

1-1-2003

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Bennett, Mary P.; Zeller, Janice M.; Rosenberg, Lisa; and McCann, Judith. (2003). The Effect of Mirthful Laughter on Stress and Natural Killer Cell Activity. *Alternative Therapies in Health and Medicine*, 9 (2), 38-45.

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THE EFFECT OF MIRTHFUL LAUGHTER ON STRESS AND NATURAL KILLER CELL ACTIVITY

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Context • A recent survey of rural Midwestern cancer patients revealed that humor was one of the most frequently used complementary therapies. Psychoneuroimmunology research suggests that, in addition to its established psychological benefits, humor may have physiological effects on immune functioning.

Objective • To determine the effect of laughter on self-reported stress and natural killer cell activity.

Design • Randomized, pre-post test with comparison group.

Setting • Indiana State University Sycamore Nursing Center, which is a nurse-managed community health clinic in a mid-sized, Midwestern city.

Participants • 33 healthy adult women.

Intervention • Experimental subjects viewed a humorous video while subjects in the distraction control group viewed a tourism video.

Main Outcome Measures • Self-reported stress and arousal (Stress Arousal Check List), mirthful laughter (Humor Response Scale), and immune function (chromium release natural killer [NK] cell cytotoxicity assay).

Results • Stress decreased for subjects in the humor group, compared with those in the distraction group ($U_{32}=215.5$; $P=.004$). Amount of mirthful laughter correlated with postintervention stress measures for persons in the humor group ($r_{16}=-.655$; $P=.004$). Subjects who scored greater than 25 on the humor response scale had increased immune

function postintervention ($t_{16}=2.52$ $P=.037$) and compared with the remaining participants ($t_{32}=32.1$; $P=.04$). Humor response scale scores correlated with changes in NK cell activity ($r_{16}=.744$; $P=0.01$).

Conclusion • Laughter may reduce stress and improve NK cell activity. As low NK cell activity is linked to decreased disease resistance and increased morbidity in persons with cancer and HIV disease, laughter may be a useful cognitive-behavioral intervention.

Over the past 20 years, psychoneuroimmunological (PNI) research has repeatedly documented that various types of stressors lead to interactions among the neurological, endocrine, and immune systems, which can decrease immune functioning and disease resistance.¹³ However, research is limited as to the interventions that effectively modify the effects of stress on immune function. Despite this lack of data, a number of interventions have been developed and marketed to the public with little or no documentation of their effectiveness or mechanisms of action.

According to a survey of rural Midwestern cancer patients, more than 87% of the respondents were currently using at least 1 complementary or alternative intervention in addition to mainstream medical care.⁴ The most common intervention cited was prayer, followed by humor. Fifty percent of the respondents reported already using humor-intervention, and an additional 13% stated that they would consider using the method.⁴ Women were more interested in use of humor than men in this survey study of local cancer patients.⁴ Consequently, we originally envisioned a study of the effects of humor in women with breast cancer.

The use of humor as an intervention has been emphasized in both consumer⁵⁻⁷ and professional publications.⁸⁻¹⁵ Unfortunately, despite the popularity of humor as a cognitive-behavioral intervention, there appears to be little scientific documentation to support the use of humor in this role. According to Fry, "as with most grass-roots movements, there is little laboratory, statistical, analytic, or otherwise scientific evidence to justify scientifically the huge degree of enthusiasm for uses of humor in this context."^{16(p119)} Several reports have demonstrated the use of humor to reduce stress, relieve pain, or as an adjuvant therapy to improve overall quality of life.¹⁷⁻²¹ According to these studies, laughter appears to improve mood, reduce psychologi-

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cal measures of stress, and reduce perception of pain. The mechanisms that underlie these effects may involve release of psychological tension, which could act as a buffer of stressful events on affect.

However, it is also possible that physiological factors may be involved. For example, laughter can lead to changes in heart rate, skin temperature, blood pressure, pulmonary ventilation, skeletal muscle activity, and brain activity, which may improve overall well-being.¹⁶

Laughter may improve immune function by blocking production of stress hormones, such as cortisol, and by increasing the release of immunoenhancers, such as beta-endorphin.^{22,25} However, data to support this aspect of humor therapy are scant. In fact, according to a published interview with Steven Locke, a psychoimmunologist at Harvard, "if there's anything newsworthy about this subject, it's how little research has been done, considering how widely accepted the ideas are."²⁶

According to PNI theory and some humor studies, humor may influence health through moderation of stress chemicals and/or immunoenhancement.^{27,34} A handful of studies have demonstrated that exposure to a humorous stimulus can result in immunoenhancement, as evidenced by increased levels of salivary immunoglobulin A (IgA).^{32,35-37} However, use of salivary IgA as a measure of immune function has been challenged by some investigators^{38,39} because of variations in individual salivary flow rate and the use of stimulated versus nonstimulated samples in some studies. In addition, the clinical significance of increased levels of salivary IgA is questionable. For this study, natural killer (NK) cell activity was used as the measure of immune function because NK cell activity assays provide some of the clearest and most replicable results in this type of research.^{1,40}

EFFECT OF LAUGHTER ON NK CELL ACTIVITY

According to earlier work,²⁷ subjects who laughed while viewing a humorous video had decreased levels of glucocorticoids. Because elevated glucocorticoid levels have been demonstrated to reduce NK cell activity,⁴¹ it appears plausible that laughter may influence NK cell activity. However, empirical evidence is scant and conflicting. To our knowledge, only 4 studies have examined the influence of a humorous stimulus on NK cell activity. The first study enrolled 22 women with breast cancer to determine the effects of viewing a both a humorous video and a distressing video.⁴² In a randomized crossover design, blood samples were taken before and after each video-viewing. The pretesting data for the second video served as an additional poststimulus test point for the first video. No significant changes were noted in NK cell numbers or activity at the end of either video. However, contrary to expectations, NK cell activity was significantly decreased 23 hours after viewing the humor video, and NK cell activity was significantly increased 23 hours after viewing the distressing film. The investigators suggested that this could be a rebound effect caused by a possible change in NK cell activity during the preceding 22 hours. Conversely, it could be related to anticipation of the film the subjects were about to view. Because data were not collected 23 hours after the second film, it was not possible to determine which of these factors may have been responsible for the potentially paradoxical findings. In addition, because this study involved running multiple group assays over a period of weeks, data on day-to-day NK cell activity assay vari-

ability would have helped to assess the reliability of the results.

In a study of 5 healthy male medical students, NK cell activity was significantly increased following a humorous video.⁴¹ For the experimental conditions, subjects viewed a 60-minute humorous video; control conditions were met by having the subjects sit quietly in the same room for the same length of time a few days later. Samples for NK cell activity were drawn at baseline and immediately after the movie or the quiet time. Because 2 separate assays were conducted for the experimental versus the control conditions, the between-groups data were subject to possible interassay variability. However, this would not affect the pre-post data from either condition, so the findings concerning NK cell activity could be considered valid using a strictly pre-post methodology.

In a third study, the effect of both humor and exam stress on T-cell subset percentages and NK cell activity was determined in 8 healthy male medical students.⁴³ The immune parameters were measured before and after an examination and before and after watching a humorous video. Contrary to findings from other studies of stress and immune function,⁴⁴⁻⁴⁶ there was no significant change in either of the immune parameters following the exam, and NK cell activity was decreased immediately following the humorous video.⁴³ Limitations of this study include the use of a small, all male sample and lack of a control group.

In the most recently reported study, the effect of exposure to a 60-minute humorous video on the activity of NK cells was examined in a controlled study of 12 healthy male medical students.³¹ Results showed that only members of the humor group had a significant increase in post-NK cell activity levels. Similar to the earlier study by this group, the sample was limited to a small number of male medical students.

Research on the effects of laughter on stress and immune function is still in the early phases. In addition, all previous work in this area, with the exception of 1 unpublished dissertation, was conducted using all male subjects. In light of these findings, a clinical study of the effects of humor and laughter in women with breast cancer was judged to be premature. Therefore, our study investigated the effect of laughter and sense of humor on stress and immune function in a sample of healthy women.

METHODS

Subjects

The study sample comprised 33 healthy women recruited from the rural Midwest using a variety of methods. The volunteers were prescreened for inclusion and exclusion criteria before being admitted into the study. Persons having symptoms of depression, taking immunosuppressant medications, having chronic illness, using antidepressants, taking herbal supplements, having sleeping difficulties, or having unexplained weight changes were excluded from the study. The use of extensive exclusion criteria was based on a thorough review of the literature, which indicated that several known factors affect immune function. Given that the sample size was to be relatively small, it was decided that the study design should control for as many immunoresponsive factors as possible.

Subjects were asked to state their preferences for 1 of 3 humor-

ous videos: *Bill Cosby, Himself*; *Tim Allen, Men are Pigs*; or *Robin Williams: Live at the Met*. This method followed the assumption that the subjects would pick the movie style that they found most humorous, and thus maximize the effect of the stimulus. The majority chose the Bill Cosby video. These subjects were scheduled into groups for the video-viewing phase. An insufficient number voiced a preference for either of the other films to put together a study group.

Subjects who met the study criteria were randomly assigned to experimental (humor) or control (distraction) groups. Subjects remained blind to their group assignment until the humor or distraction video began. The researcher conducting the laboratory assays also remained blind to group assignment during the data collection phase. Because of limitations on the number of laboratory samples that could be analyzed at one time, data collection was conducted on 4 different days spread over a 2-week period.

Instruments

The Life Experience Survey (LES),⁴⁷ was used to determine possible effects from exposure to recent negative life event stress. The Stress Arousal Check List (SACL) was used to determine both levels of stress and arousal.⁴⁸ The Center for Epidemiologic Studies Depressed Mood Scale (CES-D) was used to screen subjects for signs of depression.⁴⁹ Both the Situational Humor Response Questionnaire (SHRQ)³³ and the Multidimensional Humor Response Scale (MHRS) were used to document sense of humor.⁵⁰ All of the psychological instruments used had previously established reliability and validity.

To document mirthful laughter, the Humor Response Scale (HRS) was developed for use in this study.⁵¹ The HRS is an observer rating scale that allows subject humor responses to be recorded and quantified in a systematic manner.

Finally, to measure NK cell activity, a modification of the standard 4-hour radiolabeled chromium (51Cr) release assay was used.⁵² Interassay correlations for the daily laboratory controls ranged from .979 to .999, with a mean correlation coefficient of .991, indicating highly reliable NK cell activity assay results over the 4 days of data collection. NK cell activity was reported using lytic units (LU).⁵³

RESULTS

Despite randomization, data analysis revealed significant differences between the subjects assigned to the distraction group versus the humor group on the following measures: weight, self-reported stress arousal, and NK cell activity. At the time the baseline measures were carried out, the subjects were aware that they were in a controlled study of the effect of humor on stress and immune function, but remained blinded to their group assignment. Because of significant baseline differences in these parameters, change scores and *t* tests were used to determine between group differences in the relevant factors following the videos.

Pre-Post Video Results

Mirthful Laughter and NK Cell Activity

Paired *t* tests revealed no statistically significant difference in NK cell activity change between subjects who viewed the humor video and subjects who viewed the distraction video ($t=1.52$; $P=.138$).

Therefore, simply viewing a video or diurnal changes did not lead to statistically different post-NK cell activity.

However, when mirthful laughter was taken into consideration, using the HRS, a different picture emerged. As shown in Figure 1, scores on the HRS significantly correlated with change in NK cell activity for subjects in the humor group ($r_{16}=.774$; $P=.001$). Scores on the HRS also tended to correlate with post-NK cell activity for persons in the humor group ($r_{16}=.45$; $P=.07$). As shown in Figure 2, subjects who scored a 25 or above on the HRS, which indicated mirthful laughter responses, had significantly increased post-NK cell activity, compared with their baseline NK cell activity (mean increase of 15.77 LU, $t_8=2.52$; $P=.037$). In addition, subjects who scored 25 or above on the HRS had significantly greater positive change in NK cell activity compared with other subjects in the humor group ($t_{16}=4.85$; $P=.002$), and compared with all other participants in this study ($t_{32}=2.1$; $P=.04$).

Persons who watched the humorous video and did not display mirthful laughter had significantly decreased NK cell activity at posttesting ($t_7=4.4$; $P=.003$). These data reinforce that it is not simply exposure to a humorous stimulus that improved immune function, but the response elicited by the video. The experience of participants who viewed a supposedly humorous video without finding it funny, while in the presence of others who were amused, perhaps triggered an affectively negative response. This may have accounted for the decreased NK cell activity noted in these subjects.

Laughter and Stress Arousal

Although stress scores decreased somewhat for persons in both groups, according to the Mann-Whitney U test, stress scores decreased more for persons in the humor group, compared with persons in the distraction group ($U_{32}=215.5$; $P=.004$). In addition, as shown in Figure 3, persons in the humor group who laughed more reported lower post stress levels ($r_{16}=-.655$; $P=.004$). However, the change in stress scores appeared to be independent of the change in NK cell activity observed in persons in the humor group ($r_{32}=.21$; $P=.262$).

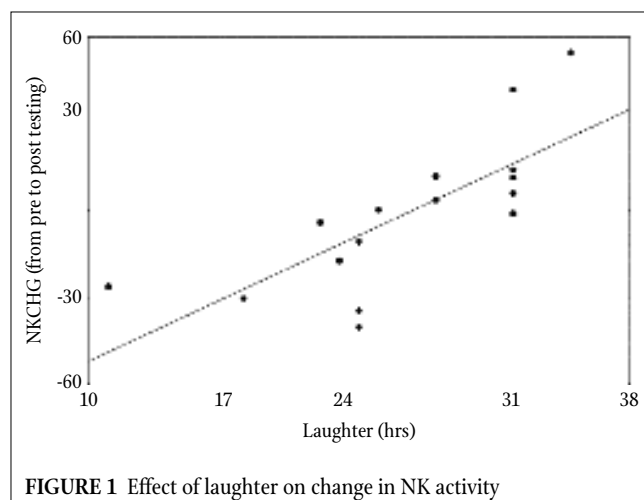


FIGURE 1 Effect of laughter on change in NK activity

Interestingly, change in stress scores tended to correlate with change in NK cell activity for members of the distraction group ($r_{15} = -.29$; $P = .081$). Decreased stress levels appeared to be related to positive change in NK cell activity, even though the distraction group as a whole did not have significantly decreased stress following the videos. For some people, taking the afternoon off work and watching a tour video was sufficient to decrease their stress levels and increase their NK cell activity, but these changes were not statistically significant.

Student's *t* test indicated that arousal decreased significantly for persons in the distraction group, compared with those in the humor group ($t_{16} = -2.82$; $P = .008$). Subjects in the distraction group reported greater agreement with feelings of sleepiness and drowsiness following the video tours. This decrease in arousal was not correlated with change in NK cell activity ($r_{15} = -.054$; $P = .84$) for members of the distraction group (Figure 2).

Post Intervention Correlations

Pre-NK Cell Activity Levels and NK Change:

Overall, a negative correlation was found between pre-NK cell activity levels and NK cell activity change ($r_{32} = -.465$; $P = .007$) for subjects in both groups, indicating that persons who had higher levels of NK cell activity at the start of the study had less positive NK cell activity change during the intervention. This could be due to a ceiling effect, similar to that noted in salivary IgA levels by Dillon and colleagues³⁵; persons who start with relatively high NK cell activity levels may not show much improvement. It could also be related to regression towards the mean, with persons having lower pre-NK cell activity scores having increased post-NK cell activity levels, and persons having higher pre-NK cell activity scores ending with decreased post-NK cell activity scores. This finding is noteworthy because, despite random assignment, persons in the humor group started with higher levels of pre-NK cell activity than persons assigned to the distraction group. This would lead us to assume that persons in the humor group should, therefore, have had less positive NK cell activity change, compared with persons in the distraction group. Yet following the videos, persons in the humor group did not have significantly different NK cell activity change than persons in the distraction group. Moreover, as noted above, persons who displayed mirthful laughter actually had higher levels of positive NK cell activity change than persons in the humor group who did not laugh and higher than the rest of the study participants. In univariate models, pre-NK cell activity levels explained 29% of the variance in NK cell activity change scores in the humor group ($r_{16} = .54$; $P = .025$) and HRS scores explained 55% of the variance in NK cell activity change scores ($r_{16} = .74$; $P = .001$). In a multivariate model controlling for pre-NK cell activity levels, HRS scores explained 67% of the variance in NK cell activity change scores in the humor group ($R^2 = .669$; $P < .005$). These data suggest that mirthful laughter can act to improve NK cell activity, even for persons who already have higher levels of NK cell activity.

Stress Levels and NK Cell Activity Change

The only other variables that correlated with change in NK cell activity scores for members of both groups were pre and post stress levels. Both prestress ($r_{32} = -.37$; $P = .034$) and poststress ($r(32) = -.43$;

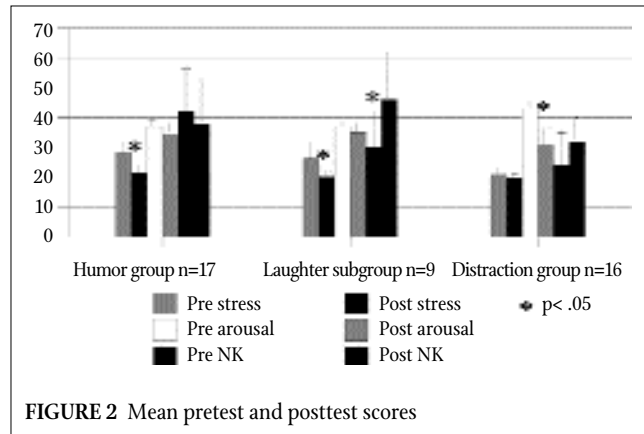


FIGURE 2 Mean pretest and posttest scores

$P = .012$) levels correlated significantly with NK cell activity change, with persons having higher stress levels also demonstrating less improvement or a decrease in NK cell activity following the videos.

COMMENT

Exposure to a Humorous Video, Mirthful Laughter, and Immune Function

As a whole, NK cell activity change scores were not significantly different for participants who viewed a humorous video compared with persons in the distraction control group. Therefore, exposure to a humorous video alone did not significantly affect immune function. Although scores on the HRS tended to correlate with post-NK cell activity for all persons in the humor group, only persons who displayed overt laughter (score of 25 or above on the HRS) had significant improvement in their posttest NK cell activity and greater positive change in NK cell activity when compared with the remainder of the study participants. These data seem to indicate that a subject's behavioral/physiological response was the operative factor leading to improved NK cell activity, not merely exposure to a humorous video.

A negative correlation was found between pre-NK cell activity levels and NK cell activity change for all subjects in this study, possibly due to the previously described ceiling effect³⁵ or regression towards

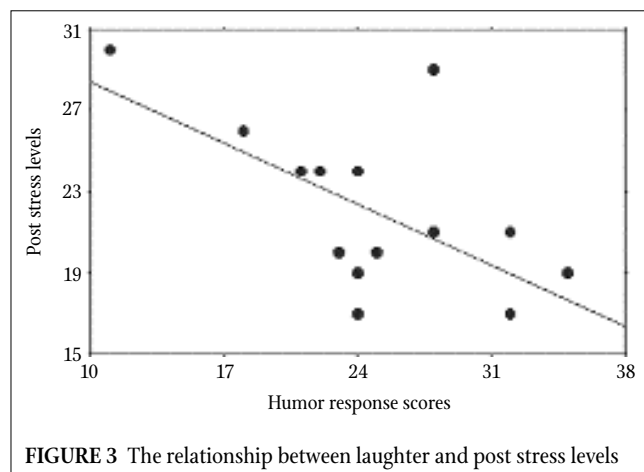


FIGURE 3 The relationship between laughter and post stress levels

the mean. Persons who started out with relatively high NK cell activity levels tended to have decreased NK cell activity following the videos. As noted before, despite random assignment, persons in the humor group started with higher levels of pre-NK cell activity than persons assigned to the distraction group. If the effects of pre-NK cell activity levels on NK cell activity change were the major factor in this study, persons in the humor group should have had less positive NK cell activity change overall, compared with persons in the distraction group. Yet following the videos, persons in the humor group who displayed mirthful laughter had higher levels of positive NK cell activity change than all other persons in the study. As demonstrated by the multiple regression results, the effect of mirthful laughter was greater than the effect of pre-NK cell activity levels on NK cell activity change for persons in the humor group. These findings of improved NK cell activity following a brief period of laughter in women add further support to similar findings by Berk and colleagues,^{31,41} who found that men exposed to a humorous video had significant increases in NK cell activity. Taken together with the results of this study, there is evidence to support that a brief period of laughter can lead to statistically significant increases in NK cell activity in both men and women.

Use of Humor as a Complementary Intervention to Reduce Stress

The results from this study indicate that exposure to a humorous stimulus can significantly decrease self-reported stress levels. However, the results should be viewed with some caution. Unfortunately, despite randomization, persons in the humor group reported higher levels of preintervention stress than persons in the distraction group, and overall, persons with higher preintervention stress scores had greater decreases in stress following the videos. This could have influenced the change in stress findings for the humor group. However, increased mirthful laughter, as measured on the HRS, correlated with decreased stress scores following the humorous video (Figure 3). As mirthful laughter was not significantly correlated with preintervention stress, or change in stress scores, the findings concerning laughter and stress levels were probably not affected by the between-group differences in preintervention stress.

The reduction of stress following mirthful laughter found in this study is supported by previous findings. A study of dental patients and stress found that patients who joked and laughed before dental surgery reported less psychological stress.¹⁹ In another study, subjects who were exposed to a brief period of mirthful laughter had significantly reduced anxiety compared with their pretest scores.²¹ Finally, a study of the effects of mirthful laughter on physiological measures of stress found that cortisol decreased more rapidly in persons who laughed compared with persons in the control group.^{29,31} As evidenced by this study, it appears that a brief period of mirthful laughter may reduce psychological and physiological measures of stress for an indeterminate amount of time.

Stress and Immune Function

Higher levels of both preintervention stress and postintervention stress correlated with less positive change in NK cell activity, no matter which group the subjects were in. Subjects who were very

stressed may not have been able to respond positively to either the distraction or humorous video. However, preintervention stress did not significantly correlate with HRS scores for persons in the humor group, so it cannot be assumed that higher stress levels led to decreased mirthful laughter in these subjects. Subjects with higher stress levels may have had higher levels of cortisol and other stress hormones, and several studies have documented the negative relationship between cortisol levels and NK cell activity.^{54,59} Therefore, baseline levels of stress hormones may have affected the subjects' physiological response to the videos, but as stress hormones were not measured in this study, this mechanism is speculative.

Members of the humor group had significantly decreased stress levels following the video, and those who displayed mirthful laughter also had significantly higher postintervention NK cell activity. However, it cannot be assumed that change in stress was related to the improved NK cell activity for these subjects, because a change in stress scores did not significantly correlate with change in NK cell activity for subjects in the humor group. Therefore, when using a humorous video as an intervention, one cannot assume that change in stress scores following the video indicates that the subjects also have improved immune function. In this study, only those subjects with a score of 25 or above on the HRS had significantly improved NK cell activity, and humor response scores were the strongest predictor of NK cell activity change in the humor group. It appears that the amount of mirthful laughter was the major contributing factor for the increased immune function seen in these subjects, rather than decreased stress levels.

Strengths, Limitations, and Recommendations

Strong points in this study include the use of an experimental design, the use of control samples to document NK cell activity assay reliability during each day of the study, and the primary researcher remaining blind to subject assignment.

The sample size was limited by practical considerations of time, lab space, and funding. An additional limitation was the between-group differences at baseline that persisted despite randomization to groups. A larger sample may have avoided this problem and increased the power of some of the correlations found in this study.

The Humor Response Scale (HRS) was a new instrument designed for this study. Although it was useful in quantifying laughter in this study, the results were dependent on the real-time observations of 1 person. Recommendations for future work with this instrument include videotaping the subjects so as to have a record of the subject responses, and rate the subject responses from the video. This would allow the researcher to have more time to rate individual subject responses, and provide a permanent copy of the subject responses. In addition, only persons in the humor group were observed in this study so it was not possible to control for the possible confound of mirthful laughter in the control group. Future studies would benefit by having information on how subjects in both groups perceive the videos.

While some subjects who were exposed to the humorous video did not respond with mirthful laughter and did not have increased NK cell activity, this might not really represent the normal humor responses of these individuals. It must be acknowledged that a controlled research project, which involves monitoring and invasive testing, can never adequately duplicate the humor responses that may take place

when a person is naturally exposed to a humorous stimulus in real life. Watching a humorous video with friends and family in a familiar setting would probably produce more laughter than watching the same video with a group of strangers in an invasive experimental setting. A more natural setting for movie viewing, such as a darkened movie theater, may have helped stimulate a more natural laughter response. Unfortunately, this would have made observation of humor responses very difficult. Having a larger number of people watching the humorous video at the same time could have led to increased laughter responses.⁶⁰ Still, the use of small video viewing groups was necessary because of the need to limit numbers of NK cell samples collected at one time. Therefore, while the humor methodology used in this study probably limited a natural humor response, these limitations were necessary for data collection. While this limitation does not affect the internal validity of this study, it does serve to make the intervention less effective than it might have been in a more natural setting.

The use of tour videos as a neutral control stimulus was somewhat problematic. Anecdotal comments from subjects in the distraction group suggest that while some persons found the distraction videos boring, others found them relaxing, enjoyable, and even funny. White and Camarena²¹ reported similar problems with their study of laughter and stress. They used a National Geographic video as a neutral stimulus, and found that even subjects in the control group had decreased stress following the control video. It appears that almost nothing can be a completely neutral stimulus in this type of research.

CONCLUSION

Humor appeals to both non-healthcare professionals and healthcare professionals alike. It seems almost intuitive that laughter can make one feel better, and therefore might help one recover. Humor workshops are being produced and marketed around the country, often in conjunction with a stress reduction or self-healing focus.^{7,15,23,61} Articles in the consumer literature and even some professional literature suggest that the effects of humor on immune function have been documented by empirical research and are therefore commonly accepted. The work of Cousins,⁶² Fry,^{9,16,63-66} Berk,^{28,31,41,67} or the field of PNI research in general are frequently cited as supporting the role of humor in healing. However, as the review of research literature noted, few studies adequately document the effects of laughter on the immune system, and no controlled studies document the effect of laughter on health outcomes. Therefore, more research is needed before broad claims for the effect of humor on healing can be made.

The results of this study support a number of basic PNI assumptions. The negative effects of stress and arousal upon immune function were supported, as persons with increased arousal levels had lower levels of NK cell activity at baseline, and persons with increased stress levels had less positive NK cell activity change following the interventions. In addition, the beneficial effects of exposure to a humorous stimulus on self-reported stress were supported, as persons viewing the humorous video reported significantly decreased stress following the video, compared with the participants in the distraction group. Taken with previous studies that document the effect of laughter on stress, it appears that a brief period of mirthful laughter may be a quick and effective method to reduce stress levels. However, more

research is needed to determine the duration of the stress reduction effect and if the effect is cumulative over time.

Another finding of this study was the apparent relationship between mirthful laughter and improved NK cell activity. Because of the role of NK cells in viral illness and various types of cancer, the ability to significantly increase NK cell activity in a brief period of time using a noninvasive method could be clinically important. The use of humor to stimulate laughter could be an effective complementary therapy to decrease stress and improve NK cell activity in persons with viral illness or cancer.

Finally, because this study was limited to healthy women, the results should not be generalized to other populations. More research is needed to determine if laughter would have the same effect on a person who has an acute or chronic illness. In addition, the results of this study only apply to the time immediately following a brief period of laughter. More research is needed to determine the effects of a longer period of laughter, how long the effects last, and whether the effects are cumulative.

Acknowledgement

Funding for this study was provided by Lambda Sigma Chapter of Sigma Theta Tau International and Indiana State University Research Committee. Lab space and equipment were provided by Indiana University—Terre Haute Center for Medical Education.

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