

The Efficacy of Laughter Yoga on IT Professionals to Overcome Professional Stress

A joint research project of
LAUGHTER YOGA INTERNATIONAL
& **SWAMI VIVEKANANDA YOGA**
ANUSANDHANA SAMSTHANA

Principal Investigators

Dr. H. R. Nagendra , ME, PhD,

President - VYASA, International
Bangalore

Dr. M. S. Chaya, MSc, PhD

Consultant – VYASA International
& Faculty Assistant Prof - SVYASA Bangalore

Co-investigators

Dr. R. Nagarathna MD, FRCP

Consultant physician - VYASA International
& Dean of Life Sciences - SVYASA Bangalore

Dr. Madan Kataria, MBBS

Founder & President - Laughter Yoga International
Founder & President - Laughter Clubs International
Mumbai India

Dr. Manjunath, M. K.

Assistant Professor - SVYASA Bangalore

Raghavendra Rao M.

Assistant Professor - SVYASA Bangalore

Published 29 September 2007 by
Laughter Yoga International
1A Denzil, 3rd Crossroad, Lokhandwala Complex
Andheri West, Mumbai 40053 India
Tel +91 22 2631 6426
info@laughteryoga.org www.laughteryoga.org

Contents

1. Introduction	7
1.1. Laughter - Different Definitions	8
1.2. Concept of Laughter	8
1.3. Types of Laughter	8
1.4. Benefits of Laughter	9
1.5. Yoga	9
1.6. Why Combine Laughter and Yoga	9
2. Review of Literature	10
2.1. Effect of Laughter on Physiological Parameters	10
2.2. Effect of Laughter on Psychological Parameters	12
2.3. Effect of Laughter on Immunological Parameters	13
2.4. Effect of Yoga Relaxation	15
2.5. Summary of literature	16
2.6. What is Missing?	17
3. Aims and Objectives	18
4. Subjects and Methods	18
4.1. Subjects	18
4.1.1. Selection of Subjects	18
4.1.2. Inclusion Criteria	18
4.1.3. Exclusion Criteria	18
4.1.4. Reasons for Selecting IT Professionals	19
4.1.5. Introductory Lecture and Registration	19
4.2. Methods	19
4.3. Details of Parameters, Equipment, Calibration, and Validation	19
4.3.1. Four Channel Polygraph	19
4.3.2. Blood Pressure Apparatus	20
4.3.3. Elisa kits	20
4.3.4. Questionnaires	24
4.3.5. Flow Chart	25
4.4. Demographic Data Collection	25
4.5. Design and Procedure for Assessments	25
4.5.1. Polygraph	26
4.5.2. Blood Pressure	26
4.5.3. Cortisol and Secretory IgA	26
4.5.4. Questionnaire	27
4.6. Intervention	27
4.7. Data Analysis: Statistical Procedure	28
4.8. Screening, Enrolment and Randomization	28

5. Results.....	29
5.1. Polygraph Data	29
5.2. Blood Pressure	32
5.3. Cortisols	33
5.4. Secretory IgA.....	34
5.5. Questionnaires	34
5.5.1 PANAS	34
5.5.2 PSS.....	36
5.5.3 TAS	37
6. Discussions.....	39
6.1. Polygraph Data	39
6.2. Blood Pressure	40
6.3. Cortisols	40
6.4. Secretory IgA.....	41
6.5. Questionnaire	41
6.5.1. PANAS.....	41
6.5.2. PSS	42
6.5.3. TAS	43
7. Summary and Conclusions.....	43
8. References	45
9. Photos of LY Research and Sessions.....	48

List of Tables

Table 5.1.	The Group Mean Values \pm Standard Deviations (SD) of the Autonomic and Respiratory Variables for Laughter Yoga and Waitlisted Control Groups.
Table 5.2.	The Group Mean Values \pm Standard Deviations of the Laughter Yoga and Waitlisted Control Group (Blood Pressure)
Table 5.3.	Cortisols – The Group Mean Values \pm Standard Deviations for Laughter Yoga and Waitlisted Control Groups.
Table 5.4.	Secretory IgA– The Group Mean Values \pm Standard Deviations for Laughter Yoga and Waitlisted Control Groups.
Table 5.5.1.	PANAS - The Group Mean Values \pm Standard Deviations for Laughter Yoga and Waitlisted Control Group.
Table 5.5.2.	Perceived Stress Scale (PSS): The Group Mean Values \pm Standard Deviations for Laughter Yoga and Waitlisted Control Group.
Table 5.5.3.	Toronto Alexithymia Scale (TAS) – The Group Mean Values \pm Standard Deviations for Laughter Yoga and Waitlisted Control Groups.

List of Figures

Figure 5.1.2.	Heart Rate Comparison Within Group
Figure 5.1.3.	Breath Rate Comparison within LY group and WLC group
Figure 5.1.4.	Comparison of Pre-post Data of LY and WLC Groups
Figure 5.2.	Comparison of Pre-post BP of LY and WLC Groups
Figure 5.3.	Comparison of Cortisol LY and WLC Groups
Figure 5.5.1A.	PANAS Comparison Of Scores Between Groups
Figure 5.5.1A.	Comparison of Pre-post PANAS Scores of LY and WLC Groups
Figure 5.5.1B.	Comparison of PANAS within Group of LY and WLC Group
Figure 5.5.2A.	Comparison of Pre-post PSS scores of LY and WLC Groups
Figure 5.5.2A.	Comparison of Pre-post PSS scores of LY and WLC Groups
Figure 5.5.3A.	Comparison of TAS of LY and WLC Groups
Figure 5.5.3B.	Comparison of TAS within the LY and WLC Groups

1. Introduction

The age old saying, 'Laughter is the best medicine' holds true as laughter is undoubtedly a positive emotion which brings about a state of well being. Laughter is favorable not only for generating health and happiness but also for improving relationships. People laugh to express joy, happiness, friendliness and acceptance.

Laughter is an expression of happiness within – though in the succeeding chapters we examine whether this is the cause or the effect of happiness. Laughter is an essential human phenomenon. Smiling in response to pleasant physical conditions occurs in early development, usually in the first month of life. As a motor reflex, laughter is usually present by the time a child is 4 months old. By the age of eighteen months, a child smiles once every six minutes, and by four years of age, the rate increases to one smile every one and one-third minutes. The ratio of laughs to smiles increases from one laugh to every ten smiles at eighteen months to one every three smile at four years. The individual differences in the rate of both laughing and smiling become greater as the children grow older. (Stearns, 1972) The instinctual development of smiling and laughing occurs very early in life suggesting a high level of importance.

Humor and Laughter are important part of our lives bringing in a lot of physical, psychological and social benefits. The positive effects of humor and laughter are gaining importance in modern society wherein people are racing against time, bound by innumerable tensions, grim faces and stiff necks. They have forgotten to laugh.

The goal of every human being is to be happy as it is a part of our survival instinct. Since the dawn of mankind we are programmed to strive for happiness and achieve a state of infinite and permanent bliss. Ancient yogis and spiritual masters sought ways and means to achieve the same and developed a methodology through the four streams of Yoga – Jnana, Bhakti, Raja and Karma (Nagendra H. R., 1998, 1999 2005).

While stress reduction is the urgent need of today's modern age of science and technology, promotion of positive health and a happy way of life is also at the core of human challenges. To build ideal social orders we have to not only prevent diseases and ill health, provide facilities for holistic treatment techniques to deal with diseases but also promote positive health. Health is a state of well being at the physical, mental, social and spiritual levels as defined by the WORLD HEALTH ORGANISATION. Such ideal social orders should also incorporate means to promote happiness and bliss, harmony and peace in society. Suitable rituals, social customs and traditions should to be promoted which are congenial for such developments.

It is therefore extremely important to discover new methods to promote lasting happiness in society. Laughter Yoga has made a very profound impact as one such attempt. The concept is fast sweeping the world and gaining tremendous popularity. Thousands of laughter clubs are among health care providers to the general public. There are many articles on the health enhancing effect of Laughter and humor in the media, in scientific journals, workshops, and in lectures promoting the health benefits of humor and laughter.

Dr. Kataria, pioneer of Laughter Yoga, in his unique way combined laughter exercises with yogic breathing (Pranayama). The concept involves stimulations through laughter and relaxation through Yoga. It is believed it works better than mere laughter. According to Dr. Kataria this 'unconditional' laughter does not rely on any jokes, humor or comedy. One laughs not because something is funny but just for the sake of laughing. What starts as an exercise regime actually leads to real and contagious laughter with the help of group dynamics and eye contact. It is laughing for the sake of laughing. The whole concept is based on the fact that fake laughter has the same physiological and psychological benefits

as real laughter. So 'fake it till you make it' is the motto of all laughter clubs around the world.

1.1. Laughter -Different Definitions

The physiological definition describes laughter as "a successive rhythmic spasmodic expiration with open glottis and vibration of the vocal folds 'or' as a series of spasmodic and partly involuntary expirations with inarticulate vocalizations. . ." These dictionary definitions emphasize a rhythmic and spasmodic expiration. While this is true, the motivations for it and its aftermath are much further reaching.

Definitions of **laughter** on the Web: laugh: the sound of laughing.

- The activity of laughing; the manifestation of joy or mirth or scorn; "he enjoyed the laughter of the crowd." wordnet.princeton.edu/perl/webwn
- Laughter is the biological reaction of humans to moments or occasions of humor: an outward expression of amusement. Laughter is sub-categorized into various groupings depending upon the extent and pitch of the laughter: giggles, clicks (which can be almost silent), chortles, chuckles, hoots, cackles, sniggers and guffaws are all types of laughter. Smiling is a mild silent form of laughing. en.wikipedia.org/wiki/Laughter
- A virtue is a soul-expanding delight that shortens time. Every laugh you manage cuts short the miles and hours and days of drudgery. You can ascend the physical body on laughter. Let a sense of humor give you balance, perspective, poise and patience. Don't take yourself too seriously. Learn to laugh at yourself and go upward. miriams-well.org/Glossary/

1.2. Concept of Laughter

Laughter is the expression of humorous experience, involving certain respiratory/vocal/behavioral pattern that also has distinctive psycho-physiological correlates (Provine & Yong, 1991). A pleasant emotional status is associated with humor and laughter (Ruch, 1993). Cognitively, humor involves the perception of incongruity or paradox in a playful context (Forabosco, 1992). Socially, humor and laughter play an important role in interpersonal relationships (communication and attraction (Murstein & Brust, 1985) and a sense of humor plays an important role in social competence (Bell, McGhee, & Duffey, 1986; Masten, 1986). Laughter involves physiological, cognitive, emotional, behavioral, psychological, and social aspects (Martin, 2000).

1.3 Types of Laughter

- Laughter as a reflex reaction to external impulses such as tickling.
- Psychosomatic response prompted by a specific stimulus. This is an informative stimulus such as a word, sentence, gesture, action, recollection, situation, etc. This requires the involvement of perception, learning, and memory. This reaction may or may not be terminated voluntarily.
- The third again is a psychosomatic response, but is differentiated because it is not caused by an adequate informative stimulus and can be terminated voluntarily.

A commonly held belief is that mirthful laughter and humor result in positive physical, psychological, and social fitness.

1.4. Benefits of Laughter

The benefits of laughter in providing a sense of positive well being are evident. Many scientific studies have shown the beneficial effects of laughter.

In 1976 Norman Cousins published an article in the New England Journal of Medicine - "Anatomy of illness". Cousins claimed that 10 minutes of hearty laughter had a reliable analgesic effect, providing two hours of pain-free sleep (pain from ankylosing spondylitis). It was not certain if the reduction of pain was due to Laughter or massive dose of vitamin C taken together. The beneficial effect of vigorous laughter is attributed to reduced muscle tension, increased oxygenation of blood, exercising of the heart muscles, endorphin production, and so on (Fry W F, 1977, 1994). But studies by Kataria et al. 1999 show that laughter need not originate from humor (e .g. forced laughter) but still have beneficial effects on health. In their study they have used yogic laughter exercises.

Zand, Spreen, & LaVallel (1999) in their book on complimentary approaches to medicine, describe the benefits of physiological and psychological laughter on the body and the mind. The research confirms that laughter be used for treatment of depression as it increases the activity of defensive immune cells, T –cells, and boosts the activity of antibodies that are a barrier to contracting infections.

During stress, the adrenal gland releases corticosteroids that are converted to cortisol in the bloodstream. Cortisol, which increases with stress response, inhibits mechanisms such as IL-2 gene expression and proliferation of lymphocytes in vitro and in vivo. (Berk, 1988) Positive emotional activities such as mirthful laughter have been suggested to reduce the classical stress response. A moderating effect like humor would lessen sympathetic stimulation due to stress and as a result, might lessen the impairment of the immune system.

1.5 Yoga

Yoga is a way of life. It is being used by millions of people all over the world and its positive effects are well documented. It is used extensively for stress management (Nagendra et al. 1977). The therapeutic effects of Yoga are also discussed by Nagarathna ((Nagarathna et al. 2000).

The effects of Yoga relaxation techniques show that it leads to hypo-metabolic states (Telles S. et al. 2000, Wallace et.al.1975, Chaya et al. 2006.) resulting in reduced sympathetic activity thereby reduction of stress.

1.6. Why combine Laughter and Yoga

Laughter is basically stimulation and physiological arousal and when combined with Yoga practice it leads to a hypo-metabolic state (Chaya et al. 2006). Relaxation techniques also lead to reduction of metabolic rate and sympathetic activity thereby reducing stress. Dr. Kataria has combined the best methods of Yoga with laughter to bring about improved health and happiness at physical, mental, emotional and spiritual levels. Laughter Yoga as envisaged by Dr. Kataria is bursts of laughter followed by certain deep breathing techniques and Yoga relaxation to get maximum benefit out of both laughter and Yoga in a short period of time. It is unique in its features as it easy to practice, safe and economical. Any person can do it anytime irrespective of age, gender, health etc.

So far clinical studies used humor to elicit laughter and concluded that exposure to any form of comedy or humor brings about positive changes in the immune system and physiological and psychological parameters. Although these studies show

positive changes, the findings are inconsistent as most of them have methodological problems.

In fact, the first ever study was conducted by Laughter Yoga on randomly selected IT professionals to test the efficacy of laughter in reducing stress in the workplace. A similar study in the US further reaffirms the findings.

2. Review of Literature

2.1. Effect of Laughter on Physiological Parameters

Norman Cousins' accounts of the therapeutic effects of laughter during his treatment and recovery from Ankylosing spondylitis added to the vast reservoir of anecdotal claims regarding the health benefits of a positive emotional state. (Cousins, 1989 as cited in Wooten, 1996) Cousins, as a result of his experience, formed a humor research task force in order to pursue studies of this connection. As a result of this renewed interest, a variety of experiments have been performed to examine the physiological changes and ramifications that result from mirthful laughter. New findings for increased popularity of humor/laughter and health were published in 1976 of Norman Cousins' article *Anatomy of an illness* in the New England Journal of Medicine. The story of how Cousins recovered from Ankylosing spondylitis, a progressive and painful rheumatoid disease involving inflammation of the spine, through laughter (and massive doses of vitamin C) has become part of recent history. Cousins claimed that 10 minutes of hearty laughter had a reliable analgesic effect, providing two hours of pain-free sleep. In addition, he reported that episodes of laughter reliably resulted in reductions in the sedimentation rate; the rate at which red blood cells descend in a test tube, which is a measure of inflammation. These observations have given rise in particular to the idea that laughter reduces pain, perhaps by stimulating the production of endogenous opioids such as beta-endorphin, and also enhances immune system functioning. Although the case of Cousins is widely cited as evidence for the health benefits of laughter, it is of course only anecdotal and suggestive at best.

The physiological changes such as cardiovascular, musculoskeletal, endocrine and immunological association with laughter can have good beneficial effect on health. (W. Fry 1994). Laughter without humor can also have beneficial effects (Kataria, et al. 1999).

Fried et al. (1998) found that electrical stimulations in the anterior part of the human supplementary motor area (SMA), located in the cortex of the left frontal lobe, stimulated mirthful laughter.

Further research explored the effects of laughter on muscle tone, respiration, and the cardiovascular system. The initial effects of laughter are stimulatory. This includes an elevated pulse, heart rate, and blood pressure. Laughter also increases respiratory rate, ventilation, and accelerates the change of residual air. Cessation is followed by a brief relaxation phase. Muscles involved in laughter undergo slight contraction while those not involved are flaccid. Muscle tone in the latter was found to diminish during laughter (Paskind, 1932). This relaxation helps ease muscle tension.

Laughter increases ventilation and aids in clearing mucus plugs. It also accelerates the exchange of residual air, increasing blood oxygen levels (Fry (1992).

Study done by Nakajima et al. (1999), has shown that mirthful laughter helps improve symptoms in rheumatoid arthritis patients. They found significant reduction of serum IL-6 levels in patients group exposed to mirthful laughter.

Hayasahi et al. (2003) have elucidated the inhibitory effect of laughter on the increase in postprandial blood glucose and glucose utilization by the muscles.

As stated earlier Norman Cousins used laughter therapy as an analgesic. Nevo (1993) et al. in their study on pain tolerance used Cold pressor task during treatment. It was found that the funniness ratings of the film significantly correlated with pain tolerance duration ($r = 0.38$) among participants in the humorous film condition, although this finding may be due to greater pain tolerance resulting from greater enjoyment of the film.

Cogan et al. (1987) in their studies using discomfort threshold with ischemic pain induced by a blood pressure cuff, found that the thresholds for laughter and relaxation conditions were significantly higher than those for the dull narrative and no treatment conditions, suggesting possible analgesic effects of laughter equivalent to the effects of muscle relaxation.

White and Camarena (1989) conducted a 6-week intervention study to examine the effects of a laughter intervention on diastolic (DBP) and systolic (SBP) blood pressure and heart rate (HR). The participants were 65 female and 28 male healthy volunteers who were randomly assigned to a laughter treatment group, a relaxation-training group and a health-education control group. The laughter intervention consisted of a variety of laughter induction exercises including watching humorous films. Each group met for 1 1/2 hours each week, and heart rate and blood pressure measures were obtained before and after each session. Frequency of laughter was not assessed although the authors noted that laughter occurred in each laughter group session. The results showed no significant pre-post session changes in DBP, SBP, or HR among participants in the laughter group and no differences between the laughter and health-education control groups. In contrast, the relaxation training group showed significantly lower post-session HR and SBP in comparison with the other groups. Thus, this study did not support the hypothesis that a laughter intervention would result in lower levels of heart rate and blood pressure.

Lefcourt et al. (1997) employed a laboratory stressor paradigm to examine possible stress-moderating effects of self-reported humor on blood pressure. They had 60 male and 49 female participants engage in five mildly stressful laboratory tasks. They measured their blood pressure before and immediately after, and three minutes after each task. Self-report measures of sense of humor were unrelated to DBP, but several findings emerged with SBP. Interestingly, significant sex differences were found in the relation between the CHS (coping humor scale) and SBP. Among females it was found that those with higher CHS scores showed a lower SBP overall as predicted. In contrast, the males with higher CHS scores related to a higher SBP opposite to the predictions. Moreover, males with higher CHS scores showed a greater increase from baseline in SBP with one of the stressor tasks (mental arithmetic). The pattern of findings with the SHRQ as a measure of humor was similar although somewhat weaker.

The authors suggested that these sex differences might be due to the inherent difference in the way in which men and women express humor. Men may be more likely to use humor in a competitive, tendentious and maladaptive manner whereas humor in women may be more tolerant, self-accepting and adaptive. These results parallel correlational findings by Martin and Kuiper (1999) that men who laughed more frequently over a three-day self-monitoring period were higher in Type A behavior

characteristics such as competitiveness, impatience, and time urgency (but not hostility), whereas women who laughed more frequently were lower in Type A traits.

Filippelli M et al. (2000) conducted a study on respiratory dynamics during laughter on 11 normal subjects. The results showed that basal pulmonary functions were within the normal range in all subjects. There was a decrease in Functional Residual Capacity (FRC) averaging to about two-thirds of the expiratory volume reserve but did not fall to RV (residual volume). When the lowest value of FRC was attained, oscillations of PES (esophageal pressure) were accompanied by only minute motions of the chest wall, indicating that there was flow limitation with near-zero flow, which was independent of pleural pressure. A rough estimation was done by the authors stating that because of the rhythmic adduction and abduction of the vocal cords during laughter, the glottis closure at the very beginning of each expiratory effort would have slowed down the expiratory flow and increased alveolar pressure. They conclude that Laughter was associated with a remarkable decrease in lung volume due to sudden and sustained increase in PGA (gastric pressure), and PES.

Boone T et al. (2000) conducted a pilot project on cardiovascular responses to laughter on 8 college students who viewed a well known comedian. They found a significant increase in stroke volume and cardiac output and significant decrease in arteriovenous oxygen differences and total peripheral resistance. The post effect showed significant decrease in oxygen consumption.

Sakuragi et al. (2002) studied the effect of laughing and weeping on the mood and heart rate variability on ten healthy female subjects. Chest electrocardiogram and respiration curve were recorded before, during and after watching a comedy or a tragedy video.

Then they filled out the profiles of moods scale. Autonomic nervous function was estimated by spectral analysis of HRV (heart rate variability) and it was found that emotions have some effect on the autonomic nervous system. Reactions like palpitations or cold sweats are generally accompanied by emotional changes. Numerous studies have reported changes in autonomic nervous function assessed by the HRV in relation to emotions. (Dishman et al. 2000, Friedman and Thayer 1998, McCarty et.al.1999, Piccirillo et al. 1997 Pine et al. 1998). Most of them have reported an ANS response to stress associated emotions like panic and anxiety.

Takahashi,K et al. (2001) studied the effect of laughter on the Natural Killer Cell Activity (NKCA) and found it significantly elevated after a comic film which was in contrast to the control film. Correlations for experiential and expressive aspects to NKCA showed a correlation of NKCA elevation with self- rated pleasantness; mood score before and after the comic film and the magnitude of laughter was statistically tested. They found that NKCA elevation was negatively correlated with the scores of negative moods scales of POMS, while NKCA elevation had no significant correlation with self rated pleasantness and magnitude of laughter. Further group analysis revealed that high scores of depression and anger hostility suppressed NKCA elevation by laughter. They also found that NKCA before and after the comic film showed a tendency of correlation with the self rated pleasantness of the comic film while NKCA had no correlation with the magnitude of laughter. This suggests that NKCA elevation and NKCA before and after the comic film seems to be related with experiential aspects of laughter rather than expressive aspects.

2.2. Effect of Laughter on Psychological Parameters

The main aspect of mechanism by which laughter may affect stress thereby affecting health is through promotion of positive emotional state (Argyle1997, Edwards 1988).It

may be this positive effect that has an analgesic effect (as happened to Norman Cousins) and an immunoenhancing effect or may have an undoing effect on the cardiovascular sequel of negative emotions.

Several studies have examined correlations between trait measures of sense of humor and levels of S-IgA, including some of the experimental studies described above. With a very small sample of 9 participants Dillon (1985) found significant correlations in the neighborhood of .75 between the Coping Humor Scale (CHS) and four assays of S-IgA. Similarly, using a sample of 17 new mothers, Dillon (1989) found a major correlation ($r = .61$) between scores on the CHS and S-IgA measured in the mothers' saliva but no correlation with S-IgA measured in their breast milk.

2.3. Effect of Laughter on Immunological Parameters

Laughter influences different aspects of immune system. Salivary IgA constitutes the body's first line of defense against entry of infections through respiratory tract and Dillon and Backer (1985) found significant increase in S IgA concentrations after watching humor videos.

Lefcourt and colleagues (Lefcourt et al. 1990) conducted three studies examining the effects of exposure to comedy on S-IgA. Two of these studies (with 45 female undergraduates as participants in one and 41 male and female undergraduates in the other) used a 30-minute comedy audio tape. The third (involving 34 female students) used a 30-minute comedy videotape. Participants were tested in small groups.

All three studies found a significant increase in S-IgA following exposure to comedy relative to a baseline measure. However, in two of the studies, the baselines were taken on different days (in one study in a different room) at least a week earlier, often at different times of the day. In addition, these studies did not include control groups, although a separate "control study" was reported in which 12 undergraduate students (sex unspecified) were shown to have no change in S-IgA over the course of a one-hour classroom lecture. The participants were asked to rate the funniness of the comedy stimuli in each study and these were found to be quite low, particularly in two of the studies.

Laughter was not monitored. Although these studies provided some evidence of increase in S-IgA following exposure to comedy materials, the lack of adequate controls and methodological problems with baseline measurement make it difficult to conclude that the observed effects were due to humor and laughter per se.

Lambert and Lambert (1995) assigned 39 male and female 5th grade students to either a humor condition (10 minutes of live comedy followed by a 15-minute segment of "America's Funniest Home Videos") or a 25-minute non-humorous condition (a 10-minute lecture on proper hand-washing and a 15-minute video about the weather). Surprisingly, participants in the humorous condition had lower S-IgA than did those in the control condition, both at the baselines and following the videos. However, a comparison controlling baseline level did show greater pre-post increase in S-IgA in the humorous than in the non-humorous condition. No manipulation checks were included, and laughter was not assessed.

Some of the results of this study were reported in a journal article (Berk, Tan, Fry, et al. 1989) while the remaining analyses was presented in conference papers, only the extracts of which have been published (Berk, Tan, & Fry, 1993; Berk, Tan, Napier, & Eby, 1989; Berk et al. 1988). The participants were 10 male medical personnel, five of whom were assigned as a single group to watch a 60-minute comedy video ("Gallagher: Over Your Head") and the other five sat quietly in a room together for 60 minutes.

Blood was collected via four catheters in the forearm at a number of intervals before, during, and after the stimulus conditions. Of 19 immunity and endocrine-related variables assayed, significant experimental effects were found in nine variables.

Participants in the humorous video group had significantly lower levels of cortisol and dopac and higher levels of growth hormone following the videotape as compared to the control participants.

In addition, in the experimental group, assays taken after the video revealed significant increases from baseline in the T cell helper/suppressor ratio, blastogenesis, IgG, IgM, NKCA and complement (C3), suggesting immunoenhancing effects of humor. However, comparisons with the control group were not reported, raising questions about whether these effects were specific to the comedy condition.

No effects were found with a number of other variables including epinephrine, prolactin, beta-endorphin, IgA, and gamma-interferon levels. Although some promising results were obtained in this study, there are a number of methodological limitations that cast a doubt on the findings. Firstly, there was a very small sample size of males only and secondly the participants were informed several days beforehand which condition they would be in. This resulted in evident differences in their mood states upon arrival and significant baseline differences in two of the physiological variables (lower epinephrine and higher growth hormone in the experimental as compared to the control group). It is not reported whether the participants were randomly assigned to groups, and there was an age difference between the two groups ($p < .10$). In addition, the no-video control group does not adequately control a variety of factors, such as the diversion of watching a videotape or general emotional arousal that might account for the findings. Manipulation checks were not included and laughter was not monitored.

Kamei et al. (1997) took blood samples before and after a group of 8 male medical students who watched a comedy video (no details given). Blood samples were taken from the same participants two hours before and one week after writing a stressful physics examination. No effects of stress or comedy were found on percentages of helper/inducer (CD4) or suppressor/cytotoxic (CD8) T-cells or CD4/CD8 ratio. However, contrary to the Berk et al. (1989) study, NKCA decreased significantly from before to after the comedy video. This finding was opposite to predictions. It suggested an immunosuppressive effect of humor. Itami et al. (1994) took blood samples from 19 volunteer participants immediately before and after a 3-hour comedy variety theater performance. Beta-endorphin, NK activity and CD 4/8 (helper/suppressor T-cell) ratio were assessed. No significant pre-post changes on any of these variables were found overall. However, when the participants were divided on the basis of pre-performance levels that were above or below a standard range, the five participants with the lowest NK activity levels before the performance showed significant increases in NK activity and CD 4/8 ratio afterwards, while the four participants with the highest CD 4/8 ratios before the performance showed a significant decrease in this variable afterwards.

Using a negative emotion control group and a larger sample size, Mittwoch-Jaffe, Shalit, Srendi, and Yehuda (1995) took blood samples from 59 male and 64 female undergraduates before and after watching either a 45-minute humorous or a 45-minute 'horror' videotape (no details given). They assayed the blood samples for TNF α (tumor necrosis factor), as well as four interleukins (IL-1 β , IL-2, IL-3, and IL-6), protein molecules that regulate and coordinate the activity of various types of immune cells. Of these five cytokines assayed, the researchers reported a pre- to post-video decrease in TNF α and increases in IL-2 and IL-3 in participants watching the humorous

video, and changes in the opposite direction in participants watching the horror video. However, no statistical tests were reported.

Yoshino et al. (1996) took blood samples from 26 female rheumatoid arthritis patients and 31 healthy women before and after they watched a one-hour performance of Japanese comedy story-telling. Of 14 endocrine and immune variables assayed (including beta-endorphin, methionine enkephalin, substance P, epinephrine, dopamine, T-cell helper/suppressor ratio, and NKCA), the arthritis patients showed only significant pre- to post-performance decrease in Cortisol, IL-6, and interferon gamma

(IFN- γ), while the healthy participants showed significant decreases only in IFN- γ .

Takahashi et al. (2001) observed the NKCA after watching a comic film on 21 healthy volunteers and the results show increased NKCA. It is negatively correlated with the score of negative mood scales on the POMS (Profiles of Mood Scale), where as NKCA elevation had no significant correlation with self-rated pleasantness and the magnitude of laughter.

Further Berk et al. (2001) in their study on immune parameters such as NKCA; plasma immunoglobulins; functional phenotypic markers for leukocytes including activated T cells, non-activated T cells, B cells, natural killer cells, T cells with helper and suppressor markers, and assessment of plasma volume and compartmental shifts; plasma cytokine - **interferon- γ** ; and total leukocytes with sub populations of lymphocytes , granulocytes, and monocytes find that there was an increase in natural killer cell activity immunoglobulins

G, A and M, with several immunoglobulin effects lasting 12 hours into recovery from initiation of the humor intervention; functional phenotypic markers for leukocyte subsets such as activated T cells , active cytotoxic T cells , natural killer cells ,B cells , helper T cells, uncommitted T cells with helper and suppressor markers, helper/suppressor ratio , with several leukocyte subset increase effects lasting 12 hours after the humor experience; the cytokine interferon- γ with increases lasting 12 hours; total leukocytes , with specific sub population lymphocytes during the intervention and 90 minutes into recovery; and granulocytes during the intervention, and 90 minutes following the intervention .

2.4. Effect of Yoga Relaxation

In the Laughter Yoga module Yoga relaxation is used to relax the mind and the body after stimulations through laughter. The effect of relaxation on the physiological, psychological and immunological parameters is well documented. The relaxation methods are meant to bring down the physiological or psychological stress since they do not involve any muscular activity. Telles S. et al. (2002) has reported reduced oxygen consumption after two types of relaxation posture (deep relaxation techniques and Shavasana). It is reported that deep relaxation techniques reduces oxygen consumption more than Shavasana.

Shavasana also leads to mental and physical relaxation in hyper reactive persons. (Bose et al. 1987).

Stimulation followed by relaxation brought down the metabolic rate more than Shavasana. (Telles s et.al.2000). Herzog et al. (1990) observed the regional glucose metabolism during Yoga meditative relaxation using Positron Emission Tomography(PET) noted that the ratios of frontal verses occipital CMRGL (regional cerebral metabolic rate of glucose)were significantly elevated ($p < 0.05$) during Yoga meditative relaxation. These alterations were caused by a slight increase of frontal CMRGL and a more pronounced reduction in primary and secondary visual centers.

This data indicates a holistic behavior of the brain metabolism during the time of altered state of consciousness during Yoga meditative relaxation. This could probably be the reason for lowered metabolic rates during relaxation or meditation.

The effectiveness of two relaxation techniques in treating snake anxious subjects who were somatically or cognitively anxious got training in progressive relaxation technique or Agni Yoga showed the response was different depending upon whether the subjects expressed anxiety somatically or cognitively. Matsumoto et al. (2001) compared the psychological effects of Progressive Muscle Relaxation (PMR) and breathing exercises on 42 students using Smith relaxation inventory before and after each sessions. PMR practitioners displayed greater increment in relaxation states, physical relaxation and disengagement, while breathing practitioners displayed higher levels of relaxation after stretch and awareness.

Andrew Newberg in their book titled 'Why God Won't Go Away' elaborates the activity of the brain through SPECT camera on meditators as subjects. It showed a marked decrease in the left orientation area of their brain as compared to the subjects in the rest period. This was surprising, as the orientation area never rests. They hypothesized that the drop in the brain activity was probably blinded deprived of the information it needed to do its job properly.

This is exactly what the generations of eastern mystics had described as their peak meditative, spiritual and mystical moments. Further studies on such mystical experiences revealed solid evidence that these experiences, which the subjects described as the altered state of mind and the absorption of the self into something larger, was not the result of emotional mistakes or simple wishful thinking. It was associated instead with a series of observable neurological events, which though unusual are not outside the range of normal brain function. In other words, the mystical experiences are biologically, observably, and scientifically real.

2.5. Summary of Literature

Fried and Wilson found that electrical stimulation in the anterior part of the human supplementary motor area (SMA) located in the cortex of the left frontal lobe stimulated mirthful laughter. In each case of stimulation, the patient attributed the laughter to an external stimulus such as people in the room, but it occurred at precisely the moment of stimulation. Also, the duration and intensity of laughter increased with increasing stimulation, ranging from a smile to robust laughter. (Fried and Wilson, 1998) This shows that at least part of the pathway runs through the cortex of the left frontal lobe, which can be triggered by an external stimulus. Moreover, the patient's identification of an external stimulus peaks to the point at which this area exists in the pathway.

Another mechanism by which mirthful laughter benefits health is the enhancement of the immune system. Mirthful laughter influences different aspects of the immune system. One effect has been observed in the body's concentration of salivary immunoglobulin A (IgA). Salivary IgA constitutes the body's first line of defense against entry of infectious organisms through the respiratory tract. It was found that IgA concentration increased significantly in ten subjects after viewing a humorous video (Dillon and Baker, 1985).

There was a significant reduction of serum IL-6 levels in rheumatoid arthritis (RA) patients after exposure to mirthful laughter. Supporting this correlation, there is recent evidence that monoclonal antibody against IL-6 receptor is effective in the treatment of RA. (Nakajima, Hirai, and Yoshino, 1999). Laughter is also thought to have an effect on the activity of Natural Killer (NK) cells. Research shows reduced levels of NK cell

numbers and activity in patients undergoing stress. (Wooten, 1996) The corollary, which suggests that levels would increase in a positive emotional state, has not been supported. In fact, a study that measured the immune responses caused by both 'stress' and 'humor' in the same patients showed a decrease in the NKCA induced by the humorous video. (Kamei, Kumano, and Masumura, 1997). Laughter is also thought to affect the endocrine system. It appears to reduce serum levels of Cortisol, dopac, epinephrine, and growth hormone. In stress inducing experiments, the change in IgA levels following stress are significantly related to those of norepinephrine. The implications of a change in endocrine hormone levels due to laughter are extensive. This enables laughter to affect any and all parts of the body.

The effect of laughter on the central nervous system is not well known. There is an increase in catecholamine levels. Current theories suggest that this may affect mental functions such as increasing interpersonal responsiveness, alertness, and memory. (Fry, 1992).

These experiments are just the beginning in the newly developed field of psycho neuro immunology. As a rapidly developing field, it explores the interactions between the central nervous system and the immune system. Studies have documented the autonomic innervations of the lymphoid tissue, both in the vasculature and in the parenchymal areas close to the lymphocytes. Moreover, there is evidence that lymphocytes contain adrenoreceptors on their surface and their responses are affected by neurotransmitters. (Halley, 1991) This anatomical connection links the brain and the immune system, allowing for communication. Another finding suggests the existence of a chemical connection. This data shows that immune cells have receptors and produce the same neuropeptides that are found in the brain and other glandular tissues. (Pert, Ruff, Weber, and Herkenham, 1985). Research also shows that this communication may be bi-directional. One study shows that after immune system activity is increased, there is increased hypothalamic neuronal activity. (Besedovsky, Rey, and Sorkin, 1985) This relationship, once elucidated, has amazing potential for practical application.

Many of the studies mentioned above have problems of methodological issues, small sample size, and array of confounding variables. The likelihood of confounding factors increases greatly in dealing with any psychological factors. As a consequence more studies are needed in this field to establish positive effects.

In the present study we propose to overcome these by using a randomized control trial, and using a very popular capsule called LAUGHTER YOGA, which is extensively used in laughter clubs in India. This will be the first study on Laughter Yoga in India. We specially selected IT professionals as there is increased sympathetic arousal in the form of personal and professional stress which makes them tense and grim. They develop a habit of tightness and 'no laugh' syndrome due to pressure of work, deadlines to meet targets etc. Such occupational stress leads to unhappy office environment, unsatisfactory interaction with colleagues, dissatisfied home environment etc. Very few people use humor as a means for releasing their tensions, though the potential of Laughter Yoga in release of tension is well documented. The sessions of Laughter Yoga on professionals may increase their interpersonal interactions leading to a healthy atmosphere conducive to higher productivity in terms of man hours, reduced infections, greater motivation and myriad of other direct and indirect benefits.

2.6. What is Missing?

Though these empirical studies give us direction to mechanism of action there are issues that need to be answered. The evidence of these studies is sometimes inconsistent or shows no evidence. There were methodological problems such as low

sample size, power problems, and control factors while conducting the study. Also, there are no studies comparing induced cognitive laughter to unconditional laughter.

There are many laughter clubs where participants practice laughter along with some exercises popularly known as Laughter Yoga. Though a study on Laughter Yoga by Dr. Kataria was presented in the workshop, no literature is available on Laughter Yoga. These issues make it difficult to arrive at conclusions as to whether the effects are due to positive emotions or laughter or any other movements associated with laughter.

3. Aims and Objectives

Specific Aim

To conduct a preliminary study on the effect of Laughter Yoga on various stress parameters such as physiological, immunological and psychological measures.

Objective

To determine the relationship between the immediate and long-term effect of Laughter Yoga practice and various measures such as physiological, immunological and psychological parameters in IT professionals aged between 20-50 years and to compare these results with a control group in the same profession/socio-economic-occupational group.

4. Subjects and Methods

4.1. Subjects

4.1.1. Selection of Subjects

IT companies were identified for recruitment of subjects and the overall assessment of the employees profile was studied.

After the initial profile assessments, the IT companies were approached and details of Laughter Yoga protocols were explained in detail during a presentation. The subjects volunteered for the project giving their names in writing. After a clinical examination and providing the demographic data the subjects were recruited for the study.

The following flow chart gives details of the recruitment of subjects.

4.1.2. Inclusion Criteria

- Normal healthy volunteers
- Men and women
- Normal weight range
- Aged 20-50
- Non Smokers

4.1.3. Exclusion Criteria

- Smokers.

- Those who had taken alcohol in the previous week.
- Those having blood pressure, asthma, diabetes or any other illness.
- Those who have had no infection for at least one week before the study.
- Those already exposed to Laughter Yoga.

4.1.4. Reasons for Choosing IT Professionals

The influx of IT companies, subsequent increase in employees and huge salaries offered by these companies have made Bangalore a hub of activities. External and internal stressors like work demands, meeting deadlines and targets, competition, race against time make IT employees the most stressed professionals today. Other peripheral factors like traveling long distances and traffic snarl-ups add to their stress levels. This affects their physiological, psychological and immunological health, resulting in losing productive days and productivity wherein both the individual and the institution are the losers. It is the accumulated stress that is more bothersome. It needs to be identified at an early stage in order to find a solution to alleviate it. There is an erosion of social life as it becomes difficult to interact under stressful circumstances. People have almost forgotten to laugh. The solution lies in how they handle their stressors. Laughter Yoga (LY) is designed to address this issue in particular. It facilitates relaxation by releasing pent up feelings and generates effective expression of emotions thereby releasing stress. It was administered to the IT professionals to document the evidence of release of stress through the intervention.

4.1.5. Introductory Lecture and Registration

For recruitment of subjects a minimum of six leading IT companies were approached. The study details were distributed through flyers (Appendix 1). Once they approved the study protocol and suitability of the research to their company, a general presentation for all those interested was given. The subjects volunteered to be either in the Laughter Yoga group or the Waitlisted control group.

Each company committed for sending their employees for the sessions also provided a closed hall for intervention to be given at their own offices.

4.2. Methods: Design

This was randomized blinding (the assessment outcome). The LY instructor was not involved in assessment or randomization. The assessment and analysis was done according to the RCT number and unblinding was done only after post assessment. The Investigator was not involved either in the intervention nor assessment/analysis.

4.3. Details of Parameters, Equipment, Calibration, and Validation

4.3.1. Four Channel Polygraph

A polygraph is an instrument that simultaneously records changes in physiological processes such as ECG, heartbeat, respiration and electrical resistance galvanic skin response (GSR).

A 4-channel polygraph (Medicaid systems, Chandigarh, India) was used to record the electrocardiogram (ECG), respiration, finger plethysmogram and skin conductance level. ECG was recorded using standard wrist and limb leads. The ECG was digitized using a 12 bit analog-to-digital converter (ADC) at a sampling rate of 500Hz. The data recorded was visually inspected off line and only noise free data was included for

analysis. The R waves were identified and used to obtain a point event series of successive R-R interval from which the beat-to-beat heart rate series was computed.

The heart rate variability (HRV) is an indicator of the cardiac autonomic control. The heart rate is controlled by neural as well as other factors. While analyzing heart rate variability two spectral components are considered

1. High Frequency (0.15 - 0.50 Hz) – Due to vagal efferent activity.
2. Low Frequency (0.05 – 0.15 Hz) – Due to sympathetic activity.

The present study aimed at analyzing the heart rate variability spectrum and to get a better understanding about the immediate effects of Laughter Yoga on the autonomic status. The study also suggests that HRV is a more useful psycho physiological measure than heart rate alone.

Skin conductance level was recorded using Ag/AgCl disk electrodes with electrode gel, placed in contact with the volar surfaces of the distal phalanges of the index and middle fingers of the left hand. A low level DC pre-amplifier was used and a constant voltage of 0.05 V was passed between the electrodes. Respiration was recorded using a belt fixed at the chest.

Electrocardiograph (ECG)

An ECG is a measurement of the electrical activity of the heart (cardiac) muscle as obtained from the surface of the skin. As the heart performs its function of pumping blood through the circulatory system, the result of the action potentials responsible for the mechanical events within the heart is the generation of a certain sequence of electrical events.

Electrodes

Electrodes are used for sensing bio-electric potentials as caused by muscle and nerve cells. ECG electrodes are generally of the direct-contact type. They work as transducers converting ionic flow from the body through an electrolyte into electron current and consequentially an electric potential measurable by the front end of the ECG system. These transducers, known as bare-metal or recessed electrodes, generally consist of a metal such as silver or stainless steel, with a jelly electrolyte that contains chloride and other ions. In this study disposable pre-gelled electrodes were used.

Respiration

Respiration transducers were used to determine the Breathing Rate (breaths per minute). The Chest Type transducer, which is a Piezo electric assembly was used, which helps record the expansion and contraction of the chest.

4.3.2. Blood Pressure Apparatus

Blood pressure was measured using ELKOMETER sphygmomanometer300 (Anita industries, New Delhi) .The blood pressure was obtained in a sitting position when the subject was relaxed. Trained nurse recorded the BP.

4.3.3. Elisa Kits

For cortisol studies the Enzyme Immunoassay Kit from Assay Designs INC USA, (Correlate-EIA™).

Description

The Assay Designs' Correlate-EIA™ Cortisol kit is a competitive immunoassay for the quantitative determination of Cortisol in biological fluids. The kit for the quantitative measurement of cortisol uses a monoclonal antibody to cortisol to bind, in a competitive manner, cortisol in a sample or an alkaline phosphates molecule which has cortisol covalently attached to it. After a simultaneous incubation at room temperature the excess reagents are washed away and substrate is added. After a short incubation, the enzyme reaction is stopped and the yellow color generated is read on a micro plate reader at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of cortisol in either standards or samples. The measured optical density is used to calculate the concentration of cortisol.

SALIVARY CORTISOL ENZYME IMMUNOASSAY

The concentration of cortisol present in the salivary samples of the subjects were analyzed by using 96 well, high sensitivity Salivary cortisol Enzyme Immunoassay Kit from Salimetrics Pvt. Ltd., (catalog 1-3002/1-3012)

Procedure

All reagents were brought to room temperature before use.

Step 1

ELISA plate layout was determined using a template i.e., pre-determining the standards, non-specific binding (NSB), zero and samples to be placed.

Step 2

Using only required number wells and the remaining wells were preserved by refrigerating in the airtight foil pouch at 8°C.

Required number of strips was arranged in the strip holder. For NSB's, the original wells of the plate were replaced with the NSB wells provided with the kit.

Step 3

25 µL of standards, controls and unknowns were pipetted into appropriate wells. Standards, controls were assayed in duplicate.

25 µL of assay diluent was pipetted into 2 wells to serve as the zero.

25 µL of assay diluent was also pipetted into each NSB well.

Step 4

24 mL of assay diluent was taken in a disposable tube.

1:1600 dilution of the conjugate was prepared by adding 15 µL of the conjugate to the 24 mL of assay diluent prepared earlier and mixed immediately.

200 µL of prepared diluted conjugate was pipetted into each well using a multi-channel pipette.

Step 5

The plate was kept on rotator for 5 minutes at 500 rpm (or tap to mix) and incubated at room temperature for an additional 55 minutes.

Step 6

Washing of the plate was done 4 times with 1X wash buffer.

Washing was done by pipetting 300 μ L of wash buffer into each well and discarding the liquid by inverting the plate over a sink.

After each wash, the plate was thoroughly blotted on paper towels before being turned upright.

Step 7

200 μ L of TMB solution was added to each well with a multi-channel pipette.

Step 8

Mixing was done on a plate rotator for 5 minutes at 500 rpm (or tap to mix) and by incubating the plate in the dark at room temperature for an additional 25 minutes.

Step 9

50 μ L of stop solution was added to arrest the reaction with a multi-channel pipette.

Step 10

The optical density was measured at 450 nm using an ELISA reader manufactured by BIORAD.

SALIVARY SECRETORY Ig-A INDIRECT ENZYME IMMUNOASSAY KIT

The concentration of secretory Immunoglobulin-A (SigA) present in the saliva of the subjects were determined by using indirect enzyme immunoassay kit manufactured by Salimetrics Pvt. Ltd., (Catalog No.1-1602, 96-Well Kit)

Procedure

All reagents were brought to room temperature before use.

Step 1

ELISA plate layout was determined using a template i.e., pre-determining the standards, non-specific binding (NSB), zero and samples to be placed.

Step 2

Using only required number wells and the remaining wells were preserved by refrigerating in the airtight foil pouch at 8°C.

Required number of strips was arranged in the strip holder. For NSBs, the original wells of the plate were replaced with the NSB wells provided with the kit.

Step 3: Serial Dilution

5 microcentrifuge tubes were labeled and other small tubes 2 through 6.

30 mL of 1X SigA diluent was pipetted in tubes 2 through 6.

Standard 3X was serially diluted by adding 15 mL of the 600 μ g/mL standard (tube 1) to tube 2.

Mixed well.

15 mL was added from tube 2 to tube 3.

Mixed well.

Continued for tubes 4, 5 and 6 so that the final concentrations of

Standards for tubes 1 through 6 becomes 600 g/mL, 200 g/mL,

66.7 g/mL, 22.2 g/mL, 7.4 g/mL, and 2.5 g/mL respectively.

Step 4

Another set of micro centrifuge tubes were taken with identical number as earlier, but without standards.

A repeater pipette was used to add 100 μ L of 1X SIgA diluent into each tube.

25 μ L of saliva was pipetted into the appropriate tube.

Step 5

One 12 x 75 mm snap-cap tube for each standard, control, unknown sample, and one tube for the zero value was labeled.

A repeater pipette was used to add 4 mL of 1X SIgA diluent to each tube.

10 L of standard (from step 3), control, or diluted unknown saliva sample (from step 4) was added to the appropriate tube.

Then 10 L of 1X SIgA diluent was added to the zero tube.

Step 6

3 mL of 1X SIgA diluent was taken in a microcentrifuge tube.

1:120 antibody-enzyme conjugate was prepared by adding 25 L of the conjugate to the 3 mL of 1X SIgA diluent prepared.

Mixed well on a plate rotor.

50 L of the diluted antibody-enzyme conjugate was added to all tubes using a repeater pipette.

Each tube was gently mixed by inversion and incubated for 90 minutes at room temperature.

Step 7

After gently mixing each tube by inversion again, 50 L of solution from step 6 was added to the microtitre plate according to the template.

50 L of 1X SIgA diluent was added to the NSB wells.

Plate was covered with the adhesive plate sealer and incubated at room temperature with continual mixing at 400 rpm for 90 minutes.

Step 8

Washing of plate was done 6 times with 1X wash buffer by gently adding 300 mL of wash buffer into each well and then decanting the liquid into a sink.

After each wash, the plate was thoroughly blotted on paper towels before turning upright.

Step 9

Add 50 µL of TMB solution was added to each well with a multi-channel pipette

Step 10

Mixed well on a plate rotator for 5 minutes at 500 rpm and incubate the plate in the dark at room temperature for an additional 40 minutes.

Step 11

50 µL of stop solution was added with a multi-channel pipette.

Mixed well on a plate rotator for 3 minutes at 500 rpm.

Step 12

The optical density was measured at 450 nm using an ELISA reader manufactured by BIORAD.

The same procedure was repeated for the Secretary IgA using the specific Assay kit.

4.3.4. Questionnaires**PANAS**

Positive and Negative Affect Schedule questionnaire standardized and validated was used to see the emotional experiences of the subjects before and after practice of Laughter Yoga. This questionnaire is tested for normality, validation and reliability (Watson D. & Clark L.A. 1994).

The PANAS (Positive and Negative Affect Schedule) consists of 10 positive affects (interested, excited, strong, enthusiastic, proud, alert, inspired, determined, attentive, and active), 10 negative affects (distressed, upset, guilty, scared, hostile, irritable, ashamed, nervous, jittery, and afraid), 4 other positive affects (happy, pleased, content and glad) and 5 other negative affects (disappointed, sad, unhappy, troubled and miserable). Participants were given sufficient time and asked to rate items on a scale from 1 to 5, based on the strength of emotion where 1 = "very slightly or not at all," and 5 = "extremely". Scores of each affects were summed up and analyzed.

PSS

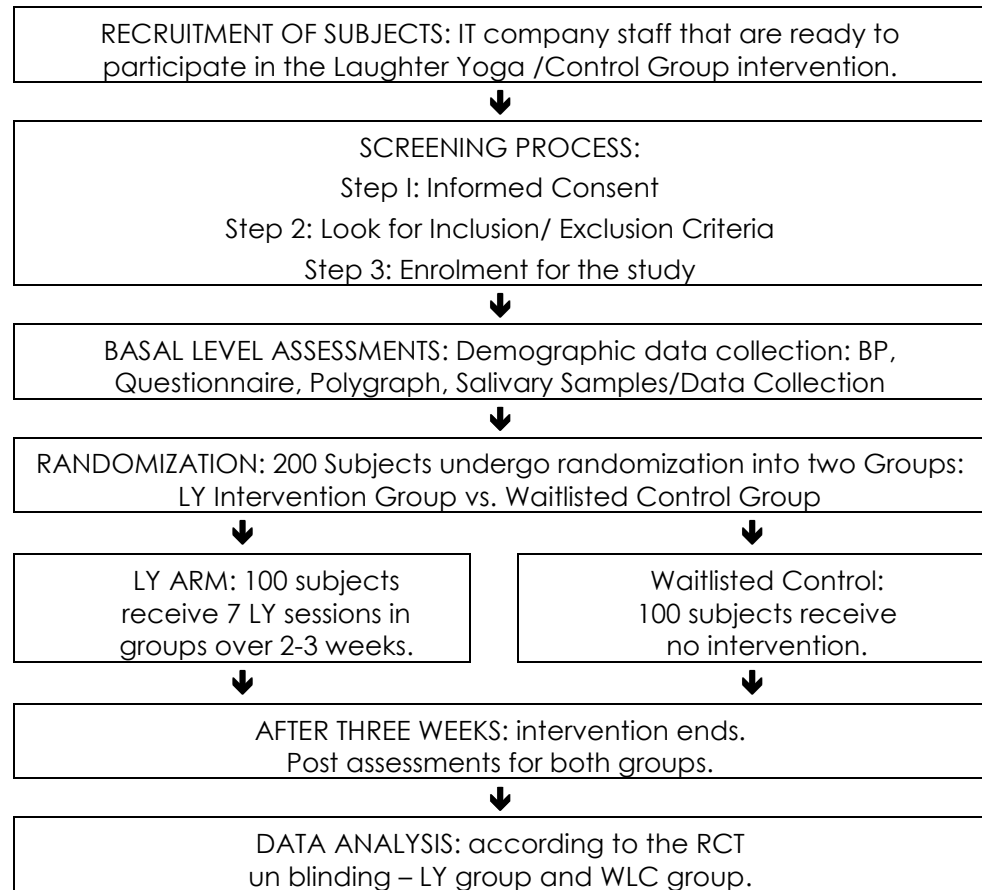
Perceived Stress Scale is an economical and simple psychological instrument to administer, comprehend, and score the degree to which situations in one's life over the past month are appraised as stressful. Items were designed to detect how unpredictable, uncontrollable, and overloaded respondents found in their lives. It poses general queries about relatively current levels of stress experienced. PSS scores are obtained by reversing the scores on the four positive items, e.g., 0=4, 1=3, 2=2, etc. and then summing across all 10 items.

The Perceived Stress Scale (PSS) assesses the degree to which the participants appraised their lives as being stressful during the past month. This provides a more subjective assessment of stress than the life events score. Internal consistency is high (Cronbach's α 0.75–0.86), and test-retest reliability as high as 0.85 has been reported (16).

TAS

Toronto Alexithymia Scale describes people who appeared to have deficiencies in understanding, processing or describing their emotions and comprises 20 questions. Deficiencies are grouped as difficulty in identifying feelings (Factor 1; questions 1, 3, 6, 7, 9, 13, 14), difficulty in describing feelings (Factor 2; questions 2, 4, 11, 12, 17) and externally oriented thinking (Factor 3; questions 5, 8, 10, 15, 16, 18, 19, 20). TAS scores were obtained by reverting the scores of question 4, 5, 10, 18 and 19. Sum of all factors were analyzed along with total factor (Factor 1+Factor 2+Factor3).

4.3.5 FLOW chart of study protocol



4.4. Demographic Data Collection

The IT companies were approached for participation for the study. They were given an initial presentation about the study, LY research and other requirements. They were given the option to volunteer for the study. Once they gave their names, they were given demographic data forms containing name, age, gender and other personal details, along with a flyer containing details of LY procedure, timing schedule, assessments details etc. (Ref Annexure 2). These forms are used for further classification based on age, height, weight, gender, activity and other details.

4.5. Design and Procedure For Assessments

Study design: Randomized double blind control trial

General Design for Assessments

LAUGHTER YOGA GROUP

Basal level assessments	Laughter Yoga intervention- 7 sessions	Post assessments

WAITLIST CONTROL GROUP

Basal level assessments	No intervention	Post assessments

4.5.1.Polygraph

Data Extraction

The following data was extracted from the digitally stored polygraph records: The breath rate (in cycles per minute) was calculated by counting the breath cycles in 60 second epochs, continuously. For each subject, the average of the values obtained during the 5-minute session was used for analysis. Frequency domain analysis of heart rate variability (HRV) data was carried out for the 5-minute recording. The mean heart rate was obtained from this record. The HRV power spectrum was obtained using Fast Fourier Transform (FFT). The energy in HRV series in the following specific frequency bands was studied, viz. the very low frequency band (0.0 - 0.05 Hz), low frequency band (0.05 - 0.15 Hz), and high frequency band (0.15 - 0.50 Hz). According to guidelines of the Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, the low frequency and high frequency values were expressed as normalized units.

Assessments

Recording of Autonomic and respiratory variables

A 4-channel polygraph (Recorders of Medicare Systems, Chandigarh, India) was used to record the electrocardiogram (ECG), respiration, finger plethysmogram amplitude and galvanic skin resistance. ECG was recorded using standard limb lead I configuration. The ECG was digitized using an inbuilt analog-to-digital converter (ADC) at a sampling rate of 1050 Hz. The data recorded were visually inspected off-line and only noise free data were included for analysis. The R waves were detected to obtain a point event series of successive R-R intervals, from which the beat-to-beat heart rate series was computed. The blood pressure was recorded with a sphygmomanometer by auscultation over the right brachial artery.

4.5.2.Blood Pressure

Blood pressure was recorded while sitting in a relaxed position by a trained nurse using ELKOMETER sphygmomanometer 300 (Anita industries, New Delhi).

4.5.3.Salivary Cortisol and Secretory IgA

Each subject was given two sterilized containers for collection of saliva samples. Evening sample was collected between 6-7 pm. The subjects were instructed to

take a morning saliva sample as soon as they got up (before brushing the teeth) and bring it to office the next day. Collected by the LY representatives, these samples were kept in a deep freeze before analysis.

4.5.4. Questionnaire

The questionnaires were administered individually when the subjects reported for assessments. They were given enough time to answer all the questions. Any doubt regarding any question was answered by the LY volunteer present at the time of assessments. The questionnaire was answered by the subjects as per their convenient time and the volunteers ensured that all questions were answered.

4.6. Intervention

The Laughter Yoga group underwent 7 sessions of Laughter Yoga classes distributed over 2-3 weeks. The sessions were conducted at the IT companies at a convenient time fixed by the HR of the company. Generally it was scheduled at 4.30 pm and each class was for 20 minutes. The practice was always done in groups. The brief description of a typical session is given below.

Laughter Yoga Protocol

Steps of Laughter Yoga Exercises: (under instruction of Laughter Yoga Leader Dr. Madan Kataria):

- Warming up: rhythmic clapping in unison, chanting 'ho ho ha ha ha' with eye contact (1 minute).
- Deep breathing: With stretching of arms, deep inhalation and exhalation (twice for 30 seconds).
- Two repetitions of deep breathing and stretching while inhaling. Holding the breath for 5 seconds and then laughing out loud while exhaling (1 minute).
- Speaking Gibberish: talking in a meaningless language. While doing this people moved around having eye contact with other group members (1 minute).
- Hand shake laughter exercise: group members move around and self induce laughter by shaking hands with each other and having eye contact followed by clapping and chanting 'ho ho ha ha' (1 minute).
- Two repetitions of deep breathing without laughing, deep inhalation and exhalation followed by chanting the affirmation 'very good, very good... YEAH' (1 minute).
- Milk shake laughter: holding two imaginary glasses of milk pretend to mix the contents while chanting 'aee..... aeee.....' then laughing out loud as if drinking it. Repeat action 4 times while expressing playfulness (1 minute).
- Two repetitions of deep breathing, inhalation and exhalation, followed by neck and shoulder rotation exercises (1 minute).
- One meter laughter: group members stretch both their arms and pretend to measure an imaginary one meter of cloth and chant 'aee.... aeee.... ha ha haaaaa.....' (laughing out loud) by spreading both arms upwards towards the sky followed by rhythm chanting, clapping 'ho ho ha ha' (1 minute).

- Deep inhalation with arms stretched upwards and laughing while exhaling followed by rhythmic chanting 'very good, very good..... yeah' twice (1 minute).
- Just laugh exercise: group members look at each other and self induce laughter as if saying, I don't know why I am laughing ... I'm just laughing (by body action) followed by 'ho ho ha ha' chanting & clapping (1 minute).
- Take two deep breaths followed by chanting 'very good, very good, yeah' (twice).
- Mobile phone laughter: group members hold an imaginary phone and laugh while moving around and having eye contact. This is followed by 'ho ho ha ha' (calmly and deeply for 1 minute).
- Again take two deep breaths and give the body a gentle self massage all over by tapping (1 minute).
- Argument laughter exercise: group members wagging their index finger at each other laugh at the same time as if they are arguing. Followed by 'ho ho ha ha' chanting and clapping (1 minute).
- Deep breathing followed by 'very good, very good yeah....' exclamation (1 minute).
- Visa card or telephone bill laughter: group members pretend to show each other their visa card bill or telephone bill and laugh while moving around followed by chanting 'ho ho ha ha' and clapping (1 minute).
- Two repetitions of deep breathing followed 'very good, very good yeah....' (1 minute).
- Gradient laughter: group members standing in close circle self induce laughter in a slow manner gradually increase the intensity (1 minute).
- Take two deep breaths and relax (1 minute).

All subjects in the LY group were given LY technique and the base line measurements recorded before the intervention started. The intervention was given for 7 sessions spread over two- three weeks for 3-4 days a week. The waitlisted group did not get any intervention till the LY group completed the course on Laughter Yoga. But they were assessed at base line and on the end of the study i.e., two- three weeks, along with the LY group. The waitlisted control group was given LY module for the next few weeks for which no assessments were made.

4.7. Data Analysis: Statistical Procedure

Paired 't' test was used to look for significance within the group, and independent 't' test for significance between the groups was used when the data was normally distributed. Non-parametric tests such as Wilcoxon signed test was used when the data was not normally distributed for within group significance and Mann Whitney tests for between subjects significance. It was considered significant if the p value was less than 0.05. SPSS version 10 was used for analysis.

4.8. Screening, Enrolment and Randomization

General health screening was done at the place of study and only normal subjects without any ailments and diseases were asked for further participation.

Before the subjects entered the study, the PI obtained written approval of the protocol from Institutional Review Board (IRB), while the Informed Consent form was obtained at the time of recruitment. The institutional HR authorized the study subjects to participate in the study.

Before any study-specific procedures were performed, all subjects personally signed the Informed Consent form. A subject was defined as enrolled in the study once she/he has been randomized to the LY group or in the Waitlisted control group.

All subjects on entering the screening period for the study (defined as the point when the subject signs the Informed Consent) received a unique subject identification number (RCT) before any study procedures were performed. This number was used to identify the subject throughout the trial and also used for all study documentation related to that subject. The subject identification number remained constant throughout the entire study period.

Randomization

The randomization was done using computerized random generator table. (Ref Saghie 2004)It was simple randomization based on the number of participants and number required in each group.

5. Results

5.1. Polygraph Data

AUTONOMIC AND RESPIRATORY VARIABLES

Heart Rate

There was no significant change in the heart rate recorded after the seventh session of Laughter Yoga compared to the before values in the Laughter Yoga group ($p > .05$, two tailed, paired 't' test). The Waitlisted control group also showed no significant change ($p > .05$). The comparisons between the two groups (Laughter Yoga and Waitlisted control) showed no significant difference ($p > .05$, two tailed, Independent-samples 't' test). On observation it was found that there was a trend of reduction in heart rate in the Laughter Yoga group (2.28%).

Respiratory Rate

The before and after comparisons in both Laughter Yoga and Waitlisted control group showed no significant change ($p > .05$, two tailed, paired 't' test). Also, the comparison between the groups showed no significant difference ($p > .05$, two tailed, Independent-samples 't' test).

Components of Heart Rate Variability (HRV) Spectrum

Low Frequency (LF) component of HRV spectrum: The within group (pre-post) comparisons for both Laughter Yoga as well as Waitlisted control groups showed no significant change ($p > .05$, two tailed, paired 't' test) in the LF component of HRV spectrum. Also, the between groups comparison showed no significant difference ($p > .05$, two tailed, Independent-samples 't' test).

High Frequency (HF) component of HRV spectrum: The within group (pre-post) comparisons for both Laughter Yoga as well as Waitlisted control group showed no significant change ($p > .05$, two tailed, paired 't' test) in the HF component of HRV

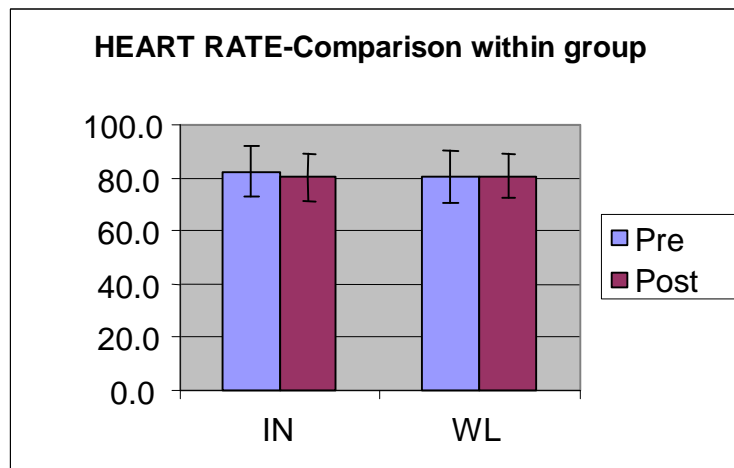
spectrum. Also comparison between groups showed no significant difference ($p > .05$, two tailed, Independent-samples 't' test).

LF/HF ratio: The within group (pre-post) comparisons for both Laughter Yoga as well as Waitlisted control groups showed no significant change ($p > .05$, two tailed, paired 't' test) in the LF component of HRV spectrum. The comparison between the groups also showed no significant difference ($p > .05$, two tailed, Independent-samples 't' test).

Table 5.1. The Group Mean Values \pm Standard Deviations (SD) of the Autonomic and Respiratory Variables for both Laughter Yoga and Waitlisted Control Groups.

Variables studied	Laughter Yoga group (n = 53)		Waitlisted Control Group (n = 51)	
	Pre	Post	Pre	Post
Heart rate	82.15	80.28 ^{ns}	80.28	80.46 ^{ns}
	± 8.56	± 8.35	± 9.23	± 9.80
Respiratory rate	15.58	16.71 ^{ns}	15.58	16.71 ^{ns}
	± 3.33	± 4.03	± 3.33	± 4.03
LF (n.u.)	54.79	54.82 ^{ns}	59.58	58.64 ^{ns}
	± 16.97	± 18.10	± 18.67	± 18.16
HF (n.u.)	45.20	45.16 ^{ns}	40.41	41.35 ^{ns}
	± 16.97	± 18.13	± 18.67	± 18.16
LF/HF ratio	1.72	2.06 ^{ns}	2.50	2.01 ^{ns}
	± 1.81	± 2.7	± 2.41	± 1.56

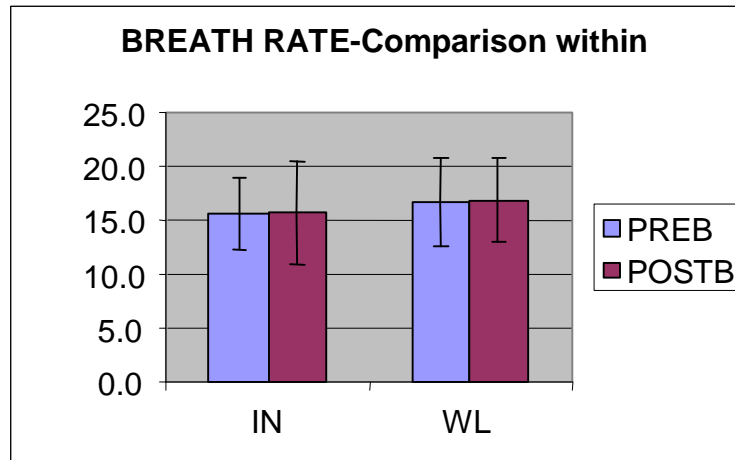
Figure 5.1. Heart Rate Comparison within Group



BREATH RATE

Table 5.1. Gives details of mean values and SD of breath rate. There was no significant difference between the group, as well as with in LY ($p>0.8$) WLC ($p>0.8$) group after the LY intervention.

Figure 5.1.3. Breath Rate Comparison within LY group and WLC group



HRV

LF

There was no significant difference between the groups in Low frequency ($p>0.2$). There was also no significant difference with in the group - LY group ($p>0.9$), WLC ($p>0.3$).

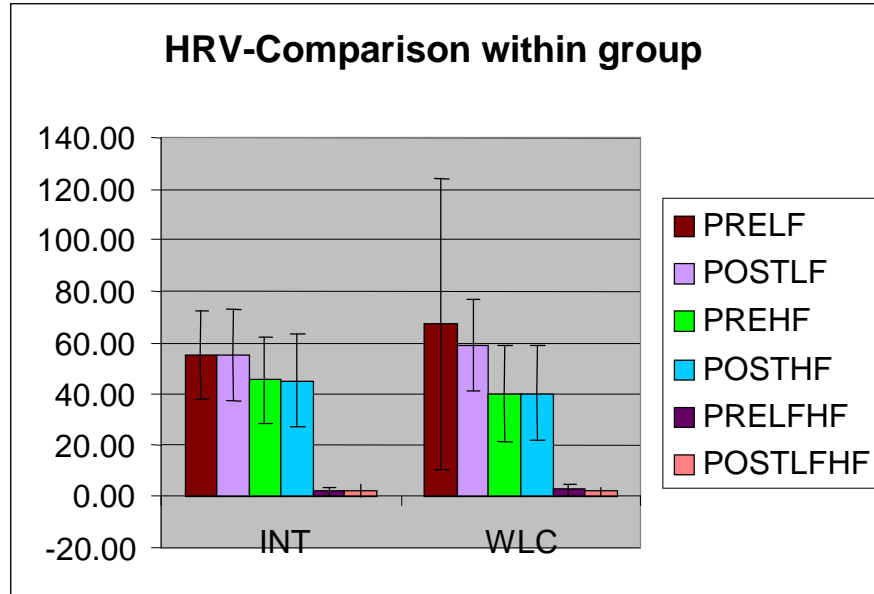
HF

There was no significant difference between the groups in Low frequency ($p>0.2$). There was also no significant difference with in the group - LY group ($p>0.9$), WLC ($p>0.7$).

LF/HF ratio

There was no significant difference between the groups in Low frequency ($p>0.9$). There was also no significant difference with in the group - LY group ($p>0.2$), WLC ($p>0.1$).

Figure 5.1.4. Comparison of Pre-post Data of LY and WLC Groups



5.2. Blood Pressure

Table 5.2. gives details of measurements and significance in BP, and Figure 5.2. gives graphical distribution of the measured parameters.

There was significant difference between Laughter Yoga group and Waitlisted control group when the post assessments was compared between groups in systolic blood pressure, LY group showing lowered Systolic pressure compared (Ly group-120.78 mm/hg, WLC group- 125.96 mm/hg ($p < 0.04$).

However there was no significant difference in diastolic pressure between the groups ($p > 0.1$)

There was also a significant difference with in the group in LY group pre compared to post –systolic- $p < 0.001$, diastolic $p < 0.000$. There was a difference/reduction of 7.46 mm/hg in systolic pressure pre compared to post in the Ly group, and 3.03 mm/hg in diastolic pressure in LY group.

WLC group showed no significant improvement in systolic blood pressure ($p > 0.1$), or diastolic pressure ($p > 0.1$).

Table 5.2. The Group Mean Values \pm Standard Deviations of the Laughter Yoga and Waitlisted Control Group (Blood Pressure)

	LAUGHTER YOGA (N=59)	WAIT LISTED CONTROL (N=56)
Pre Systolic (mm/Hg)	128.24 \pm 14.99	125.89 \pm 13.13
Post Systolic(mmHg)	120.78 \pm 14.42**§	125.96 \pm 12.80 ^{ns}
Pre Diastolic(mm/Hg)	82.37 \pm 9.18	82.34 \pm 8.28
Post diastolic(mm/Hg)	79.34 \pm 8.64*	81.81 \pm 8.50 ^{ns}

Values Mean \pm SD

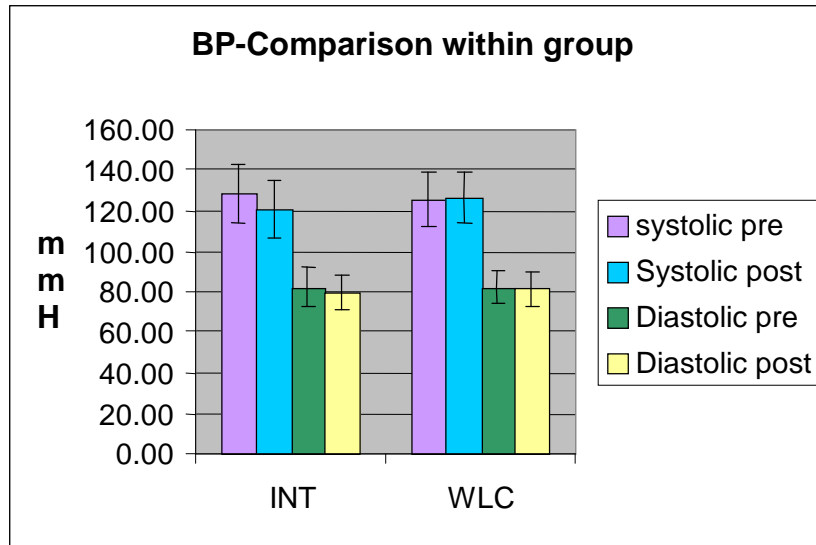
* $P < 0.05$ Paired t-test pre- compared to post

** P < 0.001 Paired t-test pre- compared to post

ns - no significant , mmHg= Millimeter of mercury,

§ P< 0.05 Independent t test comparison between LY group and WLC group.

Figure 5.2. Comparison of Pre-post BP of LY and WLC Group



5.3. Cortisol

The data was not normally distributed, and hence non-parametric tests were used.

There was no significant difference between the groups in the post assessments (Mann Whitney- $p > 0.6$).

There was significant difference in the LY group (pre compared to post)

$P < 0.001$ in Wilcoxon signed ranks test. WLC group did not show any significance in the pre- post analyses ($p > 0.2$).

Table 5.3. Cortisols – The Group Mean Values \pm Standard Deviations for Laughter Yoga and Waitlisted Control Groups.

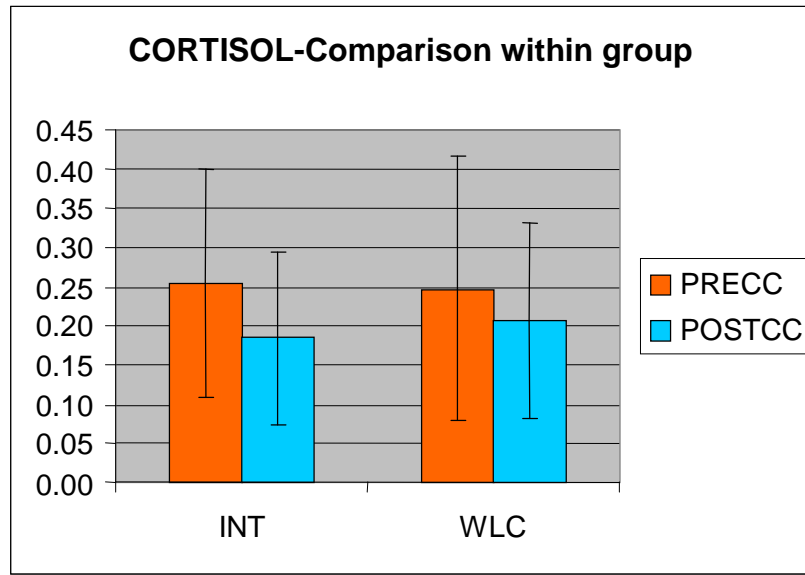
	INTERVENTION (N=32)	CONTROL (N=15)
PRE CC($\mu\text{g/dL}$)	0.25 \pm 0.14	0.24 \pm 0.16
POST CC($\mu\text{g/dL}$)	0.18 \pm 0.11*ns	0.20 \pm 0.12 ^{ns}
Difference between PRE CC – POST CC	28% (P < 0.001)	16.6% (P < 0.190)

Values Mean \pm SD

* P < 0.05 Wilcoxon signed ranks test - pre- compared to post (LY group)

ns - non significant between groups-Mann Whitney test.

Figure5.3. Comparison of Cortisol LY and WLC Groups



5.4. Secretory IgA.

The Data is not normally distributed and hence non-parametric tests were used for analyses of the results.

There was no significant difference between groups post intervention. ($p > 0.6$).

Table 5.4. Secretory IgA– The Group Mean Values \pm Standard Deviations for Laughter Yoga and Waitlisted Control Groups.

	LY group (N=20)	WLC group (N=24)
Pre SIgA	46 \pm 39.6	90.6 \pm 64.3
Post SIgA	35.4 \pm 30.1 ^{ns}	63.1 \pm 43.1 ^{ns}
Change IgA	27.5 \pm 71	10.6 \pm 40.0

ns=non significant(within group and between groups)

5.5. Questionnaires

5.5.1. PANAS

There was significant difference in total positive scores between groups ($p < 0.001$)(independent 't' test). There was also significant difference in total negative scores between groups ($p < 0.004$).(independent t test).

There significant difference in total positive scores with in the LY group ($p < 0.001$). There was also significant difference in total negative scores within LY group ($p < 0.002$).

There was no significant difference in total positive scores within the WLC group ($p > 0.05$)

There was no significant difference in total negative scores within WLC group ($p > 0.8$).

Table 5.5.1. PANAS - The Group Mean Values \pm Standard Deviations for Laughter Yoga and Waitlisted Control Group.

	LAUGHTER YOGA (N=61)	WAITLISTED CONTROL (N=56)
Pre Total Positive	33.15 \pm 10.42	30.79 \pm 9.11
Post Total Positive	38.87 \pm 9.30** §	32.64 \pm 7.69
Pre Total Negative	16.59 \pm 11.93	17.82 \pm 9.86
Post Total Negative	12.07 \pm 9.34*§	17.64 \pm 11.23
Pre-post Total Positive	17.25 %	6.0 %
Pre-Post Total Negative	27.2 %	1.0 %

Values Mean \pm SD

* P < 0.05 Paired 't'-test pre- compared to post

** P < 0.001 Paired 't'-test pre- compared to post

§ P < 0.000 independent 't' test comparing LY group and WLC group

Figure 5.5.1A. Comparison of Pre-post PANAS Scores of LY and WLC Groups

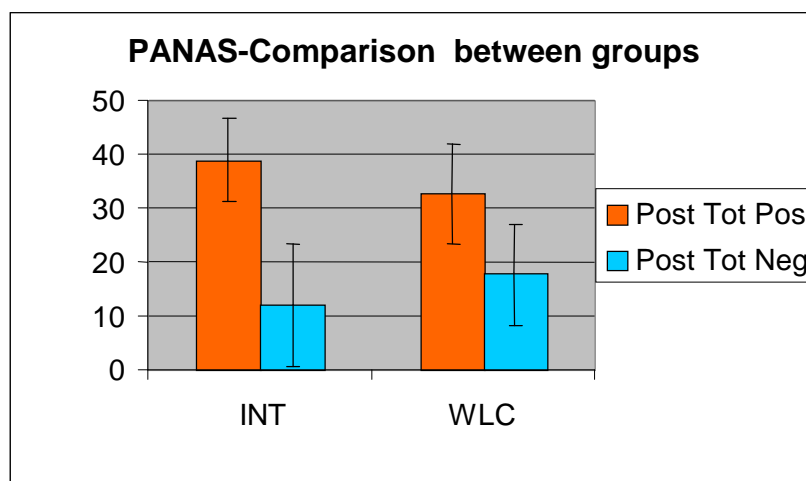
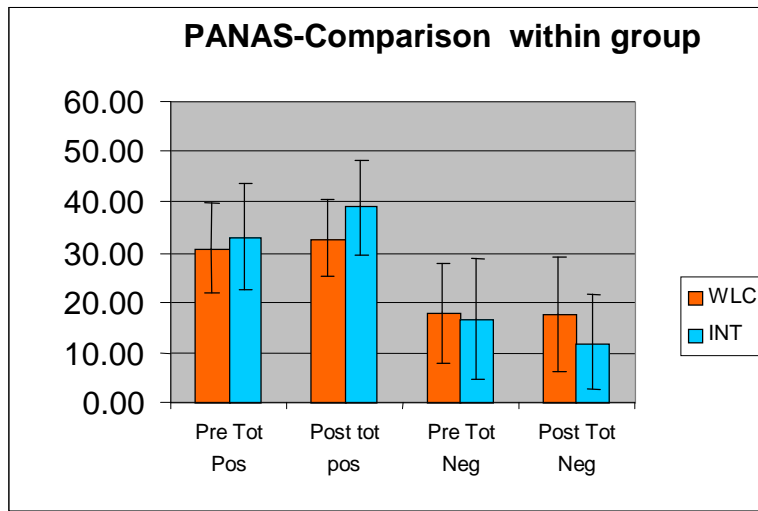


Figure 5.5.1B. Comparison of PANAS within Group of LY and WLC Group



5.5.2. PSS

There was significant difference between the groups (independent 't' test) $p < 0.01$.

There was a significant difference within LY group $p < 0.02$ (paired 't' test)

There was no significant difference within the WLC group ($p > 0.2$).

Table 5.5.2. Perceived Stress Scale (PSS): The Group Mean Values \pm Standard Deviations for Laughter Yoga and Waitlisted Control Group.

	LAUGHTER YOGA (N=61)	WAITLISTED CONTROL (N=57)
Pre PSS	17.79 \pm 6.89	19.33 \pm 5.04
Post PSS	15.80 \pm 5.28 *§	18.14 \pm 5.78

Values Mean \pm SD

* $P < 0.05$ Paired 't'-test pre- compared to post

§ $P < 0.05$ independent sample 't' test comparing LY group and WLC group.

Figure 5.5.2A. Comparison of Pre-post PSS scores of LY and WLC Groups

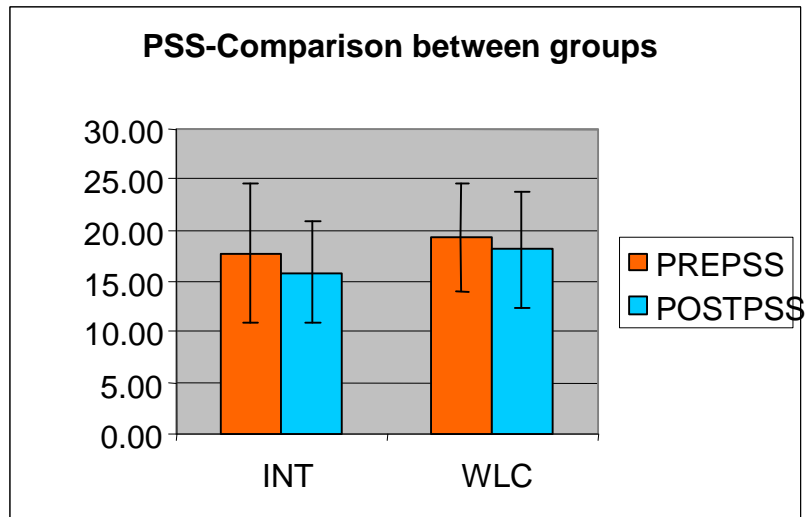
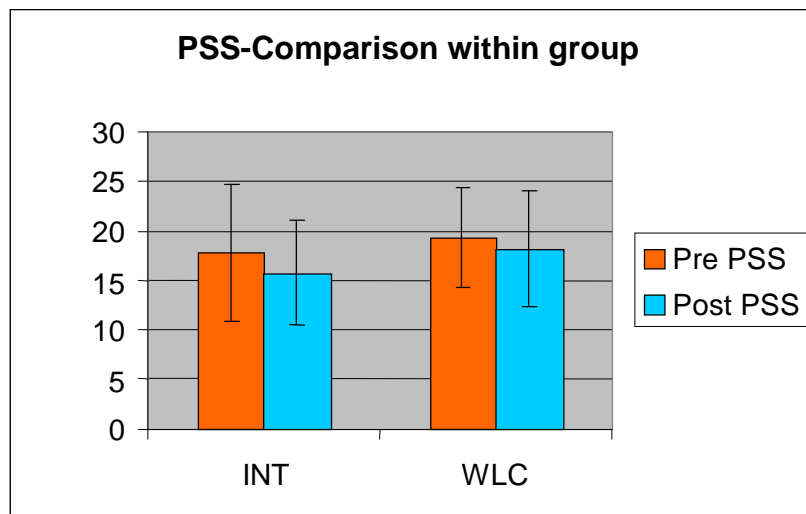


Figure 5.5.2A. Comparison of Pre-post PSS scores of LY and WLC Groups



5.5.3. TAS

There was no significant difference between groups in total factor analysis.

There was significant difference in factor 1 pre post analysis of LY group ($p < 0.001$), but not in factor 2 and factor 3. However, there was significance in total factor paired 't' test analysis in the LY group ($p < 0.02$). There was no significance in the WLC group in total factor paired 't' test. However, there were significant changes in factor 3 in the WLC group.

Table 5.5.3. Toronto Alexithymia Scale (TAS) – The Group Mean Values \pm Standard Deviations for Laughter Yoga and Waitlisted Control Groups.

	LAUGHTER YOGA (N=64)	WAITLISTED CONTROL (N=52)
Pre Total factor	50 \pm13.17	49.25 \pm12.35
Post Total factor	45.58 \pm11.20^{*ns}	49.33 \pm11.36
Pre – Post Total factor	8.84 %	0.16 %

Values Mean \pm SD

* P < 0.05 Paired 't'-test pre- compared to post

ns-Not significant between groups.

Figure 5.5.3A. Comparison of TAS of LY and WLC Groups

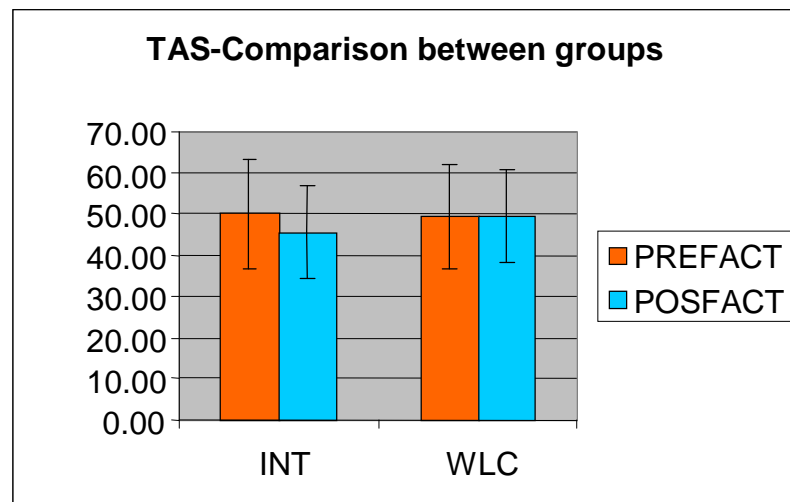
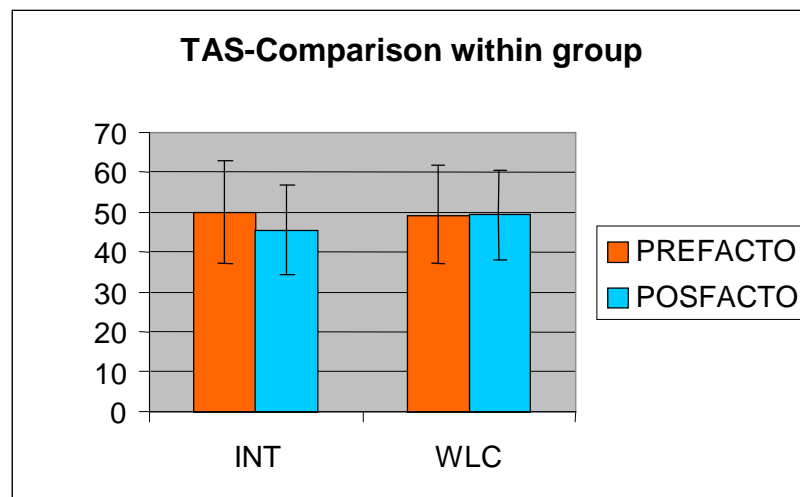


Figure 5.5.3B. Comparison of TAS within the LY and WLC Groups



6 Discussions

6.1. Polygraph Data

HRV

Seven sessions of Laughter Yoga produced no significant changes in the baseline values of the autonomic and respiratory variables. Waitlisted control group when assessed after the same duration showed similar results.

The present study was the first randomized controlled longitudinal study where an attempt was made to study the long term effects of Laughter Yoga on the psycho-physiological and immunological variables in IT professionals.

Earlier studies have shown that laughter induces transient phase of physiological arousal. Paskind (1932) in an earliest study showed that the immediate effect of laughter includes an elevated pulse, heart rate, and blood pressure. Laughter also increased respiratory rate and ventilatory capacity. A recent study by Fry and others (1998) also suggested similar results i.e., increase in ventilation and acceleration in the exchange of residual air, and increase in blood oxygen levels.

A subsequent study (Sakuragi et al. 2002) evaluated the effects of laughing and weeping on the mood and Heart rate variability on ten healthy female subjects, before, during and after watching a comedy or a tragedy video. The results suggested that watching a comedy video produces autonomic arousal (increased LF/HF ratio, suggesting a shift in the sympatho-vagal balance towards sympathetic dominance). These results were very transient, as the LF/HF ratio had come back to normal soon after they stopped watching the comedy video.

It is very clear from the above studies that laughter brings about a transient arousal in the autonomic activity as an immediate effect.

In the present study while no attempt was made to study the immediate effect of Laughter Yoga, the long-term effects were not clear. Similar attempt was made in an earlier study by White and Camarena (1989). The participants were 65 female and 28 male healthy volunteers who were randomly assigned to a laughter treatment group, a relaxation-training group, or a health-education control group. The influence of 6-week laughter intervention on diastolic (DBP) and systolic (SBP) blood pressure and heart rate (HR) was evaluated. The results showed no significant pre-post session changes in DBP, SBP, or HR among participants in the Laughter group and no differences between the laughter and health-education control groups. In contrast, the relaxation training group showed significantly lower post-session HR and SBP in comparison with both the other groups.

The results of the present study are in line with the earlier study mentioned above. The reasons behind no significant changes observed in the present study can be attributed to:

- i) The duration of intervention (seven sessions of twenty minutes each) was not enough. Probably it takes longer to bring about changes in the baseline values using Laughter Yoga as an intervention.
- ii) The sessions were not given continuously for seven days. It was either on alternate days or in some cases even after three days.

Since it was speculated earlier that laughter produces only transient changes in autonomic variables (Sakuragi et al. 2002), the results of the present study adds useful

information that to produce longitudinal changes either the duration of each session and also the number of sessions has to be increased.

6.2. Blood Pressure

It may be observed the Laughter Yoga had a significant positive effect on the systolic and diastolic blood pressure. It is evident from the basal data and many of the IT professionals had a systolic >120mm/hg and diastolic > 89 mm/hg.

Similar attempt was made in an earlier study by White and Camarena (1989). The participants were 65 female and 28 male healthy volunteers who were randomly assigned to a laughter treatment group, a relaxation-training group, or a health-education control group. The influence of 6-week laughter intervention on diastolic (DBP) and systolic (SBP) blood pressure and heart rate (HR) was evaluated. The results showed no significant pre-post session changes in DBP, SBP, or HR among participants in the laughter group and no differences between the laughter and health-education control groups. In contrast, the relaxation training group showed significantly lower post-session HR and SBP in comparison with both of the other groups. But in our study we found that both systolic and diastolic pressure reduced in LY group in comparison within group (pre- post), but no change was found in the WLC group. We also found significant difference between the groups in systolic blood pressure but not in diastolic pressure

Here again we may point out that the intervention was enough to reduce the systolic pressure and the duration (7 sessions for 20 minute) was not enough to make a significant change in diastolic pressure between the groups.

Subjects from the IT industry known for work stress showed a high basal blood pressure. But now it was clear that intervention like Laughter Yoga certainly helps bring down both the systolic and diastolic pressure by bringing down the stress levels. Such interventions should form a part of daily routine for the employees to improve their health conditions.

6.3. Cortisol

Cortisol is an important hormone in the body secreted by the adrenal glands and involved in the following functions and more:

- Proper glucose metabolism
- Regulation of blood pressure
- Insulin release for blood sugar maintenance
- Immune function
- Inflammatory response

Normally, it's present in the body at higher levels in the morning and at lower levels at night. Although stress isn't the only reason that cortisol is secreted into the bloodstream, it has been termed 'the stress hormone' because it's also secreted in higher levels during the body's 'fight or flight' response to stress and is responsible for several stress-related changes in the body.

While cortisol is an important and helpful part of the body's response to stress, it's important that the body's relaxation response be activated to help the body functions return to normal. Unfortunately, in our current high-stress culture, the body's stress response is activated so often that functioning often doesn't have a chance to return to normal thus producing chronic stress.

Higher and more prolonged levels of cortisol in the bloodstream (like those associated with chronic stress) have been shown to have negative effects, such as: Impaired cognitive performance, suppressed thyroid function, blood sugar imbalances such as hyperglycemia, decreased bone density etc .To keep cortisol levels healthy and under control, the body's relaxation response should be activated after the 'fight' or 'flight' response occurs. LY is one such technique that has a positive effect on emotions and stress. Usually the morning cortisol levels are higher but by night they decrease. The reduction of cortisol levels in the morning as compared to the basal level is as indicator of reduced stress.

The results show that the data is not normally distributed and hence non-parametric tests were done to measure the significance levels.

The results show that there was significant reduction of morning cortisol levels ($p < 0.001$) in the LY group, whereas there was no significant change in the WLC group ($p > 0.05$). However there was no significant difference between the groups. This may be due to the fact that the number was less in the WLC group who gave both pre and post samples. Overall though there seems to be a decrease in salivary cortisol and increase in secretory IgA in Yoga group, the changes were not significant between the groups.

A recent study evaluating the effects of Sudarshan Kriya (A specific Yoga technique) Yoga in alcohol addicts has shown a larger effects size with intervention for reductions in serum cortisol ($ES = 0.86$). This could be probably because of a longer duration of intervention and a different type of intervention that focused mainly on breathing. (Vedamurthachar A et al. 2006). Secondly we had inadequate sample size and Post Hoc power analysis showed that our sample had only 37% power to demonstrate change in cortisol. Though there is a trend for decrease in salivary cortisol a larger sample size and more robust intervention over a longer period could facilitate significant changes in cortisol measures between the groups. The reduced levels of cortisol in the LY group which is significant shows the effect of LY intervention in bring down the stress levels which also correlates with other stress parameters wherein we have good significance in the levels of perceived stress. It is possible that the 7 sessions were not enough to bring about changes between the groups in cortisol. Also the 20-minute session may not be enough to bring about long-term effect on the levels of cortisol.

6.4. Secretory IgA

There was no significant difference in SIgA levels in the LY or the WLC groups. This may be due to the fact that the sample size was very low in the WLC group. Similarly Post Hoc power analysis showed that our sample had only 17.8% power to demonstrate change in SIgA. The fact that some subjects had high levels of SIgA predicts the confounding effects of sub clinical infections, allergy or inflammatory conditions that can elevate SIgA levels. This could probably have confounded our results. Adequate screening with skin patch tests to rule out allergies, ESR and hsCRP (highly sensitive C-reactive protein) to rule out inflammatory conditions are necessary as a screening measure. Mere physical examination would not suffice.

6.5. Questionnaires

6.5.1. PANAS

Positive Affect (PA) and Negative Affect (NA)—have emerged reliably as the dominant dimensions of emotional experience. These factors have been identified in both intra and inter individual analyses. They emerge consistently across diverse descriptor sets, time frames, response formats, languages, and cultures. To measure these factors, Watson, Clark, and Tellegen (1988)

developed the Positive and Negative Affect Schedule (PANAS), which consists of two 10-item scales for PA and NA respectively.

In this model, the higher level reflects the **valence** of the mood descriptors (i.e., whether they represent negative or positive states), whereas the lower level reflects their specific **content** (i.e., the distinctive qualities of the individual affects).

1) Fear, Sadness, Guilt, and Hostility scales are classified as **Basic Negative Emotion Scales**. These scales are consistently and substantially inter correlated, and therefore, are strong and clear markers of the higher order Negative Affect dimension (Watson & Clark, 1989, 1992).

2) Joviality, Self- Assurance and Attentiveness are classified as **Basic Positive Emotion Scales**. These scales are also highly correlated with one another, and so are strong and consistent markers of the second-order Positive Affect dimension.

3) Shyness, Fatigue, Surprise and Serenity are grouped as **Other Affective States** because they do not strongly or consistently define either the first or the second-order factors. Shyness tends to load moderately to strongly on Negative Affect (see Table 18), but its loading generally is somewhat lower than those of Fear, Sadness, Guilt, and Hostility. That is, Shyness appears to be less strongly saturated with general Negative Affect variance than are the other scales (see also Watson & Clark, 1989). In contrast, Surprise typically has moderate positive loadings on both higher order factors. Fatigue and Serenity also tend to load significantly on both general factors: Fatigue is a marker of high Negative, low Positive Affect, whereas Serenity is a marker of low Negative, high Positive Affect.

Laughter Yoga increased the total positive affect ($p < 0.000$) and reduced the total negative affect ($p < 0.004$) compared to the WLC group. Laughter itself is a positive emotion that increases the positive affect. Since they do it in groups it invokes strong positive emotions like excitement, happiness, interest and attention. Within the LY group also there is significance in the LY group ($p < 0.000$) in total positive affect (nearly 18% increase in positive emotions) and negative emotions like being afraid, distressed, jittery, guilty, upset, nervous and hostile have decreased by nearly 28%. This clearly shows the effect of laughter on increasing the positive emotions and decreasing the negative ones. The PANAS and the Profiles of mood scale (POMS) scores are highly correlated and have similar questions.

Takahashi et al. (2001) found that watching a comedy film significantly increased the NK cell activity. Correlation for experiential and expressive aspects to NKCA showed that NK cell activity showed correlation with self rated pleasantness and mood scales of POMS. They also conclude that the elevation was more related to experiential aspects rather than expressive aspects. Our study also shows that the increase in positive emotions and a decrease in negative emotions will have a positive effect on the psychological, physiological and immunological aspects of the subject bringing in health and well being.

6.5.2. Perceived Stress Scale (PSS)

The 10 and 14-item self-report instruments have established reliability and validity ($r = 0.85$). (Cohen et.al.1983).

Perceived Stress Scale (PSS) is an economical and simple psychological instrument to administer, comprehend, and score the degree to which situations

in one's life over the past month are appraised as stressful. Items were designed to detect how unpredictable, uncontrollable, and overloaded respondents found their lives. It poses general queries about relatively current levels of stress experienced. PSS scores are obtained by reversing the scores on the four positive items, e.g., 0=4, 1=3, 2=2, etc. and then summing across all 10 items.

The results show that the perceived stress levels drastically reduced after the intervention ($p < 0.02$) in the LY group. They felt less stressful and were able to feel the reduction stress. There was no reduction perceived stress in the WLC group ($p > 0.2$) There was also a significant difference between the groups (independent t test) ($p < 0.01$). This is the first time the PSS 10 item questionnaire is being used for research on Laughter Yoga. This study shows the stress reducing effect of LY. Stress by itself has a negative effect on the health thus the reduction in stress leads to positive health and well being.

6.5.3. Toronto Alexithymia Scale (TAS)

Toronto Alexithymia Scale (TAS) describes people who appeared to have deficiencies in understanding, processing or describing their emotions and comprises 20 questions. Deficiencies are grouped as difficulty in identifying feelings (Factor 1; questions 1, 3, 6, 7, 9, 13, 14), difficulty in describing feelings (Factor 2; questions 2, 4, 11, 12, 17) and externally oriented thinking (Factor 3; questions 5, 8, 10, 15, 16, 18, 19, 20). TAS scores were obtained by reversing the scores of question 4, 5, 10, 18 and 19. Sum of all factors were analyzed along with total factor (Factor 1+Factor 2+Factor 3).

The results show that LY group showed significant decrease in the Alexithymia scales ($p < 0.02$) where as in the WLC group there was no difference. It is also interesting to note that the LY group improved in Factor 1 (identifying the feelings) ($p < 0.001$). Factor wise the WLC group showed significance in externally oriented thinking ($p < 0.03$).

However, it is important to note here that neither the LY group nor the WLC Group had the Alexithymia at base line (those who had score above 61 is identified as having high Alexithymia, and those whose scores are below 51 is rated as having Low Alexithymia.). It is probable that since they are IT professionals having high confidence levels they may not have baseline Alexithymia.

7. Summary and Conclusions

The main objective of the study was to measure the physiological, psychological and immunological benefits of Laughter Yoga.

There is tremendous awareness about the positive effects created by laughter and thereby happiness. Health care providers are getting more and more conscious of the benefits of laughter. Laughter clubs are mushrooming in major cities and every morning thousands of people especially the elderly can be seen laughing and exercising in order to improve their physical and mental health and generate a feeling of total well being. Though clubs promote laughter as being the best medicine there is little literature on the effects on physiological, psychological and immunological parameters. Few studies that have been published and are available have methodological problems.

So far most of the studies done on laughter used a humor model to induce cognitive laughter by watching comic videos. Results varied but some showed that laughter improved

NK cell activity, reduced blood pressure and reduced pain; increased blood oxygen levels released muscle tension etc.

This study was planned specially to see the effect of Laughter Yoga on the physiological psychological and immunological parameters. To control the confounding variables a randomized trial was planned. Seven sessions of Laughter Yoga were given on alternate days. There was about 40 % dropout that was well anticipated as the IT industry is one of the busiest and the employees frequently had meetings or perhaps had to travel, etc.

The results clearly suggest that Laughter Yoga has physiological, psychological and immunological benefits. Blood pressure decreased, early morning cortisol decreased indicating reduced levels of stress, less perception of stress as seen by Perceived Stress Scales, more positive affects and less negative affects were found in those attending the Laughter Yoga sessions. However, these changes were not found in the Waitlisted control group. The heart parameter showed no significant difference though there was a trend of reduction of HR after the Laughter Yoga sessions which was not found in the Waitlisted control group. This may be due to the fact that it takes a longer period of training to bring down the effect on the heart. Seven sessions might not be sufficient to make a marked difference in heart rate and the other autonomic variables like LF, HF and LF/HF ratio and the breath rate.

As hypothesized the basal blood pressure was high in the IT company employees, so also the stress parameters as seen from the questionnaires. After the intervention we did see lot of improvement in these parameters including the levels of cortisol.

The present study has broad implications for the psychologists, physiologists, educators and health professionals and Laughter Yoga teachers. This helps them formulate appropriate relaxation programs. This is the first randomized control trial on Laughter Yoga and the results are very encouraging. Further the relaxation component and the reduction of stress and the positive emotions brought out by LY sessions have tremendous implications for stress/health related ailments/management programs.

Apart from being therapeutic and reducing stress, LY has even more application for normal healthy people as a preventive measure for all physical and mental health problems.

Those who practice regularly report improved mental abilities with respect to their personal and professional endeavors, increased sensitivity and behavioral response and a general sense of joy and well being while enjoying life to the fullest.

References

1. Andrew Newberg, Eugene D'aquili and Vince Rause. (2002). *Why God Won't Go Away*. New York: Ballantine Publishing Company.
2. Argyle M. (1997). *Is happiness a cause of Health?* Psychology and Health, 12,769-781.
3. Bell, N. J., McGhee, P. E., & Duffey, N. S. (1986). *Interpersonal Competence, Social Assertiveness and the Development of Humor*. British Journal of Developmental Psychology, 4, 51-55.
4. Berk L.S, Felten DL, Tan SA, Bittman BB and Westengard J.2001. *Modulation of Neuro Immune Parameters During the Eustress of Humor–Associated Mirthful Laughter*. Alternative Therapy Health Med 7(2):62-76).
5. Berk, L. S., Tan, S. A., & Fry, W. F. (1993). *Eustress of Humor Associated Laughter Modulates Specific Immune System Components*. Annals of Behavioral Medicine, 15, Supplement, S111.
6. Berk, L. S., Tan, S. A., Fry, W. F., Napier, B. J., Lee, J. W., Hubbard, R. W., Lewis, J. E., & Eby, W. C. (1989). *Neuro Endocrine and Stress Hormone Changes During Mirthful Laughter*. American Journal of the Medical Sciences, 298, 390-396.
7. Berk, L. S., Tan, S. A., Napier, B. J., & Eby, W. C. (1989). *Eustress of Mirthful Laughter Modifies Natural Killer Cell Activity*. Clinical Research, 37, 115A.
8. Berk, L.S., Tan, S.A., Nehlsen-Cannarella, S.L., Napier, B.J., Lewis, J.E., Lee, J.W., Eby, W.C., & Fry, W.F. (1988). *Humor Associated Laughter Decreases Cortisol and Increases Spontaneous Lymphocyte Blastogenesis*. Clinical Research, 36: 435A.
9. Boone T, Hansen S, Erlandson A. (2000). *Cardiovascular Responses to Laughter: a Pilot Project*. Applied Nursing Research Nov; 13(4): 204-8.
10. Cogan, R., Cogan, D., Waltz, W., & McCue, M. (1987). *Effects of Laughter and Relaxation on Discomfort Thresholds*. Journal of Behavioral Medicine, 10, 139-144.
11. Cohen S, Kamarck T, Mermelstein R. *A Global Measure of Perceived Stress*. Journal of Health Social Behavior 1983; 24:385–96.
12. Cousins, N. (1976). *Anatomy of an illness (as Perceived by the Patient)*. New England Journal of Medicine, 295, 1458-1463.
13. David Watson and Lee Anna Clark (1994) *The PANAS-X Manual for the Positive and Negative Affect Schedule - Expanded Form*. The University of Iowa.
14. Dantzer, R., & Mormede, P. (1995). *Psycho Neuro Immunology of Stress*. In B. E. Leonard & K. Miller (Eds.), *Stress, the Immune System and Psychiatry* (pp. 47-67). Chichester: John Wiley and Sons.
15. Dillon, K. M. & Totten, M. C. (1989). *Psychological Factors, Immuno Competence, and Health of Breast-Feeding Mothers and Their Infants*. Journal of Genetic Psychology, 150, 155-162.
16. Dillon, K. M., Minchoff, B., & Baker, K. H. (1985). *Positive Emotional States and Enhancement of the Immune System*. International Journal of Psychiatry in Medicine, 15: 13-17.
17. Dishman R.K., Nakamura Y, Garcia M.E., Thomson R.W., Dunn A.L., Blair S.N.,(2000) *Heart Rate Variability, Trait Anxiety, and Perceived Stress among Physically Fit Men and Women*. International Journal Psychophysiology 37:121-133.

18. Edwards J. R, Cooper CL. (1988). *The Impact of Positive Psychological States on Physical Health: A Review and Theoretical Framework*. Social Science and Medicine, 27,1447-1459.
19. Filoppelli M, Pellegrino R, Landelli I, Misuri G, Rodarte J. R., Duranti R, Vito Brusasco, Scano G.(2001). *Respiratory Dynamics During Laughter*. Journal of Applied Physiology 90, 1441-1446.
20. Forabosco, G. (1992). *Cognitive Aspects of the Humor Process: The Concept of Incongruity*. Humor: International Journal of Humor Research, 5, 45-68.
21. Fried I, Wilson CL, MacDonald KA, Behnke EJ. (1998) *Electric Current Stimulates Laughter* (letter). Nature 392(6668): 650(1998).
22. Friedman BH, Thayer JF (1998). *Autonomic Balance Revisited: Panic Anxiety and Heart Rate Variability*. Journal of Psychosomatic Research 44: 131-151.
23. Friedman BH, Thayer J.F. (1998). *Anxiety and Autonomic Flexibility: a Cardiovascular Approach*, Biological Psychology 49:303-323.
24. Fry William F., Jr. (1992) *The Physiologic Effects of Humor, Mirth, and Laughter*. JAMA 267(13):1857.
25. Fry, W. F. (1977). *The Respiratory Components of Mirthful Laughter*. Journal of Biological Psychology, 19: 39-50.
26. Fry, W. F. (1994). *The Biology of Humor*. Humor: International Journal of Humor Research, 7:111-126.
27. Hayashi K, Hayashi T, Iwanaga S, Kawai K, Ishi H, Shiji S, Murakami K.(2003). *Laughter Lowered the Increase in Postprandial Blood Glucose*. Diabetic care, 26(11):1651-1652.
28. Herzog H, Lele VR, Kuwert T, Langen KJ, Kops ER, Feinendegen LE (1990). *Changed Pattern of Regional Glucose Metabolism during Yoga Meditative Relaxation*. Neuro psychobiology, 23(4): 182-187.
29. Hubert, W., & de Jong-Meyer, R. (1991). *Autonomic, Neuroendocrine, and Subjective Responses to Emotion-Inducing Film Stimuli*. International Journal of Psychophysiology, 11, 131-140.
30. Hubert, W., Moller, M., & de Jong-Meyer, R. (1993). *Film-Induced Amusement Changes in Saliva Cortisol Levels*. Psycho neuro-endocrinology, 18, 265-272.
31. Kataria, M., Wilson, S., & Buxman, K. (1999, June). *Where East Meets West: Laughter Therapy*. Workshop presented at the annual conference of the International Society for Humor Studies, Oakland, CA.
32. Lambert, R. B., & Lambert, N. K. (1995). *The Effects of Humor on Secretory Immunoglobulin A Levels in School-Aged Children*. Pediatric Nursing, 21, 16-19.
33. Lefcourt, H. M., Davidson-Katz, K., & Kueneman, K. (1990). *Humor and Immune-System Functioning*. Humor, 3, 305-321.
34. Marci C D, Moran E K, Orr S P.(2004). *Physiologic Evidence for the Interpersonal Role of Laughter During Psychotherapy*. The Journal of Nervous and Mental Disease 2004;192: 689–695).
35. Martin, R. A., & Kuiper, N. A. (1999). *Daily Occurrence of Laughter: Relationships with Age, Gender, and Type A Personality*. Humor: International Journal of Humor Research, 12, 355-384.

36. Masten, A. S. (1986). *Humor and Competence in School-Aged Children*. Child Development, 57, 461-473.
37. McClelland, D. C., & Cheriff, A. D. (1997). *The Immunoenhancing Effects of Humor on Secretory Iga And Resistance To Respiratory Infections*. Psychology and Health, 12, 329-344.
38. McCraty R, Atkinson M, Tomasino D, Goelitz J, Mayrovitz HN. (1999) *The Impact of an Emotional Self Management Skills Course on Psychological Functioning and Autonomic Recovery to Stress in Middle School Children*. Integr Physiol Behav sci 34:248-268.
39. Murstein, B. I., & Brust, R. G. (1985). *Humor and Interpersonal Attraction*. Journal of Personality Assessment, 49, 637-640.
40. Nakajima A, Hirai H, Yoshino S. (1999). *Reassessment of Mirthful Laughter in Rheumatoid Arthritis*. Journal of rheumatology 26(2):512-513.
41. Nevo, O., Keinan, G., & Teshimovsky-Arditi, M. (1993). *Humor and Pain Tolerance*. Humor: International Journal of Humor Research, 6, 71-88.
42. Paskind, H.A. *Effects of Laughter on Muscle Tone*. Arch Neurol Psychiatry 28(28): 623-628 (1932).
43. Piccirillo G, Elvira S, Bucca C, Viola E, Cacciafesta M, Marigliano V. (1997) *Abnormal Passive Head up Tilt Test in Subjects with Symptoms of Anxiety Power Spectral Analysis Study of Heart Rate and Blood Pressure*. International Journal of Cardiology 60: 121-131.
44. Pine DS, Wasserman GA, Miller L, Coplan JD, Bagiella E, Kovelenu P, Myers M M, Sloan RP. (1998) *Heart Rate Variability and Psychopathology in Urban Boys at risk for Delinquency*. Psychophysiology.35: 521-529.
45. Provine, R. R., & Yong, Y. L. (1991). *Laughter: A Stereotyped Human Vocalization*. Ethology, 89, 115-124.
46. Ruch, W. (1993). *Exhilaration and Humor*. In M. Lewis & J. M. Haviland (Eds.), Handbook of Emotions (pp. 605-616). New York: Guilford Publications.
47. Saghaei M. (2004). *Random Allocation Software*. Version 1.0, Isafahan University of Medical Sciences, Isafahan, Iran.
48. Sakuragi S, Sugiyama Y, Takeuchi K. (2002). *Effects of Laughing and Weeping on mood and Heart Rate Variability*. J Physiol Anthropol 21(3):159-165.
49. Stearns F.R. *Laughing Physiology, Pathophysiology, Psychology, Pathopsychology and Development*. Springfield, Ill: Charles C. Thomas; 1972.
50. Takahashi k, Iwase M, Yamashita K, Tatsumoto Y, Ue H, Kuratsune H, Shimizu A and Takeda M. (2001). *The Elevation of Natural Killer Cell Activity Induced by Laughter in a Crossover-Designed Study*. International Journal of Molecular Medicine 8: 645-650.
51. Watson, D., & Clark, L. A. (1992). *Affects Separable and Inseparable: On the Hierarchical Arrangement of the Negative Affects*. Journal of Personality and Social Psychology, 62, 489-505.
52. White, S., & Camarena, P. (1989). *Laughter as a Stress Reducer in Small Groups*. Humor: International Journal of Humor Research, 2, 73-79.
53. Zand, J., Spreen, A. N., & LaValle, J. B. (1999). *Smart Medicine for Healthier Living*. Garden City Park, NY: Avery Publishing.

9. Photographs

Laughter Yoga Research Project Team



Sitting Left –Right: 1.Sri Srikante Gowda. 2. Dr. Manjunath N.K. 3 Dr. Sudha Rani.
4. Dr. Chaya M S (principle investigator). 5. Dr. Vadiraj. 6. Dr. Raghavendra Rao.M
Standing: 1. Sri Rajesh. 2. Kum. Sneha Shekar 3.Sri Pavan Kumar. 4. Sri.Gopal Krishna D.

I Felx Solutions- IT company that participated



Software professionals filling up questionnaires



Polygraph Data being taken



Blood sample being taken



Polygraph recordings



Laughter Yoga session in progress



Dr. Vadiraj taking a blood sample



Dr. Sudha checking blood pressure



Dr. Manjunath measuring HRV



Dr. Kataria leading a LY session



Dr. Vadiraj taking a blood sample



Laughter Yoga session at software company Mphasis



Mobile phone laughter



Dr. Kataria leads LY session at KYOCERA

