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Church Attendance and CMV Herpes Virus Latency Among Bereaved and Non-Bereaved Adults

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There is widespread literature linking church attendance to physical health. However, little is known about the association of church attendance and the immune system, particularly during difficult life transitions. This study investigated the association between church attendance and CMV herpes-virus latency by assessing Cytomegalovirus (CMV) IgG antibody titers among bereaved and nonbereaved individuals. Participants included 44 bereaved individuals and 44 controls, with a mean age of 68 ($SD = 12.84$). CMV herpes-virus latency was measured using CMV IgG antibody titers. Church attendance was measured using 3 items from the Community Healthy Activities Model Program for Seniors (CHAMPS) Questionnaire. After adjusting for participant's age, gender, education, minority status, weekly alcohol consumption, smoking, depression, body mass index (BMI) and comorbidities, church attendance was associated with lower CMV IgG antibody titers among bereaved and control participants. Furthermore, there was a significant moderating effect of church attendance in the association between bereavement status and CMV IgG antibody titers, so that bereaved individuals attending church were found to have less herpes-virus reactivation (lower CMV IgG antibody titers) when compared to their bereaved counterparts that do not attend church. This study demonstrated that church attendance is associated with less herpes-virus reactivation as indexed by lower levels of CMV IgG antibody titers, particularly among the bereaved. Future studies should focus on further understanding the pathways by which church attendance impacts CMV herpes-virus latency during stressful life events, such as bereavement.

Keywords: church, Cytomegalovirus (CMV), immunity, bereavement, health

Religiosity, that is, the compliance, belongingness, and commitment to an organized tradition with a specific belief system (Thoresen, Harris, & Oman, 2001), is central to the lives of many people, particularly in the face of adversity (Wortmann & Park, 2008). A recent U.S. survey of more than 35,000 participants found that approximately 74% of Americans identified religiosity

as important in their life, with approximately 36% attending weekly religious services and 41% participating in prayer services or religious education groups several times a year (Pew Research Center, 2014). Also, research shows religious involvement and attendance at religious services to be particularly relevant in the lives of people facing disadvantage (Pew Research Center, 2014),

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and to be associated with lower mortality (Chida, Steptoe, & Powell, 2009; McCullough, Hoyt, Larson, Koenig, & Thoresen, 2000). Identifying ways in which religiosity benefits people is essential to inform program and intervention development.

An important aspect of religiosity in the health literature is church attendance, given its association with lower mortality (Chida et al., 2009) and its stress-buffering potential (Das & Nairn, 2016). Indeed, church attendance has been consistently shown to have a positive effect on health during stressful life transitions (Das & Nairn, 2016). For instance, church attendance helps individuals better cope with life-threatening illness (Matthews et al., 1998), and it serves as a protective factor against depression and anxiety after the loss of a loved one (Monserud & Markides, 2017; Woo, Chan, Chow, & Ho, 2009). Nevertheless, although church attendance has been identified as helpful during stressful life transitions, information on the potential effects of church attendance on physiological outcomes remains scant.

Among the few studies that have explored associations between church attendance and biological markers, evidence suggests that church attendance may be associated with lower blood pressure (Das & Nairn, 2016; Seeman, Dubin, & Seeman, 2003), lower heart rate (Das & Nairn, 2016), and lower prevalence of hypertension (Seeman et al., 2003). Also, in a population-based study of 1,718 participants, church attendance was associated with lower inflammation, namely lower levels of Interleukin 6 (IL-6; Koenig et al., 1997). Similarly, using nationally representative data of U.S. adults ages 57–85 from the 2005–2006 National Social Life, Health and Aging Project, results showed that church attendance was associated with lower indicators of inflammation, as well as with better immune functioning (Das & Nairn, 2016). Moreover, in this same study, researchers found that among individuals experiencing spousal loss, due to either divorce or widowhood, those who did not attend religious services had significantly elevated inflammation when compared to those that attended services regularly, but less is known about its association with immune functioning in this population (Das & Nairn, 2016).

The loss of a spouse is a particularly stressful life event (Bartrop, 2017). Consistent with this premise, recent studies examining the effects of spousal loss indicate increased risk of long-term health damage for a broad range of mental and physical health problems (Das, 2013; Hughes & Waite, 2009). For instance, the distress and negative emotions generated may dysregulate cellular immunity (Segerstrom & Miller, 2004), which in turn may lead to the development of various chronic illnesses (Black, 2003). According to biobehavioral models of adversity that emphasize the negative effects of stressful events on physiology (G. E. Miller, Chen, & Parker, 2011), the immune system is responsive to hormones (i.e., cortisol), which can be altered in the face of adversity or during difficult life transitions, such as bereavement (Buckley et al., 2012; Segerstrom & Miller, 2004). Maladaptive alterations in cellular immune function can enhance reactivation and replication of viruses, such as herpes viruses including Cytomegalovirus (CMV; Glaser & Kiecolt-Glaser, 1994; Steptoe et al., 2007). Most people have been exposed to herpes viruses, including CMV (Centers for Disease Control & Prevention, 2016). After primary infection, herpes viruses continue to reside in B lymphocytes and other white blood cells for life (Schmidt & Misko, 1995). Partial reactivation and replication of herpes viruses is controlled by the cellular response, largely by specific-memory cytotoxic T cells and

natural killer cells (Glaser et al., 1993). Although the majority of individuals remain asymptomatic, exposure to stressful events reduces the immune cellular immune response, which in turn may lead to reactivation of the viruses and increased production of specific antibodies to the expressed antigens (Glaser et al., 1993). Indeed, elevated antibody titers indicate poorer cellular immune control over viral latency (Glaser & Kiecolt-Glaser, 1994), which increases vulnerability to disease (Black, 2003).

Given that bereavement is a stressful life event associated with increased health risk, identifying protective factors associated with better health outcomes among the bereaved is essential to inform the development of interventions aimed to reduce risk associated with this difficult life transition. One such factor may be church attendance. In this study, we investigated whether or not church attendance was related to CMV herpes-virus latency among bereaved and nonbereaved individuals. Specifically, we addressed the question of whether church attendance was associated with lower CMV antibody titers, and whether church attendance moderated the association between bereavement status and CMV herpes-virus latency as measured by CMV antibody titers. We hypothesized that the association of bereavement and CMV herpes-virus latency would vary by church attendance, so that bereaved individuals attending church would have less herpes-virus reactivation as indicated by lower CMV antibody titers when compared to their bereaved counterparts that do not attend church.

Method

Study Sample

Our sample comprised participants enrolled in Project Heart, including bereaved adults and their matched controls. The primary aim of Project Heart was to examine the mechanisms that underlie increased risk for cardiovascular disease among bereaved adults. Participants were excluded because of significant visual or auditory impairment, pregnant or nursing women, autoimmune and inflammatory diseases, having experienced bereavement due to loss of a person other than their spouse or significant other, those divorced within the past year, and widowed controls. All participants were English speakers to ensure proper understanding of the questionnaires.

Procedures

Individuals who recently experienced the loss of their spouse ($M = 89.6$ days, $SD = 15.9$ days) were contacted and recruited from obituaries, support groups, flyer distribution, online postings, and community events. Control participants who had not experienced the loss of a spouse or romantic partner in the past year were also recruited through flyers, community events, and online advertisements. Bereaved participants were matched to controls on age and gender. Research assistants administered assessments at the participants' home or in the Bioscience Research Collaborative Community Research Center in the Texas Medical Center. During these visits, participants completed a questionnaire packet, which included self-report demographic questionnaires and clinical questionnaires. Furthermore, anthropometric measurements, including weight, height, waist circumference and non-fasting blood samples, were collected during the early hours of the morning. All

participants provided written informed consent, and procedures were approved by the Rice University Institutional Review Board. Out of the participants recruited, 18 were CMV seronegative and were removed from the analyses. Also, 3 participants were removed from the analyses due to missing data. Thus, our analytical sample included a total of 88 participants (44 bereaved, 44 control).

Determination of CMV IgG Antibody Titers

CMV was selected as the biomarker in this study given that there is considerable evidence suggesting that CMV contributes to immunosenescence, the gradual deterioration of the immune system brought on by natural age advancement (Pawelec, Derhovanessian, Larbi, Strindhall, & Wikby, 2009), which makes this biomarker more clinically relevant than some other herpes viruses. CMV IgG antibody titers were assessed following standard protocol (Stowe et al., 2014). Ninety-six well microtiter plates, coated with virally infected cells, were obtained from EuroImmune (Morris Plains, NJ). Antigen sources for the CMV plates were inactivated cell lysates of MRC-5 (diploid fibroblast) cells infected with the CMV AD169 strain. Plasma samples with high IFA-scored antibody titers (i.e., 2560), obtained from past studies, were used as the top standard for CMV. Eight twofold serial dilutions of the top standards were made with phosphate buffered saline in separate tubes. One hundred microliters of positive and negative controls, standards, and diluted patient samples (all dilutions were at 1:101) were pipetted in duplicate into individual microplate wells followed by a 30-min incubation (all steps were carried out at room temperature). The plates were then washed 3 times with 350 μ l wash buffer (provided) using an Embla microplate washer (Molecular Devices, Menlo Park, CA). Next, 100 μ l of enzyme conjugate (peroxidase labeled antihuman IgG) was pipetted into the wells followed by another 30-min incubation period. The plates were then washed 3 times, and 100 μ l of chromogen substrate (TMB/H₂O₂) was pipetted into the wells. The plates were then covered to protect from direct light and incubated for 15 min. One hundred microliters of 0.5 M sulfuric acid was added to each well to stop the reaction. Absorbance was then read at 450 nm (reference wavelength 590 nm) using a SpectraMax Plus 384 (Molecular Devices). The values of the unknown samples were assigned in relation to the standard curve.

Measures

Church attendance. This was assessed using three questions from the Community Healthy Activities Model Program for Seniors (CHAMPS) Questionnaire. The CHAMPS is a multiitem measure used to assess weekly frequency and duration of various physical activities typically undertaken by older adults (Stewart et al., 2001). The CHAMPS is known to have good psychometric properties, and it has been used widely in the development of interventions with the target population (Stewart et al., 2001). Specifically, in this study, church attendance was assessed using the following question: "In a typical week, during the past four weeks, did you attend church or took part in church activities?" Responses to this question was dichotomous (0 = No, 1 = Yes). Furthermore, among participants attending church, two additional questions were asked to be used in post hoc analyses to determine

if frequency of church attendance was associated with cellular immune function: (a) "How many times a week," which was assessed as a continuous variable; and (b) "How many total hours a week," which was assessed categorically using six response choices ranging from 0 = *less than an hour a week* to 5 = *9 or more hours per week*.

Covariates. Demographic factors, health behaviors, body mass index (BMI), depression, and comorbid conditions were included in the models described below as covariates. Demographics (age, gender, education, and minority status) were collected via self-report questionnaires. Age and education were used as continuous variables, whereas gender (0 = male, 1 = female) and minority status (0 = White, 1 = ethnic/racial minority) were dichotomous variables. Health behaviors included frequency of weekly alcohol consumption and current smoking (0 = no, 1 = yes). BMI was computed as weight in kilograms divided by height in meters squared. Depression was assessed using the Center for Epidemiological Studies Depression Scale (CES-D), which is a brief and widely used measure of depressive symptomatology (Basco, Krebaum, & Rush, 1997; Radloff, 1977). Studies have shown acceptable test-retest reliability and excellent construct validity (Basco et al., 1997). As the CES-D has also distinguished depressed from nondepressed participants in community and clinical samples, discriminative validity appears acceptable as well (Basco et al., 1997). Population norms provide cutoffs for varying levels of depression (Basco et al., 1997). Medical comorbidities were assessed using the Charlson index, which is the most widely used comorbidity index (Charlson, Szatrowski, Peterson, & Gold, 1994). Originally developed for predicting mortality in chronically ill patients, it has now been widely used among ill and healthy populations (Dobnig et al., 2008).

Analytic Method

Analyses were conducted using SPSS, Version 22.0. A log transformation normalized the distribution of CMV antibody titers. Zero-order correlations assessed relationships between age, gender, minority status, education, alcohol consumption, smoking, depression, BMI, comorbidities, church attendance, bereavement status, and CMV IgG antibody titers. Chi-square tests were conducted to assess for differences between bereaved individuals and their matched controls among dichotomous variables. Analyses of variance (ANOVAs) tested for group differences on all continuous variables. Using ordinary least squared multiple regression, we addressed the question of whether church attendance interacted with bereavement status to predict CMV antibody titers. All independent variables were grand mean centered. We examined residuals to confirm that they were normally distributed. To test for moderation, church attendance (0 = no, 1 = yes), bereavement status (0 = control, 1 = bereaved), and the interaction of church attendance and bereavement status were entered as predictors. For each analysis, we ran both unadjusted and adjusted regression models. When predicting CMV antibody titers in the adjusted model, we controlled for age, education, smoking, alcohol consumption, depression, BMI, and comorbidities as linear, and gender, minority status and smoking as dichotomous. We controlled for the aforementioned variables given their well-established impact on cellular immune function (O'Connor et al., 2009). All tests

were two-sided, and $\alpha < .05$ was considered to be statistically significant.

Results

As shown in Table 1, the majority of the sample was female (60%) and the mean age of participants was 68 years ($SD = 12.8$). Also, bereaved participants were more likely to be Caucasian and had lower BMI when compared to control participants. As expected, bereaved participants had greater depression scores when compared to controls. The average CES-D score for bereaved participants was 17, which is higher than the clinical depression cut-off score of 16 or higher (Radloff, 1977; R. E. Roberts & Vernon, 1983). Thus, our bereaved participants were clearly distressed. No significant differences across the groups were found in terms of gender, education, weekly alcohol consumption, comorbidities, church attendance, and CMV antibody titers. Also, among participants reporting their religious affiliation, most were associated with a Christian tradition (65.5%).

Table 2 provides bivariate correlations for all study variables. Minority status and comorbidities were significantly associated with CMV antibody titers. Neither age, gender, education, alcohol consumption, smoking, depression, BMI, church attendance, nor bereavement status were significantly associated with CMV antibody titers in bivariate analyses.

Table 3 summarizes the adjusted and unadjusted analyses that assessed for the effect of bereavement and church attendance on CMV antibody titers, as well as evaluated whether church attendance interacted with bereavement status to predict CMV antibody titers after controlling for relevant covariates. In the adjusted model, there was a significant main effect of church attendance on CMV antibody titers ($B = -.560$, 95% Confidence Intervals [CI] = -1.076 to -0.043 , $p < 0.05$). Specifically, church attendance was associated with lower CMV antibody titers. Also, the interaction between church attendance and bereavement status to predict CMV antibody titers was significant in the unadjusted model ($B = -.240$, 95% Confidence Intervals [CI] = $-.437$, $-.029$, $p < 0.05$), as well as in the adjusted model ($B = .326$, 95% Confidence Intervals [CI] = -0.002 to 0.651 , $p < 0.05$). Specifically, results from the adjusted model showed that there was a significant moderating role of church attendance in the association between bereavement status and

CMV antibody titers, so that bereaved individuals attending church had lower CMV antibody titers when compared to their bereaved counterparts that do not attend church (see Figure 1).

Post hoc analyses were conducted to assess whether frequency of church attendance interacted with bereavement status to predict CMV antibody titers. The interactions between frequency of church attendance and bereavement status to predict CMV antibody titers were nonsignificant.

Discussion

Bereavement ranks as one of the most stressful life events associated with increased health risks (Fagundes, Gillie, Derry, Bennett, & Kiecolt-Glaser, 2012). Identifying protective factors associated with better health outcomes among the bereaved is essential to guide intervention development and maintain well-being. This study investigated how church attendance is related to CMV herpes-virus latency, specifically lower CMV antibody titers, among bereaved and nonbereaved individuals. Our results indicated that after controlling for relevant covariates, church attendance was associated with lower CMV antibody titers, reflecting less herpes-virus reactivation. In addition, we provide novel data assessing the moderating role of church attendance in the association between bereavement status and CMV antibody titers. In line with our hypotheses, we found that bereaved individuals attending church had less herpes-virus reactivation, as indicated by lower CMV antibody titers, when compared to their bereaved counterparts that do not attend church. Important to note is that the association between bereavement and CMV herpes-virus latency was not significant, which is consistent with research documenting individual variation in stress-related bereavement (Bonanno & Kaltman, 1999). Prior research shows that many people exposed to loss or trauma, including bereavement, are highly resilient and show only minor and transient disruptions in functioning and wellbeing (Bonanno, 2004).

The aforementioned findings add to our understanding of the potential role of church attendance on health, particularly less herpes-virus reactivation. The association of church attendance and specific markers of cellular immune function among older adults, namely lower levels of IL-6 (Koenig et al., 1997) and EBV VCA antibody titers (Das & Nairn, 2016), has been previously documented. Nevertheless, our results are innovative in that they

Table 1
Sample Characteristics

Variable	Total ($N = 88$)			Bereaved ($n = 44$)			Controls ($n = 44$)			p
	%	M	SD	%	M	SD	%	M	SD	
CMV (log10)		2.26	.38		2.23	.42		2.29	.33	.455
Age (years)		67.67	12.84		71.78	11.17		63.56	13.20	.002
Gender (female)	60.2			70.5			69.5			.647
Minority status (Minority)	33.0			13.6			52.3			<.001
Education (years)		16.02	2.65		16.30	2.88		15.75	2.40	.338
Weekly alcohol consumption		3.65	5.65		3.76	5.68		3.55	5.68	.859
Smoker	6.80			2.30			11.4			.091
Depression		14.10	11.21		17.25	11.89		10.95	9.64	.008
BMI (Kg/m^2)		28.53	6.07		26.69	4.62		30.46	6.81	.003
Comorbidities		.31	1.04		.27	.97		.34	1.12	.761
Church attendance (yes)	41.4			37.2			45.5			.435

Table 2
Correlations Among Study Variables

Variable	1	2	3	4	5	6	7	8	9	10	11
1. CMV											
2. Age	.09										
3. Gender	.08	-.13									
4. Minority status	.10***	-.59***	-.06								
5. Education	-.11	.14	-.08	-.01							
6. Alcohol Consumption	.01	.24*	-.04	-.30**	-.30***						
7. Smoking	-.09	.21*	.02	.15**	-.29**	.12					
8. Depression	.01	-.16	.16	.13	-.04	-.04	-.14				
9. BMI	.08	-.28**	-.22*	-.23***	.41**	-.28***	-.13	.07			
10. Comorbidities	.23*	-.02	.03	.04	.04	-.11	-.22*	.01	.21*		
11. Church attendance	-.11	-.13	.07	-.07	.07	.12	-.15	.07	-.12	-.11	
12. Bereavement status	-.12	.34**	.06	.05	.12	.00	.19*	.28**	-.31**	-.04	-.06

* $p < .05$. ** $p < .01$. *** $p < .001$.

support the association between church attendance and another marker of CMV herpes-virus latency, specifically lower CMV antibody titers. CMV is an important modifier of the immune system among the elderly (Wertheimer et al., 2014), and persistently high CMV antibody titers have been found to be associated with changes in T-cell subsets, phenotype, and function in older adults. In other words, elevated antibody titers to a latent herpes virus reflect poorer cellular immune system control over virus latency (Henle & Henle, 1981) and thus provide one broad marker of cellular immune system function. Although individuals are often asymptomatic, these elevated antibody titers are not benign and have been linked to inflammation (Murdock, Fagundes, Peek, Vohra, & Stowe, 2016; E. T. Roberts, Haan, Dowd, & Aiello, 2010). In turn, research shows that inflammation is an important factor in the development and progression of aging-related diseases (Cohen, Granger, & Fuller-Thomson, 2015), including cardiovascular disease (Libby, 2002).

Findings from our study suggest that the investigation of religiosity, including church attendance and its association to health, is warranted and can have important interdisciplinary and clinical implications. Church attendance is central to the lives of many

people, particularly in the face of disadvantage, including bereavement (W. R. Miller & Thoresen, 2003). Thus, further research to identify and understand the specific ways in which church attendance influences physiology is needed. Whether it may be through psychological factors, social contingencies, or other aspects of religiosity, this information is essential to inform the development of interdisciplinary programs, interventions, and collaborations aimed to protect the health of vulnerable populations, particularly those facing adversity. Of note, the majority of bereaved individuals in this study reported not attending church; thus, identifying factors that influence church participation among bereaved individuals most at risk warrants further study.

Important strengths of our study include the inclusion of bereaved participants and their matched controls, as well as the assessment of a well-validated marker of herpes-virus latency. A limitation of our study is its relatively small sample size, which reduces the power to detect associations. For instance, it is possible that the lack of significance for the interaction between frequency of church attendance and bereavement status to predict herpes-virus latency may have been due to limited power given the small sample size. Future studies with larger samples should explore

Table 3
Summary of Adjusted and Unadjusted Regression Analyses Predicting CMV Antibody Titers

Variable	Unadjusted			Adjusted		
	B	SE	95% CI	B	SE	95% CI
Age	.073	.003	[-.004, .008]	.008	.004	[-.001, .016]
Gender	.056	.087	[-.127, .217]	.113	.091	[-.069, .295]
Minority status	.111	.085	[-.082, .258]	.125	.118	[-.110, .360]
Education	-.126	.015	[-.048, .012]	-.008	.018	[-.043, .027]
Weekly alcohol consumption	-.005	.007	[-.015, .014]	.004	.008	[-.013, .020]
Smoking status	-.096	.160	[-.460, .175]	-.013	.176	[-.364, .339]
Depression	.037	.004	[-.006, .008]	.004	.004	[-.004, .012]
BMI	.077	.007	[-.008, .018]	.002	.008	[-.013, .017]
Comorbidities	.224*	.038	[.005, .156]	.064	.040	[-.014, .143]
Church attendance	-.136	.082	[-.268, .059]	-.560*	.259	[-1.076, -.043]
Bereavement status	-.081	.081	[-.221, .100]	.011	.114	[-.215, .238]
Church attendance × bereavement status	-.240*	.102	[-.437, -.029]	.326*	.163	[.002, .651]
R ²				.181		
F(12, 84)				1.324		

* $p < .05$.

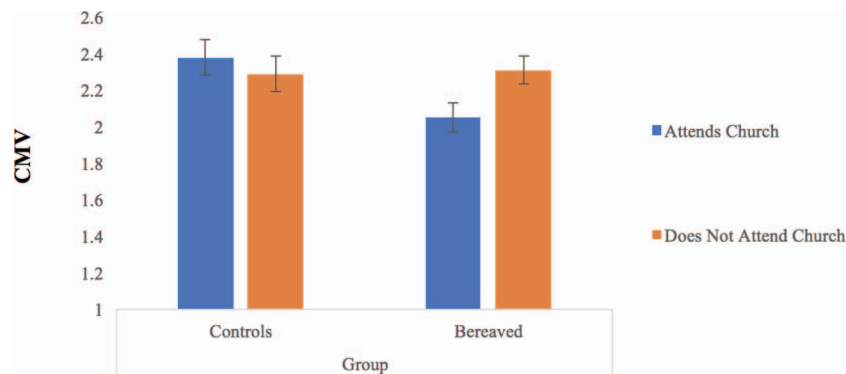


Figure 1. CMV antibody titer means and standard errors among bereaved and nonbereaved participants differing in church attendance. See the online article for the color version of this figure.

how frequency of church attendance may interact with bereavement status to predict herpes-virus latency. Nonetheless, despite our sample size, the primary results of this study identified a significant association between church attendance and a marker of herpes-virus latency. Another limitation of our study is that our sample is predominantly White and highly educated. Additional studies with more diverse samples are needed to determine the generalizability of our findings. Furthermore, assessment of church attendance was based on self-report and retrospective reporting, which may lead to biases and imprecise estimates of events (Brewin, Andrews, & Gotlib, 1993). Also, our study identified a positive association between church attendance and herpes-virus latency; however, we did not assess the specific aspects of church attendance that may be associated with less herpes-virus reactivation. Future studies should focus on identifying specific aspects of church attendance (e.g., social contingencies, religious experiences or beliefs) that may be associated with less herpes-virus reactivation. Our study is also limited by its cross-sectional design. As a result, causality cannot be inferred. However, pending prospective data, our study provides important insights on the role of church attendance as a potential protective factor in the physiological health of individuals, including those facing distressing life events such as bereavement.

Conclusion

In sum, our study demonstrated that church attendance is associated with less herpes-virus reactivation. While church attendance was associated with less herpes-virus reactivation among bereaved and nonbereaved individuals, the association between church attendance and CMV antibody titers differed by church attendance. In other words, although no differences in herpes-virus latency were observed among nonbereaved participants, bereaved individuals attending church were found to have less herpes-virus reactivation when compared to their bereaved counterparts that do not attend church. Future studies should focus on further understanding the pathways by which church attendance impacts CMV herpes-virus latency, and evaluate whether interdisciplinary interventions that incorporate religiosity may be useful to reduce risk for chronic illnesses after the death of a spouse.

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