DOES PRACTICING TRANSCENDENTAL MEDITATION HELPS IN HEALTHY AGING? A PILOT STUDY ON AFRICAN AMERICAN HYPERTENSIVE PATIENT

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INTRODUCTION:

EARLY STUDIES ON HUMAN STRESS:

Stresses occupy a central role in determining human health and longevity. The physiological/ psychological basis of the stress response involves the interaction among the brain, mind, body and cellular behavior, and has influences on healthy human aging process.

Stress is epidemic in modern life. According to an associated press poll conducted in November 2006 roughly 75% of people in USA, Australia, Canada, France, Germany, Italy, South Korea and UK said that they experience stress daily. (Luca)

Human stress has been observed not only in these modern life-times but centuries back. Celsus described stress as part of the repertoire of the inflammatory response approximately 2 million years ago. The pioneering work of Seyle-Selye in 1936, reported that rats that had been exposed to stress had enlarged adrenal glands and the thymus and lymph nodes are significantly decreased in size. Important discovery followed in the late 1940's with the observation that cortisol that was released by the adrenal glands suppressed immune system organs. In Today's scientific world, cortisol has been termed “the stress hormone” because it is 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 (Luca).
According to Selye, stress pertains to a state produced within an organism subject to a stimulus perceived as a threat (stressor). Selye actually described stress as "a common denominator underlying all adaptive responses within the body". Short time stress is called as 'acute stress' and long time stress are called as 'chronic stress'. According to Selye's stress theory of disease, there are three stages of the common disease process and he called them as general adaptation syndrome model (GAS model).

**Alarm** is the first stage. It is also referred to as the fight-flight reaction. The fight-flight response is intimately associated with an acute disease stage. A stressor causes an initial activation of the body's defense mechanism. What ensues is a complex physiological response involving several interacting systems within the body. The alarm reaction is basically characterized by the release of adrenal medullary and cortical hormones into the bloodstream. The body recognizes that it is under attack. It therefore proceeds to set into motion its first line of defense, the sympathetic nervous system response. The effect of an accelerated sympathetic nervous system response is to increase the rate of metabolism. To overcome the stressor, the body requires an increase in energy output, which is generated by increasing the activity of the thyroid and adrenal glands. Increasing one's energy level is the body's first line of defense.

**Resistance** is the second stage. If the stressor persists, it becomes necessary to attempt some means of coping with the stress. Although the body begins to try to adapt to the strains or
demands of the environment, the body cannot keep this up indefinitely, so its resources are gradually depleted.

**Exhaustion** is the third and final stage in the GAS model. At this point, all of the body's resources are eventually depleted and the body is unable to maintain normal function. The initial autonomic nervous system symptoms may reappear (sweating, raised heart rate etc.). If stage three is extended, long term damage may result as the body, and the immune system is exhausted and function is impaired..(Wilson)

The result can manifest itself in obvious illnesses such as ulcer, anxiety, post-traumatic stress disorder (PTSD), depression, diabetes, and hypertension, trouble with the digestive system or even cardiovascular problems, along with other mental illnesses.

**STRESS AND HYPERTENSION:**

Stress has long been listed as a potential and important cause of hypertension among other potential risk factors such as low potassium consumption, low physical activity and sleep abnormalities (Sparrenberger.F, 2009). Hypertension or high blood pressure is also one of the major risk factors for cardiovascular complication and death (Aviv.A, 2009). The interaction between a genetic background and environmental and behavioral exposures, such as the excess of salt, fat and alcohol consumption, accounts for most but not all cases of hypertension.

Hypertension is a public health concern due to its magnitude, risks, difficulty in management, high medical and social costs and severe cardiovascular and renal complications. The number of deaths due to hypertension as primary cause was estimated to be over 7 million in 2002, approximately 13% of all reported deaths. Hypertensive adults will reach 1.5 billion by 2025, around 30% of the world population (Gasperin.D, 2009). According to the seventh report of the
Joint National Committee (JNC) on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, the prevalence of prehypertension or hypertension was higher in African American population than whites (Wang.Y, 2004). Moreover, age and gender-specific mortality rates for African Americans are 50% higher than for white Americans. Disproportionately high rates of hypertension contribute to the excess rates of mortality and morbidity from cardiovascular and renal disease in this group. In African Americans it has a higher prevalence, incidence and severity; earliest onset; more target-organ damage; and is generally treated later and less adequately than in white Americans. Furthermore, more than 70% of elderly African Americans are hypertensive and hypertensive disease cause four to seven times more mortality than in older white Americans. For these reasons hypertension has been considered the number one health problem among adult African Americans (Schneider.R.H S. A., 1995).

Many studies have demonstrated that for middle-aged men and women, blacks have higher mean blood pressure and prevalence of hypertension than whites. For example, data from the second National Health and Nutrition Examination Survey indicated that the prevalence rates of definite hypertension (systolic blood pressure [SBP] 160 mm Hg, diastolic blood pressure [DBP] 95 mm Hg and/or taking antihypertensive medication) in persons aged 55 to 64 years were 60% and 46% for black women and men and 34% and 31% for white women and men, respectively (Liu.K, 1996). In addition, the presence of glucose intolerance and diabetes increases the cardiovascular disease (CVD) outcomes in hypertensive African Americans. Together with obesity and type 2 diabetes, hypertension significantly contributes to the two or four times higher (CVD) mortality and morbidity rates in African American than Caucasians. The Coronary Artery Risk Development in (Young) Adults Study, a biracial group of Black and White children and
young adults reported higher prevalence of hypertension at screening for Blacks at all age groups when compared with their Caucasian counterparts (Gaillard.T, 2009). A 10-year follow-up of this same cohort found that the prevalence rate of hypertension continued to be significantly higher in African American children and young adults when compared with their Caucasian counterparts for all age groups (Dyer.A.R, 1999). There also evidence that disproportionately high levels of psycho-social and environmental stress are associated with increased sympathetic tone, cardiovascular reactivity, peripheral vasoconstriction, and renally mediated sodium retention. It may be the cumulative effects of stress also contribute to the age-related increases in BP in African Americans that are not observed in traditional African societies (Schneider.R.H S. A., 1995).

**SOLUTION TO THE STRESS AND STRESS-ASSOCIATED DISEASES:**

If we look for the root cause of stress and health problem, we find that environmental and behavioral contributions are by far the most important. According to the U.S. Surgeon general, the majority of disease is self-induced and as much as 80% of medical problems can be prevented through behavioral or lifestyle change. In 1990 the U.S Department of health and Human services published an exhaustive analysis of research on prevention, concluding that “better control of fewer than 10 health risk factors- for example poor diet infrequent exercise, use of tobacco and drugs, and abuse of alcohol- could prevent between 40-70% of all premature deaths, as well as a third of all cases of acute disability and two-third of all cases of chronic disability” (Fagan.J, 1995.). A study confirmed that approximately 50% of all the deaths in the U.S are premature and preventable. These premature deaths are due to the same behavioral and environmental factors mentioned above (Ali H. Mokdad, 2004). Moreover, the seventh report of
the Joint National Committee (JNC) on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure recommends life style modifications for all patients with prehypertension or hypertension (Wang.Y, 2004).

Lifestyle modification, previously termed non pharmacological therapy has important roles in hypertensive as well as non hypertensive individuals. In hypertensive individuals, lifestyle modifications can serve as initial treatment before the start of drug therapy and as an adjunct to medication in persons already on drug therapy. In hypertensive individuals with medication controlled BP, these therapies can facilitate drug step-down and drug withdrawal in highly motivated individuals who achieve sustain lifestyle changes. In non hypertensive, lifestyle modifications have the potential to prevent hypertension, and more broadly to reduce BP (blood pressure) and thereby lower the risk of BP related clinical complications in whole population (Rainforth.M, 2007). It has been suggested that life style changes can be brought by therapies such as relaxation, meditation, or biofeedback may help patients to reduce the effects of stress by reducing physiological arousal and restoring automatic balance and thereby reducing blood pressure (Appel.L.J, 2003).

**STRESS REDUCING TECHNIQUES:**

Stress reducing technique can be divided into primary physical and primary mental.

Deep relaxation categories of methods includes

**PRIMARY PHYSICAL**

- Breathing
- Progressive muscle relaxation
• Yoga stretching
• Biofeedback-require equipment

**PRIMARY MENTAL**

• Autogenic suggestion- a special sequence of self hypothesis
• Hypnosis and self hypnosis
• Imagery and relaxing self talk
• Concentratrative - one simple stimulus or mantra
• Mindfulness    - quiet observation with detachment (Smith JE, 2005).

**RESEARCH EVIDENCE ON RELAXATION RESPONSE (RR) TECHNIQUE:**

With regard to RR techniques, we find a significant number of studies pointing to the usefulness of the RR as a treatment option in hypertension. In particular yoga, Buddhist meditation, Transcendental Meditation (TM), progressive muscle relaxation (PMR), and autogenic training (AT) have been shown to be effective.

TM is a well structured relaxation technique that is associated with the Maharishi Vedic Approach to Health. In this context, TM has been demonstrated to have a beneficial impact upon cardiovascular functioning (including blood pressure regulation) at rest and during laboratory stress in adolescents at risk for hypertension. Additionally, TM may reduce an overall (elevated) cardiovascular risk and decrease carotid atherosclerosis. In a recent long-term study, TM has been demonstrated to increase exercise tolerance, maximal workload, to delay the onset of ST-segment depression. (Tobias Esch, 2003).
TRANSCENDENTAL MEDITATION:

Meditation practices aim to cultivate a state of inner awareness and calm. The most widely researched form of meditation is the Transcendental Meditation ™ technique, which was introduced to the west by Maharishi Mahesh Yogi, a scholar of the Vedic tradition of India. It is simple, psycho physiological procedure practiced for 20 minutes twice a day (Rainforth.M, 2007). It is unique and effortless process of taking the attention to successively finer states of a thought, until thought is transcended and the mind experiences pure awareness (Fagan.J, 1995). During the practice, a reduction in mental and physical activity occurs as a result of experience of a state called “transcendental consciousness”, which is different from walking, dreaming or sleeping states. This experience is thought to be responsible for the normalization, that is, restoration of normal function to be responsible for the particularly those involved in adapting to environmental “stressors” or challenges (Robert K.W, 1970).

According to Maharishi’s Vedic knowledge, the human physiology is structured in the pattern of Vedic literature. Maharishi’s knowledge about Vedic science and Dr. Tony Nadar extensive research on human physiology structured in Vedic literature bring to the light that unified field of consciousness is structured in all level of existence. Therefore, it is very important to understand how the gene expression and regulation is structured in Maharishi’s Vedic knowledge.
VEDIC KNOWLEDGE ON GENE EXPRESSION AND REGULATION:

The etheric or energy body of the human being is an integral part of the etheric body of the planet itself and consequently of the solar system. Through this medium, every human being is basically related to every other expression of the divine life from minute to great. Therefore, from the very minute level of subtle expression we can understand the expression of whole creation. 

I would suggest starting with an introduction to the Unified Field of Natural Law, from Hagelin, then making a strong bridge to the unified experience of pure consciousness, also from Hagelin and Maharishi and Nader. You will need to build the argument step by step, showing the parallels. The ‘etheric body’ is not clearly defined in Maharishi Vedic Science, so I would avoid that term.

Maharishi describes the minute level as a unified field of pure consciousness, which every individual can experience through Transcendental Meditation and TM-SIDHI program. The mantra and sutras for practicing above technique have been taken from Rik Veda. These scientifically proven technologies are derived from the Veda and Vedic Literature (Yoga) by Maharishi Mahesh Yogi.
Rik *V*veda is the holistic aspects of total knowledge. It represents reverberating dynamism and infinite silence. Tony Nadar in his *book Human Physiology: Expression of Veda and Vedic Literature*, *vedic physiology book said explained* that the whole universe is very clearly available as the structure of *V*veda. *These ideas need systematic development. They will be unfamiliar to many readers.*

You will need to carefully define *Veda* (always capitalized) and the *Vedic Literature*, with some concrete examples (e.g. *Yoga*, with which most people are at least vaguely familiar.)

This *truth-correspondence* revealed by maharishi's commentary has made the entire structuring dynamics of creation available in Rik *V*veda and the *V*edic literature. *A very big statement, which needs a good, logical, systematic explanation.*

The same structure of sequential unfoldment of the self-interacting dynamics of natural law is available in the 3 major level of its physical manifestation in the physiology.

1. In the DNA within each cell
2. In each cell within human physiology
3. In the entire physiology with its cells, tissue, organ and organ system.

This exact correspondence between the structure and function of human physiology and the structure and function of *Veda* shows that *Veda* is the blue print of creation-the blueprint that evolves into physical creation. (Nadar). *We need some concrete examples here. And we should probably say that Maharishi explains that, … so we don’t say it as a fact, as if it were common*
knowledge. We want to convince step by step, scientifically, not emotionally.

In Rik veda, Apaurusheya Bhashya (again this abstract concept needs careful definition and description) represents Totality-infinity of silence containing within it all dynamism.

Likewise, the genetic code contains all the information to create the whole human body and also it remains silence until they allowed expressing and producing functional protein. Hence gene represents Totality in the human body.

According to Maharishi's Apaurusheya Bhashya of the Veda, the first syllable of Rk Veda contains the whole essence of the Veda, just as a seed contains the whole tree. The first syllable of Rk Ved is Ak. ‘AK’ describes the collapse of the fullness of consciousness (A) within itself to its own point value (K). This collapse, which represents the eternal dynamics of consciousness knowing itself, Describe a little more so the reader can understand the concept. Then go on to the 8 stages.

occurs in eight successive stages.

We will need to explain above how the Apaurusheya Bhashya is structured; how it sequentially unfolds the knowledge of natural law in successive steps….

In the next stage of unfoldment of the Veda these eight stages of collapse are separately elaborated in the eight syllables of the first pad (define pada), which emerges from, and provides a further commentary on, the first syllable of Rik Ved, 'AK'. These eight syllables correspond to the eight 'Prakritis' (Ahamkar, etc.) or eight fundamental qualities of intelligence. The eight qualities of intelligence are:….

• The first pad expresses the eight Prakritis with respect to the knower, observer or 'Rishi'
quality of pure consciousness.

- The second pad expresses the eight Prakritis with respect to the process of knowing, process of observation of 'Devata' (dynamism) quality of pure consciousness.
- The third pad expresses the eight Prakritis with respect to the known. Observed or 'Chhandas' quality of pure consciousness (Dillbeck, 1989).

Therefore, in the language of the Veda, the three aspects of awareness are called: Rishi, Devata and Chandas and the togetherness is called samhita. It is the basic law that governs unfoldment manifestation. These ideas should be introduced before the concept of the three padas in the first richa.

The gene expression of human also represents these 3 forms. Rishi is the knower, Devata is the knowing and Chandas is the known. Likewise, gene promoter represents Rishi, gene structure represents Devata and terminator represents Chhandas in gene expression. Gene promoter is a region of DNA that facilitates the transcription of a particular gene. The knowing and known is not possible without the knower, the same way, gene expression and termination is not possible without the gene promoter. Gene structure contains the information to be expressed or transcribed and terminator will allow shutting down the gene expression once the necessary functional protein has been produced. Good points here. Clear.
FIGURE: THREE ASPECTS OF AWARENESS AND ITS ASSOCIATION WITH GENE REGULATION
Maharishi explained knowledge is different in different state of consciousness. Likewise, the level of gene expression and their action are also different in different cell, tissue type. For instance, in case of HTERT gene expression, it is seen higher in embryonic stem cells, cancer cells than normal somatic cells (Vicki Lundblad).

According to Maharishi Vedic Science, there are seven state of consciousness. They are sleeping, dreaming, waking, transcendental consciousness, cosmic consciousness, God consciousness, Unity consciousness. These states of consciousness will help us to understand the transfer-development of consciousness in each level to reach the pure consciousness through the practice of Transcendental Meditation, which realize the This is the systematic unfoldment of the inner self, or Atman.

As Maharishi says, the above knowledge is also structured at the level of gene expression and regulation. The central dogma of molecular biology deals with the detailed residue-by-residue transfer of sequential information. The dogma is a framework for understanding the transfer of sequence information between sequential information-carrying biopolymers. There
are 3 major classes of such biopolymers: DNA and RNA (both nucleic), and protein.

Here we will discuss how the seven states of consciousness correspond to the level of DNA and gene regulation.

1. **SLEEPING STATE OF CONSCIOUSNESS**: No Awareness

   If there is no regulatory proteins to bind to specific sequences of DNA, the gene cannot be switched on to regulate the transcription. At this stage the gene can be considered to be in state of sleep.

2. **DREAMING STATE OF CONSCIOUSNESS**: Illusory Awareness with little or no conscious control

   The dreaming state is similar to sleeping state except that the information required to express and to produce functional protein is still stored in the gene. This give us an illusory reality that gene is in the manifestation form. But in reality, the manifestation form exists only when the gene expression takes place and produce functional protein.

3. **WAKING STATE OF CONSCIOUSNESS**: Awareness of outside reality. Inner reality not clear; Identified with the object of perception

   Signal transduction is a local process by which signaling molecules outside the cell interact with cellular receptors to initiate a cascade of biochemical reactions inside the cells, ultimately stimulating a protein transcription factor to activate gene expression. These signals could be hormone or neurotransmitter or heat shock factors that have a specific receptor molecule to bind either on the surface or inside the cells. Even though a signal from outside has come to switch on the gene, the gene cannot be completely expressed unless the signal from outside translated inside the cells through the transcription factors. It is like the lamp is lit outside but not inside.
4. **TRANSCENDENTAL STATE OF CONSCIOUSNESS**: pure inner awareness; no awareness of outside phenomena

At this state the absolute wholeness is maintained and the pure consciousness is experienced and also expressed on its own. It is like lamp is lit inside but not outside. (reference)

Similarly in gene regulation, once the signal from outside translated inside the cell, and then the process started taking place inside the cell. Transcription factor attach to particular stretch of gene of DNA for transcription into RNA. Genes can be activated by transcription factor attach to the nucleotide sequence of the gene's promoter-the stretch of DNA lying upstream of the coding region.

5. **COSMIC STATE OF CONSCIOUSNESS**: Inner pure awareness, along with waking, dreaming and sleeping states

In cosmic state of consciousness both the inner and outer are seen as separate values—one non-changing (the inner, the self) and the other changing (the outer, the non-self). (a little more description of CC and give References).

The same way, gene expression is changing always according to the environment provided by the human physiology but the gene structure is always remain the same and hence it is non-changing.

6. **GOD STATE OF CONSCIOUSNESS**: Inner pure awareness along with perception of the finest relative values

In God state of consciousness, the outer perceptions become more and more refined and sharp. (Define more completely, with references)

Similarly, when there is a steady state of mind (which can be achieved by practicing TM), it helps to balance the nervous system, which thereby regulate hormonal balance (reference).
When the outer environment balances, then there will be steady state of balance inside the cell in terms of gene expression.

7. **UNITY STATE OF CONSCIOUSNESS**: Everything is perceived in terms of the unbounded Self

In this state, we have merged all individuality back into the ONE. There is no duality whatsoever. The ONE has awakened with full consciousness of SELF within the creation, which is now also experienced as SELF. Our former individuality is now the same as all other individualities: one more of the infinite experiences of IT of ITSELF. The unified field of Natural law is the only experience. The inner reality is never overshadowed by any outer experience, yet sharpness of perception of all specific qualities is maintained and enhanced *(very nice; give a Maharishi quotes and references)*. Likewise, each gene which is considered as inner reality will maintain specific information and it produce corresponding functional protein.

Good. This should go first in this section, and all subsequent discussion should refer back to elaborate the correlation between the UF of modern physics and the field of pure consciousness, with lots of quotes and references from Maharishi and Hagelin.
According to The Constitution of the Universe by Maharishi Mahesh Yogi, “modern science has systematically revealed deeper layers of order in nature; from the atomic to the nuclear and sub nuclear levels of nature's functioning the ancient Vedic wisdom identifies a single, universal source of all orderliness in nature. Both understandings, modern and ancient, locate the unified source of nature's perfect order in a single, self-interacting field of intelligence at the foundation of all the laws of nature. The self-interacting dynamics of this unified field constitutes the most basic level of nature's dynamics. The laws governing the self-interacting dynamics of the unified field can therefore be called the **Constitution of the Universe.**

We have seen the theoretical correlation between the development of higher states of consciousness through the Transcendental Meditation program and the process of gene expression. In the next section, the potential effects of Transcendental Meditation on gene expression will be described. It is expected that these effects may lead to more healthy aging.

Now we have a clear understanding and experience about Vedic science through transcendental meditation and TM-SIDHI program. In other hand, we also have clear understanding about the gene regulation through modern science. Hence, it is the time to reveal the effect of transcendental meditation on gene expression, which might lead to healthy aging.
REVIEW OF LITERATURE:

RESEARCH STUDIES ON TRANSCENDENTAL MEDITATION:

The superiority of the method in comparison to other mind-body techniques stems from the more restful state that the body enters in during TM as evidenced by EEG changes and peculiar physiological findings. Transcendental Meditation also been reported to have practical therapeutic value in relieving mental and physical tension (Dillbeck.MC, 1977) (Robert.K.W, 1970). According to evidence in more than 600 published articles from 200 universities and research institutions in 30 countries, the beneficial effects are apparent at the psychological, physiological behavioral and sociological levels. Moreover, earlier studies (clinical and observational) have reported positive benefits of TM, such as reduction in oxygen consumption (Wallace.RK, ), decreases in cardiac output (Wallace.RK and Jevning.R), improved respiratory efficiency (Allison.J), decreased metabolic rate and respiratory rate (Dhanaraj.V.H), increase in plasma prolactin and cortisol (Jevning.R W. V.), increased plasma phenylalanine (Jevning.R P. W.), increased skin resistance (Wallace.R.K ) and reduced systolic and diastolic blood pressure (Benson.H) (Blackwell.B) (Lovell-Smith.H.D), improve mental activity and decrease anxiety (Yunesian.M, 2008).

RESEARCH STUDIES ON TM IN HYPERTENVE PATIENT:

Extensive research has been done to show the positive impact of Transcendental Meditation ™ Program on the treatment of hypertension in African Americans. For instance, two randomized
control studies of TM intervention in African Americans reported reduction in left ventricular mass and a regression of carotid atherosclerosis compared to controls. Another study explore the effectiveness of TM as a secondary prevention tool in African Americans with New York Heart Association (NYHA) class II or class III heart failure (Jayadevappa R, 2007). Another randomized controlled trial was conducted for over one year period. The result of the study was the Transcendental Meditation program significantly decreased diastolic BP more than PMR (Progressive muscle relaxation) or HE (Health Education), and there was a trend for a greater reduction in systolic BP. In addition there was a significant reduction in anti hypertensive medication use in the TM group compared to relaxation and education controls (Schneider.R.H A. S.-J.-R., 2005).

GENETIC KNOWLEDGE ABOUT HYPERTENSION:

Even though many research studies provide evidence for the positive effect of Transcendental meditation on the treatment of hypertension especially in African Americans, no genetics studies have been done till date. It is because the genetics of essential hypertension is poorly understood. Why is this? For one thing, essential hypertension is a complex epigenetic disorder with poor phenotypic expression, and an accurate phenotypic characterization is central to dissecting the genetics of any heritable disease. In addition, the search for genes responsible for heritable disease is based on the fundamental premise that such genes are defective; i.e, they express mutations and encode dysfunctional proteins, which poorly serve the cell and the organism. This paradigm has guided successful investigations of a host of monogenetic diseases, and to some extent, it may also apply to essential hypertension (monogenic form of hypertension results from major gene mutations that primarily influence one biological system) (Aviv.A,
2002). However, it is also plausible that not all genes that are directly involved in the pathophysiology of essential hypertension and other complex traits are abnormal. For instance, rearrangements of networks of genes, evoked by environmental factors, might give rise to different phenotypic expressions in health and disease states (Aviv A. A., 1997).

Any variant gene predisposing to essential hypertension would be difficult to identify because its effect on the overall increase in systemic blood pressure is relatively small and obscured by effects of other genes and the environment. What this argument fails to acknowledge, however, is that in industrialized societies the systemic blood pressure—particularly the systolic blood pressure—is largely age-dependent and primarily an index of vascular aging. In this regard, the search for variant genes that cause human diseases is based on the premise that gene segregation in a population follows principles of evolution by natural selection. But such principles are applicable only during the reproductive years, whereas essential hypertensions, and particularly systolic hypertension, primarily occur during the post-reproductive period. Therefore, it is necessary to incorporate the factor of age (aging) into models of essential hypertension (Aviv A., 2002).

**AGING:**

Ryff and Singer provide two definitions of “healthy aging” corresponding to biological and behavioral/medical orientations: (a) fending off cellular and molecular damage for the longest possible period of the life course and (b) the maximal delay of illness, disease, disability, and hence mortality—factors that keep the organism functioning optimally for the longest period of time. (Vern L. Bengtson). In some organ systems, the loss of reserve capacity with increasing age can be attributed to the loss of cell function. Chronic localized stress to specific tissues/cell types may result in increased cell turnover, focal areas of replicative senescence followed by
alterations in patterns of gene expression. This can result in reduced tissue regeneration, culminating in some of the clinical pathologies that are often associated with increased age (Shay.J.W, 2007).

Genetics is clearly important in determining cellular aging *in vitro* and *in vivo* and part of organism aging may be dependent on cell division, with total cellular lifespan measured by the number of cell divisions (i.e., generations), not necessarily by chronological time. This means that there is an intrinsic process occurring during cell growth which culminates in the cessation of cell division. If cellular age is regulated by a genetically determined counting program that controls the number of cell divisions, then it is important to determine and understand the molecular pathways and regulation of this mechanism (Shay.J.W, 2007). Telomere attrition is the intrinsic process occurring during cell growth which culminates in the cessation of cell division. It is also considered as one of the aging theories.

**HISTORY OF THE TELOMERE:**

The foundations for the telomere study have been created decades back. In 1908, A. Carrel a Nobel Prize winning surgeon became interested in the growth of cells in culture, in 1912; he established a culture of chick heart fibroblast cells, which he then *grew* in the laboratory for 34 years. His groundbreaking work, made the scientific world to *realize* that vertebrate cells can divide indefinitely in culture. Carrel reasoned that “aging is an attribute of the multicellular body as a whole” (Carol.W.Greider, 1998).

Then, in the 1930's, McClintock and Muller showed that if a chromosome breaks, the broken
end usually becomes unstable. However, they showed that soon after fertilization in specific cell types, a broken end can heal in a genetically determined process.

Later in 1961, Leonard Hayflicks and Paul Moorhead, (REALLY?) came with a new concept. They discovered that human cells derived from embryonic tissues can only divide a finite number of times in culture. They divided the stages of cell culture in three phases: Phase I is the primary culture, when cells from the explants simply multiply to cover the surface of the culture flask. Phase II represents the period when cells divide in culture. Cells divide vigorously and can often be subcultivated in a matter of a few days. Eventually, however, cells start dividing slower, which marks the beginning of Phase III. Eventually they stop dividing at all and may or may not die. They noticed that cultures stopped dividing after an average of fifty cumulative population doubling (CPDs). This phenomenon is known as Hayflick's limit, Phase III phenomenon, is called as replicative senescence (RS). (Hayflick.L,1968).

On the other side of the world, a Russian scientist, Alexei Olovnikov was the first to recognize (1971) the problem of how chromosomes could replicate right to the tip, as such was impossible with replication in a 5' to 3' direction. This is termed as the 'end replication problem'. To solve this and to accommodate Leonard Hayflick's idea of limited somatic cell division, Olovnikov suggested that DNA sequences would be lost in every replicative phase until they reached a critical level, at which point cell division would stop. An important question arises after these discoveries. How do the cells stop dividing? Is there any connection between limited capacity of cells and human aging?
These questions were answered after a breakthrough discovery at Gall's laboratory by Blackburn. In 1978, she determined the DNA sequence that allowed the Tetrahymena rDNA molecule to be maintained as a linear chromosome. Her work led to the finding that chromosome ends, or telomeres, are made of simple repeat sequences. It was soon understood that this should be a common mechanism existing even in eukaryotes. Later in 1984, Carol W. Greider identified an enzyme, telomerase that added telomere repeats onto chromosome ends. Later on many scientists all over the world contributed in finding the structure and function of telomere and telomerase and also their association with aging and age related diseases.

**TELOMERE STRUCTURE:**

Telomeres are repetitive DNA sequences at the ends of linear chromosomes that protect the termini from being recognized as double-strand breaks. Without telomeres, the ends of the chromosomes would be ‘repaired’, leading to chromosome fusion and massive genomic instability (White.L.K, 2001). Telomeric DNA consists of non-coding double-stranded repeats of G-rich tandem sequences (TTAGGG in humans) that extend several thousand base pairs and end in a 3’-overhang (G-strand overhang) (Edo. M.D, 2005). Electron microscopy has revealed that the single-stranded 3’ end overhangs invades the duplex telomeric DNA repeat array to form a D-loop and T-loop structure in vitro (Cong.Y.S, 2002).

With each cell division, telomeres shorten by 50-200bp because the lagging strand of DNA synthesis is unable to replicate the extreme 3’ end of the chromosome (the end replication problem) (White.L.K, 2001). The end replication problem leads to chromosome shortening with each round of cell division. Therefore, the main function of human telomere is to cap the
chromosomal ends and to maintain genome stability and integrity. When the telomere reaches a critically short length, they lose capping function at the chromosomal ends, resulting in activation of DNA damage checkpoint. These checkpoints limit cell survival by induction of senescence or apoptosis (Yang.Z, 2009). But how does this end replication problem resolve in vivo? The answer to this question was resolved after the discovery of the Telomerase enzyme by Carol W. Greider and Elizabeth Blackburn in 1984 (Vicki Lundblad).

FIGURE 2: END REPLICATION PROBLEM

DNA polymerase requires an RNA primer to initiate synthesis in the 5'-3' direction. At the end of a linear chromosome, DNA polymerase can synthesize the leading strand until the end of the chromosome. In the lagging strand, however, DNA polymerase's synthesis is based on a series of fragments, called Okazaki, each requiring an RNA primer. Without DNA to serve as template for a new primer, the replication machinery is unable to synthesize the sequence complementary to the final primal event. The result is the "end-replication problem" in which sequence is lost at
each round of DNA replication.

TELOMERASE: THE FACTOR INFLUENCING TELOMERE REGULATION

Telomerase is an RNA-dependent DNA polymerase that synthesizes telomeric DNA sequences and provides the molecular basis for unlimited proliferation potential. Telomerase consists of two essential components: one is the functional RNA component (HTR), which serves as a template for telomeric DNA synthesis; the other is a catalytic protein (HTERT) with reverse transcriptase activity. (Yusheng Cong, 2008).

In humans, telomerase is regulated in a tissue-specific manner during development; the enzyme is present in early embryogenesis but is repressed upon cell differentiation in somatic tissues. The enzyme is present in early embryogenesis but is repressed upon cell differentiation in somatic tissues (Silvia Misitl et al 2000). Due to absence of telomerase activity in most somatic cells, incomplete synthesis of the lagging strand leads to telomere shortening every time these cell divides. Hence ectopic expression of telomerase in normal human cells leads to extension of life span. (Osiewacz, 2002) (Cong Y, 2008).

When purified HTERT is mixed with HTR, it is sufficient to reconstitute telomerase activity in vitro. Moreover, mutations in either HTERT or HTR are associated with many premature aging phenotypes leading to human disease. Therefore, the above facts indicate that for telomerase activity, expression of HTERT and HTR gene is an essential component (Kyo S, 2002), (C.J.Cairney, 2007), (Cong Y, 2008).
**HTR GENE:**

The gene encoding HTR is a single copy gene, which was localized to chromosome 3 at 3q26.3 by fluorescence *in situ* hybridization. The cloning of the HTR promoter in 1998 opened up the opportunity to investigate the molecular details of HTR regulation. HTR promoter contains a CCAATT box in close proximity to the transcription start site in addition to a TATA box consensus in the reverse orientation, consistent with transcription of this gene by RNA polymerase II. Several putative binding sites, such as those for the glucocorticoid, progesterone and androgen steroid hormone receptors in addition to those for the transcription factors AP1 and Ets, were found in the 5' flanking region of the HTR gene. A minimum region of 272bp upstream of the transcription start site was shown to be required for promoter activity. This region contains 4 Dnase I protected sites, which encompass 4 consensus binding sites for the sites SP1 family of transcription factors in addition to the CCAAT box. (C.J.Cairney, 2007).

**HTERT GENE:**

In human diploid cells, the HTERT gene is present as a single copy on chromosome band 5p15.33, the most distal band on the short arm of chromosome 5p. The HTERT gene consists of 16 exons and 15 introns and extends over 40kb. Sequence analysis indicates that the HTERT promoter has no TATA or CAAT boxes but is highly GC-rich. The HTERT promoter contains binding sites for many transcription factors that may involved in its regulation. The minimum sequence requirement for promoter activity is contained within the 330bp upstream of the ATG
(the translational start site). (Yusheng Cong, 2008)

**FIGURE 3: TELOMERE REPLICATION:**

![Diagram of telomere replication](image)

- **Parental Strand:** TTGGGGTTGGGGTTGGGTTG 3'
- **Incompletely Newly Synthesized Lagging Strand:** AACCCC

**Telomerase Binds**

- **Telomerase Extend 3’End (RNA Dependent DNA Template):** TTGGGGTTGGGGTTGGGTTG 3' AACCCC

**Telomerase with Bound RNA Template**

**Completion of Lagging Strand by DNA Polymerase (DNA Templated DNA Synthesis):**

- **DNA Polymerase:** GGTTGGGGTTGGGTTG GGTTGGGGTTG 3'

ACCCCAACCCCAACCCCA5'
AGING AND TELOMERE:

During the past 15 years, there has been mounting evidence that the progressive loss of the telomeric ends of chromosomes is an important intrinsic timing mechanism in the aging process, both in cell culture and in vivo (Cawthon RM, 2003), (Jiang.H, 2007), (Benetos.A, 2001)(Ahmed.A, 2001), (Shay.J.W, 2001) (Hanna.C.W, 2009), Klapper.W et al, 2001 and (Edo. M.D, 2005). As a self-preservation mechanism, in order to avoid genomic instability and protect the organism from catastrophic diseases, such as cancer and cardiovascular disease, cells shut them down by entering a state of replicative senescence—the hallmark of aging (Skordalakes, 2008).

Even though many research significant evidence provides insight about into aging and telomere attrition, there is no clear clue for the mechanism behind age related diseases like hypertension and telomere attrition. However, there are accumulating evidence states indicates that oxidative stress could cause both telomere length and hypertension, which provide a molecular framework for the pathway which could possibly be involved in telomere shortening.
FIGURE: MECHANISM OF TELOMERE SHORTENING

Stress

Reactive oxygen species (ROS)

INCREASE

CAUSE TELOMERE DAMAGE

CAUSE HYPERTENSION

DOWN REGULATE TELOMERASE GENE EXPRESSION

Telomere shortening

Cell senescence
OXIDATIVE STRESS AND REACTIVE OXYGEN SPECIES:
A free radical is any species capable of independent existence that contain one or more unpaired electron, thus making them highly reactive. These radical include superoxide anion and hydroxyl radical, and they have implicated in the pathogenesis of cardiovascular diseases. Reactive oxygen species (ROS) are chemically-reactive molecules and metabolites of univalent or divalent reduction of molecular oxygen, specifically superoxide and hydrogen peroxide. Examples include oxygen ions and peroxides. ROS form as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling. However, during times of environmental stress (e.g. UV or heat exposure), ROS levels can increase dramatically. This may result in significant damage to cell structures. This lead to a situation called as oxidative stress.

The term Oxidative stress is a biomolecular damage caused by attack of reactive species (RS) upon the constituents of living organisms. Increased oxidative damage can result not only from more oxidative stress but also from failure of repair or replacement system.

In principle oxidative stress can result from:

1. Diminished antioxidants
2. Depletion of dietary constituents
3. Exposure to elevated oxygen
4. Presence of toxins that produce RS
5. Excess activation of 'natural' system produces RS like inappropriate activation of phagocytic cells in chronic inflammatory diseases (Barry Halliwell).

ASSOCIATION BETWEEN OXIDATIVE STRESS AND HYPERTENSION

Experimental evidence indicates that oxidative stress like reactive oxygen species (ROS) plays an important pathophysiological role in the development of hypertension. The first data to implicate ROS in hypertension came from a study by Wei et al, which showed that in response to acute hypertension, superoxide accumulated in the extracellular space and contributed to the impairment of cerebral vascular response by inactivation of endothelium-derived relaxing factor, now known as NO (Satomi Miwa, 2008). In another study, accumulation of ROS by-products from oxidized genomic and mitochondrial DNA have been demonstrated in hypertensive individuals (Rhian M. Touyz, 2010). In a study by Nakazono et al, suggested that superoxide near endothelium contributes to the pathogenesis of hypertension, presumably due to reductions in bioavailable nitric oxide (NO). Based on their findings proposed that hypertension is a “free radical disease” (Satomi Miwa, 2008). Collectively these findings indicate that the alterations in ROS would be predicted to have a profound influence on blood pressure.

TABLE 1: (Grossman, 2008)

POSSIBLE MECHANISMS BY WHICH OXIDATIVE STRESS MAY CAUSE HYPERTENSION

- Quenching of the vasodilator nitric oxide
- Generation of vasoconstrictor lipid peroxidation products
- Depletion of tetrahydrobiopterin (BH4)
- Damage to endothelial cells
- Damage to vascular smooth muscle cells
- Increase in intracellular free calcium concentration
- Increased endothelial permeability
HOW DOES OXIDATIVE STRESS CAUSE TELOMERE SHORTENING?

The contribution to telomere loss by oxidative DNA damage was, in many cases, reported to overrate the contribution by the end-replication problem. Thomas von Zglinicki showed that telomere shortening is strongly modulated by the ratio of oxidative stress and antioxidant defense. It appears that due to their high content of guanines, telomeres were demonstrated highly sensitive to damage by oxidative stress. (Zglinicki, 2002). In addition senescent cells were found to contain 30% more oxidatively modified guanines in their DNA and four times as many 8-oxodG bases. Moreover, when compared to genomic DNA, telomeric DNA was reported to be deficient in the repair of single strand breaks. Consequently, telomeres appear to be especially sensitive to the accumulation of ROS-induced 8-oxodG DNA-strand breaks. (Houben JM, 2008).

There is accumulating evidence that oxidative stress is associated with telomere length attrition. Elissa S. Epel research group found that chronic psychological stress is correlated with higher oxidative stress and shorter telomere length. They also proposed that telomere length as a useful psycho biomarker linking stress and disease. (Elissa S. Epel et al., 2004). As the researchers anticipated; the caregivers had significantly shorter telomeres. Furthermore, those with the shortest telomeres also had lower serum levels of the antioxidant vitamin E, and higher levels of a cellular metabolite known as isoprostane, which is an indicator of increased cellular levels of toxic oxidants. In other words, psychological stress increased oxidative stress, which destroys tissues and may be at least partly responsible for the

- Stimulation of inflammation
- Stimulation of growth signaling events
decreased length of the telomeres.

In a cohort study, they tested whether oxidative stress-related gene polymorphisms contribute to variance in both telomere length and physical biomarkers of aging in humans. Telomere lengths were calculated for 190 (82 men, 108 women) participants aged 79 years and associations with 384 SNPs, from 141 oxidative stress genes, identified 9 significant SNPS, of which those from 5 genes (GSTZ1, MSRA, NDUFA3, NDUFA8, VIM) had robust associations with physical aging biomarkers, respiratory function or grip strength. (Starr JM, 2008). In another study, they conducted an experiment in which a human telomere insert and a similar-sized control fragment were exposed to hydrogen peroxide in the presence of iron and ethanol, the telomere insert acquired sevenfold more strand breakage than the control. (Lara Pizzorno, 2010) These data indicate that oxidative stress genes may be involved in pathways that lead to both telomere shortening and physiological aging in humans.

ASSOCIATION BETWEEN TELOMERE LENGTH, HYPERTENSION AND OXIDATIVE STRESS:

From Framingham Heart study conducted at Boston University, explored the relations of leukocyte telomere length, expressed by terminal restriction fragment (TRF) length, with insulin resistance, oxidative stress and hypertension. Their observation lead to a conclusion that hypertension, increased insulin resistance and oxidative stress are associated with shorter leukocyte telomere length and that shorter leukocyte telomere length in hypertensives is largely due to insulin resistance. (Demissie S, 2006). Another study was conducted in Chinese patients with essential hypertension, which revealed that the leukocyte telomere length of the hypertensive subjects was significantly shorter than the normotensive subjects. (Zhewi Yang et
According to the studies reviewed in the above sections, it is understood that ROS and hypertension negatively influence telomere length and antioxidant positively influence telomere length. However, these research findings revealed one possible mechanism of telomere attrition through environmental factors like oxidative stress. We should also take into account that genetic factors like telomerase gene expression also plays an important role in maintaining telomere length. This also raises a question whether oxidative stress might affect telomerase gene expression; thereby it will also leads to telomere shortening.

**OXIDATIVE STRESS AFFECTING GENE EXPRESSION**

Gene expression is modulated by both physiological signals (hormones, cytokines, etc.) and environmental stimuli (physical parameters, xenobiotics, etc.). Oxidative stress appears to be a key pleiotropic modulator which may be involved in either pathway. (Barouki, 1999). A large-scale gene expression profiling study on hypertension patient leukocytes investigated whether leukocytes of hypertensive patients differentially express oxidative stress and inflammation-related genes. Indeed, expression of several pro-oxidant genes was increased, whereas antioxidant genes decreased (Helena Cohn et al 2004).

The conceptual relationship between psychological stress and gene expression have also been studied and showed that stress could down regulate the gene expression. In one such study stress related changes in genomic expression profiling, GEP have been demonstrated by microarray
analysis in healthy subjects, including up-regulation of several cytokines/chemokines and their receptors, and in individual suffering from post traumatic stress disorder, including inflammation, apoptosis and stress response as well as RNA processing pathway. (RH Segman et al, 2005).

**POSSIBLE MECHANISM OF TMREGULATING TELOMEREASE GENE EXPRESSION:**

Steve W. Cole have described, the possible mechanism involved in change in gene expression due to sociological stress, which gives a clue how relaxation technique like Transcendental Meditation could up regulate telomerase gene expression. Socio-environmental processes regulate human gene expression by activating central nervous system process that subsequently influences hormone and neurotransmitter activity in the periphery of the body. Peripheral signaling molecules interact with cellular receptors to activate transcription factors, which bind to characteristic DNA motifs in gene promoter to initiate (or repress) gene expression (Steve W. Cole, 2009, Steve W. Cole et al, 2007).

There is increasing evidence which showed the effect of Transcendental Meditation on EEG coherence, which activates the central nervous system. This needs to be explained more completely.

Moreover in five days integrative body-mind training (IBMT) study, better regulation of autonomic nervous system by a ventral midfrontal brain system seen in IBMT group than relaxation group. (Yi-Yang Tang et al 2009).

This activation may lead to increase in neurotransmitters and hormones, which thereby activate transcription factors (which are present in both HTR and HTERT gene promoter) and regulate the telomerase gene expression and maintain telomere length.
Furthermore, there is increasing evidence that relaxation techniques will increase telomerase activity. Herbert Benson study indicates that there are distinct differences in the Gene expressing profile (GFP) between individuals with many years of relaxation response practice and those without such experience. In a longitudinal wait list control study design, they found that 3 months intensive meditation training; significantly increases the telomerase activity than wait list control group. (Tonya L. Jacobs et al 2010). Moreover, Ornish also showed that adopting a multi modal healthy lifestyle approach increased the concentration of telomerase in peripheral blood mononuclear cells (PBMCs) (Ornish et al 2008).

Moreover, HTERT gene helps not only in maintaining the telomere length and also has anti apoptotic and anti neurotic activity; thereby they also protect the cell from apoptosis.(Gorbunova V, 2002)(Jin X, 2010). Hence, analyzing HTR and HTERT gene expression and measuring telomere length in African American Hypertensive patient practicing TM will reveal whether this technique helps in healthy aging and decrease age related disorder.

Finally, analyzing HTR and HTERT gene expression is more sensitive and accurate than measuring telomerase enzyme activity, because presence of inhibitors may lead to false negative results in telomere repeated amplification protocol (TRAP assay).(Tomomi Yajima et al 2000,Susan et al 2004).
HYPOTHESIS:
The goal of the research is to determine whether practicing Transcendental Meditation (always capitalized) will help in healthy aging and to overcome age-related disease like hypertension. In order to understand this, we plan to test the following hypothesis.

1. Practicing Transcendental meditation for 3 months could possibly increase the gene expression of telomerase HTERT and HTR.

2. Telomere length increase could be seen within 3 months of practicing Transcendental Meditation.

3. Since increase in HTERT gene expression leads to anti-apoptotic anti-neurotic activity and also helps to maintain telomere length, it could possibly consider as an aging gene marker.

4. Transcendental meditation could be implemented as a non-pharmacological treatment for hypertensive patients.
HEALTHY AGING

CELL SURVIVAL

HYPOTHESIS OF THE STUDY

MAINTAIN TELOMERE LENGTH

UP REGULATION OF HTR GENE REGULATION IN NORMAL SOMATIC CELL

ANTI-APOPTOTIC ACTIVITY

ANTI-NEUROTIC ACTIVITY

UP REGULATION OF HTERT GENE REGULATION IN NORMAL SOMATIC CELL

PRACTICING TM
**AIM OF THE STUDY:**

To test the hypothesis we propose studies with the following specific aims.

1. To analyze the gene expression of HTR and HTERT in peripheral blood using real time PCR technique at baseline and post test in TM and HE group.

2. To analyze the telomere length in peripheral blood using real time PCR at baseline and post test in TM and HE group.
RESERCH DESIGN AND METHODS:

a). Study Population:

The study material was part of the NHLBI funded study and consisted of 30-40 hypertensive African Americans participating in blood pressure reduction through Transcendental Meditation program plus conventional health education program and conventional health education program ‘alone’ control for a study period of four months. The age span of both the controls and Hypertensive African Americans will be ≥ 40 years. All subjects of the study will be documented stage I hypertension, i.e. SBP 140-159 mm Hg and/or DBP 90-99 mm Hg. Subject will be tested at Howard University Clinical Research Center (CRC) for a total of three days of physiological and behavioral testing. Genetics study will be conducted at Maharishi University of Management in collaboration with Genetics id company, Fairfield, USA. Telomerase activity and telomere length should be assayed at the beginning of the TM program plus conventional health education program group and health education program ‘alone’ control group and at the end of the four month intervention study period (baseline and 4 months). The subject and the control are divided into four groups according to their age (40-50, 50-60, 60-70 and 70 and above)

Subjects:
All subjects will meet the following inclusion and exclusion criteria

1. **Inclusion criteria:**
   - Ethnicity: Self-identified African Americans
   - Gender: Male or female
   - Age: 40 years or older
   - Residence: Washington, DC or surrounding communities
   - Blood pressure: Stage I hypertension by JNC VII criteria (6) defined as systolic BP of 140-159 mm Hg and/or diastolic BP of 90-99 mm Hg average without antihypertensive medication documented in the study clinic on two or more occasions; or BP within this range with diuretic only.

2. **Exclusion criteria:**
   - Blood pressure: <140/90 mm Hg or ≥ 160/100 mm Hg
   - A history of clinical cardiovascular disease (defined as myocardial infarction, angina, intermittent claudicating, congestive heart failure and stroke) or chronic renal failure.
   - Two or more antihypertensive medications or sympatholytic antihypertensive monotherapy e.g. beta blocker, alpha blocker, central adrenergic agent, ACE inhibitor.
   - Any other life threatening illness e.g. advanced malignancy.
   - History of major psychiatric disorders, e.g. psychosis, dementia or substance abuse disorder.
• Inability or unwillingness to give informed consent.

1. Subject recruitment:

• Subjects who meet the above eligibility requirements will be recruited from specialized clinics at Howard University Medical Center in Washington, DC, including Dr. Randall’s hypertension clinic, medicine, family practice, and private physicians’ clinics.

• Subjects who are interested in participating in the proposed study and who have a history of stage I hypertension but who are taking antihypertensive medications will be offered the opportunity to taper off these medications to determine if they are eligible by BP criteria for the study.

b). Transcendental Meditation Program instruction:

The Transcendental Meditation program has been taught worldwide since 1957. Qualified and experienced African American Transcendental Meditation teachers in the Washington, DC area have been implementing this intervention in clinical trial in the Washington, DC area. These teachers will be available for the current study.

The instruction involves a seven-step course over six days, which will follow the standard format offered throughout the USA by Maharishi Vedic Universities and Maharishi Vedic Schools

➢ Introductory Lecture:

Review of previous scientific research on the Transcendental Meditation Program and a vision of possible benefits through practice;

➢ Preparatory Meeting:

Review of mechanics and origin of the Transcendental Meditation technique;
- **Personal Interview:**
  Interview with a qualified teacher of the Transcendental Meditation program;

- **Personal Instruction:**
  Individual learning of the Transcendental Meditation technique;

- **First day checking:**
  Verifying the correctness of the practice and further instruction;

- **Second day checking:**
  Understanding of the mechanics of the Transcendental Meditation technique from personal experiences;

- **Third day checking:**
  Understanding the mechanics of the development of higher states of wellness and health;

Most sessions will last 1 to 1.5 hours with the exception of the personal interview (about 10 minutes). The general format of most sessions will be lecture/discussion.

Follow-up program:
Following the initial phase of the intervention, there will be follow-up program twice a month for the duration of the four-month intervention period that will include:

a) Checking of correct practice of the Transcendental Meditation technique and
b) Advance lecture and seminar to ensure complete understanding of benefits of the practice for physiological, psychological, and behavioral health.

Health Education (HE) Program:

Both groups will receive a course of didactic health education twice a month on the prevention of CHD through lifestyle modification of conventional CHD risk factors (e.g. high blood pressure, serum cholesterol, smoking, weight, physical activity). Subjects will learn about the value and approaches to making and maintaining reductions in primarily body weight, dietary fat and salt. The saturated fat, cholesterol, and sodium reduction segments will be event-oriented, emphasizing knowledge and approaches to controlling dietary intake in a variety of situations. Participant will learn to identify the source in their food and the value of planning, reading labels, shopping and modifying recipes. The weight management focuses on the value, knowledge and approaches to decreasing caloric intake, counting fat intake, and increasing physical activity. Participant will learn mild to moderate intensity physical activity. Participants will learn the value to identify major source of high calorie foods and make appropriate substitutions. Participants will learn the value of mild to moderate intensity physical activity on a daily basis. The sessions will include discussions of stress as it relates to weight management and physical exercise.
Practical Methods:

Isolation of RNA from frozen blood clot using TRIZOL Reagent

Materials required for RNA extraction from Frozen blood clot

- Porcelain Motor and pestle.
- Vortex
- Petri dish
- Forceps
- Blade
- Dry ice
- weigh boats
- Weighing machine
- pipette and tip
- Micro centrifuge tubes
- Micro centrifuge
- Beaker
- 100ml Conical flask
- Gloves
- paper towel
- Marker
- Mask
**Chemicals required for RNA extraction**

- Tri reagent solution (Ambion. Cat no: AM9738)
- Chloroform
- 100% isopropanol
- 100% ethanol
- RNA storage solution (Ambion Cat no:AM7001)
- DEPC treated water.(Ambion Cat no:4387937 )
- Nuclease free water (Ambion Cat no:4387936)
- TURBO DNase kit (Ambion Cat no:AM1907)
- RNAase ZAP solution (AmbionCat no:AM9780)
- RNAase Wipes (Ambion Cat no:AM9786)

**75% Ethanol preparation for RNA extraction**

25ml of nuclease free water added to 75ml of 100% ethanol in RNAase free conical flask.
RNA EXTRACTION PROTOCOL

HOMGENIZATION:

- 50-100 mg Blood clots weighed and transfer them into 2ml micro centrifuge tube.
- 1ml of TRIZOL reagent was added to the blood sample.
- Homogenization done using motor and pestle. The motor was washed throughly with Rnase away, followed by water.
- The samples were incubated at Room Temperature (RT) for 5-10 minutes after homogenization.

RNA EXTRACTION:

- The homogenate was centrifuged at 12,000 g for 15 minutes at 4°C. The resulting pellet contains extra-cellular membranes, polysaccharides and high molecular weight DNA while the supernatant contains RNA.
- The supernatant were transferred to a new micro Centrifuge tube.
- 0.2 ml Chloroform per 1 ml TRIZOL reagent was added to the supernatant. The tubes were shaken vigorously for 15-30 seconds by hand and incubated at RT for 5-15 minutes.
- Then they subjected to centrifugation at 12,000 g for 15 minutes at 4°C. Carefully the upper aqueous phase was removed, and transfers them to a new micro centrifuge tube.
• The bottom layer was preserved at 4°C for subsequent isolation of DNA and proteins.

**RNA PRECIPITATION AND WASH:**

• 0.5 ml Isopropyl alcohol per 1 ml of TRIZOL reagent was added and incubated at room temperature for 10 min. Then they subjected to centrifuge at 12,000 g for 8 min at 4°C.

• The pellet were washed 2 times with 1 ml of 75% ethanol per 1 ml TRIZOL reagent, and then centrifuge at 12,000 g for 5 min at 4°C.

• The pellet was dried at room temperature and re-suspended the RNA pellet in 80 μl of RNA storage solution.

**DNASE TREATMENT:**

A frequent cause of concern among investigations performing quantitative RT-PCR is inaccurate data due to DNA contamination in RNA preparation. Although DNA contamination is easily detected by performing a 'no RT control', it is vital to give DNase treatment. Turbo Dnase treatment has consistently proven to be the most effective method for removing DNA contamination from RNA samples.

**TURBO DNase TREATMENT PROTOCOL:**

• 50ul of RNA sample was treated with 5ul 10x turbo reaction buffer

• incubate at 37oc for 30 minutes

• 5 ul of DNase inactivation reagent added

• Vortex them for every 10 seconds for 2 minutes.
• Centrifuge at 10,000xg for 3 minutes.
• 40ul were removed and used for further analysis.

QUANTIFICATION OF RNA BY SPECTRAL ABSORPTION:
The traditional method for assessing RNA concentration and purity is UV spectroscopy. RNA was quantified using absorption of light at 260 and 280 nm (A260/A280). A A260/A280 ratio of 1.82 is indicative of high purified RNA.

TELOMERAISE GENE EXPRESSION ANALYSIS USING QUANTITATIVE REAL TIME PCR:
REAL TIME PCR TECHNIQUE:
RT PCR is the most sensitive techniques for mRNA detection and quantification currently available. Compared to the two other commonly used techniques for quantifying mRNA levels, northern blot analysis and RNase protection assay, RT PCR can be used to quantify mRNA levels from much smaller samples. Moreover, this technique is sensitive enough to enable quantification of RNA from single cell.

One step RT PCR reaction method is followed, where reverse transcription and amplification of cDNA takes place in one reaction well.

Materials Required:
• Primer and Probe for HTERT, HTR, GAPDH were made from Integrated DNA technologies, USA
• RT PCR Master mix (Qiagen Cat no: 204954)
• Nuclease free water (Ambion Cat no:4387936)
• Real time PCR (ABI biosystem 7500)
• Universal RNA (amsbio Cat no:R1234148-10)

**PRIMER AND PROBE DESIGN:**

Primers and the TaqMan probe for HTR, HTERT and GAPDH were designed as described in (Yajima T, 2000). GAPDH was used as an internal control gene and they also considered as a housekeeping gene. According to the RT PCR Kit manufactured by Qiagen, length of PCR product should not exceed 60-150bp. Hence, we modified GAPDH forward primer and HTERT reverse primer using http://frodo.wi.mit.edu/primer3/. The sequences are as follows.

**hTR FW:** 5'-GGTGGTGCGCATTGGTTTGTCT

**hTR RV:** 5'-CTAGAATGAACGGTGGAAGGC

**hTR probe 5'-FAM-CGCCGCTGTTTTTCTCGCTGACTTTC- Iowa Black FQ**

**HTERT FW:** 5’-ACGGCGACATGGAGAACAA

**HTERT RV:** 5’-GGGTGCTGTGGAAGGTTTTC

**HTERT probe: 5’-6-FAM-CTCCTGCGTTGGATGATTGTTCAGCC-Iowa Black FQ**

**GAPDH FW:** 5’-CAATGACCCCTTCATTGACC

**GAPDH RV:** 5’-GAAGATGTTGATGGGATTTC

**GAPDH probe 5-Cy5-CAAGCTTCCCGTTCTCAGCC-Iowa Black RQ-Sp**
**One step RT PCR:**

Twenty five micro liters of reaction mixture were used. They are as follows.

**TABLE 1:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x Quantifast RT PCR Mix</td>
<td>12.5µl</td>
</tr>
<tr>
<td>50x ROX dye solution</td>
<td>0.5µl</td>
</tr>
<tr>
<td>20x primer-probe mix</td>
<td>1.25µl</td>
</tr>
<tr>
<td>Reverse transcriptase (RT) mix</td>
<td>0.25µl</td>
</tr>
<tr>
<td>RNase free water</td>
<td>0.5µl</td>
</tr>
<tr>
<td>Template RNA or Universal RNA</td>
<td>10µl</td>
</tr>
<tr>
<td>TOTAL reaction volume</td>
<td>25µl</td>
</tr>
</tbody>
</table>

**TABLE 2:**

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
<th>Temperature</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse transcription</td>
<td>20 min</td>
<td>50°C</td>
<td>RNA is reverse transcribed cDNA</td>
</tr>
<tr>
<td>PCR initial activation step</td>
<td>5 min</td>
<td>95°C</td>
<td>HotStarTaq Plus DNA Polymerase is activated by this heating step</td>
</tr>
<tr>
<td>Denaturation</td>
<td>15 s</td>
<td>95°C</td>
<td></td>
</tr>
<tr>
<td>Annealing/extension</td>
<td>30 s</td>
<td>60°C</td>
<td>Combined annealing/extension step with fluorescence data collection</td>
</tr>
</tbody>
</table>
The number of cycles depends on the amount of template RNA and the expression level of the target gene.

| Number of cycles | 40 |

**Procedure**

- 2x QuantiFast Multiplex RT-PCR Master Mix, template RNA, primer and probe solutions, and RNase-free water were allowed to thaw on ice.
- The above individual solutions mixed, and kept on ice.
- Reaction mixes are prepared according to the above reaction mix protocol (Table 1).
- The reaction mixes are mixed thoroughly, and appropriate volumes are dispensed into PCR tubes or the wells of a PCR plate.
- Template RNA and Universal RNA added at the concentration of 50ng per reaction.
- No RT control (to ensure DNA free sample), No template control (to ensure primer dimer formation, contamination etc) are the two control used in the experiment.
- Real time PCR cycle is programmed according to the table2.
- Data analysis was set to measure using delta delta ct method.
TELOMERE LENGTH ANALYSIS:

The traditional method of measuring telomere length in samples of total human genomic DNA determines a mean terminal restriction fragment (TRF) length. The method requires large amounts of DNA (0.5-5µg-5µg) and takes 3-5 days to complete the experiment. Moreover, the result cannot be accurate because the relative mean TRF length of individuals can vary by as much as 5% depending on the particular restriction enzymes used (Cawthon, 2002).

Likewise, southern blotting and Q-FISH (fluorescent in situ hybridization) have their own limitation and time consuming.

The recent technique developed by Richard M. Cawthon at Utah University using monochrome multiplex real time PCR to measure telomere length, require small amount of DNA and require much less time than the traditional methods.

MATERIALS REQUIRED:

- DNA sample
- TE Buffer
- Bio-Rad MyiQ single color Real time PCR detection system.
- Primer for telomere and beta globin
- SYBR GREEN
- PCR reaction mix
• Reference DNA

**PRMIER DESIGN:**

The primers were designed according to Richard.M Cawthon, 2009 protocol.

Telg, AACAAGGTTTGGGTGTTGTTGTTGTTGTTGTTGTTAGTGT

Telc, TGGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA

beta-globin,Fw CGCGCGCGGCGGCGGCGGCTGGGCGGcttcactcaegttcactttg

beta-globin, Rw GCCCCGCCCCGGCGCCTCCGCGgaggagaagtctgcgcgtt

**PREOCEDURE:**

The procedure is very similar to telomerase gene expression but the Data analysis was set to measure using standard curve method.
**EXPECTED RESULT:**

Practicing transcendental meditation for 3 months expected to increase the expression of HTERT and HTR and also telomere length in subject's post test peripheral blood sample. Moreover, our findings will be supported by a research study conducted in the cancer cell line model and human lung fibroblast; where over expression of HTR and HTERT gene greatly induce the telomerase activity and telomere length elongation. (C.J. Cairney and W.N. Keith, 2007). However in another study, HTERT and HTR gene expression are not related to telomere length, which indicate that it is necessary to speculate the relation between telomerase gene and telomere length (Susan J.J Swiggers et al 2004, Yining et al, 2003).
SIGNIFICANCE OF THE STUDY

If subsequent randomized controlled trials substantiate that Transcendental Meditation might increase the telomere length and telomerase activity, then increase in HTERT gene also indicate that they protect the cells from stress-induced apoptosis and necrosis ((Gorbunova V, 2002). If the further research provide an evidence for the up regulation of HTERT gene, then they could possibly considered as an aging gene bio marker. Furthermore, this research also establishes whether increase in telomere length and telomerase activity might be associated with decrease in the incidence of age related risk factors and diseases.

Finally, this study will also help us to move beyond the correlation studies on physiological, biochemical analysis on Transcendental meditation technique and could be a platform to reveal the pathway or mechanism involved in relaxation response and telomere length and gene expression.
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DEFINITION OF TERMS:

FIGHT OR FLIGHT RESPONSE:
It is the biological response of animals to acute stress

GENE EXPRESSION:
It is the process by which information from a gene is used in the synthesis of a functional gene product.

ALLSOSTASIS:
The Ongoing adaptive effort of the body to maintain stability (homeostasis) in response to stressors.

ALLOSTATIC LOAD:
It is defined as the physiological consequences of chronic exposure to fluctuating or heightened neural or neuro-endocrine response that results from repeated or chronic stress

WEAR AND TEAR:
Wear and tear is damage that naturally and inevitably occurs as a result of normal wear or aging.

OXIDATIVE STRESS:
It is the bio molecular damage caused by attack of reactive species upon the constituents of
living organism.