi-Ethnic

[g]Categories based on a quintile assessment for functional form

[h]Recent flu vaccine was defined as having a flu vaccine for the 2007-2008 flu season at baseline
[i]Variables considered statistically significant at the P<0.05 level

Table 3.6 Multivariate linear regression model for influenza A/H3 viral load over the follow-up period[a]

Variable	n	ICC[b]	β[c]	95% CI[d]	P
Perceived Stress (linear term)[e]	41	0.64	-0.15	(-0.25 to -0.04)	0.01[f]
Perceived Stress (quadratic term)[e]			0.007	(0.001 to 0.01)	0.02[f]
Flu Vaccination Prior to 2008 Season					
Ever			3.02	(0.26 to 5.78)	0.03[f]
Never			Ref		
Sleep Quality					
Fairly/Very Good			-1.95	(-5.09 to 1.19)	0.22
Very/Fairly Bad			Ref		

[a]Multivariate analysis includes variables that were predictive of viral load using P < 0.10 in univariate analyses
[b]ICC=Intracluster Correlation Coefficient
[c]β=The expected change in the outcome for a unit increase in the independent variable of interest; a negative regression coefficient corresponds to a higher viral load
[d]CI=Confidence Interval
[e]Modeled including a stress-by-stress interaction (i.e. quadratic term) based on a quintile assessment of the functional form of perceived stress
[f]Statistically significant at P < 0.05 for the Wald statistic

3.4 Discussion

Young adults with above average levels of perceived stress had a substantially greater risk of acquiring laboratory-confirmed influenza infection compared to those with moderate levels of stress who were protected against infection. Among participants with confirmed influenza A H3N2, viral load increased with increasing psychological stress but then slightly decreased when levels of stress surpassed the 80th percentile of stress scores. Contrary to initial study hypotheses of a positive linear relation between

perceived stress, infection rates, and viral load, a statistically significant curvilinear (i.e. U-shaped) association was observed.

This study is the first to establish an association between perceived stress, rates of naturally acquired influenza A infection, and influenza A viral load in young adults living in a high-risk community setting for transmission of infection during a season with high influenza activity.[27] The findings suggest that increased stress differentially influences the risk of naturally acquired infection and viral pathology. Therefore, not only has this dissertation research identified stress as an important predictor of ILI incidence, but also as a risk factor for PCR-confirmed influenza A infection and underlying disease pathology. The observed association between stress and infection parallels the curvilinear association previously reported for stress and ILI using data from the same study collected during the 2007-2008 flu season. In this current dissertation study, participants with slightly higher than average stress levels were protected against infection; however those with the highest levels of perceived stress were placed at a substantially increased risk for acquiring infection. Sub-analyses show that participants with high compared to moderate perceived stress had a greater than 2-fold increased risk for acquiring influenza infection and those with moderate compared to low stress had a 57% reduced incidence of infection. No differences in rates of infection were observed between participants with low or high perceived stress.

The observed curvilinear association may be attributable to differences in the emotional and behavioral response to perceived stress as well as differences in individual coping behavior.[30, 111, 112] Participants with moderate perceived stress may have simply had better stress management techniques such as relaxation, for example, compared to

participants with very high stress. Indeed, relaxation has been associated with an increased production of salivary immunoglobulin A which helps to protect against respiratory infection including infection with influenza.[12, 120, 121] Evidence that stress-reduction interventions may be beneficial for respiratory health has also been documented. One such stress intervention study reported a reduction in the number of days with upper respiratory tract symptoms among a small sample of university students who were treated for exam anxiety compared to controls (N=27 in treatment group, N=25 in control group).[122] Current research has not, however, examined the impact of individual coping styles for stress and stress-reduction interventions effective for reducing the burden of naturally acquired flu infection in large young adult populations. Hence, epidemiologic studies should aim to identify the most effective coping responses (e.g. problem-oriented vs. emotion-oriented) and stress management techniques for reducing the burden of infection among young adults in the university setting. Examining stress-induced immune changes influenced by coping mechanisms should also be incorporated in future studies to better elucidate the mechanistic pathways by which stress differentially impacts rates of seasonal influenza in young adults within the community setting.

Viral load increased with increasing psychological stress but then slightly decreased when levels of stress exceeded the 80th percentile of stress scores. Viral load showed no statistical association with self-reported symptom severity during clinical examination, but parameter estimates did suggest greater severity to be positively correlated with viral load. However, additional sub-analyses showed that reported stress levels inversely corresponded to the number of days that had elapsed between reported

illness onset and specimen collection for virus detection (range: 0-7 days). On average, participants with high stress were clinically examined within 1 day of symptom onset and the majority of participants with moderate stress were examined 2 to 3 days following reported symptom onset. Participants with lower than average stress scores were clinically examined between days 4 and 7 following symptom onset. Interestingly, Nilsson et al.[123] showed that mean influenza A H3N2 viral load on day 1 of presentation was low, peaked on days 2 and 3 after presentation and then decreased after day 3 in an adult population. Hayden et al.[124] reported a similar finding of viral titers peaking at 2 days post-inoculation with influenza A/Texas/36/91 (H1N1) virus in a healthy adult population (median age: 21 years; age range: 19 to 40 years). Nonetheless, this dissertation still managed to detect a statistically significant upward- and downward effect in viral load among participants with the lowest and highest levels of perceived stress, respectively, even though these students, on average, provided throat swab specimens on days when viral load may not have peaked.

No studies have examined the extent to which perceived stress is a risk factor for confirmed influenza infection or viral load in young adults living within a university community setting. Clover et al.[15] and Cluff et al.[21] both used a prospective study design and found no statistically significant relation between stressful life events and confirmed influenza B infection and no association between psychological "vulnerability" and rates of infection, respectively. Of note, Cluff et al.[21] examined all male employees in a military research installment and Clover et al.[15] examined rates of influenza B infection across households. Many methodological differences between these earlier studies and this dissertation study are also noteworthy. For example, Clover et al.[15] examined the

association between stressful life events and infection whereas this dissertation examined psychological stress and infection. Research findings have shown that scales of life event stress and psychological stress tap into different constructs of the infection-illness spectrum despite some similarities between these measures.[10, 14, 50] For example, life event stress has been shown to predict increased rates of clinical disease through an increased number of symptom reporting among persons inoculated with a common cold virus.[10] On the other hand, perceived stress has been shown to predict increased rates of clinical disease through increased rates of infection.[10] Hence, when examining risk factors for respiratory infection, measures of perceived stress appear to be more sensitive.

To the best of my knowledge, this study is the first to examine the impact of perceived stress on viral load among participants with confirmed infection. Only one influenza viral-challenge study[16] detected an association between perceived stress and another biomarker of disease severity, namely mucus production, following inoculation in a sample of healthy adult volunteers. This earlier study[16] was similar to this dissertation study since it also used the Perceived Stress Scale.[66] Both this dissertation and the Cohen et al. study[16] identified stress as a novel risk factor for underlying disease among participants with confirmed influenza A infection. However, unlike the Cohen et al.[16] study, this dissertation examined naturally acquired infection and viral load in young adults at high risk for pandemic influenza in the community setting.

Multivariate analyses showed that females had more than a 2-fold rate of influenza A infection than males. Although seasonal influenza infection, and even infection from the 2009 pandemic A H1N1 virus, does not discriminate between males and females,[125] coping mechanisms used more often by males may be more effective for

controlling stress than those typically used by females. Some research suggests that men use more problem-solving coping whereas women more often use emotion-based coping[126-128] and also tend to perceive their sources of stress as more severe than men.[129] Attributing the increased rate of infection observed in this dissertation between males and females to differences in their coping strategies is supported by Kemeny et al.[130] who found that poorer immune function was more strongly influenced by emotion-based versus problem-based coping in response to stress. Interestingly, the two-fold increased risk among females was apparent even though they had better hand washing habits compared to males throughout the study period (observed in a sub-analysis).

Multivariate analyses also showed that participants who had "ever" received flu vaccination had significantly lower viral load than participants who had "never" been vaccinated. This is not unexpected since annual immunization provides the best protection against infection and, even during influenza seasons when vaccine strains provide a sub-optimal match to circulating strains such as in the 2007-2008 season,[131, 132] vaccination from previous influenza seasons can produce cross-reactive antibodies.[133] Hence prior influenza vaccination among laboratory-confirmed cases of influenza A in this study may have induced limited viral replication and therefore lower levels of viral load.

There are several strengths and limitations to the work performed in this study. A major strength of this work is that it elucidated, for the first time, the role of perceived stress as a risk factor for incidence of influenza infection and viral load of seasonal influenza A in a young adult cohort. Utilizing a young adult cohort also controlled for many factors that are known to influence susceptibility to influenza such as age. In

addition, transmission of infection was enhanced due to participants living in shared residence hall units. This work also utilized a validated and reliable instrument for perceived stress that was internally consistent within the study population. Limitations include utilizing influenza viral load as a primary outcome. The level of virus detected in this study may have been affected by the method of specimen collection (i.e. throat swabs).[84, 86, 87] In addition, the true concentration of influenza A RNA was not represented due to the use of semi-quantitative PCR methods. These results do, however, provide very useful information regarding the impact of perceived stress on virus-host interactions. Other limitations include the reliance on self-report data and utilization of young adults from a randomized intervention trial in a university setting. Therefore, these results may not be generalizable to other study settings and designs. Finally, this study was unable to examine stress coping mechanisms that may be responsible for the associations identified.

3.5 Conclusion

Among young adults living in residence halls within a university setting during the 2007-2008 flu season, as levels of psychological stress approached slightly higher than study participant average stress levels, a reduced incidence of infection was observed; however, once students' stress level exceeded this threshold, incidence of infection increased significantly. The relationship between psychological stress and influenza A/H3 viral load followed a similar curvilinear pattern, however the findings suggested that higher levels of perceived stress were associated with greater viral load. This research is the first to establish an association between perceived stress, rates of naturally acquired influenza A infection, and influenza viral load in young adults at high risk for pandemic influenza.

Replication of these novel results and further studies on coping mechanisms on the observed pathway and stress management interventions within the influenza context are warranted.

Chapter 4

Influenza-like Illness is Associated with Sub-optimal Compliance with Non-pharmaceutical Measures for Influenza

4.1 Background

In April 2009, the WHO declared the circulation of a novel strain of A H1N1 influenza. Similar to some seasonal influenza viruses, H1N1 spread rapidly between humans and dispersed globally leading the WHO to declare a pandemic in June 2009.[134] The demand for rapid vaccine production led to a limited vaccine supply worldwide at the beginning of the pandemic. This delay resulted in calls for the use of non-pharmaceutical measures such as personal hygiene (i.e. hand washing and use of protective equipment such as face masks), isolation of sick persons, and quarantine of persons exposed to sick individuals to mitigate disease spread. Hence, the emergence of the 2009 H1N1 flu pandemic and lessons from past pandemics (i.e. Spanish flu of 1918, the Asian flu of 1957, and the Hong Kong flu of 1968) laid the groundwork for enhancing pandemic preparedness globally.

Despite the urgency of pandemic preparedness today, there is little scientific literature on compliant behavior with recommended non-pharmaceutical measures for limiting transmission of influenza, particularly during flu outbreaks in the community setting.[22, 96] Aledort et al.[97] evaluated the most feasible non-pharmaceutical measures for pandemic flu based on expert opinion. In their review,[97] proper hand hygiene such as hand washing, especially before and/or after critical periods like coughing or sneezing,

and use of alcohol-based hand sanitizers to disinfect hands; and, limited social interactions and/or contact with others to minimize exposure to or transmission of disease were among the most feasible measures. These "less invasive voluntary efforts"[97] are highly recommended for stemming an influenza epidemic. Certain behavioral responses to an influenza outbreak and disease onset and an optimal level of compliance with these non-pharmaceutical measures for influenza are therefore key components of the pandemic preparedness agenda.

Few studies have provided empirical evidence on public compliance with these non-pharmaceutical measures in response to an outbreak. For example, one cross-sectional study[24] surveying adults in May 2009 in the United Kingdom found that in response to the 2009 H1N1 flu pandemic, 38% of all participants reported following at least one of three recommended behavior changes including increased hand washing frequency or surface cleaning and engaging in "avoidance behavior." Data from a Harvard School of Public Health opinion poll in June 2009 showed that 20% of respondents reported reducing their contact with people outside their household "as much as possible" during the 2009 H1N1 flu pandemic.[23] A recent modeling study also showed that a reduction in the number of social contacts led to a significant reduction in attack rates of influenza during an influenza outbreak.[135] However, no studies have ever examined how influenza illness affects the hand hygiene behaviors and social interactions of those who are sick.

The objective of this university-based study was to examine if confirmed ILI was associated with an immediate and sustained adherence to key non-pharmaceutical measures including hand washing, use of alcohol-based hand sanitizer, and one's

reduction in their exposure to social contacts. Compliance was quantified and compared among confirmed (i.e. clinically verified) cases of ILI, self-reported cases of ILI reported only on web-based surveys, and control subjects who never reported ILI throughout an eight week study period during the influenza season. It was hypothesized that university students seeking clinical examination of their ILI along with laboratory testing would report greater adherence to these non-pharmaceutical measures compared to participants not seeking clinical verification of their reported ILI and participants with no reported ILI throughout the study. It was also hypothesized that a greater severity among those with confirmed ILI would be associated with greater adherence to these measures due to some participants' health-seeking behavior compared to those participants with only reported ILI and control subjects who were not ill.

4.2 Methods

Study population and design

The data utilized for this research came from the second year of the M-Flu study, a 2-year university-based cluster randomized intervention trial conducted during the 2007-2008 influenza season. A description of the original study has been published.[102] Overall, 1,111 young adults residing in five randomly selected university residence halls were eligible for study. Randomization was based on a total of 37 residence houses located throughout the selected residence halls. Each house was randomly allocated to one of three groups, namely, the control, face mask only, or face mask and hand hygiene group. Participants in the control group were not given any intervention materials. All subjects were, however, given instruction on appropriate hand sanitation and respiratory hygiene/cough etiquette via an on-line video link and the study website.

Baseline and weekly surveys

On-line questionnaires were distributed to all subjects following the occurrence of the initial case of laboratory-confirmed influenza on the university campus, which transpired throughout the week of 21 January 2008 as part of surveillance. Baseline surveys were administered before the intervention period began on 28 January 2008. Self-reported data on subjects' age, gender, race/ethnicity, consumption of alcohol, quality of sleep, physical activity,[105] employment, recent and history of influenza vaccination, and previous diagnosis of either asthma or allergies was collected. Self-reported data on hand hygiene habits, systemic and respiratory illness symptoms in the previous week, the number of roommates participants lived with, perceptions of influenza, and exposure to social contacts were also collected. In addition, data on exposure to psychological stress was obtained and measured using a validated scale.[66]

Eight web-based surveys were distributed on-line during the intervention period. Data on reported illness symptoms experienced within the previous week, hand hygiene habits, and exposure to social contacts were also collected.

Clinical specimens

All students in participating residence halls were invited to provide specimens for virus identification when experiencing ILI during the study. Each time ILI was reported, the participant would immediately be contacted to see study personnel for their symptoms and clinical specimen collection. All throat swab samples were tested for influenza by reverse transcriptase polymerase chain reaction (Rt-PCR). A monetary incentive of $25.00 was given to all study participants providing a throat swab specimen.

Variables of interest

Three outcome variables, all continuous, measured at baseline and throughout the follow-up period were examined. Two variables quantified hand hygiene: (1) the average number of times participants washed their hands with soap and water in the past week, including the day of clinical illness verification or when the ILI survey response was completed; and, (2) the average number of times participants used alcohol-based hand sanitizer to disinfect their hands in the past week, including the day of clinical illness verification or when the ILI survey response was completed. The third outcome aimed to capture participants' voluntary reduction in their exposure to social contacts as measured by the number of hours spent in one's own residence hall room in the 24-hour period prior to clinical illness verification or when the ILI survey response was completed. All outcomes were self-reported on the web-based surveys and utilized for this study because of their role in mitigating transmission of infection.

The predictor variable of interest was a 4-level indicator variable dividing the study population into four comparison groups (0 = control subjects with no reported ILI either clinically or on any survey, 1 = ILI cases who never contacted a study clinician but had reported ILI through the on-line surveys, 2 = confirmed ILI cases with symptoms that were lower than or equal to the average symptom severity score recorded by the clinical assistant attending to the case, 3 = confirmed ILI cases with symptom severity that was higher than the average symptom severity score recorded by the clinical assistant attending to the case). Control subjects never reported ILI either clinically or on weekly surveys and therefore did not have reported ILI or confirmed influenza infection during the study. Each case of ILI was matched to a control subject using a one-to-one matching

algorithm[136] based on gender and residence house (i.e. proximity of residence location). Controls were selected retrospectively.

The following baseline covariates were considered potential confounders for all analyses: gender, race, vaccination status for the 2007-2008 flu season, diagnosis of allergies and asthma, the participant's number of roommates, alcohol consumption, employment, level of physical activity, psychological stress,[66] and perceptions of influenza. Intervention group based on randomization at the outset of the study was deemed a confounding variable a priori due to possible intervention effects on adherence with the recommended non-pharmaceutical measures considered. Distributions regarding students' preferred method of hand hygiene (i.e. hand washing vs. using hand sanitizer) were also examined.

Analytic methods

An ILI-free cohort at baseline was utilized (total N=938). Initial analyses examined the distribution of potential confounders at baseline between participants with confirmed ILI, those reporting ILI but who were not clinically examined, and control subjects. Chi-squared tests and ANOVA accounting for the clustered study design[108] were used to compare categorical and continuous data, respectively. Variables with observed differences between groups in baseline characteristics at $P < 0.05$ were included as potential confounders in regression models.

To predict adherence with the preventive measures of interest (Y) for a given subject, *i,* at time t_2 based on reported information at time t_1, lagged regression using analysis of covariance while correcting for the phenomenon of regression toward the mean[137-141] was performed. To compare the immediate effects of illness on compliant

behavior, the absolute difference between Y_{t2} (i.e. mean response of the outcome measure during the week of illness confirmation or control selection) and Y_{t1} (mean response of the outcome during the week prior to illness confirmation or control selection), while controlling for the value of Y_{t1}, was calculated. To examine sustained effects of illness on compliance (i.e. a sustained improvement or worsening in behavior over the weeks subsequent to illness confirmation or control selection), changes were modeled between two outcome measurements at consecutive time points, controlling for the value of the outcome at the beginning of the specified time period of interest.[138] Analyses were performed using a wide data set format. All model-based regression analyses accounting for clustering at the residence house level and specifying an independent covariance structure were performed using Proc Genmod in SAS (SAS V.9.1, Cary, NC.). The CONTRAST statement in SAS was used to compare the mean response in compliance between ill and non-ill participants.

4.3 Results

At baseline, there were 540 ILI-free participants available for analyses and 277 ILI cases from either clinical ascertainment or survey-based reporting (see Table 4.1). Five of the 277 ILI cases had no controls that matched by gender and place of residence at the time illness was reported and nine additional ILI cases were unable to be uniquely matched to a control subject using a one-to-one matching. Of the 159 confirmed ILI cases, 54 had a high ILI symptom severity score and 105 had a low symptom severity score (mean severity score = 5.01; range: 2 to 12). No statistically significant differences in baseline characteristics between confirmed ILI cases, reported ILI cases, and controls were observed (all $p>0.05$; see Table 4.1).

Table 4.1 Demographic characteristics among confirmed ILI cases, reported ILI cases, and control subjects who remained ILI-free throughout the study (N = 540)

	No. (%) of Participants				
Characteristics	Overall	ICC[a]	Confirmed ILI	Reported ILI	Controls	P
Total number of participants	540		159	118	263	
Intervention Randomized to						
Mask/ hygiene	137 (25)		38 (24)	31 (26)	68 (26)	
Mask only	202 (37)		57 (36)	46 (39)	99 (38)	
Control	201 (37)		64 (40)	41 (35)	96 (37)	
Gender		0.13				
Female	327 (61)		98 (62)	70 (59)	159 (60)	0.95
Male	213 (39)		61 (38)	48 (41)	104 (40)	
Race		0.03				
White	348 (67)		100 (66)	77 (67)	171 (67)	0.98
Other[b]	175 (33)		52 (34)	38 (33)	85 (33)	
Current Employment		0.06				
Yes	198 (37)		59 (38)	46 (39)	93 (36)	0.88
No	331 (63)		95 (62)	72 (61)	164 (64)	
Perceived Stress[c], mean (SD)	22.6 (7.7)	0.01	21.8 (8.74)	23.7 (7.9)	22.6 (6.9)	0.14
Current Drinker		0.01				
Yes	173 (33)		50 (33)	42 (37)	81 (32)	0.65
No	351 (67)		102 (67)	73 (63)	176 (68)	

Exercise[d]			< 0							
Low Rate	398	(76)		113	(72)	91	(79)	194	(77)	0.41
High Rate	126	(24)		43	(28)	24	(21)	59	(23)	
Roommates in Room Assignment[e]			0.12							
0	122	(23)		30	(19)	25	(21)	67	(25)	0.51
1	370	(69)		111	(71)	81	(69)	178	(68)	
2 or more	44	(8)		15	(10)	11	(9)	18	(7)	
Recent Flu Vaccine[f]			0.03							
No	430	(82)		130	(84)	91	(80)	209	(81)	0.71
Yes	96	(18)		25	(16)	23	(20)	48	(19)	
Prior Allergy Diagnosis			< 0							
No	371	(69)		100	(64)	79	(67)	192	(73)	0.14
Yes	166	(31)		56	(36)	39	(33)	71	(27)	
Prior Asthma Diagnosis			< 0							
No	447	(83)		129	(83)	102	(86)	216	(82)	0.57
Yes	90	(17)		27	(17)	16	(14)	47	(18)	

[a]ICC=Intracluster Correlation Coefficient

[b]Includes black or African American, Asian, Hispanic, Hawaiian or Pacific Islander, American Indian or Alaskan Native, Multi-Ethnic

[c]Total of 32 participants were missing a perceived stress score; 11 with clinically verified ILI, 6 with non-clinically verified ILI, and 15 ILI-free controls

[d]High rate was defined by a very or extremely hard rate for 4 or more times per week for at least 20 minutes each time or an easy, medium, or hard rate for 5 or more times per week for at least 30 minutes each time

[e]Categories compared for 0 vs. 1 or more roommates

[f] Recent flu vaccine was defined as having a flu vaccine for the 2007-2008 flu season at baseline

[g]P-values computed using cluster-adjusted chi-square test for categorical characteristics and cluster-adjusted ANOVA for continuous characteristics; no variables were statistically significant at the P<0.05 level

Figure 4.1 displays the reported hand hygiene characteristics and hours spent in one's own room at baseline between confirmed ILI cases, reported ILI cases, and control subjects. No statistically significant differences were observed. More than 70% of participants reported having poor hand hygiene and spent an average of 10 hours per 24 hour period in their residence hall rooms at baseline.

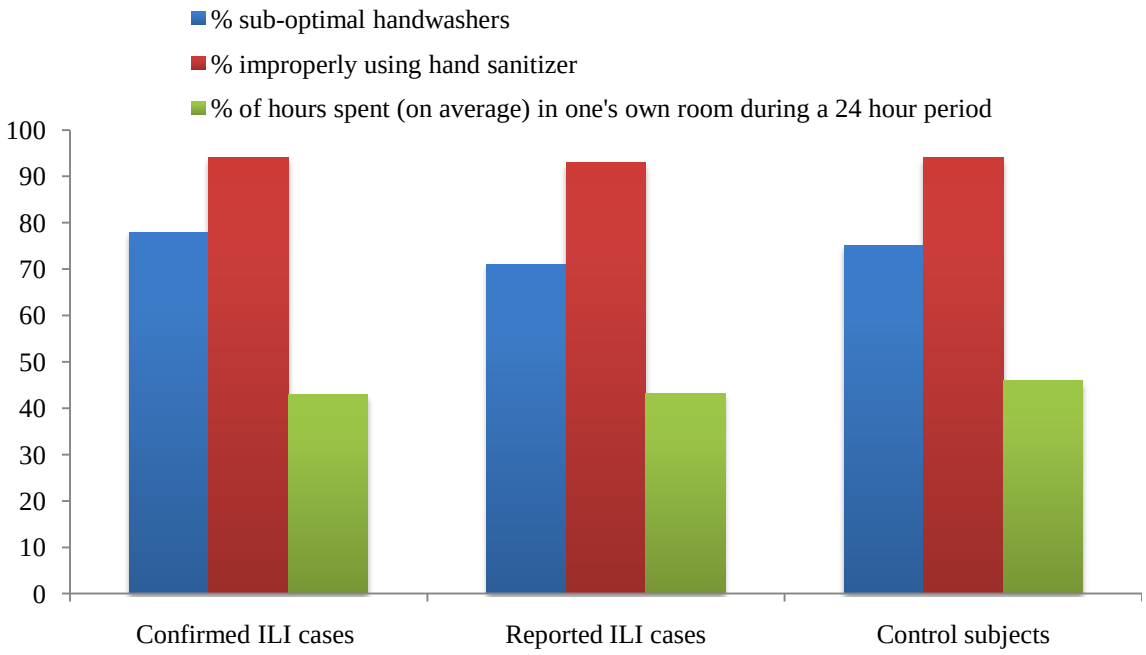


Figure 4.1 Reported hand hygiene characteristics and hours spent in one's own room at baseline

Note. Analysis was conducted among an ILI-free cohort at the study outset. There were 159 confirmed cases of ILI, 118 reported cases of ILI on survey data, and 263 control subjects; a composite variable for optimal hand washing based on CDC recommendations of duration and the average number of hand washes (mean number = 5) among participants (i.e. optimal hand washing was defined by washing with soap and water at least 5 times on average per day for at least 20 seconds each time); a composite variable for use of alcohol-based hand sanitizer (proper use/otherwise) defined by the use of at least a quarter-sized amount of hand gel (recommended by the CDC) twice daily (the average number of uses among study participants).

Figure 4.2 shows no statistically significant difference (p = 0.08) between confirmed ILI cases, reported ILI cases, and control subjects at baseline in response to the question "Are you concerned that you or someone in your immediate family or group of

friends may get sick from influenza during the next 3 months?" In total, only 34% of respondents (n = 176/521) reported being concerned.

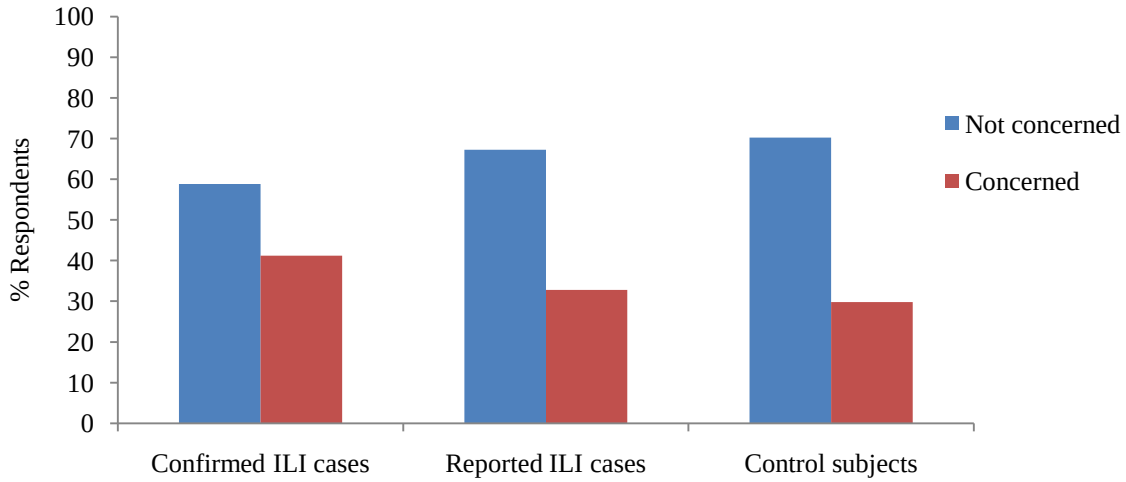


Figure 4.2 Perceptions of influenza at baseline (N=521)

Figure 4.3 shows the baseline distribution in students' responses to questions regarding their preferred method of hand hygiene. A substantially larger proportion of students reported that hand sanitizer was easier and faster than washing with soap and water, but a larger proportion also reported that hand washing was milder on hands, more effective at removing "germs", and the preferred method compared to using hand sanitizer. These trends parallel the trends observed when examining baseline responses according to whether participants had confirmed ILI, reported ILI, or were control subjects.

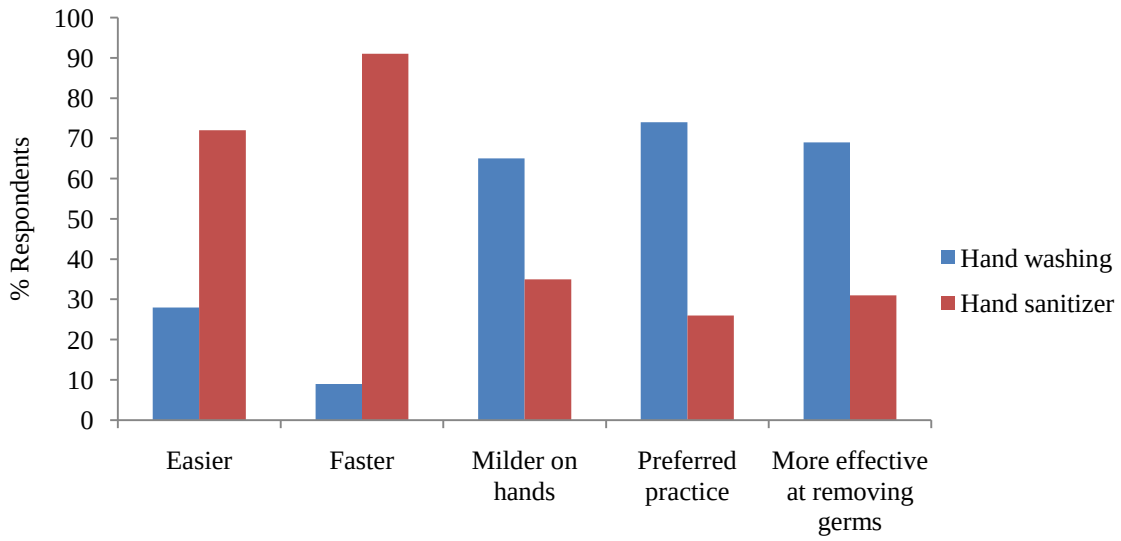


Figure 4.3 Distributions regarding students' preferred method of hand hygiene at baseline

Note. Total N=507 for respondents to the question regarding which practice is easier, N=522 for respondents to the question regarding which practice is faster, N=417 for respondents to the question regarding which practice is milder on hands, N=489 for respondents to the question regarding which hand hygiene method is preferred, and N=355 for respondents to the question regarding which hand hygiene method is more effective at removing germs.

Figures 4.4a–4.4c show trends in reported hand washing frequency up to two weeks post symptoms being present or, in the case of control subjects, up to two weeks post control selection.

Figure 4.4a Mean Hand Washing Frequency:
Week Symptoms Were Present VS. One Week Prior

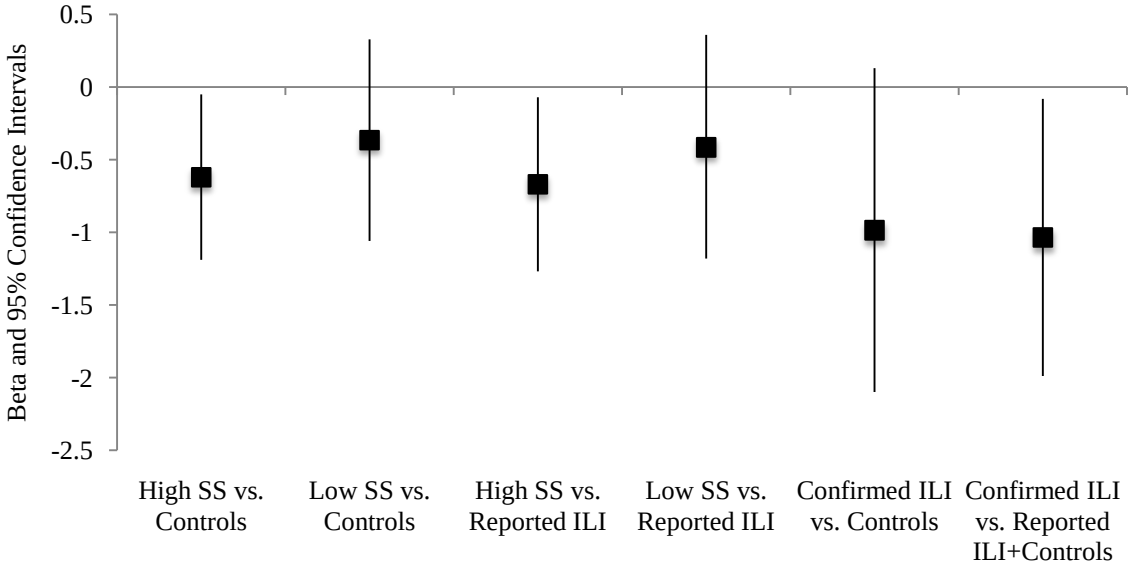


Figure 4.4b Mean Hand Washing Frequency:
1-Week Post-Symptoms VS. Week Symptoms Present

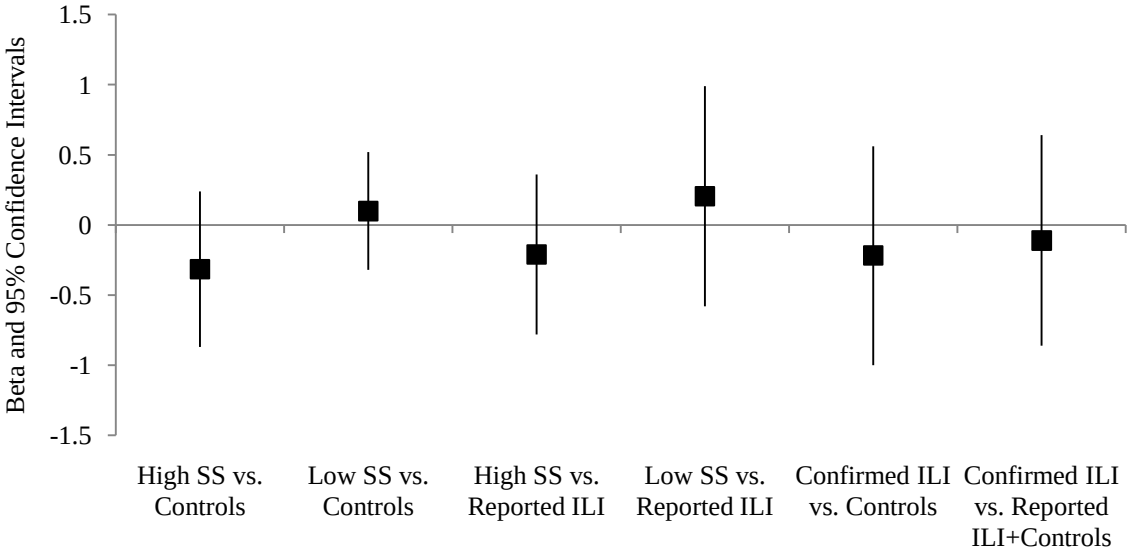


Figure 4.4c Mean Hand Washing Frequency:
2-Weeks Post-Symptoms VS. 1-Week Post-Symptoms

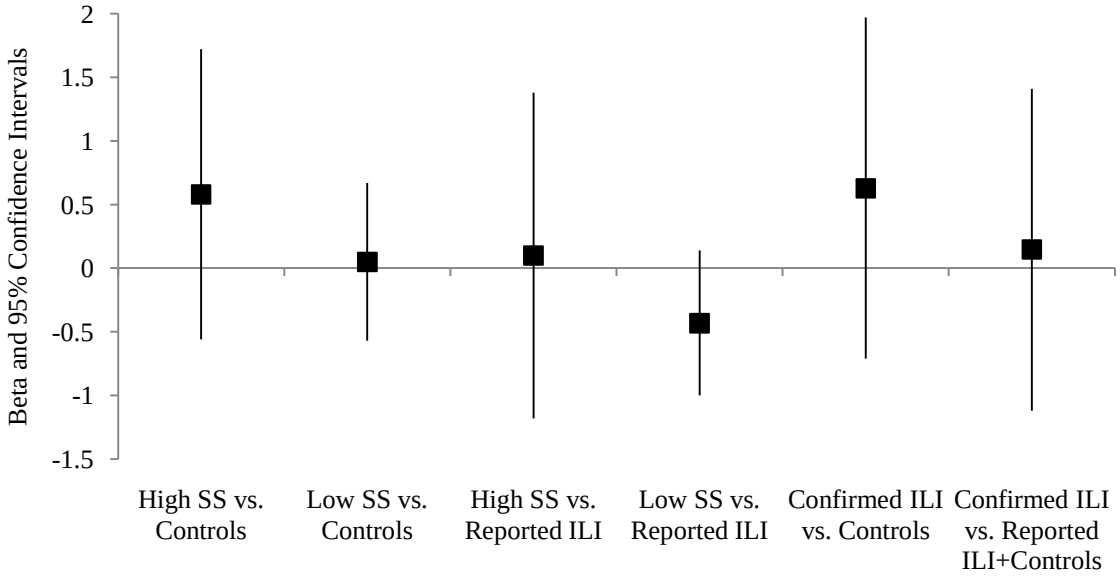


Figure 4.4 Trends in reported hand washing frequency up to two weeks post symptoms being present or, in the case of control subjects, up to two weeks post control selection

Note. SS = symptom severity among confirmed cases of ILI based on the mean severity score; of the 159 confirmed ILI cases, 54 cases had high ILI symptom severity and 105 cases had low symptom severity (mean severity score = 5.01; range: 2 to 12); the numbering of the vertical axes in these figures differ based on parameter estimates obtained from regression models; the main predictor variable in each regression model was a 4-level categorical variable separating the study population into (1) cases of confirmed ILI with high SS, (2) cases of confirmed ILI with low SS, (3) cases of reported ILI, and (4) control subjects who did not report ILI either clinically or on weekly surveys; all lagged regression models adjusted for intervention or control group assignment (face mask and hand hygiene, face mask only, no intervention) and the mean response in hand washing at the beginning of the specified time period (i.e. Y_{ti-1}) of interest for the continuous outcome analyzed (i.e. $Y_{ti} - Y_{ti-1}$); models were stratified by week.

Figure 4.4a shows that during the week ILI was present or the week when controls were selected compared to the week prior, the mean hand washing frequency among confirmed ILI cases was significantly lower than the mean hand washing frequency for both reported ILI cases and control subjects, adjusting for intervention group and hand washing in the week prior (adjusted Beta coefficient [aβ]=-1.04, 95% CI: -1.99 to -0.08). A negative correlation between symptom severity and hand washing frequency was identified such that confirmed ILI cases with low symptom severity had a significantly lower hand washing frequency than controls during the week of illness verification (aβ=-

0.37, 95% CI: -1.06 to 0.33; see Figure 4.4a) and an even lower hand washing frequency was observed among confirmed ILI cases with high symptom severity compared to controls (aβ=-0.62, 95% CI: -1.19 to -0.05; see Figure 4.4a). Figure 4.4a also shows that hand washing frequency and symptom severity followed a negatively correlated trend when comparing confirmed ILI to reported ILI cases during the week symptoms were present (confirmed ILI with high symptom severity vs. reported ILI cases: aβ=-0.67, 95% CI: -1.27 to -0.07; and, confirmed ILI with low symptom severity vs. reported ILI cases: aβ=-0.41, 95% CI: -1.18 to 0.36). Thus, high or low symptom severity was associated with poorer hand hygiene compliance among clinically verified ILI cases. Parameter estimates suggested an improvement in hand washing among participants with confirmed ILI compared to reported ILI cases and control subjects during the 2 weeks post-illness verification (see Figures 4.4b and 4.4c; all $p > 0.05$).

No statistically significant trends were observed when examining compliance with alcohol-based hand sanitizer use or the reduction in exposure to social contacts during the week illness symptoms were present (see Table 4.2). Trends in compliance were also not observed throughout the study (all $p > 0.05$; see Table 4.2).

Table 4.2 Influence of influenza-like illness (ILI) and symptom severity (SS) on compliance with non-pharmaceutical measures for influenza (N=540)

		Frequency of Alcohol-based Hand Gel Use	Exposure to Social Contacts
Outcome[a]	Classification of Participants[b]	β (95% CI)	β (95% CI)
$Y_{t2} - Y_{t1}$	High SS vs. Controls	0.77 (-0.59 to 2.12)	-0.93 (-1.84 to -0.02)
	Low SS vs. Controls	0.44 (-0.16 to 1.05)	-0.05 (-1.03 to 0.93)
	High SS vs. Reported ILI	0.78 (-0.70 to 2.26)	-0.69 (-1.95 to 0.57)
	Low SS vs. Reported ILI	0.46 (-0.38 to 1.29)	0.19 (-0.98 to 1.35)
	Confirmed ILI vs. Controls	1.21 (-0.49 to 2.92)	-0.98 (-2.13 to 0.17)

	Confirmed ILI vs. Reported ILI + Controls	1.22 (-0.54 to 2.98)	-0.74 (-2.03 to 0.55)
$Y_{t3} - Y_{t2}$	High SS vs. Controls	-0.22 (-1.00 to 0.55)	-0.07 (-1.20 to 1.06)
	Low SS vs. Controls	-0.09 (-0.74 to 0.57)	-0.28 (-1.04 to 0.47)
	High SS vs. Reported ILI	-0.54 (-1.50 to 0.42)	0.48 (-0.50 to 1.47)
	Low SS vs. Reported ILI	-0.41 (-1.21 to 0.40)	0.27 (-0.56 to 1.10)
	Confirmed ILI vs. Controls	-0.31 (-1.54 to 0.91)	-0.36 (-2.03 to 1.31)
	Confirmed ILI vs. Reported ILI + Controls	-0.63 (-1.76 to 0.50)	0.20 (-1.20 to 1.60)
$Y_{t4} - Y_{t3}$	High SS vs. Controls	0.13 (-0.77 to 1.03)	-0.71 (-1.57 to 0.15)
	Low SS vs. Controls	-0.02 (-0.75 to 0.71)	-0.14 (-1.07 to 0.78)
	High SS vs. Reported ILI	-0.07 (-0.99 to 0.86)	0.33 (-0.77 to 1.43)
	Low SS vs. Reported ILI	-0.21 (-0.94 to 0.51)	0.90 (-0.06 to 1.85)
	Confirmed ILI vs. Controls	0.12 (-1.27 to 1.50)	-0.86 (-2.11 to 0.40)
	Confirmed ILI vs. Reported ILI + Controls	-0.08 (-1.40 to 1.24)	0.18 (-1.02 to 1.39)

Note. Hand hygiene based on on-line weekly survey responses of health practices within the past week, including the day the survey was completed and illness verification was made; the measure for a reduction in social contacts was based on on-line weekly survey responses within the past 24 hours from survey completion; of the 159 confirmed ILI cases, 54 cases had high ILI symptom severity and 105 cases had low symptom severity (mean severity score = 5.01; range: 2 to 12).

[a]Change in the mean response for outcome measure listed per column during consecutive weeks; $Y_{t2} - Y_{t1}$ = change between week of illness/control selection and week prior; $Y_{t3} - Y_{t2}$ = change between one week following illness/control selection and week of illness/control selection; $Y_{t4} - Y_{t3}$ = change between 2 weeks following illness/control selection and 1 week post-illness/control selection; all models controlled for intervention or control group assignment (face mask and hand hygiene, face mask only, no intervention) and the mean response in hand washing at the beginning of the specified time period; models were stratified by week.

[b]Main predictor variable in model is a 4-level categorical variable separating the study population into (1) cases of clinically-verified ILI with high ss, (2) cases of clinically-verified ILI with low ss, (3) cases of reported ILI, and (4) controls who did not report ILI either clinically or on weekly surveys; symptom severity was based on the mean value among clinical ILI cases; a CONTRAST statement in SAS was used to obtain parameter estimates.

4.4 Discussion

This study investigated if confirmed ILI among young adults during the 2007-2008 flu season was associated with changes in behavior with recommended non-pharmaceutical

measures for mitigating transmission of influenza in university residence halls. The findings show that participants seeking clinical verification of their symptoms washed their hands with soap and water significantly fewer times than participants not clinically examined for their symptoms and controls during the week of illness onset. Evidence of a negative correlation between increasing symptom severity and lower hand hygiene compliance during the week of illness confirmation was also observed. An improvement in hand washing among confirmed ILI cases in the two weeks subsequent to symptom onset was suggested. No statistically significant differences between groups were observed when comparing reported compliance with hand sanitizer use or students' reduction in exposure to social contacts, although the findings indicated that confirmed ILI cases were more likely to use hand sanitizer when symptomatic compared to reported ILI cases and controls. Contrary to the initial study hypotheses, young adults in the university setting seeking clinical verification of their ILI reported sub-optimal hand hygiene, a key non-pharmaceutical measure recommended for mitigating influenza transmission, following illness confirmation.

To the best of my knowledge, this study is the first to provide longitudinal data on the influence of ILI on hand hygiene practices and other health habits among university students living in shared residence halls. No studies have provided empirical evidence thus far regarding the influence of ILI on health behavior that can affect disease transmission, particularly in crowded community settings. Influenza outbreaks in the institutional setting play a significant role in disease transmission, morbidity, and illness.[142-144] Outbreaks of influenza especially within the university setting can also produce a considerable amount of stress on students and university health services.[145] For

example, Nichol et al.[146] examined the impact of colds and ILI on academic performance and health care use in a large university student population and found that illness resulted in a significant number of school/work absence days, days in bed, poor academic performance, and an increased utilization of health services and antibiotics. Hence, detecting an outbreak via surveillance and compliant behavior with non-pharmaceutical measures for influenza can help limit the challenges university health care systems face.[147-149] Given the recent A H1N1 pandemic, identifying the most effective interventions[150-152] for behavioral change in response to future influenza pandemics and outbreaks among university students is warranted.

Scientific evidence on the relative contribution of improved hand hygiene when ill within the university setting is needed. A gradual improvement in hand washing among those participants with confirmed ILI in the two weeks subsequent to symptom confirmation was observed. Interestingly, though, is the fact that when symptoms were present, clinical ILI cases reported using more hand sanitizer than survey reported ILI cases and control subjects, albeit statistically non-significant. This finding may primarily reflect the convenience of using hand sanitizer. Nonetheless, no sustained adherence with hand sanitizer use among confirmed ILI cases two weeks post-illness was observed, suggesting that the slight increase in hand sanitizer use among these participants would not have been carried forward once illness symptoms subsided. It is therefore possible that students perceived hand sanitizer to be easier to use or more effective than hand washing when they were ill. Indeed, among the confirmed ILI cases at baseline, a larger proportion of cases said using hand sanitizer was easier and faster than washing with soap and water, but a larger proportion of cases also reported that hand washing was milder on

hands, more effective at removing "germs" (in general), and the preferred method compared to using hand sanitizer.

Pittet et al.[152] has reported poor adherence to hand hygiene guidelines among healthcare workers in the hospital setting and raised key questions regarding hand hygiene for infection control that are pertinent to this study, such as the relative contribution of hand hygiene versus other non-pharmaceutical measures, risk factors that affect compliance, and thresholds of compliance for impacting transmission. Although proper hand hygiene practices are critical for mitigating transmission of infection in crowded settings, immediate and direct contact with contaminated surfaces (e.g. door handles) following proper hand washing may still greatly contribute to the spread of viral pathogens. In addition, influenza can spread via aerosols and the relative contribution of aerosols versus direct contact transmission is still unclear, making it difficult to quantify the independent impact of hand hygiene on attack rates of influenza and similar respiratory infections.[37]

There are several limitations to this study. First, some of the variables were based on self-report. The literature does however support self-report of health practices as a reliable proxy for objective measures. For example, a study in the clinical setting found that self-report and direct observation of nurses' hand hygiene practices in two intensive care units were highly correlated, indicating no statistically significant difference between nurses' self-report of hours spent wearing gloves and hand hygiene episodes per hour and direct observation over a 22-month period.[153] There are, however, no US-based studies examining the level of concordance in the community setting. Although attack rates of influenza were high during the 2007-2008 flu season, several viruses can cause

ILI; indeed, only 37 of the 159 (23%) confirmed cases of ILI were cases with influenza A or B by Rt-PCR in the M-Flu laboratory. Nonetheless, the attack rates from clinically verified ILI and confirmed influenza infection in the M-Flu study were very similar. Since underreporting of illness or selection bias may have also occurred, non-differential misclassification of controls and reported cases of ILI cannot be ruled out and may have biased these results towards the null hypothesis. In addition, all study participants would have received a monetary incentive for providing a throat swab specimen during their clinical exam. Nonetheless, inherent differences between those participants with reported ILI and those with confirmed ILI were unable to be determined and/or measured. Finally, the measure for participants' reduction in their exposure to social contacts represents a surrogate marker for self-isolation/quarantine and does not account for the specific duration participants were unexposed to others, including roommates. It is also possible that measurement error in this variable biased the results for exposure to social contacts towards the null.

4.5 Conclusion

University students who sought clinical verification of their ILI reported sub-optimal compliance with hand washing and other health practices during the 2007-2008 influenza season. This is the first study to provide epidemiologic data on the behavioral response to confirmed ILI in the university setting. Additional studies should identify the most effective interventions for promoting long-term behavioral change in response to seasonal influenza among university students. Future research should also aim to identify risk factors for compliance and a compliance threshold resulting in an adequate control of infection in the university setting.

Chapter 5

Conclusion

This dissertation demonstrated temporal, curvilinear associations between perceived stress and rates of ILI, naturally acquired influenza A infection, and influenza A viral load in an understudied population of young adults at high risk for pandemic flu within a large university setting. Perceived social support did not modify the observed association between perceived stress and ILI. Confirmation of ILI among participants seeking clinical verification of their symptoms was associated with sub-optimal hand washing, a non-pharmaceutical recommendation for mitigating the spread of influenza, when symptoms were present. An improvement in hand washing was suggested in the two weeks post-clinical examination of symptoms. These novel findings are timely and a major contribution to the current epidemiologic literature because they establish a precedent for future well-controlled, longitudinal studies to further explore the behavioral and biological pathways by which these associations exist in understudied human populations within a community setting. Young adults at high risk for pandemic influenza may greatly benefit from stress-reduction and behavioral interventions aimed at improving respiratory health and mitigating transmission of influenza, respectively.

5.1 Aim 1

University students with the highest levels of perceived stress had a 52% greater incidence of ILI compared to participants with moderate stress, but those with moderate

stress had a 32% reduced incidence of ILI compared to participants with low stress. No statistically significant difference between those with high and low perceived stress was observed. Differential exposure to psychological stress among young adults in the university setting therefore proved to be an independent risk factor for flu-like illness. Social support networks did not modify the observed stress-ILI association, thus indicating that perceived social support did not impact coping among participants with the highest burden of stress.

Aim 1 highlights several gaps in our current understanding of the mechanisms by which perceived stress is associated with ILI incidence in young adults within a university setting. For example, how do different stress thresholds predict variability in rates of ILI? The human stress literature primarily suggests that increasing levels of perceived stress predict increasing rates of respiratory illness and infection. The findings discussed for aim 1 are the first to report a statistically significant curvilinear association and, more importantly, in a population considered at low risk for influenza until the recent 2009 influenza A H1N1 pandemic.

Coping styles in response to specific psychological stressors experienced by young adults in a university setting is one possible pathway linking perceived stress to ILI. Coping responses for stress were not collected in this study and therefore limit the ability to examine these as possible mediators of the observed stress-ILI association. This renders researchers to, first, identify the stressors that are pertinent to young adults in a university environment and, second, to properly measure well-defined behavioral and coping responses to these stressors. Examining stress-induced immune changes influenced by coping mechanisms should be incorporated in future studies to better

understand the mechanistic pathways by which stress differentially impacts rates of ILI in young adults. Use of validated instruments for stress that are internally consistent and well-defined coping mechanisms for the study population of interest are fundamental study design aspects that should be considered. In addition, students living in a university setting most likely do not experience the same stressors or have the same coping mechanisms as persons in other community settings. Perceived stress among various study populations is most likely caused by different stressors and therefore the incidence of ILI is variable between different populations and also seasons with varying flu activity. Hence, data regarding behavioral and coping responses for perceived stress must be specifically examined for the study population within the community setting of interest.

Future epidemiologic research should aim to identify the most effective stress-management interventions for reducing the burden of ILI in university students under high stress. Currently there are no studies, to my knowledge, that examine this relation in a high risk setting for transmission of infection. Relaxation, time management techniques, and exercise can all help young adults cope with stress; however, consistent data on these interventions for alleviating the problem of poor respiratory health on campus communities is lacking. Earlier mention of the Reid et al.[122] study showed a reduction in the number of days with upper respiratory tract symptoms among a small sample of university students treated for exam anxiety compared to controls. The current literature can therefore gain from examining the influence of varying stress-reducing interventions on rates of ILI in a large cohort of university students within an influenza context.

This dissertation did not examine perceived stress as a predictor of multiple case definitions of ILI. ILI case definitions vary between studies and are a major source of

variability in study findings. Their sensitivity and specificity for influenza infection can be impacted by the amount of seasonal flu activity as well. Hence, a comparison of multiple ILI case definitions in future studies of perceived stress within the community setting would produce a more comprehensive analysis for understanding this association. In addition, accurately measuring and controlling for seasonal vaccination is needed. This research examined self-reported flu vaccination which is subject to recall bias and can be inaccurately measured since participants reporting vaccination are enrolled in an influenza study. This confounder of the stress-ILI association could be measured using medical records, however the resources needed to obtain accurate vaccination history can prove costly in observational studies. Nonetheless, because young adults are at high risk for pandemic flu due to a lack of prior immunity and their ability to mount a vigorous immune response, controlling for vaccination using a measurement superior to self-report is certainly needed.

5.2 Aim 2

In aim 2, young adults with above participant average levels of perceived stress had a significantly greater risk of acquiring confirmed influenza infection and those with moderate levels of stress were protected against infection. Novel findings of viral load showed an increase in response to increasing psychological stress but a slight decrease when stress levels exceeded the 80^{th} percentile of stress scores among 46 participants with confirmed influenza A H3N2. This dissertation is the first to establish curvilinear associations between stress and naturally acquired influenza A infection and viral load. Due to the small number of incident cases and the functional form of stress, perceived stress was modeled continuously and a stress-by-stress interaction was examined. Had

there been a larger number of incident cases, categorizing participants into low, moderate, and high levels of perceived stress as in aim 1 would have been done to facilitate interpretation of study results. Nonetheless, the results clearly indicate that exposure to psychological stress differentially influences the risk of naturally acquired infection and viral pathology. Understanding the underpinnings of these associations in young adult populations remains to be determined in future epidemiologic research.

Epidemiologic studies have yet to examine the impact of coping for stress and stress-reduction interventions for mitigating the burden of naturally acquired flu infection in large young adult populations. Researchers need to identify and understand specific coping responses such as problem-oriented vs. emotion-oriented coping that can mediate the stress-infection relationship found among young adults at high risk for pandemic flu. Since previous research has documented that the onset of colds and ILI result in a significant number of school/work absence days, days in bed, poor academic performance, and an increased utilization of university health services and antibiotics, the respiratory health of young adults can be beneficially impacted by future stress intervention studies. Examining stress-induced immune changes resulting from coping mechanisms should also be incorporated in future infection studies to understand how stress differentially impacts rates of seasonal influenza infection in young adults within the community setting.

Although viral load showed no statistical association with self-reported symptom severity during clinical examination, the findings suggested a greater severity to be positively correlated with viral load. Discussion was made regarding the fact that a slight decline in viral load among participants with the highest levels of stress may have been

observed because these students, on average, provided throat swab specimens 1 day following illness onset when viral load may not have peaked. Additional research is needed to disentangle whether this is in fact a real biological pattern in young adults with confirmed influenza A infection. If future studies can replicate the findings in aim 2 with respect to viral load, then perceived stress plays a significant role in increasing viral replication and delaying viral clearance from one's immune system. Hence, infection control protocols in crowded university settings may need to address psychological risk factors such as perceived stress. Stress-reduction interventions may therefore play a vital role in infection control within the university setting.

Since this work focused on influenza A infection, a pandemic variant strain, it can be informative if future studies examine the temporal relation between perceived stress and naturally acquired influenza B infection. Seasonal influenza A viruses are distinctly different from influenza B viruses due to antigenic variation in their genetic structures. However, influenza B viruses can, like influenza A, cause severe illness in those at high risk. Unlike influenza A virus, influenza subtype B has never caused a global pandemic. Nonetheless, influenza B epidemics have occurred. Therefore, perceived stress should be examined as a possible risk factor for acquired influenza B infection, particularly in crowded community settings.

5.3 Aim 3

In aim 3, university students who sought clinical examination of their reported ILI along with laboratory testing for influenza washed their hands with soap and water significantly fewer times than participants not clinically examined for their symptoms and control subjects during the week illness symptoms were present. Evidence of a negative

correlation between increasing symptom severity and lower compliance during the week ILI was clinically verified was also found and an improvement in hand washing among confirmed ILI cases in the two weeks subsequent to symptom confirmation was suggested. Well-controlled prospective studies within the community setting are needed to address risk factors for compliance with these non-pharmaceutical measures in young adults. Once risk factors can be identified, solutions to improve hand hygiene for mitigating the spread of influenza can be achieved.

Identifying the most effective interventions for promoting long-term compliant behavior in response to influenza among university students, particularly those with symptoms of ILI, is necessary to limit the spread of infection. Part of the effort to reduce secondary attack rates in this population begins with seeking medical attention for symptoms, which was the case for participants with clinically verified illness, but more importantly is taking action in physically intervening with the spread of illness. Physical interventions such as proper hand washing and overall hand hygiene are critical in this process especially in crowded settings. Therefore, examining which behavioral multi-level interventions (i.e. individual- and institutional-level interventions) are more effective for eliciting optimal compliance with non-pharmaceutical measures for influenza in this study population is warranted.

Quantifying the relative contribution of improved hand hygiene and limited social interaction in crowded community settings would greatly enhance the current epidemiologic literature. For example, what is the relative contribution of a sustained improvement in hand hygiene and adherence to other preventive measures in predicting influenza transmission within university residence halls? Although proper hand hygiene

practices are critical for mitigating transmission of infection in crowded settings, immediate and direct contact with contaminated surfaces following proper washing still plays a vital role in the spread of infectious disease. Hence, what is the relative contribution of contaminated surfaces to transmission of infection? Is there a compliance threshold that would result in adequate infection control within university residence halls? Future epidemiologic studies are needed to address these complex issues.

5.4 Dissertation summary

Utilizing prospective data from the M-Flu study, a randomized intervention trial conducted among students living in residence halls at the University of Michigan during the 2007-2008 flu season, this dissertation demonstrated that differential exposure to psychological stress influences the risk of ILI, naturally acquired influenza A infection, and influenza A viral load, a possible biomarker of disease severity. This is the first study to establish these novel and timely findings for a pandemic variant strain of influenza in an understudied human population living within a high risk community setting for infection. Research examining stress reduction and management as preventive measures for influenza in young adult populations is warranted. Further studies of the biological mechanisms that influence changes in immune susceptibility to influenza are needed. In addition, ILI confirmation was associated with sub-optimal compliance with hand hygiene among young adults who sought clinical examination of their ILI symptoms. Future research should aim to identify the most effective interventions for promoting long-term behavioral adherence to non-pharmaceutical measures in a community setting where influenza is known to be circulating. Timely identification of risk factors for

compliance with non-pharmaceutical measures and determination of a compliance threshold resulting in adequate infection control in university residence halls are needed.

5.5 Strengths and limitations

Utilizing a prospective, observational study design and young adult population strengthened this research by allowing for statistical control of many risk factors for influenza such as age, socioeconomic position, and seasonal variation. This work also used a validated stress instrument for measuring perceived stress that was internally consistent in the study population examined. Therefore, a valid measurement of perceived stress was utilized to examine this novel risk factor for three integral influenza outcomes in an understudied population at high risk for pandemic flu. In addition, this was the first study to identify a clear trend over time in hand hygiene behavior in response to flu-like illness. Thus this work has novel implications for future intervention studies aimed at improving the respiratory health and control of infection among young adults in a large university setting.

Several limitations are also noteworthy. One such limitation is that some cases of ILI may have stemmed from viruses other than influenza. Nonetheless, the distribution of primary attack rates from clinically verified ILI and confirmed infection in the M-Flu study population were comparable, indicating a reliable case definition of ILI for influenza. Of note, future studies should be aware that the sensitivity and specificity of ILI case definitions are influenced by the level of flu activity present and therefore analysis of multiple ILI case definitions may enhance study findings. In addition, the measure for viral load was based on cycle threshold values obtained from Rt-PCR and serial dilutions of two standard H3N2 viral strains. Since semi-quantitative Rt-PCR

methods were performed on this data, the measure of viral load did not represent the true concentration of influenza A RNA in laboratory confirmed specimens. This dissertation also utilized self-report data which is subject to some degree of bias. The results from this dissertation may also not be applicable to non-university settings and other study designs and populations. However, generalization of the results to similar study designs and populations residing in crowded community environments is possible. Finally, the goal of the M-Flu study from which this dissertation work was based was not to examine the associations between perceived stress, influenza, and subsequent compliance with non-pharmaceutical measures for influenza. Thus, key variables such as stress coping mechanisms and risk factors for compliance with these measures were unable to be examined.

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