Acupuncture and Traditional Chinese Medicine in Treating and Preventing Insomnia and Associated Co-Morbidities

A Capstone Project Submitted in Partial Fulfillment of the Requirements for the Degree
Doctor of Acupuncture and Oriental Medicine

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Abstract

Insomnia is a very common sleep complaint that often goes undiagnosed and untreated. In the last few decades people who suffer from insomnia have become more numerous. The problem has become more acute worldwide and keeps growing at a fast rate.

Independent of other sleep disorders, insomnia was found to affect approximately 20 million Americans yearly, with an estimated treatment cost and lost work cost of about $100 billion dollars per year. Judging from recent changes in the economy and increasing stress levels in the USA and worldwide, these estimates have grown considerably and will likely keep growing.

The causes of insomnia appear to involve a complex combination of biochemical, physiological, psychological, and social factors. Insomnia is understood to be a state of hyper-arousal, in which cellular oxidative stress seems to play a leading role in causing chronic insomnia, which sets the stage for chronic co-morbidities the nature of which depends on each individual's propensity.

The objectives of this study were: (a) to gather information about the role(s) of sleep, and understand the devastating effects of sleep-loss on the organism, (b) to find out insomnia's relation to aging, and substantiate our growing knowledge of sleep as a critically essential basic need without which serious health problems set in. When insomnia becomes chronic, it becomes a major risk factor for chronic diseases such as hypertension, heart attack, stroke, Alzheimer's disease (AD), Parkinson's disease (PD), cancer, affective conditions such as major depression/anxiety, as well as mental illnesses, including schizophrenia and other psychoses, and accelerated aging. The third objective of this study was (c) to gather information on the effectiveness of acupuncture and other TCM modalities for treating and preventing insomnia.

Traditional Chinese Medicine has treated insomnia for millennia during which time it has accumulated great knowledge and deep understanding of sleep as a vital basic need, with a central role in restoring depleted substrates in the human body to restore homeostasis and balance between yin and yang.

Severe insomnia tends to be chronic, with about 85 percent of the patients continuing to report the same symptoms and impairments for months or years after having been diagnosed as having insomnia. Insomnia was found to be more prevalent in among women and in older persons starting in middle age. In younger adults insomnia can be reversed more readily by allowing the recovery of sleep in a timely manner before too much damage has been caused, so it becomes chronic. This recovery is much more difficult to achieve in older people, due to the impairment of sleep regulating mechanisms which are greatly affected by aging.

Sleep deprivation studies in humans and animals highlight the severe effects of sleep-loss on health. In order to decipher the mechanisms of how this resulting health deterioration takes place, animal studies have been performed which clearly indicate the greatly accelerated aging caused by chronic sleep-loss.

Since the elderly population is expected to double in the next few years, treatments that can improve sleep quality are very much needed. Pharmaceutical therapy can help, but is weighed down by side effects and a heavy risk of dependence and the ensuing loss of effectiveness over time, as well as possible contraindications with patients other medications. It is hence urgent to find good alternatives, such as acupuncture and other traditional Chinese modalities that have no side effects or very mild ones, and are much better at addressing the root causes of insomnia.

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Approval Signatures Page

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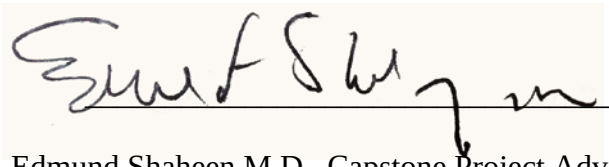

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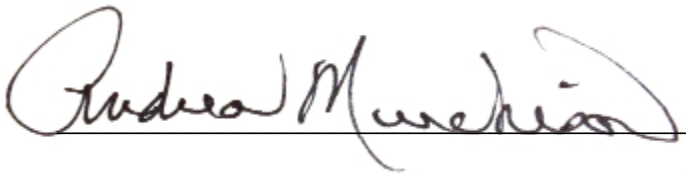

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Chapter 1: Introduction

Background of Sleep and Sleeplessness

Insomnia is a very common sleep complaint that has been frequently ignored by health practitioners, who used to almost never ask their patients how their sleep was. Not surprisingly, therefore, insomnia has often not been diagnosed and remained untreated (Benca, 2005). Recent studies show that insomnia might be a two-tailed story in that both people who sleep too short a duration and those who sleep too long a duration have a greater risk to have co-morbidities and a shorter lifespan. In a relatively large longitudinal study of older women - 444 women with an average age of 67.6 years, a U-shaped relationship was found between survival and sleep duration as measured by actigraphy (Kripke, Langer, Elliott et al., 2011). The study lasted from 1995 to 2009. Persons sleeping longer than 7.5 hours per night or less than 6.5 hours, had a greater mortality risk and died younger. Sleep quality and duration seems to greatly affect the quality of life and the epidemiological studies life span.

Chronic insomnia as defined by the DSM-IV criteria manifests in ongoing complaints such as difficulty falling asleep or staying asleep, short sleep duration despite adequate opportunity for a good night sleep, waking up early in the morning and being unable to resume sleep, or waking up tired in the morning and having a non-restorative sleep, with any of these symptoms occurring at least sometimes, or often, for one month or more (APA, 2000).

Daytime consequences of insomnia include tiredness, lack of energy, difficulty concentrating, and/or irritability (Simon and von Korff, 1997), which often result in daytime impairment, including missed days at work or at school, or reduced productivity by half or more at work, school or household, as well as by missed social or leisure activities – all due to sleeping problems.

The American Psychiatric Association (APA, 1994) diagnostic criteria for primary insomnia included the following: difficulty initiating or maintaining sleep, that occurs not due to the direct physiological effects of a substance or a medical condition, nor exclusively occurring during the course of another sleep disorder, yet causing significant clinical distress or impairment in social, occupational, or other important areas of functioning.

Severe insomnia tends to be chronic, with about 85 percent of the patients continuing to report the same symptoms and impairments for months or years after having been diagnosed as having insomnia (Hohagen, Rink, Kappler et al., 1993; Katz and McHorney, 1998). Insomnia was found to be more prevalent among women and in older persons (Mellinger, Balter & Uhlenhuth, 1985; Ford and Kamerow, 1989; Foley, Monjan, Brown et al., 1995).

The causes of insomnia seem to involve a complex combination of biochemical, physiological, psychological, and social factors. Insomnia is understood to be a state of hyper-arousal (Perlis, Smith & Pigeon, 2005), in which stress may play the leading role in activating the hypothalamic-pituitary-adrenal axis, to set the stage for chronic insomnia.

Vgontzas, Bixler, Lin et al., (2001) indicate that when compared with normal sleepers, adults with insomnia show higher levels over a 24-hour period of cortisol and ACTH (the adreno-corticotropic hormone), the hormone released by the hypothalamic-pituitary-adrenal axis after being exposed to stress. However, the 24-hour patterns of cortisol and ACTH secretion in insomnia patients are different from those of persons who are chronically stressed.

Cognitive factors such as worry, brooding and fear of not being able to sleep, seem to perpetuate the problem of insomnia through behavioral conditioning. Other perpetuating factors include exposure to light during sleep at night, as well as irregular sleep schedules (Partinen and Hublin, 2005).

Neuroimaging studies of insomnia suggest that multiple neural systems that are hierarchically arranged in the central nervous system (CNS) contribute to the arousal as well as to the insomnia. Disturbances in these hierarchical neural systems may differ according to the nature of insomnia. Structures that regulate sleep and wakefulness, such as the brainstem, hypothalamus and basal forebrain of primary-insomnia patients, were shown to be abnormally overactive during sleep (Nofzinger, Buysse, Germain et al., 2004).

In addition, the limbic and paralimbic structures that regulate basic emotions and instinctive behaviors, such as the amygdala, hippocampus, ventromedial prefrontal cortex and the anterior cingulate cortex, have all been shown to be abnormally active during sleep in persons with primary insomnia, as well as in secondary insomnias associated with depression (Nofzinger, Buysse, Germain et al., 2004).

Insomnia's main risk factors seem to be advanced age and female gender (Edinger and Means, 2005). A large, population-based study found insomnia to be nearly twice as common among women (Ford and Kamerow, 1989). Other risk factors for insomnia appear to be family history of insomnia (Dauvilliers, Morin, Cervena et al., 2005), shift work, stressful life styles, and medical and psychiatric disorders (Edinger and Means, 2005). Persons with schizophrenia, for example, manifest insomnia from the very earliest stage of being diagnosed (Lou, Dey & Wiseman, 2000).

Brief History of Modern Western Sleep Research

In 1916 the Viennese neurologist Baron von Economo started seeing patients with a new type of encephalitis, that was later termed 'von Economo's sleeping sickness' or 'encephalitis lethargica', since it caused most of his patients to sleep twenty or more hours per day, waking up briefly to eat and drink. Their cognitive function did not seem to be affected, and it took them many weeks to recover from this condition.

On the other hand, some of his patients manifested a severe insomnia, and they slept for just a very short time every day, then woke up and were unable to sleep. No virus that supposedly caused any of these brain lesions was found, and by the early 1930s this disease came to a stop.

Dr. von Economo accurately identified the brain stem lesions that caused these profound changes in wake-sleep regulation (Saper, Scammell & Lu, 2005). He located the lesions causing the prolonged sleepiness at the junction of the brainstem with the diencephalon, and correctly identified the lesions resulting in a severe insomnia at the anterior hypothalamus. He also suggested that narcolepsy (as the prolonged sleepiness disorder came to be later termed) was caused by lesions in the in the posterior lateral hypothalamus.

Dr. von Economo additionally suggested the existence of an ascending arousal system that originates in the brainstem and keeps the forebrain awake. Such a pathway was indeed found later by other researchers, who located its starting location at the rostral pons, and its ascending course passes through the midbrain reticular formation. It was named "The Ascending Reticular Formation System", but its nature was clarified much later, in the 1970s and 1980s (Saper, Scammell and Lu, 2005).

Wakefulness was shown to depend on a network of cell groups that activate the thalamus and cerebral cortex. During sleep a switch in the hypothalamus shuts off this arousal system. Other hypothalamic neurons stabilize this switch, and its absence results in an inappropriate switching of behavioral states, such as in narcolepsy, a sleep disorder in which the individual feels sleepy during much of the day.

In 1923 EEG (encephalography) was discovered and the development of brain wave recording ensued, which enabled the later discoveries of the "sleep architecture" in the early 1950's, with REM (rapid eye movements) sleep and NREM (non-rapid eye movements) sleep determined to be the major two types of sleep by Kleitman, Aserinski and Dement (Dement, 2003).

Insomnia and Chronic Insomnia

In 2005, insomnia was finally counted among the sleep disorders by the International Classification of Sleep Disorders of the American Academy of Sleep Medicine (AASM, 2005). Sleep loss in adults now generally refers to sleep duration that is shorter than seven to eight hours per night, the average basal need of sleep in adults. The American Academy of Sleep Medicine does not distinguish between acute and chronic insomnia, both being considered insomnia disorders, despite finally admitting that chronic insomnia has very serious consequences for health, performance and safety (Colten and Altevogt, 2006; Luyster, Strollo, Zee & Walsh, 2012).

As many as 92 distinct sleep disorders have been officially defined by APA, most of which present at least one of the following symptoms: excessive daytime sleepiness, difficulty initiating or maintaining sleep, and/or abnormal movements, behaviors, or sensations occurring during sleep (Colten and Altevogt, 2006).

Although sleep disorders are among the most common health problems, sleep disorders and sleep-loss are frequently overlooked by health professionals (Namen, Wymer, Case & Haponik, 1999; Namen, Landry, Case et al., 2001). This failure to recognize insomnia's serious health and social consequences precludes its timely diagnosis and treatment, and forestalls taking adequate measures for preventing its grave and costly public health consequences.

Colten and Altevogt (2006) state that the previous ten years have produced research findings that "overturned the dogma that sleep-loss has no health effects except for daytime sleepiness. Hence the case can now be confidently made that chronic sleep-loss and sleep disorders have profound and widespread effects on human health."

The main symptoms of sleep loss are considered to be excessive daytime sleepiness, as well as a depressed mood and poor memory and/or concentration (Dinges, Rogers & Baynard, 2005). These are indeed the main symptoms of primary short-term insomnia, but do not address the much wider and more severe symptoms of chronic insomnia, which seem to be a risk factor for serious chronic diseases. Sleep has to be recognized as the vital basic need that it is, just like oxygen, warmth, food and water, the chronic deficiency of which has severe effects on health (Luyster, Strollo, Zee & Walsh, 2012; Everson & Szabo, 2011).

As we shall see later on, many of these scientific findings and more recent ones indicate that cumulative effects of chronic sleep-loss greatly increase the risks of serious diseases such as hypertension, heart attack, stroke, Alzheimer's disease (AD), Parkinson's disease (PD), cancer, affective conditions such as major depression/anxiety, as well as mental illnesses, including schizophrenia and other psychoses.

Chronic sleep deprivation seems to tip the moderate oxidative cellular stress towards a more severe oxidative cell stress, which initiates a process of pathological chemical, immune and

neural changes at the cellular and molecular levels that cause serious co-morbidities to develop. These serious chronic illnesses result in a poor quality of life and a shorter life span, independent of the primary sleep disorder.

Luyster et al. (2012, AASM) cite an estimate of 50 to 70 million adult Americans having a chronic sleep disorder (among which they include insomnia), which contributes to poor health, so that approximately one in three adult Americans (37.1%) sleep fewer than seven hours per night, "an amount [of sleep] at which physiological and neuro-behavioral deficits manifest and become progressively worse under chronic conditions" Luyster et al. (2012, AASM).

Sleep deprivation studies in both humans and animals highlight the severe effects of sleep-loss on health. In order to decipher the mechanisms by which this resulting health deterioration takes place, animal studies have been performed which clearly indicate the greatly accelerated aging caused by chronic sleep-loss.

Insomnia has now become a highly prevalent problem globally, and it seems to keep growing at a fast rate. Insomnia, independent of other sleep disorders, affected approximately 20 million Americans yearly. The estimated cost of treatment and lost productivity is about $100 billion dollars per year (Daley, Morin, Leblanc et al., 2009; Kessler, Coulouvrat, Hajac, et al., 2010; Roth, 2007). However, judging from the significant recent changes in the economy and stress levels in the USA and worldwide, these estimates have considerably grown and will likely keep growing.

Sleep Architecture and Regulation

Human sleep – as appears to be the case in most other mammals and birds – has two distinct states: REM sleep (rapid eye movements) which is the dream sleep, also called "paradoxical sleep", is characterized by rapid eye movements yet a relaxed muscle tone; and non-REM sleep (NREM), with no rapid eye movements, which is a typically more synchronized cortical neuronal activity, with a more stable autonomic nervous system (ANS) activity.

In 1953 a regularly recurring phase of rapid eye movements was discovered during sleep by Kleitman and Aserinski at the University of Chicago. Kleitman and Dement later termed this phase REM sleep, during which most people reported dreaming when woken up 10-15 minutes after REM skleep onset, as determined by polysomnography (PSG).

The other sleep stage was collectively termed NREM sleep, which was then differentiated into four consecutive stages, N1-N4, to later be reduced to three phases, N1-N3. This was the beginning of sleep research as a modern quantitative science (Dement, 2003).

A typical night involves four to six repeated cycles of alternating NREM and REM sleep periods, each cycle lasting 90-110 minutes. NREM sleep is restful and dreamless, or with dreams that are much less vivid than durin REM, with decreased blood pressure and respiratory rate. NREM sleep is now further divided into three phases, N1, N2 and N3, each with a progressively deeper sleep. NREM seems to normally occur first, at sleep, then it alternates with REM sleep three to six more times per night. In the morning we normally wake up from REM sleep.

Sleep deficiency due to short sleep duration accumulates to result in 'sleep debt', which manifests in an increased drive to fall asleep in order to recover the lost sleep. This sleep-recovery (SR) sleep is characterized by having a shorter sleep latency, of greater intensity and

with a longer total sleep duration, as well as with enhanced EEG synchrony of NREM sleep in the following night. The major changes following sleep deprivation are longer bouts of REM sleep, which delay the recovery of NREM sleep.

Homeostatic Sleep Drive and the Circadian Timing Mechanism

There seem to be two separate processes which work together to regulate sleep-wakefulness: 1) the homeostatic sleep drive, and 2) the circadian timing mechanism. The basic homeostatic process ensures adequate sleep time, and its intensity depends on the duration of time elapsed since the last sleep period. The longer this time interval, the stronger the homeostatic drive to sleep. When wakefulness is very extended, such as after a prolonged sleep deprivation, the brain blocks attempts to remain awake, so that sleep occurs even when the person is active and resists sleep, such as during driving. If sleep debt is substantial, transitions into very short "micro-sleeps" of 3-30 seconds take place, of which the person is unaware, although the electroencephalography (EEG) recording clearly indicates it.

The circadian process is greatly affected by a central circadian timing mechanism, which in mammals was found to be located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus. It is termed the "master circadian pacemaker" since it was shown to generate circadian rhythmicity of about 24 hour. It controls and orchestrates all the other numerous 'local clocks' in other brain regions and in peripheral tissues. Regardless of their locations, these circadian clocks were found to be cell-autonomous and self-sustainable, so they create 'rhythmic oscillations' in a variety of biochemical and metabolic processes (Valko, 2007).

Sleep Deprivation Studies

Experimentally-induced sleep deprivation was found to cause impaired sleep homeostasis, thus leading to sleep-debt, when the body attempts to recover the lost sleep by forcing the body to sleep. A recent key study in rats indicated that this sleep-debt mechanism works through adenosine stimulation of adenosine-A receptors, which then leads to nitric oxide (NO) being released, all of which take place in the cholinergic basal-forebrain area (BF) of the brain. This study indicates that the sensitivity level of this nitric-oxide-mediated recovery sleep becomes gradually attenuated with aging, starting at middle age and progressing with age (Rytkönen, Wigren, Kostin et al., 2010).

Insomnia in Traditional Chinese Medicine (TCM)

TCM (Traditional Chinese Medicine), of which acupuncture and Chinese herbal medicine are two major modalities, have been used to effectively treat insomnia for thousands of years. This section records Dr. Maoshing Ni's summarized view on the basics related to insomnia in TCM (Ni, personal communication, 2015).

Esoteric Daoism, the way of life from which evolved the theoretical foundation of TCM, sees the human essence as composed of three basic components: a) Shen (the heart channel spirit), b) Ching (the physical essence), c) Qi (the concept of energy that is used in acupuncture and is active in every animate living being in the world). It is this concept of Shen that underlies insomnia. Normally, Shen is housed in the heart and becomes quiet during sleep at night. During the day Shen emerges and is active in conducting our daily activities, as well as guiding our Qi and our thought processes in our life.

Hence all insomnia, regardless of its type and manifestation, results from disharmony in the heart. As long as the heart is off balance, Shen too will not be calm. When the heart is not calm

and improperly nourished and housed, our Shen becomes restless. In more extreme conditions, Shen becomes disturbed or agitated, or even deranged, in which cases it may result in a mental illness manifesting as psychosis, schizophrenia, depression and/or anxiety, or as some other mental condition.

According to TCM, insomnia could basically arise for the following reasons: liver Qi stagnation, phlegm heat disturbing the heart, heart Blood and spleen Qi deficiency, heart Yin deficiency, Yin deficiency fire, blood stasis, heart and gall bladder deficiency, liver fire, heart fire blazing, disharmony of heart and kidney, food stagnation, or from both liver Yin and blood deficiency.

The objectives of this study

The main objective of this study was to examine our current knowledge about sleep processes, as well as the role(s) of sleep and the regulatory mechanisms of sleep in health and in pathological processes of sleep, particularly in sleep loss through insomnia or due to experimentally-induced sleep deprivation. The investigator of this work hypothesizes that chronic sleep loss is the root cause of the chronic co-morbidities associated with prolonged or chronic insomnia/sleep loss.

A second major objective was to examine the effects of sleep and sleeplessness on aging. Shedding light on these two central basic processes, of sleep-loss causing aging and co-morbidities, could guide us in slowing down these co-morbidities, or even stopping their progress for a good number of years, to lengthen the life span of such individuals with improved health and quality of life.

There have been many epidemiological studies indicating a clear association between chronic sleep-loss and chronic diseases, however, this investigator found very few

epidemiological studies which enable us to draw causal conclusions about which came first. Was it the chronic disorder that caused the insomnia? Or did insomnia lead to the development of the chronic disease, such as major anxiety disorder, a major depression, schizophrenia, Parkinson's disease, Alzheimer's disease, hypertension, heart failure, diabetes, cancer, and other chronic diseases?

It was therefore the intent of this investigator to review the current evidence for the causal relationship between chronic sleep-loss as in chronic insomnia, or as in experimentally induced extended sleep deprivation, that were shown at the cellular and molecular levels to trigger cellular oxidative stress which began the pathogenesis of serious chronic illnesses, some of which develop late in life, and result in poor life-quality, with serious effects on a patient's daily functioning. Chronic insomnia may greatly affect daytime functioning, with conditions such as constant fatigue, difficulty concentrating, poor memory, as well as fatal car and work accidents.

The third major objective of this work was to study the effectiveness of TCM treatments on chronic insomnia and co-morbidities, including treatments of acupuncture, acupressure, cupping, exercise, and nutrition.

Glossary of Key Terms

Insomnia – sleeplessness. An experience of inadequate or poor quality sleep characterized by one or more of the following: difficulty initiating sleep, difficulty staying sleep, waking up too early in the morning and unable to resume sleep, or nonrefreshing sleep.

(1) Classes of Insomnia (mostly based on Keenan, 2014)

Transient insomnia – lasts less than one week. Usually caused by acute events including changes in sleep environment, jet lag, changes in a work shift, environmental issues such as excessive noise or extreme temperatures.

It may also result from stressful life events, such as an acute medical or surgical illness, use of substances or prescription medications that may be addictive and/or CNS-sedative or stimulant, such as coffee, corticosteroids, decongestants, bronchodilators, amphetamines, heroin, or cocaine, or caused by withdrawal from a CNS (central nervous system) depressant substances, including alcohol or benzodiazepines.

Short-term insomnia – Lasts one to three weeks, with causes resembling those of transient insomnia.

Chronic insomnia – Defined by DSM-IV criteria (APA, 2000) as insomnia for at least one month or more, with ongoing complaints of sleeplessness. Often starts with an acute event. When insomnia persists, it is generally more serious and related to a broader range of deeper problems.

Short sleeper – A person who has a decreased TST (total sleep time), but no significant daytime consequences. Considered a normal genetic variant.

Irregular sleep schedules – Frequently include significant differences between sleep on weekdays and on weekends, which contribute to shifts in sleep phase and sleeping difficulties.

Primary insomnia – Insomnia that is not due to medical, mental, or any other factors.

Secondary insomnia – when in addition to insomnia, the person suffers from other co-morbidities (other disorders/diseases).

Psychophysiological insomnia – Learned or conditioned insomnia. This subtype of primary insomnia usually arises from an episode of acute situational insomnia. The person then associates the bed with non-sleeping and instead of falling asleep he/she becomes hyperaroused whenever attempting to sleep, when they would normally be fast asleep. When the acute situation resolves, the conditioned insomnia persists.

Adjustment sleep disorder – insomnia associated with acute life events, such as medical or surgical illnesses, bereavement, divorce, or stress from other causes.

Co-morbid insomnia – Insomnia associated with a co-morbid medical and/or psychiatric illness, medication use, or another primary sleep disorder/s. Substantial recent evidence points to chronic insomnia as the initial trigger and risk factor for chronic diseases.

Advanced sleep phase syndrome: A circadian rhythm disorder, in which a person has difficulty with early awakenings but no difficulty initiating sleep early at night, with normal sleep quality and duration.

Delayed sleep phase syndrome – A circadian rhythm disorder in which a person has difficulty falling asleep but has normal sleep quality and duration once sleep is initiated.

(2) Instruments of Subjective or Objective Outcome Measures of Sleep

Pittsburgh Sleep Quality Index (PSQI**):** The most frequently used questionnaire for self-reporting sleep-difficulties and sleep-loss. Considered a subjective outcome measurement (Tsai, Wang, Wang et al., 2005).

Insomnia Severity Index (ISI**)** – Together with PSQI, one of the two most frequently used questionnaires for self-reporting sleep-difficulties and sleep-loss. Like PSQI, it is considered a subjective outcome measurement.

Polysomnography – A typically all-night recording of EEG. May simmultaneously record other brain or body activities, such as EOG**,** EMG, ECG (electrocardiogram). Airflow can be simultaneously measured by a nasal thermistor (Budhiraja, Roth, Hudgel, et al., 2011).

Polysomnogram – A recording during sleep of brain activity of a single person, usually in a sleep laboratory. Considered an objective outcome measurement.

EEG - Electroencephalography, an objective measure of brain activity typically during sleep. Measures brain wave forms and duration of each sleep-stage – REM, NREM, and wakefulness at nighttime.

EMG – Electromyography, recording of movements of skeletal muscles of the chin/neck, leg or other skeletal muscles.

EOG – Electrooculography - for recording an electrooculogram of eye-moving muscles.

Actigraphy - A wristband for recording movements of the upper limbs mostly during sleep at night. Considered an objective outcome measurement.

3) Sleep Stages

REM – REM sleep, also termed "Paradoxical Sleep", characterized by rapid eye movements and a relaxed muscle tone. This is considered to be the main dreaming sleep phase. Its EEG is of low-voltage and mixed frequency, resembles EEG during relaxed wakefulness. Periodic bursts of rapid eye movements, with variable autonomic activity and a relaxed muscle tone. Constitutes about 20-25% of total sleep, with REM sleep episodes becoming longer over the night (Carskadon and Dement, 2005; Luyster, Strollo, Zee & Walsh, 2012).

NREM – Non-REM sleep, is considered dreamless and restful (some people report dreams during NREM, which are cosiderably less lively than during REM sleep). Characterized by having a more synchronized cortical neuronal activity, and a more stable autonomic nervous system (ANS) activity, such as a decreased heart rate and blood pressure, and stable breathing, especially during the deeper sleep stages (Luyster et al., 2012). NREM sleep is considered to be a more restful and restorative sleep stage, for restoring depleted cellular substances such as ATP and antioxidants. NREM occurs first, when we just fall asleep, then it alternates with REM sleep three to six more times per night. In the morning we wake up from REM sleep. NREM Sleep is comprised of three stages: N1, N2 and N3, each with a progressively deeper Luyster, Strollo, Zee & Walsh, 2012sleep.

N1 – characterized by being very brief. Its EEG resembles EEG of a drowsy wakefulness, with high frequency low amplitude wave forms. Has a low threshold for arousal, and easily wakes up.

N2 – Similar to N1, with a higher threshold for arousal, hence a deeper sleep, and of a longer duration.

N3 – also termed “Slow Wave Sleep” (SWS). Characterized by high-voltage slow wave activity, and considered to be the deepest sleep in humans. Believed to be restorative since it shows a markedly decreased sympathetic nervous system (SNS) activity, such as a decreased heart rate and blood pressure, and a stable breathing. Believed to play a major role in both learning and memory consoidation.

Chapter 2: Literature Review

This chapter begins with three recent representative examples of epidemiological studies of insomnia and co-morbidities, with typically large numbers of participants. Next this chapter presents the more modern recent studies, on sleep deprivation and its effects on the organism at the cellular and molecular levels, in an effort to study the mechanisms at work and the role(s) of sleep through the effects of sleep-loss on the organism. It then continues with the relation of sleep-loss to pathogenesis of chronic diseases and to the aging process of the organism.

I. Western Medicine Research Approaches to Chronic Insomnia

1) Epidemiological Studies of Insomnia

Three examples of relevant studies are reviewed here.

a) Budhiraja, Roth, Hudgel et al. (2011). Prevalence of Polysomno- graphic Correlates of Insomnia Co-morbidities with Medical Disorders

Budhiraja et al. examined the association between self-reported medical disorders, a diagnosis of chronic insomnia, and a polysomnographically (PSG) recorded sleep in a large community sample.

The researchers' two hypotheses were: a) participants with a history of medical disorders have a higher prevalence of chronic insomnia than those without any reported medical disorders, and b) the risk of having chronic insomnia would depend on the number medical disorders of the person, with greater risk when having more medical disorders. Both were confirmed by these researchers' outcomes.

Participants were recruited from the general population of the tri-county Detroit area by telephone survey, using a random digit dial. At this first stage the researchers excluded individuals who could not answer the questionnaire because of sensory or mental impairments, and those younger than 18 or older than 65. From the 4,682 persons recruited, 3,283 (70.1%) participants completed the telephone survey.

Based on the US Census of the year 2000, the socio-demographic characteristics of their sample were similar to the USA general population, except for a higher proportion of African-American participants and a lower proportion of Hispanic participants. 668 participants of the total interview sample were then randomly selected to participate in the sleep laboratory study.

Chronic Insomnia was assessed using DSM-IV (2000) criteria, so that respondents had to have chronic sleep complaints with daytime functional impairment. All the subjects had to have reported difficulty falling asleep, staying asleep, or having a non-restorative sleep at least sometimes or often for one month or longer.

To comply with the DSM-IV criteria, respondents were then requested to complete a validated subjective self-report scale of sleepiness, the "Epworth Sleepiness Scale", as well as answer the following three questions:

a) How many days in the last three months had they missed work or school because of sleep problems, or had reduced productivity by half or more at work/school due to sleep problems?

b) How many days in the last three months did they not do any household work due to sleep problems – or had productivity reduced by half or more in household work?

c) How many days in the last three months did they miss social or leisure activities due to sleep problems?

If they answered any of the above three questions with “more than twice a week”, or their score on the Epworth Sleepiness Scale was greater than 10, they were considered as having daytime impairment due to insomnia. Three months were used to ensure that they had significant daytime chronic functional impairment due to sleep problems. All remaining Ss from the overall interview who scored high on the validated Epworth Sleepiness Scale were asked to participate in the sleep laboratory study (n = 668).

Medical Disorders

Respondents were then asked whether they had any of the following medical disorders: heart disease, hypertension or high blood pressure, diabetes, thyroid problems, cancer, ulcers, colitis, arthritis, migraines, asthma, COPD (chronic obstructive pulmonary disease), or neurological problems. Women were additionally asked if they currently had menstrual problems. Responses of No/Don’t Know were collapsed together for analyses.

Sleep Objective Measurement Outcomes

The diagnostic PSG included recordings of EEG (electroencephalogram), EOG (electrooculogram) to measure eye movements during sleep, submental and leg EMG (electromyograms) to evaluate skeletal muscle movements during sleep, and EKG (Electrocardiogram), to assess the heart function during sleep, with airflow measured by a nasal thermistor, to measure breathing during sleep. Both cardiovascular and breathing measurements aimed at assessing the ANS (autonomic nervous system) activities during sleep.

All studies were scored according to previously published criteria. Time in bed was set at 8:30 pm, sleep efficiency was calculated as total sleep time divided by the time in bed multiplied by 100. Sleep latency was defined as time from lights off to the first epoch of any sleep stage.

Findings

1. Ss with chronic insomnia were slightly younger: 40.2 ± 11.5 *versus*. 42.1 ± 12.9 (P< 0.001) and more likely to be women, since chronic insomnia prevalence was 24.7% among women *versus* 18.0 among men (P< 0.001).
2. Participants reporting medical disorders tended to be older: 43.2 ± 12.4 *versus* 37.1 ± 12.5 (P< 0.001).
3. Chronic insomnia prevalence among Ss with any medical disorder was significantly higher than among those with no medical disorders: 26.3% *versus* 14.8% (P< 0.001), and increased with increasing number of medical disorders (P< 0.001).
4. Logistic regression indicated that participants with any medical disorders and women had higher odds of having chronic insomnia (P< 0.001), while older respondents had lower odds of having insomnia (P< 0.04).
5. 54.9% of the respondents reported at least one medical disorder.
6. Of all the respondents, 45.3% reported no medical disorders, 29.4% reported one medical disorder, 14.9% reported two medical disorders, 6.7% reported three medical disorders, and 3.7% reported more than three medical disorders.

Significantly Greater Sleep Efficiency (SE) Found in Women:

Among the participants who underwent PSG (n = 621), the mean SE (sleep efficiency) was 83.9% ±12.4%, and SE was found to be inversely correlated with age: r = - 0.44, (P< 0.001). Women (n = 320) had a significantly higher mean of SE than men (n = 301), 84.9% *versus* 82.8% (P< 0.04).

Sleep Efficiency (SE) was inversely correlated with SL (Sleep Latency), r = -0.48 (P< 0.001), and SL was inversely correlated with continuous sleep: r = -0.61 (P< 0.001). Participants with any medical disorder had a lower proportion of REM sleep than did participants without medical disorders.

Prevalence of chronic insomnia in this community-based sample of 3,282 participants was 21.4%. The adjusted odds of insomnia were 2.2 times greater in persons with any medical disorders, compared with persons reporting no medical disorders.

Odds of chronic insomnia were 1.6 fold higher in people with heart disease (P < 0.005), 1.5 fold higher (P< 0.001) in people with hypertension, 1.5 fold higher (P< 0.001) in people with diabetes, 2.1 fold higher (P< 0.001) in people with stomach ulcers, 1.8 fold higher (P< 0.001) in people with arthritis or migraine, 1.6 fold in asthma (P<0.05), 1.9 fold in COPD (P< 0.001), 2.0 fold in neurological problems (P< 0.001), and 1.7 fold in menstrual problems (P< 0.001) than in people without these disorders.

Chronic insomnia frequency increased with increasing number of medical disorders. However, for most of the assessed medical disorders, PSG-recorded sleep was not significantly different in persons with medical disorders versus persons without medical disorders. The researchers therefore concluded that chronic insomnia is highly prevalent in diverse chronic

medical disorders. However, PSG evidence of disturbed sleep was present only in a subset of co-morbid insomnia populations.

The greatest problem with such epidemiologic studies is that there is no way to know which associations are causal, and what exactly causes chronic insomnia, or vice versa, if chronic insomnia causes any of these diseases or others. Hence the researchers state in their summary that they chose to further investigate these questions using other methods.

b) Taylor, Lichstein, Durrence et al., 2005. Epidemiology of Insomnia, Depression, and Anxiety.

The second epidemiological study (Taylor et al., 2005) investigated insomnia's relation to depression and to anxiety. About 9-12% of the USA population was estimated as suffering from chronic insomnia, which appears to be more widely spread than heart disease, cancer, AIDS, neurological disease, breathing problems, urinary problems, diabetes or gastrointestinal diseases, with an estimated annual cost of $30-35 billion.

These researchers in their previous research (2003) found not only a strong association between chronic insomnia, depression and anxiety disorders, but also a possibly causative inter-relation, so that chronic insomnia could possibly be a risk factor for depression and anxiety.

The researchers state they have carefully designed and rigorously controlled this study, to rule our any possible confounders, in order to find out the relation between chronic insomnia, depression and anxiety. Their findings confirm the close relation of chronic insomnia with both depression and anxiety.

This was a community-based sample of the Shelby County of Tennessee. Participants were first contacted using a random digit dialing protocol, and 1,769 volunteers responded. To

remove any confounding variables, their exclusion criteria included: BMI (body mass index), cigarette smoking, diabetes, pain, neurological problems, PLM (periodic limb movement), RLS (restless leg syndrome), cancer, etc.

Of the remaining 534 participants, aged 20-89, 150 had insomnia, and 384 had no insomnia. The researchers then randomly assigned the participants to various 10-year age groups, with at least 50 men and 50 women in each age group.

Each participant received a self-report questionnaire packet that included 14 sleep diaries for two weeks. Participants were asked to assess their own sleep from the previous night, as well as general information about health, sleep, depression and anxiety. Sleep diaries were previously shown to correlate quite well with PSG ($r = 0.63$-0.87), and were found to be better than single-point retrospective estimates of typical sleep.

Daytime mood was evaluated using the BDI (Beck Depression Inventory), one of the most commonly used depression measures, with extensive reliability and validity data. It is comprised of 21 items, and scores range from 0 to 63, with higher scores indicating greater depression.

Anxiety was assessed using the STAI (State Trait Anxiety Inventory), one of the most frequently used self-reported anxiety measures, with adequate reliability and validity. It has 20 items, with a total score ranging 20-80, with higher scores indicating greater anxiety. Exclusion criteria at this point included: returned questionnaires with no ethnicity data, persons of Asian or Hispanic descent (authors explained they had too few volunteers - 1 Hispanic, 7 Asian), persons reporting sleep disorders but no insomnia complaint (e.g. having apnea or frequent leg jerking during sleep).

A “health problem” was operationally defined as an affirmative answer to one of the global disease categories: heart disease, AIDS, high blood pressure, cancer, neurological disease (e.g. seizures, Parkinson’s Disease, breathing problems, urinary problems, diabetes, chronic pain, or gastro-intestinal (GI) problems).

Findings

a) Participants with insomnia had higher depression and anxiety levels than those having no insomnia. People with insomnia had a 9.82 fold greater likelihood of having a clinically significant depression, and had a 17.35 fold likelihood of having a clinically significant anxiety compared to people with no insomnia.

b) Increased insomnia frequency was associated with increased depression and anxiety. Only two sleep variables were found to significantly relate to depression and anxiety: (1) increased number of awakenings was associated with increased depression, and (2) people with combined insomnia (both delayed sleep onset and difficulty staying sleep) had greater depression than did people with delayed sleep onset, sleep maintenance difficulties, or a mixed insomnia. No differences were found between other insomnia types.

c) African Americans had a 3.43 fold likelihood of having a clinically significant depression, and a 4.8 fold likelihood of having a clinically significant anxiety than did Caucasians.

d) Women had significantly higher levels of depression than men.

c. Cappuccio, Cooper, D'Elia et al. (2011). Sleep Duration predicts Cardiovascular Outcomes: a Systematic Review and Meta- Analysis of Prospective Studies.

Objectives

Assess the relationship between duration of sleep, morbidity and mortality from CHD (coronary heart disease), stroke, and CVD (total cardiovascular disease).

Methods and results

The researchers conducted a systematic search of publications using MEDLINE (1966–2009), EMBASE (from 1980), the Cochrane Library, and manual searches without language restrictions.

Inclusion Criteria:

Criteria used included the original article, prospective cohort design, assessment of sleep duration at the baseline exposure, cause-specific death or non-fatal incident case of CHD (Coronary Heart Disease), stroke or CVD (Cardiovascular Disease) recorded prospectively as outcome, followup of at least three years, adult population, and indication of the number of subjects exposed and the rate or number of events in different sleep duration categories, studies which presented the RR (relative risks) and had a 95% confidence interval (CI), with data pooled using a random-effect model.

Exclusion Criteria

Case-control design. If multiple published reports of the same study were available, these researchers included only the one with the most detailed information for both exposure and outcome.

Data were extracted independently by two investigators (F.P.C. and D.C.), and references were resolved by discussion with a third investigator (M.A.M.). In each study the researchers

identified the reference category, being 7-8 hours per night in the majority of studies. In most studies, 'short' sleep was defined as <(5-6 hours/night), and 'long' sleep as >8-9 hours per night.

Statistical Analyses

The studies' quality was assessed by the Downs and Black Quality Index score system. The feasibility of creating a checklist for the assessment of the methodological quality both of randomized and non-randomized studies of health care interventions.

Relative risks (RR) or hazard ratios were extracted as a measure of the relation between sleep duration and disease-incidence. Pooled RR and 95% confidence interval (CI) were estimated using a weighted random-effect model.

The researchers state they had carried out the following tests to prevent bias: 1) They separately estimated the pooled RR and 95% CI of disease for the 'short' and 'long' sleep categories. 2) They then tested for heterogeneity among studies. 3) Publication bias by funnel plot asymmetry and Egger's test, 5) Sensitivity and subgroup analyses. All the statistical analyses were performed using MIX software version 1.7, and they state that their study adheres to the PRISMA Statement Guidelines (supplementary material online, Appendix S2).

Overall, 15 studies were included, with 24 cohort samples. Participants were 474,684 male and female participants, with a follow-up range of 6.9–25 years, covering 16,067 events, of which 4169 were of CHD (coronary heart disease), 3478 of stroke, and 8420 of total CVD (cardiovascular disease).

Sleep duration was assessed by the subjective self-reported questionnaires, and incident cases were found through the official registers of certifications and events.

Findings

Short duration of sleep was associated with a greater risk of developing or dying of CHD ($P < 0.0001$), stroke ($P < 0.047$), but not of total CVD ($P = 0.52$), with no evidence of publication bias ($P = 0.46$).

Long duration of sleep was also associated with a greater risk of CHD ($P < 0.0005$), stroke ($P < 0.0001$), and total CVD ($P < 0.0001$), with no evidence of publication bias ($P = 0.79$).

Conclusions

On the basis of this study's results, the researchers concluded that there seems to currently be no evidence that habitual sleep of 6-8 hours per day in adults is associated with harm or long-term health consequences. However, sleeping 9 hours or more per night may represent a useful diagnostic tool for detecting sub-clinical or an undiagnosed co-morbidity.

On the other hand, persons reporting consistently sleeping 5 hours or less per night, should be regarded as having higher risk for cardiovascular morbidity and mortality. The researchers summarize that this study implies that both short and long duration of sleep are predictors, or markers, of cardiovascular outcomes.

d. Buysse, Angst, Gamma et al., 2008. Prevalence, Course, and Co- morbidity of Insomnia and Depression in Young adults.

Study Design:

Longitudinal prospective investigation of insomnia in young adults having infrequent short episodes of depression, employing the assessment of psychiatric, physical and sleep symptoms in a community sample of young adults (n = 591), with six interviews over 20 years, to observe the changes in insomnia and in depression over 20 years.

Study objectives:

1. Describe the prevalence and prospective course of insomnia in a representative sample of young adults.

2. Describe the cross-sectional and longitudinal associations between Insomnia and depression.

Setting: The Community of Zurich, Switzerland.

Ss: A representative stratified population sample

Interventions: None.

a) **Measurements**: Prospective assessment of psychiatric, physical and sleep symptoms in a community sample of young adults (n = 591) with six interviews over 20 years.

b) The researchers differentiated four duration-based subtypes of Insomnia:

1). A one-month insomnia associated with significant distress.

2). A recurrent brief insomnia of 2-3 weeks.

3). Occasional brief insomnia.

a. The annual prevalence of the one-month insomnia gradually increased over time, with a cumulative prevalence of 20% and a greater than two-fold risk among women.

b. In 40% of the Ss, insomnia developed into more chronic forms over time.

c. Insomnia, either with or without co-morbid depression was highly stable over time. d. Insomnia lasting two to three weeks predicted major depressive episodes and a major depressive disorder in subsequent interviews (17-50% of these Ss developed a major depressive episode in a later interview).

e. “Pure” insomnia and “pure” depression were not longitudinally related to one another, whereas insomnia co-morbid with depression were longitudinally related.

Conclusions

a. This longitudinal study confirms the persistent nature of insomnia and the increased risk of subsequent depression among individuals with insomnia.

b. The data support a spectrum of insomnia (defined by duration and frequency) co-morbidities with, rather than secondary, to depression.

II. Sleep Deprivation Studies at the Cellular and Molecular Levels

In the last ten years intensive research has been conducted to discern the pathological outcomes of chronically disrupted sleep, in both humans and in experimental animal models, including at the cellular and molecular levels. These studies became possible thanks to recent technological advances in molecular biology and biotechnology, such as gene cloning and DNA sequencing, the development of complex combinations of delicate techniques such as micro dialysis, unit recording, axonal tracers and immunohisto-chemistry, which gave researchers the tools for much more advanced and specific research.

Everson and colleagues at the Medical College of Wisconsin in Milwaukee, methodically studied CSD (chronic sleep deprivation, or experimentally-induced prolonged sleep deprivation) in rats for over a quarter of a century since the 1980's, attempting to unravel the different outcomes of experimentally induced sleep loss. The present work reviews three of Dr. Everson and colleagues' recent studies on chronic sleep deprivation (Everson, Laatsch and Hogg, 2005; Everson, Thalacker and Hogg, 2008; and Everson and Szabo, 2011).

Although there has been no consensus thus far regarding which biomarkers define a chronically sleep deprived (SD) state, for enable assessing the physiological impact of chronic SD on the organism, Dr. Everson and colleagues state they have seen a unique clinical profile emerging in their animals after exposure to chronic SD.

The principal signs include: a) abnormally high food consumption, b) overeating with no weight gain (and even weight loss), c) an early yet sustained decreased anabolic hormones at the hypothalamic level, such as TSH (thyroid stimulating hormone), and d) early temporary infections of internal organs by opportunistic microorganisms, e) three weeks later the opportunistic microorganisms develop into an advanced morbidity and lethal septicemia if not

reversed by enabling the animals to sleep and recover before too much damage was done to their vital internal organs.

a) Everson, Laatsch and Hogg, 2005. Antioxidant Defense Responses to Sleep Loss and Sleep Recovery.

In their 2005 study, Everson, Laatsch and Hogg produced TSD (Total Sleep Deprivation) or PSD (Partial Sleep Deprivation) of five- or ten-days in young adult male rats. This study design (Appendix C) employs four experimental groups, five days of TSD or PSD, and ten days of either TSD or PSD.

Their two control groups consist of a baseline conditions group, and a SR (Sleep Recovery) of 48 hour group when sleep *ad libitum* was allowed to the TSD animals following 10 days of sleep deprivation (Appendix C). The researchers traced the uncompensated oxidative stress and the antioxidant response in three peripheral tissues: liver, lung and heart.

Ten days of SD were chosen since 10 days were found to be sufficient time to produce clear effects of adaptation in response to chronic TSD, yet with no deep irreversible morbidity.

Their objectives were to study how much oxidative stress these three vital organs were exposed to by this study's TSD and PSD protocols, and how these rats' bodies responded to it.

GSH (glutathione) is considered the major free radical scavenger in the organism, and is normally under tight regulation by enzymatic control. Since GSH content normally varies within a narrow range for the organism to maintain homeostasis, following GSH levels, as well as studying the activities of GSH's main regulating enzymes, catalase and glutathione peroxidase (GPX), and some other indices of glutathione recycling, could reveal the organism's antioxidant

response to oxidative stress resulting from CSD (chronic sleep deprivation) in the three peripheral tissues under study.

To produce TSD (total sleed deprivation) and PSD (partial sleed deprivation), the researchers employed the Bergmann-Rechtschaffen disk method (Bergmann et al., 1989; Appendix C), a programmable housing apparatus attached to a computer, to reliably produce the required amount of SD.

Each experiment consisted of procedures conducted on two animals at the same time, in either a totally sleep-deprived (TSD) animal and its paired yoked animal of PSD of the experimental condition, or in two control animals under baseline conditions. The floor platform of the housing apparatus could be rotated so as to compel both housed animals to get up and walk in order to stay in a comfortable position and keep dry by not having to step into a pan of shallow water (Appendix C).

During the SD (sleep deprivation) phase of this study, the platform rotated 18% to 22% of the time, compelling the animals to get up and walk, thus preventing them from sleeping. The platform was programmed to rotate every time the TSD-rat were ready for their sleep-onset, which was sensed by the attached computer. The PSD paired rat had to likewise walk, but this platform rotation did not coincide with the PSD rat's sleep onset, so the PSD animal could still sleep while the TSD rat was engaged in other behaviors except for attempting to fall asleep.

For the TSD animals, the TST (total sleep time) obtained during the SD phase was about 10% of the baseline TST (5.4%), compared with 54% of TST in the control rats, and their obtained sleep was a mostly transitional sleep and very fragmented high-amplitude NREM sleep, with only 1% of REM sleep, compaired with 6% REM in controls. PSD animals' sleep was highly fragmented as well, but with 38% of NREM and 3% of REM sleep. Yoked rats (PSD)

typically exhibited several similar tendencies as the TSD rats, but with weaker effects that did not always reach statistical significance.

Findings

Glutathione (GSH) content in the liver significantly decreased (-30% GSH decrease in TSD animals by both day 5 and 10) compared to the baseline controls ($P<0.04$). GSH being the major marker for antioxidant depletion and tissue repair, these animals seem to have been greatly affected by this experimental CSD intervention even by day 5, manifesting this early and sustained decrease in GSH as very different from the relatively unchanged GSH content in the PSD animals at the same time points.

The activity of catalase, an enzymatic antioxidant, in the liver was also strongly decreased by the CSD ($P<0.001$), by 23% in TSD rats by day 5, and by 36% by day 10, compared to the baseline controls. The PSD animals showed the same tendency, but did not reach significance. SR was marked by TSD rats returning to normal concentrations of liver GSH within 48 hours, and catalase activity in the liver likewise increased to almost normal activity levels. The restored content of liver GSH during the SR 48 hour stage was associated with significantly increased GPX activity in TSD rats.

In the heart muscle, SR was marked by supranormal levels of GPX activity of 30% in PSD and 40% in TSD above baseline.

GPX and catalase activity in liver, heart and lung, are two major enzymatic antioxidants and indices of GSH recycling. Normally both GPX and catalase activities maintain tight enzymatic regulation of GSH to maintain homeostasis.

In the lung no significant treatment-effects were found for antioxidant parameters, and the researchers explain this as a likely result since the lung does not contain much GSH

compared to the liver. No changes from baseline levels were found in the lung during the SD period nor during the SR 48 hours.

Plasma amino transferases, considered markers of cell injury indicating cell-membrane damage, allow leakage of the cell content. In this study the researchers traced the changes of the three aminotransferase: ALT (alanine), AST (aspartate aminotransferase), and GGT (gamma-glutamyltrasferase). Their findings were as follows:

a) AST and ALT significantly differed between TSD and PSD animals: both increased in TSD animals, AST to 264% ($P<0.001$) of the baseline controls, and ALT to 219% ($p<0.01$) of baseline controls' concentrations, without a significant change in their ratio.

b) At each point across cumulative SD, values in the TSD animals were significantly different from those of the PSD (yoked) animals, as well as from their own baseline values. Although PSD levels were not as high as in TSD animals, yet PSD animals demonstrated a progressive increase in plasma ALT and a late yet significant increase in plasma AST.

c) GGT did not differ from basal levels in either TSD or PSD animals until later, when GGT was significantly increased in TSD animals.

These results indicate the early great damage caused to the cell membranes by this regeme of sleep deprivation, with the cellular oxidative stress damaging the cell membranes by TSD day 5, when the braking of the cell membranes resulted in great leakages of the cellular content, including these amino transferases, into the plasma, thus their increased quantities in the plasma.

Other indices of GSH recycling in the cells are two enzymes of the oxidative pentose phosphate pathway. G-6-PD is the first enzyme in the oxidative pentose phosphate pathway, and 6-PGD is the second enzyme in this pathway. Restored liver GSH and catalase content were

associated with marked increases of both G-6-PD and 6-PGD, which enable the recovery of GSH recycling so it can once again act as an anti-oxidant, to resolve the oxidative stress in the cells.

Sleep recovery (SR) from PSD in the yoked animals showed high activity levels of both G-6-PD and 6-PGD, which were even higher than in the TSD animals.

In the heart muscle, SR 48 hours resulted in significantly increased G-6-PD 66% beyond baseline levels in the TSD rats at 5 days, and 82% at 10 days of TSD, and in yoked animals at 5 days of PSD it initially increased by 54%. GPX increased activity was observed on day 10 in TSD.

In the heart muscle no detectible changes were observed in total GSH content or in catalase activity, neither in TSD nor in PSD animals.

SR 48 hours in the heart muscle were marked by a strong yet nonsignificant trend towards decreased G-6-PD levels in TSD animals, which still averaged 35% above baseline, whereas PSD animals still showed activity levels of 60% above baseline.

The changes of G-6-PD were essentially mirrored in 6-PGD, although group differences from baseline control were nonsignificant for 6-PGD in TSD or in PSD animals at 5 days.

GSSG-R activity, as determined by the GSSG-R recycling assay, is a kinetic method of absorbance spectrophotometry. GSSG is glutathione disulfide, the oxidized glutathione, whereas GSSG-R is glutathione reductase, which catalyzes the reaction of reducing GSSG back to GSH, so it can again resume its role as an anti-oxidant.

In the heart an accelerated rate of the oxidative pentose phosphate pathway was observed during the SD stage, which determines the NADPH supply for GSH reduction, coinciding with a marked increase in the levels of all three aminotransferases (ALT, AST, GGT), that indicates cell membrane damage resulting in leakage of the cellular-content. Day 10 in TSD animals was

marked by a GPX activity increase of 30-40% above the baseline control animals, indicating an increased oxidative stress.

The restored content of liver GSH during SR 48 hours was also associated with a nonsignificant tendency of increased GSSG-R activity from sleep deprivation levels, and a significantly increased GPX activity in the TSD group.

Interpretation of the Oxidative Stress Resulting from Chronic Sleep Deprivation (CSD).

Depletion of antioxidants is considered a disease-associated oxidative stress, since attenuated defenses have been associated with increased vulnerability to disease. GSH depletion of 20–30% below normal can impair cellular defense against ROS (reactive oxygen species), and may lead to disrupted cell communication, aberrant protein degradation, and to serious cell injury, the cell being exposed to increased oxidative strsss.

In animal models of SD, diminished antioxidant capacity takes place concomitantly with profound decreases in energy balance (exhausting of ATP), as well as host defense impairments (anti-oxidants' exhaustion), pointing to at least two sources of heavy oxidative burdens resulting from the current study's CSD protocols.

SR 48h (sleep recovery after 10 days of TSD) tended to normalize the cellular antioxidant activities, as well as the oxidant capacity in both liver and heart, as shown by the increase in GSH content and increased catalase activity to almost normal levels. Marked increases were also seen in the enzymes G-6-PD and 6-PGD, the first and second enzymes in the oxidative pentose phosphate pathway, and GSSG-R reactivity showed the same tendency for increased activity, yet not reaching statistical significance. All these findings during the SR (sleep recovery) indicate an almost complete recovery from the extensive oxidative stress seen in these animals following the 10 days of TSD.

Body Weight and Food Intake

Both changes in TSD and PSD rats showed a strong effect of the experimental treatment. Significant body weight reduction despite significant hyperphagia, especially in TSD ($P < 0.001$), together with a marked negative energy balance (ATP exhaustion), both support these researchers' previous findings (Everson, Bergmann and Rechtschaffen (1989); Everson and Crowley, 2004; Everson and Wehr, 1993).

TSD rats progressively lost 16% of their baseline body weight by the second day of SR. PSD rats showed similar tendencies but to a lesser extent, although their food consumption was higher during SR ($P < 0.016$) compared to the TSD animals.

Food intake

Baseline food intake of both TSD and PSD groups was similar. TSD food intake increased during the SD stage by 177% of baseline amounts by day 10. Their food intake dropped back to near-normal food intake by SR (sleep recovery) day 2, but was still 6.3% above baseline.

Summary and Conclusions

The decreases of both GSH content and catalase activity in the liver by day 5 of TSD rats occurred early relative to the expected survival time of TSD animals, and were sustained or worsened by prolonging the SD period.

The researchers interprete the 30% decreases in liver glutathione (GSH) content and in catalase activity, without increasing the GPX (glutathione peroxidase) or G-6-PD activity levels, as strong indications that in these animal models of CSD, diminished antioxidant capacity takes place concomitantly with profound decreases in energy balance (greatly decreased ATP), as well

as host defense impairments (decreased anti-oxidants), so that the SD in the current study clearly produces uncompensated oxidative stress.

These researchers focused on measuring glutathione (GSH), which is the main marker of antioxidant depletion or repair, and the enzymatic antioxidants associated with the GSH recycling pathways, GPX (glutathione peroxidase), which reduces hydrogen peroxidase (H2O2) and other hydroperoxidases, such as lipid hydroperoxides in a reaction coupled to the oxidation of reduced glutathione (GSH). GSSG-R is glutathione reductase, which catalyzes the reaction of reducing GSSG back to GSH, so that it can participate in antioxidant activities, to counteract the cellular oxidative stress, to re-establish the cellular homeostasis.

The co-factor for GSSG reduction is NADPH, which is provided by means of the oxidative pentose phosphate pathway. In this pathway, the two key enzyme indicators of the rate of NADPH regeneration from available NADP are G-6-PD (glucose-6-phosphate dehydrogenase) and 6-PGD (6-phosphogluconate dehydrogenase).

Catalase is an antioxidant enzyme, that catalyses the conversion of hydrogen peroxidase (H2O2) into water and oxygen (O2). Catalase is located in the peroxisome and is the counterpart of GPX (glutathione peroxidase), which is mainly located in the cytosol.

The appearance of increased plasma aminotransferases (ALT, AST, GGT) indicates cell membrane damage and leakage from the cytoplasm, by means of which many investigators measure oxidative stress to cell injury. In this study increased plasma aminotransferases was the earliest sign of cell damage, appearing first by SD day five.

The researchers emphasized that they focused on effects resulting from sleep deprivation on peripheral tissues, as opposed to most other studies attempting to find SD consequences in the CNS, with little success. Their focus on systemic effects of SD is due to their extensive

experience with SD in the rat animal model, where the first signs of SD are systemic. Additionally, most co-morbidities in humans that are linked to disturbed or impaired sleep – such as cardiovascular diseases, diabetes and arthritis – are foremost described by their peripheral manifestations.

The researchers explain that as with other basic needs – food, water, oxygen and warmth –to enable the study of sleep regulation, the deprivation of sleep must be prolonged and fairly constant, since all the physiological mechanisms in the body strongly protect basic needs from depletion or impairment, to ensure survival of the organism, even at the cost of maladaptations that are less than optimal for its health.

Hence they focus on the antioxidant status of peripheral tissues during SD and during subsequent SR, hypothesizing that prolonged SD disrupts the homeostatic balance in the body by increasing the oxidative stress. Their findings in this study support this hypothesis.

Comparable Tissue Injuries

The injuries seen in these sleep-deprived rats are of comparable severity with other animal models of pathogenesis, such as toxicity from chronic cyclosporine-A administration, which was found to result in a 30-37% decrease in rat liver GSH, 72 h of starvation and the streptozolocin-induced diabetes in rats, each associated with 25% decreases in rat liver GSH. Skin burn injury-induced damage was associated with a 50% decrease in rat liver GSH, and intoxication by the herbicide paraquat resulted in a 25% decrease in liver GSH.

Weight loss continued into the SR 48h phase, after antioxidant concentrations had already become normalized and GSH recycling appeared accelerated, suggesting that additional factors may be involved beyond the weight loss.

Depletion of GSH content and decreased catalase activity during SD occurred despite a progressive rise in food consumption. The high food intake rate during SD period was shown by other studies to be adaptive (Everson and Wehr, 1993), in addition to food being a major source for antioxidants. This suggests that uncompensated stress would be considerably worse under nutritional limitations, where the animals cannot consume much more food and water than they normally would.

Oxidation of foodstuffs may also produce toxic intermediaries. Even so, liver GSH content was already diminished by the fifth day of TSD, a time when food intake and body weight changes were not yet different from the PSD group, suggesting involvement of additional factors.

Incomplete compensation for the negative energy balance or the immune-related activities would be expected to result in an imbalance between pro- and anti-oxidants, which is how imbalances are expected to result in health impairments. Cell injury is expected whenever antioxidant activities are not sufficient to balance the oxidative stress.

SD-induced increased in plasma aminotransferases by SD day five, and progressed when SD was prolonged, results which provide evidence for peripheral cell-membrane damage as an early consequence of SD, relative to the later developments of advanced morbidity and lethality. Increases in both plasma AST (aspartate aminotransferase) and ALT (alanine aminotransferase), without a change in their ratio, suggest involvement of tissues besides the liver, such as muscle and possibly the heart.

Both body weight and food intake showed a strong effect of TSD (as well as of PSD) on the energy level, indicating a marked negative energy caused by oxidative stress (which exhausted the ATP), clearly resulting from the prolonged chronic sleep loss. Significantly

lowered body weight ($P<0.001$ on SD day ten) and increased food intake ($P<0.001$ were observed on both TSD days 5 and 10), when there were still no observable difference in body weight nor in food intake between the experimental and control groups, indicating great damage to the organism due to impaired homeostasis.

By SD day 10, TSD greatly increased the rats' food intake to 177% (±SE 16) above baseline levels. TSD animals recovered by the second SR day, and their food intake dropped, yet it still was 6.3% (±SE 5) above baseline. TSD rats progressively lost body weight while consuming large amounts of food, which clearly indicates a negative energy balance (energy exhaustion due to the great cellular stress).

The marked increases in both liver glutathione content and catalase activity in sleep-deprived rats yet no detectable increases in recycling activities suggest an ongoing process of uncompensated oxidative stress.

In the heart, both the accelerated rate of the pentose phosphate pathway, which determines the NADPH supply for reducing glutathione to produce GSH during the SD stage, coincide with marked increases in serum aminotransferase concentrations, indicating cell damage.

Sleep recovery stage (SR 48 hour) was associated with restoration and exaggeration of antioxidant activities and oxidant capacity in both liver and heart.

Valko, Leibfritz, Moncol et al., 2007. Free Radicals and Antioxidants in Normal Physiological Functions and Human Disease,

The following paragraphs are additional background explanations based on the Valko et al. (2007) review on free radicals and causes of oxidative stress. It provides a wider perspective on homeostasis and some mechanisms of conditions that lead to imbalance in the organism, such as oxidative stress. Also reviewed is how oxidative stress could result in pathogenesis, such as seems to operate in prolonged chronic sleep deprivation.

ROS (reactive oxygen species) and RNS (reactive nitrogen species), are both well known for their dual role as both beneficial and injurious to living cells. Normally their production in the cell is tightly controlled by enzymes such as NOS (nitric oxide synthase) and NAD(P)H oxidase isoforms, respectively. However, stress conditions could easily upset this delicate balance. For example, overproduction of ROS arising from the mitochondrial electron transport chain for energy production in the body, or due to over-stimulation of NAD(P)H), result in oxidative stress, which is an injurious process capable of mediating serious damage to cell structures.

Free radicals can damage lipids, proteins, and DNA, as well as disrupt the cell membrane functions and the cellular organelles. Chronically increased ROS- or RNS-induced oxidative stress have been associated with pathogenesis of cancer, cardiovascular disease, atherosclerosis, hypertension, ischemia/reperfusion injury, diabetes mellitus, neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, rheumatoid arthritis, and aging.

As mentioned above, oxidative stress is associated with increased formation of ROS, which in turn augments the oxidative stress even more. In addition to cellular protein and lipid damage, abnormalities in myocyte function due to increased oxidative stress are considered to be

associated with the effects of ROS on cellular organelles that modifies phospholipids and proteins, leading to peroxidation and oxidation of thiol groups and causing dysfunction of cardiac and vascular myocytes. Assaults by ROS result in disruption of the cells' lipid bilayer membrane, thus changing membrane permeability, resulting in leakage of cell content.

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electron in atomic or molecular orbitals. Such unpaired electrons usually lend the free radical considerable reactivity. Radicals derived from oxygen represent the most important class of radical species generated in living systems.

One example is the dioxygen (O2, molecular oxygen), which has a unique electronic configuration and is itself a radical. The addition of one electron to dioxygen forms the superoxide anion radical. Arising either through metabolic processes or from oxygen activation by physical irradiation, the superoxide anion is considered the primary ROS, and can further interact with other molecules to generate secondary ROS, either directly or indirectly through catalyzed processes by enzymes or metals. Superoxide production occurs mostly within the mitochondria of a cell.

The mitochondrial electron transport chain is the main source of ATP in the mammalian cell, thus it is essential for life. Yet, not infrequently, a few electrons might prematurely "leak" during the energy transduction, about 1-3% of all the electrons in the transport chain, to form superoxide, the oxygen free radical which has been implicated in the pathophysiology of a variety of diseases.

The redox state (of oxidation-reduction) of the cell is largely linked to an iron (and copper) redox couple, and is maintained within narrow physiological limits, like the pH in different locations of the body, or like the core body temperature. It has been suggested that

iron's strict regulation ensures no free intracellular iron; however, under *in vivo* stress conditions, excessive superoxide releases "free iron" from iron-containing molecules. The released Fe2+ can generate highly reactive hydroxyl radicals.

The beneficial effects of ROS and/or RNS occur at low to moderate concentrations, and include some very important physiological roles, such as in cellular responses to obnoxious stimuli, or in defense against infectious agents, their role in several cellular signaling pathways, and in inducing mitogenic responses. Various ROS-mediated actions in fact protect cells against oxidative stress induced by ROS, to re-establish or maintain redox (oxidation-reduction) homeostasis.

Glutathione and other Antioxidants

Exposure to free radicals from various sources has led organisms to develop a series of defense mechanisms against free-radical-induced oxidative stress, including preventive mechanisms, repair mechanisms, physical defenses, and antioxidant defenses. Enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT). Important non-enzymatic antioxidants are ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids, and other antioxidants.

Under normal conditions, there is a balance between the intracellular levels of these antioxidants and their actions' effects. This balance is essential for the organisms' health and survival. The major thiol antioxidant and redox buffer of the cell is the tripeptide glutathione (GSH).

GSSG (Glutathione Disulphide) the Oxidized Form of Glutathione

Glutathione is highly abundant in the cytosol, nuclei and mitochondria, and is the major soluble antioxidant in these cell compartments. Because GSH is synthesized in the cytosol, its

mitochondrial presence requires inner membrane transport. Two mitochondrial electro-neutral protein carriers have been shown to have the capacity to transport GSH, the dicarboxylate carrier protein and the 2-oxoglutarate carrier protein. Externally added GSH was shown to be readily taken up by mitochondria against the concentration gradient, despite the 8mM GSH present in the mitochondrial matrix.

GSH in the nucleus maintains the redox state of the critical protein sulphydryl that is needed for DNA repair and expression. Oxidized glutathione is accumulated inside the cells and the ratio of GSH/GSSG is a good indication of the oxidative stress in the organism. Too high a GSSG concentration may oxidize and damage many enzymes.

The major protective functions of glutathione against oxidative stress are the following:

(i) Glutathione (GSH) serves as a co-factor of several detoxifying enzymes against oxidative stress (e.g., glutathione peroxidase (GPX), glutathione transferase, and others).

(ii) GSH participates in amino acid transport across the plasma membrane.

(iii) GSH scavenges hydroxyl radical and singlet oxygen directly, detoxifying hydrogen peroxide and lipid peroxides by the catalytic action of GPX.

(iv) Glutathione can regenerate vitamins C and E, the most important antioxidants, back to their active forms. Glutathione can directly reduce the tocopherol radical of vitamin E, or indirectly via reducing the semi-dehydroascorbate into ascorbate.

Glutathione's capacity to regenerate the most important antioxidants is associated with the redox state caused by the action of the glutathione disulphide-glutathione couple, GSSG - 2GSH.

ROS and Maintenance Mechanisms of Redox Homeostasis

Free radicals, as well as reactive diamagnetic species derived from radicals, operate at low but measurable concentrations in the cells. Their steady-state concentrations depend on the balance between their production rates and their removal rates by various antioxidants.

Everson, Thalacker and Hogg, 2008. Phagocytic Migration and Cellular Stress induced in the Liver, Lung and Intestine During Sleep Loss and Sleep Recovery.

In this second study by this team of researchers to be discussed here (Everson, Thalacker and Hogg, 2008), Dr. Everson et al. investigated the SD effects on the migratory traffic of phagocytes into peripheral tissues, a process that may indicate an inflammatory response to cellular stress.

Since the early 1920s almost a century ago, studies demonstrated that SD in humans resulted in leukocytosis, the progressive increase in circulating white blood cells, mainly granulocytes. The finding has been repeated in later studies, and is one of the few consistent physiological findings in humans following chronic SD (sleep deprivation).

This study design is similar to the Everson et al. 2005 study (Appendix C). Like their 2005 study, this study employs four experimental groups of young adult male rats: five or ten days TSD, five or ten days PSD, and two control groups - a forced ambulation group kept under baseline conditions, and a control group that was allowed 48 hours of ad-libitum SR following ten TSD days.

Myeloperoxidase (MPO), an Enzyme Virtually Exclusive to Neutrophils

MPO was extracted from three internal organs – liver and lungs, the two sites affected by systemic inflammation, and intestinal tissues, since the intestine was implicated as a site of

bacterial translocation in SD rats. MPO activity was determined by spectro-photometry. MPO acts here as a tracer of Neutrophils' location and quantity.

MPO activity in the lungs of TSD rats on SD day 10 nearly doubled the baseline controls' MPO activity ($P < 0.001$), and was more than 1.5-fold of the MPO activity on TSD day five ($P < 0.01$).

Using immunohistochemistry, these results strongly suggest a neutrophil migration into extravascular liver and lung tissues, concomitant with cellular stress, which is consistent with tissue injury or an SD-induced infection.

Heme oxygenase-1, A Cellular Stress Marker in the Liver, Lung, Heart & Intestine

The enzyme heme oxygenase-1 (heat shock protein-32) and corticosterone, both markers of cellular stress, were traced. Phagocytes were localized using immunohisto-chemistry in the liver and lungs.

In the liver, total HIS48-positive populations were greater than baseline levels in TSD rats, reaching 59% above baseline on TSD day five, and 80% above baseline on TSD day ten.

In the lungs, counts of HIS48-positive labeling indicated that ten TSD days nearly doubled the number of granulocytes ($P < 0.001$), that were over 1.5 fold the baseline on TSD day five ($P < 0.01$).

Heme oxygenase-1 increased over 2.5 fold in the liver on TSD day ten ($P <0.001$) and in the lung too it showed a strong experimental effect on TSD day 10 ($P < 0.016$).

Most HIS48-positive cells (over 90%) were found in the extravascular spaces, indicating an SD induced neutrophil migration into extravascular tissues of both liver and lungs, which suggests an inflammatory response to sleep deprivation (SD), concomitant with cellular stress, and is consistent with SD-induced tissue injury or infection which outlasted the SR 48 hours.

Plasma corticosterone levels remained unchanged throughout the SD phase, but were then decreased 50% below baseline levels following the 48 hours of SR, supporting a similar finding observed in a previous study (Everson et al., 1989).

The authors interpreted this post-SR abnormally low plasma corticosterone as a compensatory response to SD, indicating an augmented negative feedback with both the brain and the pituitary gland, prompting both to send more regulating hormones to replenish the shortages or depletion brought about by SD, to re-establish homeostasis.

Yoked rats (PSD) showed tendencies in the same direction as TSD animals - of both plasma corticosterone decreased levels during SR, and of increased MPO activity during the SD phase on both days five and ten, but none of these tendencies reached statistical significance.

The traced cellular effects of SD thus resulted in findings that show undeniably the greatly injurious effects of chronic TSD.

Most 10-day TSD rats appeared healthy, with a great appetite that masked the severe internal organ abnormalities, which would lead to severe pathology only three weeks later if not reversed in time by prolonged recovery sleep.

The changes produced in MPO activity in these animals by TSD after 5 or 10 days of TSD in the lung and liver were as severe as those reported for burn injury and for hepatic ischemia-reperfusion, for septic shock, or for severe acute pancreatitis with infected necrosis.

In this study the researchers prove that the progressive post SD increase in circulating leukocytes is indeed an inflammatory process in rats in a nearly identical manner to humans, hence it signifies an ongoing infectious disease.

Under the present method of producing SD, rats show a progressive increase in circulating leukocytes, due mostly to an early increase in the population of immature neutrophilic

granulocytes, a “left shift” towards earlier stages of maturation, and days later to an additional increase in monocytes.

Leukocytes are considered to be the organism’s first line of defense against infection due to viruses, bacteria, fungi or parasites, and include neutrophils, eosinophils and basophils. They multiply as needed, and pass through capillary walls to pursue invading organisms within the body’s tissues.

Neutrophils are the first granulocytes to arrive at the invaded location, to phagocyte and destroy foreign particles and cells. Monocytes arrive at the invaded site a few days later, and are immobile granulocytes which phagocyte in lymph nodes, in the spleen, liver and lungs. They give rise to macrophages, which attach themselves to lymph- and blood vessels, and have a greater capacity to engulf and digest much larger particles and organisms than neutrophils.

In recent years researchers have reported increases in various circulating pro-inflammatory molecules, with different time courses, in both SD humans and animals. There is yet very little evidence of any physiological causes, consequences, or sequelae to explain this phenomenon.

Except for a marked hyperphagia, the profile of SD in the laboratory rat resembles chronic systemic inflammatory response syndrome in humans, including poor microbial control and eventual lethal septicemia.

Because inflammatory processes are generally considered the etiological root of several diseases, the main purpose of this study was to investigate whether the increased phagocytes in the circulation implies a level of migratory traffic into the tissues, which would constitute an inflammatory response.

PSD rats showed trends in the same direction as TSD animals, of increased MPO activity during SD days five and ten, which were statistically insignificant.

Everson and Szabo, 2011. Repeated Exposure to Severely Limited Sleep Results in Distinctive and Persistent Physiological Imbalances in Rats.

In their third study reviewed here these researchers further investigated whether a considerably more prolonged duration of chronic SD would result in physiological adaptations and phenotypic changes that are not seen following a relatively shorter-term of SD.

Now with stronger evidence that repeated exposure to deprivation of sleep causes severe damage to TSD rats. At the end of 72 days, the researchers added a final extended sleep recovery of almost four months, of sleep *ad libitum*.

The rational for their present study is based on the findings of a previous study (Everson and Szabo, 2009) with the same design except without the final LSR (lengthy sleep recovery) of almost four months. That study revealed that prolonged chronic TSD in laboratory rats resulted in increased energy expenditure, connective tissue abnormalities, and increased weights of major organs relative to body weight, all of which suggest that high metabolic demands may have preserved the size of vital organs relative to expectations of severe energy deficiency alone. Low plasma corticosterone and leptin concentrations seem to reflect low substrate availability and diminished adiposity.

The researchers extended the SD period to 72 days, consecutively repeating six times each cycle of ten TSD days and two SR days, 72 SD days total, with a final LSR of sleep *ad libitum* lasting almost four months (Appendix C). The LSR period was added to examine the

extent to which a prolonged TSD resulted in internal organs abnormalities, which became long-lasting, or even permanent.

The computerized housing apparatus previously described (Appendix C) was re-programmed to allow more sleep for the PSD group, which here too proved to reliably produce greatly fragmented sleep in both PSD and TSD groups. Measurements of TST in their previous study (Everson and Szabo, 2009) of the same design indicated that in any 10-day TSD cycle, NREM sleep was reduced from 54% sleep during baseline to 34-40% TST in TSD animals, whereas REM sleep time was reduced from 7.3% of baseline TST to 2.4-3.4%.

During the 48h of SR, NREM sleep was consolidated and did not statistically differ from the baseline in terms of percentage of time. On the other hand, REM TST (total sleep time) rebounded and was increased by 50-67% above baseline (Everson and Szabo, 2009).

Food and water intake, as well as body weights, were recorded daily. Food and stool waste were collected in the middle of both the fifth and sixth 10-day cycles to measure their caloric value, which were reported to rule out malabsorption and feeder waste as explanations for weight loss in the previous study (Everson and Szabo, 2009). Measurements were performed on tissues from the present study groups, as well as on preserved tissues from the previous study (Everson and Szabo, 2009) experimental and forced ambulation control groups.

In the two previous studies (Everson et al., 2005, 2008), a single ten-day session of SD in rats resulted in clear signs of metabolic, hormonal, and immune system injuries, yet those conditions were well tolerated in those previous studies, and the 48 hour SR periods seemed to normalize the energy expenditure and antioxidant parameters in the SD rats.

In contrast, food and water intake in the current study were both increased in TSD rats, with a pronounced progressive loss of body weight across cycles of SD. On the last two days

before the LSR phase, TSD animals' body weight averaged 15% below controls, a highly significant change ($P < 0.0001$). During the LSR period, body weights of both TSD and controls rebounded to the same long-term growth pattern, reaching on average 95% of controls' body weight by LSR day 33, and 98% by LSR day 88.

The major findings for TSD rats after almost four months of LSR indicated they were still consuming 20% more food and 35% more water than forced ambulation controls, despite normalized body weight, normalized adipocytes, elevated plasma leptin levels, and decreased plasma cholesterol levels, which taken together indicate that internal processes remained modified long after the severe prolonged SD had ended.

Another finding in TSD rats with LSR was that adipocytes in the omentum, epididymus, and surrounding the mesenteric lymph nodes did not differ in size from those of control animals. Only one multilocular (divided into many small vesicles) region of adipose tissue of a TSD rat was found in the mesentery during LSR.

Hormonal and Clinical Chemistry Results

Among TSD rats with no LSR (Everson and Szabo, 2009), plasma corticosterone was significantly lower than in controls ($P = 0.01$). In contrast, plasma corticosterone in TSD rats with LSR did not differ from controls.

Plasma insulin was lower than 1.8 ng/ml in all animals, except one value of 3.4 ng/ml in a TSD rat with no LSR, which showed a more advanced morbidity than the other rats.

Plasma leptin was significantly higher in TSD rats with LSR than in ambulation controls ($P = 0.03$), despite equivalent body weights.

Plasma albumin was 14% lower in TSD rats with no LSR, compared to TSD with LSR (2.5 g/dl *versus* 2.9 g/dl, respectively, $P = 0.009$), or to ambulation controls with no LSR ($P =$

0.0003), in which plasma albumin values were considered normal (3.1 g/dl). Rats from all four groups were considered hyperlipidemic, being fed an atherogenic diet to induce hypercholesterolemia (Joris et al., 1983). Plasma cholesterol values ranged between 258-978 mg/dl among all four groups, compared with normal plasma cholesterol values (115 mg/dl) for Sprague-Dawley rats (Harlan Laboratories, 2010). In TSD rats with LSR, plasma cholesterol was 31% lower in TSD rats than in controls. TSD animals had 449 mg/dl plasma cholesterol, compared to 649 mg/dl

($P = 0.047$) in ambulation controls.

Plasma low-density lipoprotein (LDL) levels were determined independently, not by calculation, and were significantly lower in TSD rats with LSR compared to ambulation controls, TSD rats having 347 mg/dl *versus* 503 mg/dl in ambulation controls ($P = 0.05$).

No differences were detected for the following body substances measured in plasma: glucose, phosphorous, total protein, HDL, triglycerides, urea, nitrogen, and creatinine. Similarly, no differences were found for osmolality, which represents the total molar concentration of solutes in the blood.

The first SD stage (prior to LSR) replicated the failed growth despite the extraordinary food and water intakes observed during the SD phase in TSD animals without LSR (Everson and Szabo, 2009), thereby enabling adaptations and pathologies to develop, for comparison with the values before and after LSR**.**

Physiological adaptations could be expected to give the organism some survival benefit and sustain health during SD, yet the findings indicate that repeated SD sessions resulted in increasingly severe cumulative changes, including deep negative energy balance, increased intestinal length and remodeling of adipose tissues.

In the researchers' two previous studies (Everson et al., 2005; Everson et al., 2008), a single ten-day session of SD in rats resulted in clear signs of metabolic, hormonal, and immune system damage, yet those conditions were well tolerated, and the 48 hour SR periods seemed to normalize the energy expenditure and antioxidant parameters in the SD rats.

In contrast, in the current study food and water intake were both increased in TSD rats, together with a pronounced progressive loss of body weight across cycles of SD. On the last two days before the LSR phase, TSD animals averaged 15% (± 6% standard deviation) below controls, which was highly significant ($P < 0.0001$). During the LSR period, body weights of both TSD and controls rebounded to the same long-term growth pattern, reaching on average 95% of the controls' body weight by LSR day 33, and 98% by LSR day 88.

Before the LSR, peak values of food intake in TSD rats were typically double or triple the controls', rising in a nearly cumulative manner across the six SD periods, compared to stable food intake in controls. The hyperphagia due to chronic TSD is equivalent to a single prolonged TSD period in rats produced by the same method in past studies (Everson, Bergmann and Rechtschaffen, 1989; Everson and Rowley, 2004; Pilcher et al., 1990; Everson and Wehr, 1993).

Because a single 10-day TSD seemed well tolerated by the rats, and the two SR days appeared to normalize the TSD effects, the investigators state they did not expect the progressive deep negative energy balance seen in the this 2011 study.

Another unexpected finding was the significant 20% increase in food intake during the early SR 48h periods, indicating adjustments and compensations for the previous sleep loss. These investigators state they also did not expect the prompt increase in food consumption from the start of the SD period. The greatly disrupted and fragmented sleep is the most likely explanation for these dramatic metabolic effects.

Compared to findings of nonspecific stress, the dramatic increase in food intake concurrent with suppressed plasma corticosterone in TSD rats contrasts the findings of aphagia with high corticosterone levels typically seen in nonspecific stress.

The increased total energy expenditure appears to be the major effect of disrupted sleep, so that some effects of SD may have resulted from malnutrition and due to hormonal responses to the prolonged chronic TSD.

Overall, food and water intake of control animals before LSR, replicated those of the previous study with no LSR (Everson and Szabo, 2009). Peak daily food intake, based on 48-hour averages, ranged between 179-495% of baseline food intake in TSD rats during the SD phase.

The initial food intake increases were seen in all animals at the beginning of the initial SD phase, increasing 1.24-fold in TSD animals and 1.18-fold in controls, supposedly due to the scheduled forced ambulation. The positive slope of food intake of TSD animals during the SD phase was significantly steeper than the control animals

($P< 0.0001$). Thereafter food intake continued to rise in TSD animals by 0.77% every SD day, whereas in the control animals it remained stable throughout the study.

During the 48h SR of the first cycle, food intake in TSD animals was elevated 1.2-fold relative to baseline ($P=0.001$), while food intake in control animals remained at baseline levels.

Water intake initially dropped in TSD rats by 0.58-fold ($P = 0.0001$), but then rose at an average rate of 1.12-fold on every day of the 10 SD days ($P=0.013$). The 48h SR periods in TSD rats were marked by high water intake (1.5-fold) relative to their previous 10-day SD periods ($P<0.0004$). High water intake in TSD rats continued through the LSR at a 1.6-fold of baseline amounts (95%, $P<0.0001$). This magnitude was 1.35-fold higher than controls during the LSR

(P=0.008). In controls, water intake rose 1.17-fold of the baseline during the forced ambulation phase, but stably remained at that elevated baseline level.

Thirst became progressively abnormal after the initial increase in water intake during SR periods. The LSR did not normalize water intake, which remained persistently elevated 35% above controls. Disturbed dipsogenic hormone cannot be ruled out, especially since the hypothalamic supraoptic nucleus, which is one of the two brain sites of ADH (antidiuretic hormone) synthesis, is reportedly damaged by TSD (Eiland et al., 2002).

The increased water intake can be reasonably viewed as compensatory for the chronic excessive loads of solutes due to greater prandial intake, and because of the anabolic and catabolic cellular demands. Osmoregulation by the brain is supported by the normal plasma osmolality found in this study.

Additionally, the nonsignificant changes in plasma urea nitrogen and creatinine concentrations is evidence that the waste products of metabolism are not elevated in the circulation, which indirectly indicates that ADH's effector's functions were compensatory, and that no renal disease was apparent. Hence the abnormal thirst seen during TSD appears to be adaptive rather than dysfunctional.

Results in TSD Animals with No LSR

TSD animals with no LSR showed 22% less lipid in the liver ($P < 0.02$), 12% less protein in the kidney ($P < 0.002$), and 26% less protein in the intestine ($P = 0.04$) than ambulatory controls. Coincidental increased water content in kidney ($P = 0.02$) and intestine ($P = 0.04$) were observed in TSD rats compared to controls. Average lipid and protein contents per gram of skeletal muscle tended to be non-significantly low in TSD rats.

TSD animals with no LSR differed from ambulatory controls in heart- and spleen-ash content, as did intestinal ash contribution of TSD rats with LSR. In TSD rats with LSR, lipid, protein, and water content of the liver, heart, kidney, spleen, intestine, and muscle did not differ from ambulatory controls with LSR.

Furthermore, in groups with LSR, neither intestinal length nor internal organ weights of TSD and PSD were significantly different, both showing similar tendencies of increased intestinal length as did animals without LSR, 110 cm in TSD animals *versus* 103 cm in controls.

As in the previous study, liver, heart, kidney and large intestine each accounted for a significantly greater proportion of body weight in TSD animals than in controls, where spleen weight was maintained (Everson and Szabo, 2009).

The above changes to viscera from TSD and the ensuing energy deficit, clearly differ from those resulting from experimental caloric restriction. Internal organs of chronically TSD rats appeared to be associated with the high energy demands and production (Everson and Szabo, 2009). Adipose tissue mass was decreased and remodeled, which included increased incidence of multilocular adipocytes (divided into many small vesicles), which were shown by others to be associated with increased mitochondria biogenesis and increased catabolic activity.

The small intestine was 30% longer in TSD rats than in matched controls, which is consistent with increased need for nutrient absorption. Other pathologies suggest inanition (exhaustion due to lack of food and water), and included loss of fat in the connective tissues that separated the major internal organs, hair loss, and skin lesions on the paw's sole.

The weights of most vital organs in TSD rats without LRS were increased relative to body weight, in contrast to experimental caloric restriction, that was shown to induce decreased mass of internal organs during energy deficiency in rats.

Compared to the 15% loss of body weight in TSD rats, a starvation-induced loss of body weight was 17.5%, associated with an approximate 60% loss of liver weight, 55% loss of small intestine weight, 15% loss of kidney weight, and 20% loss of heart weight.

By decreasing energy demands of these organs, caloric-restricted rodents can survive for many months at body weights that are 60% of fully fed controls. Instead, TSD rats die at body weights averaging 22% below controls after only 16 to 27 days of SD (Everson, 1993).

The current results indicate that lipids were diminished only in the liver (by 22%), hence the lipid amount did not appear to be overly or selectively drawn from the vital organs, implying the preservation of lipids as local messengers and substrate sources. Proteins too appear to be maintained in the liver, heart, and spleen, implying their preservation for enzymatic, mechanical, and structural roles. Proteins were moderately reduced in the kidney by 12% and by 22% in the intestine.

In marked contrast, a 17% loss of body weight in rats due to experimental caloric restriction resulted in protein losses of over 50% in the liver and small intestine, over 17% in the kidney, and over 18% in the heart.

Although skeletal muscle protein and lipid levels were lower in TSD rats compared to controls, these were not significant. This may be due to a limited sample size, since the contribution of skeletal muscle in order to meet energy production demands during chronic SD would be likely considering the major contribution of skeletal muscle to the body mass.

The liver, kidney, heart, GI tract (gastrointestinal tract) and brain normally are the organs with the greatest drain on energy expenditure, with 30%-50% of energy expenditure attributable to the liver and intestine alone.

Whereas adipose tissue, skin and muscle normally drain much less energy of the total energy demands relative to the body mass, they proved here to result in the greatest atrophy in TSD rats. Although the etiology of skin lesions is unknown, the investigators believe they are signs of inanition. This concept is supported by previous studies in which skin lesions on TSD rats were less severe or more delayed in rats fed with a diet dense in calories from fat, or in rats in which basal metabolism was lowered by experimental hypothyroidism.

The burden of TSD on skeletal muscle and connective tissues would also be expected to be much greater if food consumption had been limited so the TSD rats were unable to double and triple their food intake.

Regarding the brain, regional metabolism in both humans and rats is either unchanged or decreased by acute SD, therefore, the brain is an unlikely source of an excessive and progressive drain on energy reserves during chronic SD. Candidate cell functions that may drive the metabolic intensity of the vital organs during SD include anabolic processes, implicating mitochondrial reactions, substrate cycling, and membrane integrity.

Plasma leptin concentrations in TSD rats without LSR were consistent with a large body of empirical evidence indicating that leptin is low during energy deficiency and is possibly related to the amount of body fat, which appeared exhausted in these animals.

On the other hand, in TSD rats with LSR, elevated leptin was associated with a persistent elevation of food intake by 20% throughout the nearly four-month LSR period, despite a catch-up growth and normal adipocyte morphometrics. This is abnormal, because elevated leptin is expected to suppress appetite, except in individuals with insensitivity to leptin effector functions. Together, the elevated food consumption concurrent with elevated leptin concentrations, point to perturbed negative feedback control over food intake.

Cholesterol levels in these SD rats were completely opposed to those expected from energy deficit due to caloric restriction, in which food restriction induces increased circulating cholesterol due to mobilizing lipids from adipocytes, despite decreased cholesterol biosynthesis.

On the other hand, cholesterol and LDL levels in TSD rats, with or without LSR, are considered low in view of two- and four-fold increases in the consumption of an atherogenic diet. This supposedly reflects accelerated cholesterol metabolism.

Plasma cholesterol decreases of 78% and 88% of controls have been found in healthy men following four and five days of sleep loss, respectively, although not necessarily during shorter SD durations or from poor sleep efficiency. A role for sleep in the synthesis of cholesterol and the proteins of lipid transport has been previously suggested on the basis of gene expression studies.

LDL primarily functions to transport cholesterol from the liver to the tissues that incorporate it into cell membranes. Corresponding plasma concentrations of insulin and glucose in TSD rats were normal for the most part, which suggests normal insulin sensitivity and effective management of fuel for energy.

Corticosterone concentrations in TSD rats without LSR were greatly decreased, as opposed to the five-fold increases reported for caloric restriction. Energy deficiency is expected to stimulate the adrenal cortex to produce large quantities of glucocorticoid hormones, above all cortisol, resulting in the mobilization of proteins from essentially all body cells to provide substrates for conversion into glucose.

The low circulating corticosterone concentration in unrecovered TSD rats might serve to protect the vital organs by minimizing the lipolytic and proteolytic actions.

Low circulating corticosterone is a consistent finding in both TSD and PSD rats studied under similar experimental conditions, or unchanged cortisol concentrations in nearly all human studies of sleep loss.

Small, statistically significant increases in cortisol in humans have been reported only for certain times of the day in a seven-day sleep restriction protocol (Buxton et al., 2010).

Humans SD for over 24 hours show signs consistent with those of laboratory rats, suggesting comparable underlying factors and evolving adaptations and maladaptations. For example, citations of hunger in SD humans are fairly abundant, as are those of decreased plasma leptin, which is an early and convincing marker of nutritional or energy deficiency in humans as well as in laboratory rodents.

Findings in humans include inappropriately low TSH (thyroid stimulating hormone), suppressed growth hormone, and perhaps suppressed prolactin; these changes are also observed in SD rats and mice. Various signs of sympathetic nervous system activation are typically reported in humans, as well as in laboratory rats.

SD normal humans and lab rodents without increased corticosterone also share signs of increased circulating white blood cells and proinflammatory molecules, known as cell injury respondents and metabolic drivers, yet without the classical clinical signs of fever or identifiable localized inflammatory reactions.

In light of these many similarities, hypermetabolism and weight loss in TSD lab rodents seem at odds with results from human studies that have pointed to glucose intolerance and insulin insensitivity as consequences of TSD, that would promote obesity and pose a risk for diabetes type 2.

However, body weight in humans is expected to be an extraordinarily insensitive measure of cellular functions, whereas for lab rats it is much more useful because they operate closer to their maximal aerobic capacity. Despite differences of severity of SD that may underlie differences in outcomes, the effects of SD in humans may be viewed as conceptually consistent with those in rats.

The authors therefore hypothesize that glucose intolerance and insulin insensitivity observed in humans may be parsimoniously explained as "hunger diabetes" or "injury diabetes", reflecting a shift in dominant fuel selection from glucose to lipids, as their data on rats suggest.

Furthermore, tests of glucose utilization in humans primarily reflect a metabolic response by skeletal muscle and not necessarily the status of the visceral tissues, due to differential autonomic enervation and region-specific alterations.

The present tissue composition analyses show differential effects of SD in muscle and in vital organs, implying that muscle may be used to support metabolic demands during inadequate sleep. Comparably, a recent report of combined caloric and SD in humans cited loss of fat-free body mass and changes in substrate utilization.

Morbidity

One TSD rat was removed from the current study at the start of the 5th SD period due to lethargy, apparent weakness, and inability to handle the platform rotations. The same clinical picture was seen in the previous study in two TSD animals that did not survive the 6th cycle (Everson and Szabo, 2009).

Only 24 hours prior to its removal from the formal study, the current study's lethargic rat showed robust food intake of 131% of baseline, with reduced water intake to 47% of baseline, and body weight loss of 12% of baseline, which should not have been lethal.

Observations indicated that this animal was awake most of the time during the SR 48h periods, when sleep was allowed *ad libitum*. It did not regain health during subsequent days and was euthanized.

Dermatoses began to develop on the paws in all TSD rats of this study at about the same time, and at the same sites as those of TSD rats in the previous study (Everson and Szabo, 2009). Typically, initial small papules appeared on the main pad and in the area beneath the calcaneus's bone, developing into large (8–10 mm diameter) lesions by the 6th SD cycle. In contrast, control animals' paws were typically free of pathology, or had an occasional isolated reddish spot or small papule without necrosis.

The fur of TSD rats before LSR became lackluster with oiliness and denuding along the back. After LSR the fur had regained its normal appearance upon visual inspection, but lesions on the hind paws of five of the seven SD rats were still present as firm, circular, plaques of a deep maroon color, with or without an inflamed periphery.

During LSR, two TSD rats developed ulcerous growths with necrotic centers either near the mandible (1 cm in diameter), or on the back (2 cm in diameter), whereas no such lesions were found in controls during LSR. Necropsy evaluations of rats with LSR revealed healthy internal appearance, except for one abnormal mass in the lower retroperitoneal region of a control rat, which did not appear to interfere with organ functions.

The present outcomes point to dynamic and fundamental physiological adjustments in response to repeated exposure to inadequate sleep. Peripheral organs and systems are largely ignored by most recent approaches to the study of sleep, which typically are brain-centered, believing the brain to be the sole recipient of benefits from sleep.

These researchers provide evidence that signals for altered appetite drives and negative feedback to brain foci during inadequate sleep originate in the periphery. Brain function may be considered responsive and adaptive, given the circumstances, rather than dysfunctional.

The outcomes point to relatively well preserved compositions of the internal organs during repeated SD despite an energy imbalance heavily tipped toward catabolism. This indicates a priority for carrying out the tissue and cellular functions of vital organs at high energy costs when sleep functions are interrupted. This outcome is in marked contrast to the energy-deficient state of food restriction on every aspect examined.

The outcomes of the present studies show that recovery from chronic TSD takes a long time and that some of the physiological adaptations and potential maladaptations that arise in response to LSR lasting nearly four months after a long bout of repeated exposure to limited sleep produced a return of an overall healthy-appearing countenance, that was belied by signs of imbalance, including prominent elevated food and water intake, indicative of elevated metabolism, elevated leptin, which has many effector functions, as well as signs of altered substrate demands.

The present evidence prompted the authors to speculate that sleep facilitates certain cellular processes to take place efficiently during relative immobilization, because without normal sleep, metabolic imbalance and physiological outcomes result. This speculation has its origins in long-standing ideas and theories about sleep.

While adaptive changes may increase survivability following severe TSD, changes to the mediators of the observed signs that persist during apparent recovery, could increase the likelihood of various disease processes to arise, the nature of which would be expected to depend on the unique susceptibilities of the individual.

III. Sleep Deprivation and Pathogenesis of Chronic Diseases

a) Thompson, Larkin, Patel et al., 2011. Short Duration of Sleep Increases the Risk of Colorectal Adenoma.

Short sleep duration and poor sleep quality have been associated with increased risks of obesity, cardiovascular disease, diabetes mellitus, and mortality. However, few studies have investigated their associations with the risk of colorectal neoplasia.

Methods

In a screening colonoscopy-based case-control study, PSQI was administered to 1,240 study participants prior to their colonoscopy.

Results

338 (27.3%) of the participants were diagnosed with incident colorectal adenomas. Although there was no appreciable difference in the overall PSQI score between cases and adenoma-free controls (5.32 vs. 5.11; P = 0.37), the researchers found a statistically significant association of colorectal adenoma with the PSQI sleep duration (P = 0.02).

Cases (diagnosed with incident colorectal adenomas) were more likely to average less than 6 hours of sleep per night (28.9% vs. 22.1% in controls, P = 0.01). In multivariate regression analysis adjusted for age, gender, race, smoking, family history of colorectal cancer, and waist-to-hip ratio, individuals averaging less than 6 hours per night had an almost 50% increase in risk of colorectal adenomas (P = 0.02) as compared with individuals sleeping at least 7 hours per night.

Cases were also more likely to report being diagnosed with sleep apnea (9.8% vs. 6.5%, P = 0.05) and more likely to have worked alternate shifts (54.0% vs. 46.1%,

P = 0.01), although these differences were not significant in multivariate models.

Materials and Methods

1240 patients scheduled for routine screening colonoscopy at University Hospitals Case Medical Center in Cleveland, Ohio, and affiliated Gastroenterology practices in the surrounding areas were recruited prior to their colonoscopy examinations.

These patients were asymptomatic, and were referred for colorectal cancer screening because they were at least 50 years old and had no colonoscopy in the last 10 years, or they had a positive family history of colorectal cancer that met the American Cancer Society colorectal cancer screening recommendations to undergo screening colonoscopy at a younger age, but were at least 30 yo., did not have any inflammatory bowel disease (such as Crohn's disease or ulcerative colitis), and had never been diagnosed with colorectal cancer or polyps or any form of cancer.

The interview included a comprehensive inquiry of lifestyle factors, such as diet, physical activity, alcohol consumption and smoking, as well as a self-reported medical history including diagnosis of metabolic syndrome components and family history of colorectal and other cancers. For this project, all responses other than Caucasian and African American were collapse into one "other" category.

Fasting blood samples were obtained at the time of colonoscopy, prior to the procedure and refrigerated immediately. Height, weight, hip and waist circumferences, and blood pressure measurements were also obtained just prior to colonoscopy by nursing staff at the participating endoscopy laboratories. Waist-to-hip ratio (WHR) was calculated as waist circumference divided by hip circumference. Body mass index (BMI) was calculated as weight (in kg) divided by height (in meters) squared.

Patients were classified as a "case" if their colonoscopy results indicated at least one pathologically confirmed colorectal adenoma. All others were considered as "controls". The recruitment rate was 64.9% for all eligible patients, and those who completed the study did not differ from those who refused to participate with regard to age, gender and race ($p > 0.05$).

Sleep Quality Data

In addition to the risk factor questionnaires, the PSQI was administered via in-person phone survey to all participants to obtain information on the subject's overall sleep quality in the past month. PSQI includes four open-ended questions asking the participant to report what time they typically go to bed, how long it takes them to fall asleep, what time they wake up, and how many hours of actual sleep they get on a typical night. Additional select questions not included in the PSQI were added to the phone survey. These questions include self-report of a diagnosis of sleep apnea (yes/no), and if they have ever worked alternate (2nd or 3rd) shifts (yes/no).

Responses to the PSQI were then coded into seven components representing different aspects of sleep quality, including self-reported quality, sleep latency, duration, sleep efficiency, disruptions, sleeping aid use and daytime sleepiness.

Homeostasis Model Assessment – Insulin Resistance (HOMA-IR)

Serum levels of insulin and glucose were determined on fasting blood samples. Glucose was measured using colorimetric reflectance spectrophotometry and had an overall QC range <5%. Insulin was measured using a standard ELISA assay with a mean inter-assay CV of 4.7%. Insulin resistance was calculated using the homeostasis model assessment – insulin resistance (HOMA-IR) model.

These cutoffs were based on the PSQI component 3, sleep duration which scores each individual as 0 (>7 hours), 1 (6-7 hours), 2 (5-6 hours) and 3 (<5 hours). Because very few

individuals (N=122) reported sleeping less than 5 hours per night, we collapsed the two shortest duration categories into one as < 6 hours.

To evaluate risk association of sleep quality parameters with colorectal adenoma, two main logistic regressions were performed on each component of the PSQI, as well as the total PSQI and the additional sleep measures. The base model included age, gender, and race as covariates (338 cases and 902 controls). To control for potential confounding by other known risk factor for colorectal neoplasia, the full model also included smoking, family history of colorectal cancer, and WHR (338 cases and 902 controls).

Furthermore, because of the known association between sleep quality and obesity, which is strongly linked to insulin resistance, and the importance of sleep in the metabolic syndrome, we hypothesized that associations of sleep quality and duration with colorectal adenomas may be mediated by insulin resistance. To test this hypothesis, we further adjusted for HOMA-IR in the full model above (266 cases and 745 controls with available data). Finally, since sleep apnea patients are known to have different sleep patterns, we repeated all logistic regressions excluding subjects who reported a diagnosis of sleep apnea.

Recently, it was reported that the association between sleep duration and measures of obesity was stronger in women compared to men. Therefore, we carried out stratified analyses to explore potential gender differential associations between sleep quality or duration with colorectal adenomas. All statistical tests were two-sided. SAS (version 9.1, SAS Institute, Inc.) was used for all statistics. A P-value < 0.05 was considered statistically significant.

Results

Of the 1,240 participants, 338 were diagnosed with incident colorectal adenomas at their colonoscopy. In general, cases were older and more likely to be male compared with controls.

There were also significant differences in the distribution of race, with African-Americans more likely to be cases compared with Caucasians.

Interestingly, while there was no statistically significant difference in BMI between cases and control ($p = 0.10$), on average, WHR, as a proxy for central obesity, was significantly higher among cases as compared to the controls ($p < 0.0001$). Current smoking was significantly more common in cases than controls, with 22.9% of cases and 14.3% of controls reporting current smoking.

Family history of colorectal cancer was reported in over 20% of the study population and did not differ between cases and controls. While not statistically significant, on average, cases scored higher (corresponding to poorer sleep quality) on the PSQI, as well as most of the individual components.

However, the score of component 3 of the PSQI, which corresponds to the hours of sleep per night, was statistically significantly different between cases and controls (univariate $P = 0.005$). Cases reported sleeping fewer hours on average than controls (6.35 versus 6.54, $P = 0.038$), which remained borderline significant after adjustment for age, gender, and race ($P = 0.07$).

Further adjustment for family history, smoking, and WHR did not materially change the risk estimate. When individuals were grouped into three categories of sleep duration, our analysis showed that individuals averaging less than six hours of sleep per night had a statistically significant increase in the risk of colorectal adenomas compared with individuals sleeping at least seven hours per night.

Both the base and the full models showed a statistically significant linear dose-response relationship between sleep duration and risk of adenoma. Cases were also more likely to report

ever having worked alternate shifts than controls (P = 0.01), although this association reduced to non-significance after adjustment for potential confounders in the regression models.

Self-reported diagnosis of sleep apnea was also more prevalent in cases (9.8%), compared to controls (6.5%) (P = 0.04), but the small numbers limited our statistical power to detect an association after adjustment for covariates.

Further adjustment for HOMA-IR did not alter our results. Indeed, a statistically significant linear trend for the categorical group of hours of sleep per night remained after adjustment for HOMA-IR, suggesting the influence of sleep duration on colorectal adenomas is independent of insulin resistance.

Discussion

We found that shorter sleep duration is associated with an increased risk of colorectal adenomas in a population of patients undergoing routine screening colonoscopies. In contrast, we found no evidence for an association of overall sleep quality with colorectal adenomas.

This is in line with the current literature on breast cancer reporting evidence for an association of sleep duration, but not quality of sleep (6,16-18). Individuals sleeping less than six hours per night have a nearly 50 percent increase in the risk of developing colorectal adenomas compared to those sleeping more than seven hours.

This association is independent of central obesity and insulin resistance. To our knowledge, this is the first study to report a significant association of sleep duration and colorectal adenomas, supporting short duration of sleep as a novel risk factor for the development of early colorectal neoplasia.

The magnitude of increase in risk observed here is comparable to the increase in risk of colorectal cancer associated with having a first degree relative affected with colorectal cancer,

and high red meat intake. Furthermore, this estimate is similar to those observed with respect to short sleep duration and breast cancer.

Thus, short sleep duration is a public health hazard leading not only to obesity, diabetes and coronary heart disease, but also to, as we have now demonstrated, colorectal adenomas. Our data suggest that even a modest increase in sleep duration could have a substantial impact at the population level because of the high prevalence of colorectal adenomas, a well established precursor of colorectal cancer.

Increasing evidence strongly suggests that disruption of circadian rhythm and suppression of nocturnal production of melatonin may be the key mechanism underlying the shift work cancer link. Melatonin has been shown in animal models to reduce the number of DNA adducts and also to promote DNA repair and thus reduce the overall amount of DNA damage and to decrease cellular proliferation via cell cycle inhibition.

Conclusions

Shorter sleep duration significantly increases risk of colorectal adenomas.
Our results suggest sleep duration as a novel risk factor for colorectal neoplasia.

Valko et al., 2007 – Ischemia/reperfusion injury

Ischemia/reperfusion injury results from damage caused to the myocardium following blockage of the coronary artery when blood circulation was restored. Despite the low tension of oxygen during the ischemia, moderate ROS generation proved to occur, most likely from a mitochondria source. The massive burst of ROS found during reperfusion may have been caused by a different cellular component yet unidentified.

The maasive ROS production during Ischemia/reperfusion leads to tissue damage due to the massive oxidative stress, which results in considerable complications in organ- transplanting, stroke, and myocardial infarction.

Ischemia/reperfusion that was induced in the rat model of cardiac myopathy, activated the redox-sensitive transcription factors NF-kB and AP-1 as well as the MAPKs JNK and p38 in the presence of minimal activation of ERK. This activation may have occurred due to the inflammatory responses and the apoptotic cell death in the affected tissue.

Neutrophils proved to be the main effector cells of reperfusion injury. During the ischemia condition, the greatly augmented ATP consumption results in the accumulation of purine catabolites hypoxanthine and xanthine, which are metabolized by xanthine oxidase in the subsequent reperfusion and oxygen influx, thus generating great amounts of hydrogen peroxide and the superoxide radical.

Using varied pharmacological interventions, many studies have investigated the injurious effects of the exaggerated production of oxidants induced by ischemia-reperfusion.

When the role of cellular antioxidant enzymes in the pathogenesis of myocardial injury i*n vivo* were studied in gene-targeted mice, neither deficiency nor overexpression of Cu–Zn-SOD changed the extent of myocardial necrosis. Nor did the overexpression of GPX (glutathione peroxidase). On the other hand, overexpression of Mn-SOD significantly attenuated myocardial necrosis after myocardial injury/reperfusion, which indicates the important role of MnSOD, as opposed to Cu/ZnSOD or glutathione peroxidase.

Valko et al., 2007 – Rheumatoid Arthritis (RA)

Rheumatoid arthritis is an autoimmune disease that causes chronic inflammation in the joints and tissues surrounding the joints with infiltration of macrophages and activated T cells. The pathogenesis of this disease is predominantly linked to free-radical formation at the inflammation site.

The oxidative injury and inflammatory status have been confirmed in various rheumatic diseases, by increasing the levels of isoprostanes and prostaglandins in the serum and the synovial fluid compared to controls. Oxidative conditions in synovial tissue were shown to also be associated with a higher incidence of p53 mutations.

T cells isolated from the synovial fluid of RA patients show signs of decreased intracellular GSH levels, impaired phosphorylation of the adaptor protein LAT (linker for activation of T-cells), and the "primed" CD45RO phenotype. Altered subcellular localization of LAT has been shown to be caused by the decreased intracellular GSH level.

The migration of monocytes and lymphocytes into the rheumatoid arthritis synovium is mediated by the abnormal expression of several adhesion molecules (ELAM-1, VCAM-1, ICAM-1, ICAM-2), which can be explained by the abnormal induction of redox-sensitive signalling pathways.

Naidoo, 2009 – Cellular stress/The unfolded protein response: Relevance to sleep and sleep disorders.

BiP is the key cellular marker and master regulator of the signaling pathway. (BiP is also called "The ER stress response", or "Unfolded protein response). BiP is a chaperone residing in the ER (endoplasmic reticulum), which seems to increase with SD (sleep deprivation) in all the species studied.

Aging tilts the response to sleep deprivation, going from an adaptive protective action, to a maladaptive response. Due to recent extensive research of the cellular and molecular correlates of sleep (largely through the methods of subtractive hybridization, differential transcript, and DNA microarray studies), our understanding of the molecular changes occurring in sleep, wakefulness, and in extended sleep deprivation (SD) has grown considerably growing.

In his article, this researcher provides evidence that even a modest SD induces cellular stress that activates an adaptive response. Hoever, aging tilts the organism's response to SD from an adaptive and protective one into a response that is maladaptive.

Hopefully understanding the pathways activated by extended sleep loss, as well as the mechanisms by which they take place, will allow the developing of therapies that will protect the brain during prolonged wakefulness, and specifically in sleep disorders, including those associated with aging, such as insomnia.

Among the great number of genes (some 3,000) which were shown to change with the sleep state, a change in one particular class of genes, the heat-shock proteins/chaperones, was found to be highly conserved in all the species studied. BiP is one gene of this class, that was found to increase with experimentally induced chronic SD, or with extended wakefulness in all

species studied: in the drosophila, in themouse and rat cerebral cortex, and in the telencephalon of the white crowned sparrow.

BiP, the “Immunoglobulin-Binding Protein”, which has also been called in a variety of other names, including the “Pittsburgh compound B”, the GRP78 (“Glucose regulated protein 78”), or HSP5A (“Heat shock protein 5A”). BiP serves as a sentinel for the up-regulation of several signaling pathways, collectively called the “unfolded protein response” (UPR), or the “ER (Endoplasmic Reticulum) Stress Response”. BiP increases with SD in all the species studied.

The UPR responds to cellular stress, and this response has three phases – depending of the severity of the cellular stress:

1. Adaptation - occurs during moderate stress, when UPR is healthy, protective and adaptive - at which point it is cytoprotective.
2. Alarm –takes place during greater stress, when adaptation fails, which then activates genes that mediate defense host mechanisms that lead to activating an inflammatory response.
3. Apoptosis – occurs during a persistent extended stress condition, when ER stress burden becomes too great and prolonged, it then activates the executioner pro- apoptotoic signaling pathways.

ER is a sub-cellular organelle comprised of a reticular membranous network, that extends throughout the cytoplasm and is contiguous with the nuclear envelope. This is where all the secretory and integral membrane proteins are folded, and modified after translation in an ATP-

dependent processes (for they consume energy) that is largely mediated by the above mentioned chaperone, BiP.

ER is also where the biosynthesis of steroids, cholesterol and lipids takes place. It also is the storage site of calcium ions in the cell. Additionally, ER is the major organelle which continuously responds to environmental cues to release calcium ions, and the organelle which transduces signals in the cell. ER clearly has several very central roles in the cell. BiP is the most abundant chaperone of ER, for being the major chaperone of ER and the key regulator for protein folding. It is the first chaperone that a nascent peptide meets when it comes off the ribosome during protein translation - when the protein/ peptide is destined for the ER. BiP binds to the hydrophobic domains on the peptide, in order to prevent misfolding, while the rest of the peptide/protein continues to be synthesized in the ribosome.

Once the entire peptide is synthesized, it is folded with the aid of BiP and other chaperones. If properly folded, the new protein is escorted out of the ER. If a protein is misfolded, it is re-folded with BiP's help, but if this is not possible, the protein is escorted by BiP to the cytosol to be degraded by the proteosomes.

ER Stress Response and Unfolded Protein Response (UPR)

Due to its reticular nature, ER can sense and transmit signals originating from any cellular sub-compartment. Disturbances that alter ER homeostasis also disrupt the folding of protein, and lead to the accumulation of unfolded proteins and protein aggregates, which are detrimental to the cell survival.

These disturbances include perturbations of calcium ion homeostasis, redox status, elevated secretory protein synthesis, and glucose- or energy deprivation - all of which occur coincidentally following a prolonged SD (Everson et al., 2005, 2008, 2011). As a consequence,

the cell has evolved an adaptive coordinated response to limit the accumulation of unfolded proteins in ER, and restore the normal ER function and homeostasis at the soonest possible.

This signaling system is termed “the ER response to ER stress”, and is composed of the above mentioned three phases: adaptation, alarm, and apoptosis, depending on the duration and intensity of the cellular stress.

In case of alarm, the cellular level UPR triggers additional three types of adaptive and protective cellular responses, to facilitate the fast return to homeostasis:

a) Up-regulation of BiP/GRP78 (Glucose regulated protein 78)

b) Attenuation of protein translation

c) Degradation of misfolded proteins (in the cytosol in proteosomes, by a process termed the ER-associated degradation = ERAD).

Excessive and prolonged stress leads to a maladaptive response and apoptosis. Likewise, if cell protective changes (mediated by UPR) fail to restore the protein folding capacity, apoptosis takes place (mediated by the CHOP gene and other pro-apoptotic signaling factors).

In addition to handling the protein load, the adaptive response of the UPR serves in both anti-apoptotic and antioxidant roles. For example, it has been shown that over-expression of BiP leads to CHOP suppression, and to the inhibition of CHOP-mediated cell cycle arrest and apoptosis.

All the Components of the UPR Pathway Appear in Chronic Sleep Deprivation

In mice, a prolonged wakefulness of over 6 hours, was found to induce ER stress followed by the UPR response in the mouse cerebral cortex. BiP protein levels were found to increase with 6,9 and 12 hours of extended wakefulness. Immunoprecipitation studies indicate that in a SD of six hours or more, BiP is dissociated from the kinase PERK, while

phosphorylation of PERK and elF2 alpha take place, indicating the inhibition of protein translation.

Ribosome profiles from mice subjected to 6 hours of SD indicate a de-segregation of polysomes into monosomes, suggesting an attenuation of protein translation (active translation is associated with increased numbers of polysomes, as seen in control mice).

Additional studies in rats during prolonged wakefulness showed that there is an increased cortical mRNA expression of the UPR-associated chaperones BiP and GRP 94. PERK cerebellar transcript levels were also found to be higher in wakefulness than during sleep.

Additional studies demonstrated that SD is associated with attenuated protein translation, with a likely increased protein synthesis during SR (sleep recovery). On the other hand, protein expression was shown to decrease during SD. Thus, a decreased protein translation is a key consequence of SD, with recovery of this process occurring during recovery sleep.

The increased expression of BiP during SD in drosophila is followed by a decline in BiP during SR. Also in the drosophila, following an almost 3-fold increase of BiP protein during a 6 hour SD, BiP protein levels returned to baseline levels during 24 hour of SR (recovery sleep).

Changing the amount of functional BiP alters the amount of recovery sleep in flies following SD: a) Flies with high levels of BiP had more recovery sleep (30% more sleep in the first 4 hours post SD) than flies with normal BiP levels, b) In contrast, flies with inactive BiP had 60% less sleep in the first 4 hours post SD, and less recovery sleep than flies with normal BiP. In mammals too, there is evidence of a similar effect of these pathways on sleep-wake control.

Interactions of ER Stress Signals with Sleep Control

Methippara, Kumar, Alam et al. (2007) used the small molecule inhibitor salubrinal, which prevents dephosphorylation of elF2 alpha, in order to test whether a build up of p-elF2 alpha (phosphorylated elF2-alpha) had any effect on sleep. Salubrinal (administered ICV for 12 hours) significantly increased the SWS2, and suppressed active wakefulness for the first three hours. For the remaining nine hours salubrinal had no further effect on sleep or wakefulness.

During the first three hours, salubrinal increased the number of SWS2 episodes, increased delta power as well, and also increased the number of p-elF2-alpha labaled neurons in the basal forebrain region (FB). Salubrinal activated the GABAergic rostral-median preoptic nucleus neurons as well, as indicated by FOS expression.

Taken together, these findings suggest that ER stress signaling may play a role in sleep control through p-elF2-alpha, and suggests that sleep is part of the compensatory response to ER stress in the brain.

Effect of Aging on the UPR Response to Chronic Sleep Deprivation

In eukaryotic cells the UPS (ubiquitin-proteosome system) is the main pathway for eliminating misfolded proteins. Misfolded or damaged proteins are marked for UPS-mediated degradation by a covalent attachment of ubiquitin molecules.

Thus, in older mice, in the absence of a robust protective UPR, the pro-apoptotic signaling pathway is turned on in older mice even at baseline, and is much more increased by SD. Other studies showed a dramatic increase in the pro-apoptotic factor CHOP with advancing age, that was even more greatly increased in older animals with prolonged wakefulness (SD).

CHOP's role is mediating apoptosis in response to ER stress is well established. Increased CHOP expression was shown to induce the down-regulation of BCI-2 expression, depletion of cellular glutathione, and an greatly increaed production of reactive oxygen species

(ROS), thus in the presence of CHOP antioxidant systems are suppressed, while at the same time free radical production is increased. If ER stress is not alleviated in a timely manner by the adaptive arm of UPR, the pathway switches to a mechanism that promotes cell death (apoptosis).

In both humans and rats, aged animals exhibit a more fragmented sleep. Surprisingly, SR (recovery sleep) following SD is reduced in older animals relative to young ones, which has been seen in both humans and rats.

Given that SD in older animals is more injurious, one might have expected more, not less, recovery sleep. That older animals, who exhibit reduced SR following SD, have no BiP response to SD, which is compatible with the concept that BiP itself may play a direct role in determining the magnitude of SR, as discussed above.

Neurodegenerative Diseases, ER Stress and Sleep Disorders

Misfolded proteins and the associated ER Stress are both common emerging features of neurodegenerative diseases. Neuronal loss in both familial and sporadic forms of neurodegenerative diseases is often accompanied by an aggregation of misfolded proteins. For example, accumulating evidence suggests that both ER dysfunction and aberrant protein degradation play a role in dopamine neuron loss in Parkinson's disease (PD).

Extended polyglutamine tracts were also shown to stimulate ER stress-induced cell death. Neurons over-expressing mutant presenilin-1 have been shown to be more sensitive to ER stress-induced apoptosis. Whether or not mutation in presenilin-1 down-regulates BiP and induces CHOP is still uncear.

The ER stress response has also been implicated in Alzheimer's Disease (AD), Amyotrophic Lateral Sclerosis (ALS), Huntington's Disease (HD) and in Spino-Cerebellar Ataxias. An accumulation of misfolded proteins, that leads to alterations in organelle structure

including the ER, has been described in transgenic animal models of ALS, AD and Huntington's Disease. These diseases usually develope late in life and are associated with aging.

Patients with neurodegenerative diseases such as PD or AD were shown to manifest sleep fragmentation, which could place an additional burden on an already stressed protein folding and degradation system. It thus could greatly increase protein aggregation, and act as a positive feedback in these conditions. One needs therefore question whether sleep fragmentation in neurodegenerative disorders accelerates their progress, which recent studies support.

Conclusions

Both SD and the cyclical intermittent hypoxia that occurs in sleep apnea lead to ER stress, during which the protein production machinery is compromised and protein misfolding takes place. ER Stress responds to this stress by upregulating a series of coordinated cellular protective signaling pathways called UPR, a response that protects the cell against the effects of misfolded proteins, which can form toxic protein aggregates. This response appears to be highly conserved as SD upregulates BiP, the sentinel marker of the UPR in all species studied so far, including fruit flies, birds and rodents.

This protective response can be overwhelmed by additional stress, and if the cumulative burden is too great, then proapoptotic signaling is activated. This takes place in older animals with short-term sleep deprivation, with cyclical intermittent hypoxia in certain motor neurons, and even in young animals when undergoing a prolonged SD.

Valko et al., 2007 - Parkinson's disease (PD)

Parkinson's disease (PD) involves a selective loss of neurons in the substantia nigra of the midbrain. The substantia nigra neurons uses dopamine as the chemical messenger between the brain and the nerve cells to communicate with the nerve cells in the stratium, and a reduction in nigral dopamine levels results in decreased stratial dopamine, which is believed to cause the PD symptoms.

Serious Sleep disruptions, as well as neuronal loss and Lewy bodies, are well known pathological characteristics of PD. Neuronal loss and Lewy bodies were found in the cerebral cortex, anterior thalamus, hypothalamus, amygdala and basal forebrain.

Lewy Bodies are tiny spherical protein deposits found in nerve cells. Their presence in the brain was shown to disrupt the brain's normal functioning, interrupting the chemical messengers' action, including acetylcholine and dopamine. Lewy Bodies are most likely formed as the neurons try to protect themselves from attack. The major component of intracytoplasmic Lewy bodies are filaments which consist of alpha-synuclein. The genetic causes of PD seem to be two recently identified point mutations in the alpha-synuclein (Jin and Yang, 2006).

A majority of studies explored the effect of oxidative stress that contributes to the cascade of events leading to dopamine cell degeneration in PD. The occurrence of oxidative stress in PD is supported by both postmortem studies and by studies demonstrating the capacity of oxidative stress to induce nigral cell degeneration. There is evidence that there are high levels of basal oxidative stress in the substantia nigra pars compacta (SNc) in the normal brain, but that this increases in PD patients.

However, other factors involving inflammation, excitotoxic mechanisms, toxic action of nitric oxide, and mitochondrial dysfunction play roles in the etiology of PD. Since it is known

that the c-Jun N-terminal kinase (JNK) pathway plays an important role in regulating many of the cellular processes which are affected in Parkinson's disease, the possible importance of JNK pathwa in pathogenesis of PD is being increasingly recognized.

One of the earliest detectable changes in the PD brain is a dramatic depletion in substantia nigra levels of the glutathione. It has been demonstrated that glutathione depletion in dopaminergic cells in culture results in a selective decrease in mitochondrial complex I activity (a major hallmark of PD) and a marked reduction in mitochondrial function.

Current evidence suggests that mitochondrial complex I inhibition may be the central cause of sporadic PD and that derangements in complex I cause Alpha-synuclein aggregation, which contributes to the demise of dopamine neurons. The complex I inhibition appears to be due to production of nitric oxide (NO•), which can interact with the proteins within complex I and thereby inhibit its activity. Treatment of glutathione-depleted, cultured dopaminergic cells with inhibitors of nitric oxide synthetase (NOS), the enzyme that makes NO•, prevents mitochondrial complex I inhibition. In addition, increased iron levels have been reported in the Parkinsonian midbrain.

Interestingly, genetically or pharmacologically chelated iron (e.g. Fe–clioquinol complex, see also above) in a form which cannot participate in oxidative events prevents degeneration of dopaminergic midbrain neurons. This suggests that increased level of iron is actively involved in subsequent neurodegeneration and that iron chelation may prevent or delay PD progression.

The above mentioned biochemical abnormalities, such as mitochondrial complex I deficiency, depletion of intracellular thiols, and increased nia gral iron result in

aberrant oxidation of dopamine to 6-hydroxydopamine or dopamine-quinone, neurotoxic either directly or indirectly.

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In addition, increased iron levels have been reported in the Parkinsonian midbrain. Interestingly, genetically or pharmacologically chelated iron (e.g. Fe–clioquinol complex) in a form which cannot participate in oxidative events prevents degeneration of dopaminergic midbrain neurons. This suggests that increased levels of iron is actively involved in subsequent neuro-degeneration and that iron chelation may prevent or delay PD progression.

The above mentioned biochemical abnormalities, such as mitochondrial complex I deficiency, depletion of intracellular thiols (including glutathione), and increased nigral iron result in aberrant oxidation of dopamine to 6-hydroxydopamine or dopamine-quinone, both neurotoxic either directly or in conjugation with cystein (Sayre et al., 2001). The entry and release of iron from iron-storage protein, ferritin, occurs via the "free iron (ferrous) labile pool", which are active in Fenton chemistry. Besides superoxide, ferritin iron can be released by 6-hydroxydopamine, a neurotoxin implicated in PD.

It has been recently shown that the loss of inherited PD gene DJ-1 leads to striking sensitivity to the herbicide paraquat and the insecticide rotenone, which suggests that DJ-1, may have a role in protection from oxidative stress from environmental toxins. Thus exposure to various environmental toxins acting through oxidative stress seems to be associated with PD.

Levodopa (l-dopa) (often combined with carbidopa) is a dopamine precursor and the most commonly used medicine to treat Parkinson's disease. It is possible that the use of l-dopa for prolonged periods causes oxidation and toxicity to brain cells. If this turns out to be true, it

would further justify the recommendations to add antioxidants to the standard Parkinson's disease therapy.

Since oxidative stress appears to represent a portion of a cascade of biochemical changes leading to dopaminergic cells' death, one of a major problem in understanding the pathogenesis of PD is separating out the effect and extent of oxidative stress from other components of the cascade, that themselves can play a primary role in the initiation of ROS and RNS.

IV. Aging-Related Decline of Sleep

a) Valko, 2007 - Aging

Aging may be defined as a process of progressive decline in physiological functions of an organism. The free radical theory of aging was first introduced in 1956 by Denham Harman, who proposed that free radicals play a role in the aging process. His work gradually triggered intense research of the role of free radicals in biological systems.

There are basically two main theory-types describing the aging process: a) the damage-accumulation theories, and b) genetic theories.

a) The damage accumulation theories include the following theories: “free radical theory”, “glycation theory”, “error catastrophe theory”, “membrane theory”, “entropy theory” and others, among which “free radical theory” is probably the most complex approach to explain the aging process.

b) The “free radical approach” is based on the fact that random injurious effects of free radicals produced during aerobic metabolism cause damage to DNA, lipids, and proteins which accumulates over time.

Aging begins with oxygen, which occupies the final position in the electron transport chain. Even under ideal conditions, some electrons “leak” from the electron transport chain and interact with oxygen to produce superoxide radicals, so that under physiological conditions, about 1–3% of the oxygen molecules in the mitochondria are converted into superoxide.

The primary site of oxygen radicals (ROS) damage due to superoxide radicals is in the mitochondrial DNA (mtDNA). The cell is able to repair much of the damage caused to nuclear DNA (nDNA), but it is difficult to repair mtDNA injuries, hence extensive mtDNA damage accumulates over time and shuts down mitochondria, causing cell-death which results in aging.

Studies revealed an interesting correlation between oxygen consumption and aging: (i) lowered oxygen consumption explains why queen bees live 50 times longer than actively flying worker bees; (ii) houseflies prevented from flying by removing their wings lived much longer than normally flying insects because of decreased oxygen consumption; (iii) larger animals consume less oxygen per unit of body mass than smaller ones and live longer; (iv) different rates of ROS generation influence the life span of animals.

For example, a rat and a pigeon have similar metabolic rates, yet they have strikingly different life spans – whereas a rat lives three years, the pigeon can live 30 years. *in vitro* experiments showed that pigeon tissues generate ROS more slowly than the rat's mitochondria;

(v) in rodents, caloric restriction plays an important role in aging, since it is associated with increased DNA repair capacity thus a decreased levels of damaged DNA, lipids, and proteins; in addition, it is also linked with decreased production of superoxide. (vi) animal species that live longer have more efficient antioxidant protective mechanisms relative to their rates of oxygen uptake – as opposed to short-lived species. This mainly applies to their levels of SOD (superoxide dismutase), carotenoids, GSH (glutathione), glutathione peroxidase, and Vitamin E in animals.

In humans, the oxidative DNA damage level can be modulated by caloric restriction and dietary composition, as measured by urinary biomarkers. Consequently, longevity may depend not only on the basal metabolic rate, but also on the dietary caloric intake.

Accumulation of free radical-induced damage to biomolecules may be illustrated by an age-related increase in the serum 8-OH-dG levels in disease-free individuals over an age range of 15–91 years.

Numerous studies have reported the accumulation of 8-OH-dG as well as other lesions with age, both *in vivo* and *in vitro*, in nuclear and in mitochondrial DNA. DNA repair capacity correlates with species-specific life span.

Repair activity appears to decline with age. However, several studies in animals reported that age-related increase in 8-OH-dG in nuclear and mitochondrial DNA is due to the tissue increased sensitivity to oxidative damage rather than to a decreased repair capacity with age.

Interestingly, antioxidant status does not change significantly with age. Human studies show no alteration over the age-groups 35–39, 50–54, or 65–69 when comparing the levels of SOD, GSH, catalase, and ceruloplasmin (a blue copper-binding serum oxidase, that appears to catalyze the conversion of ferrous iron (Fe2) in tissues into ferric iron (Fe3), and is deficient in Wilson's disease, a genetic disease characterized by abnormal copper metabolism which therefore tends to accumulate in the liver and in the brain).

The concept of aging is supported by studies in many different animals showing that aging is frequently associated with the accumulation of oxidized forms of proteins.

Generally, a role of protein modification in aging was highlighted by the result that many different enzymes isolated from younger animals were catalytically more active and more heat-resistent than the same enzymes isolated from older animals. Since exposure to enzymes from young animals to metal-catalyzed oxidation led to changes in activity and heat-stability similar to those observed during aging, it was proposed that this process involve ROS-mediated protein damage.

Telomeres are unique DNA-protein structures that contain noncoding TTAGGG repeats and telomere-associated proteins. These specialized structures are essential for maintaining the

genomic integrity. Telomere dysfunction has been proposed to play a critical role in aging as well as in cancer progression.

Aging is a multifactorial process, and DNA and protein damage cannot be responsible for all of the patho-physiological changes seen.

Klerman, Wang, Duffy, et al. (2013). Survival analysis indicates that age-related decline in sleeo continuity occurs exclusively during NREM sleep.

A common complaint of older persons is disturbed sleep, typically characterized as inability to return to sleep after waking up. Since every sleep episode includes multiple transitions between wakefulness and sleep, these investigators applied "survival analysis curves" to their sleep data, by appropriate probabilistic curve fitting, to determine whether changes in the probability ("hazard") of awakening from sleep and/or returning to sleep, underlie the aging-related sleep purturbances. The "hazard rate" is the duration-dependent probability of transitioning out of a sleep phase.

The probability of awakening from sleep from NREM sleep was found to be considerably greater in older than in young adults. However, once the person woke up spontaneously, the probability of falling back to sleep was not greater in younger persons.

Independent of bout length (the length of time within each state), the number of transitions between NREM and REM sleep stages relative to the number of transitions to wake, was approximately 6 times higher in young than older persons, which explains the difficulty of maintaining sleep in older persons. Interventions to improve age-related sleep should thus target this change in awakenings.

Subjective complaints of disturbed or unrefreshing sleep are frequent in the US population, especially among older persons (National Sleep Foundation, 2002, 2003). Insomnia affects approximately 20 million Americans yearly, with an estimated treatment and lost work cost of $100 billion (Daley et al., 2009; Kessler et al., 2010; Roth, 2007).

Accurate assessment or definition of the exact phenotype(s) of poor sleep quality is crucial for designing and assessing treatment. Most methods for assessing sleep quality focus on the total number of minutes of various sleep stages across the night, but seldom quantify the dynamic processes that occur within a sleep episode.

These sleep dynamics are hypothesized to contribute to subjective sleep quality, yet until recently they have been difficult to quantify. Two statistical approaches for assessing sleep dynamics are the rate of transitions between states (e.g., between sleep and wakefulness), and bout duration (the length of time within each state) analyses.

Although these two approaches are related, they describe different aspects of sleep dynamics. An additional barrier to quantifying changes in sleep with aging or pathology is that some measures, such as sleep and wake bout durations being correlated within an individual, but not following a statistically normal distribution.

Therefore, statistics such as mean and standard deviation, and tests based on normal distributions of independent data, are not appropriate for describing these data, nor for comparing between conditions or subject populations. In addition, they do not take advantage of the wealth of information within the collected data, such as transition rates, bout durations, and other measures of sleep structure and dynamics.

Within a sleep episode, transitions between sleep, which can be subdivided into the physiologically different sub-states of NREM and REM sleep, as well as into wake, do occur.

We used survival-based analyses of sleep and wake bout lengths, and transition analyses to quantify age-related changes in sleep dynamics.

This probabilistic method is based on the concept that a state (e.g., sleep or wake within a sleep episode) "survives" until there is a transition into another state.

Survivor analyses can be used on data with non-normal statistical distributions, and they also allow use of "censored" data which, in the present analysis, occur when the end time of a bout is unknown due to data loss from recording difficulties, scheduled termination of the sleep episode by laboratory personnel, or for other reasons.

These methods can also be used to determine if transition rates are similar at all the bout durations, or if the transition rate for very short bouts is different from that of long bouts. The "hazard rate" is the duration-dependent probability of transitioning out of the state, which therefore supplies information about the stability of the state.

Survival-based analyses allow the quantification of the relative distribution of bout lengths, and can provide information about the underlying physiology involved in initiating, maintaining, and terminating each sleep state.

This method has been used in individuals with sleep apnea to quantify the differences in the hazard of awakening and falling back to sleep (Penzel et al., 2005), compared with unaffected individuals. These investigators applied these analyses to data from groups of healthy young persons, and groups of older persons in two types of protocols, to quantify changes in sleep dynamics with healthy aging, which we have previously found to impair consolidation of NREM sleep.

In a forced desynchrony protocol, the sleep/wake cycle length is not 24 hours in length and therefore sleep and wake can be studied at all circadian phases so that the influence of circadian rhythms as well as length of time awake or asleep can be investigated.

In the other protocol type, sleep occurred only at the habitual times for each individual, which is a restricted subset of all circadian phases.

Subjects were healthy as judged by their medical history, physical examination, EKG (electrocardiogram), and clinical tests of blood and urine, and no Ss were taking prescription nor nonprescription medications. Subjects were also psychologically healthy, as determined by questionnaires and an interview with a clinical psychologist. Older subjects had no clinically significant sleep abnormalities, as determined by screening questionnaires and diagnostic PSG (polysomnogram).

Additionally, no caffeine, alcohol, or nicotine use were allowed for at least one week before, and during the inpatient portion of the protocol. The data come from four different studies that utilized two types of protocols:

1) Forced desynchrony protocol:

13 older (64–74 years) and 11 young (21–30 years) subjects, who participated in a month-long inpatient forced desynchrony protocol, during which they were on a 28-hour activity/rest schedule, with PSG recordings during each scheduled 9.33-hour sleep episode. This protocol was designed to allow for sleep episodes to start at many different circadian phases across the 360° circadian cycle. There were a total of 229 sleep episodes from young subjects and 265 from older subjects.

2) Habitual sleep time protocols (data came from four different studies):

a) Data from the third baseline 8-hour sleep episode, before begining the forced desynchrony protocol, in 13 older Ss (9 male; 4 female) and 11 young Ss (all male), (Dijk et al., 1999).

b) Data from the third baseline 8-hour sleep episode in 14 (4 M; 10 F) older (65–75 years) and 5 (all M) young (18–25 years) subjects (Klerman et al., 2001).

c) Data from the third baseline 8-hour sleep episode in 12 older Ss (65–76 years, 9 M and 3 F), and 26 young (18–29 years, 17 M; 9 F) (Duffy et al., 2009).

d) Data from the first baseline 7-9 hour sleep episode (depending on the individual subject's habitual sleep duration) in 17 older Ss (60–76 years, 11 M, 6 F) and 18 young Ss (18–27 years, 10 M, 8 F) (Klerman and Dijk, 2008).

All inpatient studies were conducted at the Brigham and Women's Hospital General Clinical Research Center - Intensive Physiological Monitoring Unit, or at the Environmental Scheduling Facility. All events were scheduled relative to each individual's habitual sleep and wake times.

Sleep was recorded and scored using standard criteria (Rechtschaffen and Kales, 1968). Each 30-second epoch was classified as wake, NREM sleep, or REM sleep. Standard summary sleep statistics are presented in the Supplementary portion.

Survival Analyses

No data from before the first epoch of any sleep stage within a scheduled sleep episode were included in the survival statistics. These researchers defined a "bout" as a series of consecutive 30-second epochs at the same state (wake, sleep, NREM sleep, or REM sleep), and the bout (defined as Wake, Sleep, NREM Sleep or REM Sleep, respectively) lasted until a bout

of another state began. To explore the effect of bout length, they tested minimum bout lengths of 1.0, 2.0, 3.5, 5.0, and 7.5 minutes in the forced desynchrony data set.

Cox proportional hazards regression models for multiple events data were applied to study the "survival" probability— the probability of a bout length greater than a specific value.

To account for the fact that they were modeling multiple events - sleep bouts for each subject, with the possibility that there is a correlation between the bouts within each subject, and in order to fit a model which accounts for correlated observations within subjects, a robust sandwich estimate for the covariance matrix was used which resulted in a robust standard error for the parameter estimates.

Hypotheses testing of the regression parameters were carried out based on the robust sandwich covariance matrix estimates, and did not need to assume an independence of observations within a subject. 95% confidence intervals were calculated for the estimated "survival" probability. Note that for this analysis, by definition, there must be another state between bouts.

Competing risk approaches were also applied, to study the "survival" probability of a bout to transition to another stage using the stratified extension of the Cox proportional hazard models. All analyses were performed for Wake and Sleep bout categories (without subdividing the type of sleep), and separately for NREM Sleep and REM Sleep bout categories.

One, two, and three exponential curves were fitted to the survival curves, using MatLab v7.8 Curve Fitting Toolbox v 2.0 (MathWorks, Natick, MA, USA), and selection of the best fit was obtained by adjusted $R2$ values.

Discussion and Conclusions

Their findings (which are not brought here), of age-related changes in the statistical distribution and transitioning of sleep state bouts, confirm that a primary cause of sleep maintenance problems in aging is a decreased ability to remain in NREM sleep. The increased amount of wake within scheduled sleep episodes in older persons is due to more frequent awakenings, not due to a decreased ability to fall back to sleep.

These results using survival analysis are consistent with and help account for these researchers' previous more limited reports based on a subset of these data, in which they analyzed the frequency and duration of awakenings relative to recent history of NREM and REM sleep (Dijk et al., 2001) and interstate transition rates using Markov transition analyses (Klerman et al., 2004).

The current survival-based method, however, adds additional information about the bout length-dependent distribution of transitions to/from wake, sleep, NREM sleep, and REM sleep stages, as well as the differences in transition probabilities depending on the state from and to which the transition is occurring.

The advantage of survival analyses is that they allow more thorough, bout-dependent and nonnormal-distribution based analyses of these very complex data.

Because sleep timing and content is regulated by both circadian and homeostatic influences, the changes in sleep with aging may be due to changes in circadian rhythms, sleep homeostasis, and their interactions with aging.

This new analysis method may be potentially used to address the relative importance of circadian phase and/or sleep homeostasis with aging. However, the appropriate metric for

whether sleep homeostatic pressure at sleep onset changes with aging is the build-up rate, not decay rate; therefore application of this method to sleep dynamics within a sleep episode is not appropriate for that question.

Instead, additional experiments, including dose–response curves of wake duration from relatively short (e.g., with a nap midday) to long (after a sleep deprivation), are required to determine whether homeostatic influences on sleep change with healthy aging.

Their survival methods also do not assume a specific distribution; such *a priori* assumptions may affect the results obtained, and also are able to account for the multiple observations from each individual. Median statistics, while they do not assume a particular statistical distribution, summarize the data with a single number rather than including the bout length-dependent changes quantified by survival analyses.

These investigators conclude that their findings point to the continuity of NREM sleep bouts, especially those of short duration, as the most promising for developing effective therapies the sleep disruptions associated with healthy aging.

The types of analyses used here will also be useful in understanding the physiological basis of sleep problems in other patient groups, such as individuals with insomnia and narcolepsy; such analyses may provide insight into how sleep maintenance is affected in those conditions.

The participants in this study were healthy, taking no medications, and without sleep disorders and therefore did not have causes of sleep disturbances frequently observed in older people, including pain, sleep disordered breathing, or apnea with arousals, periodic leg movements, or even a bed partner with these conditions.

Sleep dynamics of older persons with sleep disorders or other medical conditions may have even more differences than the sleep dynamics of younger individuals. Each medical condition will therefore have to be studied separately to determine its impact on sleep.

Survival analyses can also be used to better understand the effects of medications (e.g., hypnotics or stimulants), other substances (e.g., caffeine or melatonin), or other interventions (e.g., yoga or behavioral) on sleep.

Rytkönen, Wigren, Kostin et al., 2010. Nitric oxide mediated recovery sleep is attenuated with aging.

Aging was shown to be associated with decreased sleep quality, including increased fragmentation, attenuated amplitude of the sleep–wake rhythm, and decreased sleep intensity. One of the best documented features of aging proved to be an impairment of the homeostatic sleep regulation.

Sleep homeostasis is the process by which an extended waking period brings about an increased intensity (measured by EEG delta power of 0.7-4.0 Hz) and duration of the subsequent NREM recovery sleep. The NREM delta response is significantly attenuated in aged individuals. The molecular mechanisms responsible for such impairments are not clear, and need to be further elucidated, which the objective of this study.

Substantial evidence points to the involvement of several endogenous sleep factors in the homeostatic control of sleep. Levels of endogenous sleep-inducing factors, such as adenosine (AD) and nitric oxide (NO), increase during a prolonged wakefulness, to induce a recovery sleep. Both AD and NO mainly exert their effects on the cholinergic basal forebrain (BF), an

area known to be involved in the regulation of sleep/wakefulness as well as regulation of cortical arousal.

Sleep inducing effects of AD in the BF are mediated by inhibition of wake-active cells, through the AD A1 receptors. A number of previously published studies reported a decline in AD receptor sensitivity with age, and this reduced receptor sensitivity in the BF was proposed to be one of the potential mechanisms underlying the significant age-related decline in sleep homeostasis.

These researchers have recently showed that NO in the BF is indeed involved in sleep homeostasis, and that that the effects of NO on sleep are mediated via AD. NO levels increase in the BF during sleep deprivation, while the blocking of NO synthesis reduces both AD release and the recovery sleep response. An infusion of a NO (nitric oxide) donor stimulates AD release, and promotes sleep.

NO is produced in the CNS by three types of nitric oxide synthases (NOS): the constitutively expressed endothelial (eNOS) and neuronal (nNOS) isoforms, and iNOS, the inducible NOS.

These researchers found that NO release in the BF during SD is mediated by the activation of iNOS. Thus, according to their model, iNOS-mediated NO production in the BF, followed by AD release, is one of the important mechanisms underlying the homeostatic increase in sleep intensity and duration following SD.

Several studies have shown that NO is involved in the regulation of spontaneous sleep–wake cycle, and that some aspects of this regulation are changed by aging. In the present study they have addressed the question whether attenuation of the homeostatic sleep response seen in the elderly could be due to changes in production of iNOS, NO and AD in the BF.

They compared iNOS level as well as NO and AD concentrations in the BF during three hours of SD, and measured sleep responses following SD, and after infusion of a NO-donor (DETA/NO) into the BF (the cholinergic basal forebrain) in three different age groups of rats, young, middle-aged, and old male rats.

Materials and Methods

Male rats, of three age-groups – young 4 months old; middle-aged, 14 months of age; and old rats, age 24 months, were housed individually with a constant temperature in a 12:12 hour light-dark cycle, with lights on at 8:30 am, and with food and water *ad libitum*.

Efforts were made to minimize the number of animals used and their suffering. The rats were habituated to handling starting at least 4 days before surgery under general anaesthesia, with dosages of anesthetics adjusted according to age, and the rats were placed in a stereotaxis for the surgery.

Two gold-coated screws were threaded into the skull for frontoparietal epidural recording of the EEG. A unilateral guide cannula for the micro-dialysis probe was aimed at the BF area, including the horizontal diagonal band of Broca (HDB), substantia innominata (SI) and mango-cellular preoptic area (MCPO). For EMG recording, two silver electrodes were inserted into the neck musculature. Finally, the guide cannula and screw electrodes were secured with acrylic dental cement.

Following recovery and adaptation to recording conditions (1–2 weeks, depending on age and recovery rate), a 2–3 day recording was obtained from each rat, to ensure that rats have adapted to the recording conditions.

In Vivo Microdialysis

Microdialysis probes were inserted through the guide cannula 20 hours before the first experiment. Rats were connected to microdialysis leads before 10:00 am, after which a continuous infusion of one microliter per minute of pure artificial cerebrospinal fluid (aCSF) was started. Samples were collected at 30-minute intervals for 6 hours starting at 10:00 am.

Samples were stored at minus 80° Celsius until assayed. EEG and EMG were recorded simultaneously during the 6 hours of microdialysis, and continued for additional 17 hours (total EEG recording of 23 hours).

Experiments

Reference day (RD)

RD served as an EEG baseline day, to which subsequent EEG recordings during experimental days were compared. During reference day, *in vivo* microdialysis was performed as described above.

Sleep deprivation (SD)

Sleep deprivation took place on the following day. The SD experiment was otherwise similar to the RD, except that rats were deprived of sleep for three hours (1-4 pm) by gentle handling. Novel objects were introduced to the cages to keep the rats awake (wake time >95%, as determined by the EEG, with no statistical differences found between age groups).

NO-donor infusion

The first 3 hours (10 am -1 pm) of aCSF infusion were followed by three hours (1 pm - 4 pm) of infusion of the NO-donor DETA/NO at one milli-Molar (mM) concentration dissolved in aCSF. This dose has previously been shown to be effective for increasing AD level and sleep in

young rats. After 1-2 days of recovery, the experiment was repeated using a higher dosage of DETA/NO (10 mM). EEG and EMG were recorded as in the other experiments.

EEG recording and Analysis

EEG recordings were digitally high-pass filtered (0.7 Hz) using the Spike 2 program, to avoid low frequency artefacts, and then semi-automatically scored in 30 sec epochs for NREM sleep and wakefuless using custom made scripts.

REM sleep was manually scored in Spike 2 program according to standard criteria: REM sleep was distinguished as a state with regular theta (5–9 Hz) activity in the EEG and decreased muscle tone (i.e., decreased EMG), as compared with wakefulness.

The amounts of NREM sleep and REM sleep were calculated separately as the number of 30-second epochs in each 6 h time bin, and presented as percentages of time. NREM delta power was calculated as the sum of NREM delta power values in each time bin, divided by the number of NREM epochs during that time bin. We call this the 'NREM sleep intensity'.

All NREM intensity values were normalized to EEG total power (frequency bands between 0.7 and 35 Hz) for each experiment. The effects of SD and DETA/NO were then quantified by calculating the difference in sleep measures (NREM and REM sleep, NREM sleep intensity) between RD and SD, and between RD and DETA/NO, respectively.

Statistical Analysis

Statistical analyses were performed using SigmaStat 3.0 statistical software. The effects of treatments within age groups were tested using paired t-tests or one-way repeated measures (RM) ANOVA, followed by Holm-Sidak post hoc test. Differences between age groups were tested using one-way ANOVA or two-way repeated measures (RM) ANOVA (age, time,

age×time), both followed by Holm-Sidak post hoc test. Equivalent non-parametric statistical tests were used if the data were not-normally distributed.

Results

Spontaneous sleep

There were no major differences between age groups in NREM or REM sleep duration, except for the slight nonsignificant reduction in NREM sleep duration in the old group as compared to the young one at time point 10:00–16:00 hours. N

REM sleep intensity was significantly reduced in both middle-aged (n = 12) and old (n = 9) animals, compared to the young animals (n = 12) during the reference day (one-way ANOVA, $P = 0.005$).

Recovery Sleep Following SD

The researchers studied age differences in sleep homeostasis by three hours SD (13:00–16:00 h), which invariably leads to a recovery sleep response (increased sleep duration and intensity). NREM sleep duration and intensity increased significantly in all age groups during the recovery sleep period (16:00–09:00 h) compared to the reference day (paired t-tests, $P < 0.01$).

However, between-group comparison revealed a significant age-related difference in the recovery sleep response: old rats (n = 9) had a significantly smaller increase in NREM sleep intensity during the first six hours (16:00–22:00 h) of recovery sleep, compared to the young rats (n = 10, two-way RM ANOVA, $P < 0.008$).

Nitric oxide levels

Basal levels of NOx (nitrogen) in the BF, as measured by *in vivo* microdialysis in micro Molar concentrations, differed significantly between the age groups. Old rats (n = 9) had higher concentrations of NOx than young rats (n = 12) (one-way ANOVA,

P = 0.042). There was also a trend for middle-aged rats (n = 12) to have higher NOx levels than young, but this difference did not reach significance (P > 0.05).

Basal iNOS

iNOS is a transcriptionally regulated protein not present in the brain tissue under normal conditions. Basal iNOS levels (optical density), as measured by immunoblotting, did not significantly differ between the age groups in the BF, nor in the cortex (CX) (one-way ANOVA, P = 0.284 and 0.472 for the BF and CX, respectively).

NOx− and iNOS during SD

NOx-concentrations (in micro Molar concentrations) increased significantly in the BF during SD when compared to pre-SD baseline (BL) in both young (*n* = 10) and middle-aged (*n* = 11) rats (paired t-tests, P = 0.027 and 0.039 for young and middle-aged, respectively). In contrast, no significant increase was detected in the NO*x* concentration in the BF of old rats (n=9, P = 0.581).

Accordingly, iNOS protein levels, as measured by immunoblotting, increased significantly in the BF of young (*n* = 5) and middle-aged (*n* = 6) rats, but no significant increases were found in the BF of old SD rats (n=4) (P = 0.038, 0.037 and 0.146 for young, middleaged and old rats, respectively).

Changes in iNOS level in young rats were consistent with previously published data. There was no increase in iNOS protein level in the CX of young animals sleep deprived for 3 hours. In the CX of middle-aged (n = 6) and old (n = 4) rats SD induced a slight, although statistically non-significant, increase in the iNOS protein levels, compared to control animals.

AD (Adenosine) Levels During SD

It has been previously shown that NO in the BF is necessary for SD-induced AD release, thus, it was important to see whether AD release in aged animals is impaired following the impairments in NO production.

In their study, the AD levels in the BF of young rats increased significantly during the 3 h SD (n = 9, paired t-test, P = 0.027), confirming several previous studies, while no statistically significant increases were found in AD levels of the BF in old rats.

Sleep responses following DETA/NO infusion

The NO-donor DETA/NO (1 mM) was used to experimentally increase NO levels in the BF during the spontaneous sleep–wake cycle without SD. As previously shown, in young rats (*n*=8) DETA/NO infusion increased NREM sleep intensity during the post-treatment period (16:00–09:00 h). This increase in NREM sleep intensity resembles the recovery sleep (recorded between 16:00 and 09:00 h) following SD ($P < 0.05$).

Contrary to young rats, neither middle-aged (n = 10) nor old rats (n = 7) showed significant increases in sleep during the post-SD period (16:00–09:00 h) following DETA/NO infusion with 1mM concentrations, nor with 10 mM.

Discussion

The present study findings indicate a clear age-dependent impairment in the molecular mechanism underlying homeostatic sleep regulation, which is mediated by NO release in the BF.

The researchers demonstrate for the first time that aging attenuates the SD-induced iNOS and NO production in the BF, which correlates with a decreased recovery sleep response, and

with a reduced sleep response to an infusion of NO-donor into the BF, suggesting a reduction in the BF sensitivity to NO. Old rats also demonstrated impaired AD accumulation during SD.

Sleep deprivation invariably leads to an increase in NREM sleep intensity during the recovery sleep period. In this study, the NREM sleep intensity response was reduced in middle-aged and old animals, signifying disturbances in the homeostatic sleep regulation, a finding consistent with previous studies in both animals and humans.

The researchers tested whether these impairments in sleep homeostasis in aged rats could be induced by changes in production of iNOS, NO and AD in the BF, a mechanism shown to be critical in homeostatic sleep control.

Conclusions

a) Aging attenuates SD-induced iNOS and NO production in the BF, which correlates with the decreased recovery sleep response, and reduces sleep response to an infusion of their NO-donor into the BF, suggesting an age-related decreased sensitivity of this area to NO. Old rats also showed impaired AD (adenosine) accumulation during SD.

b) Aging-related disturbances begin gradually in middle age.

c) Taken together, the inability to produce NO during a prolonged waking period (chronic SD) and the agng-dependent insensitivity of the BF to the sleep promoting effects of NO, lead to a diminished homeostatic sleep response in aging.

Chapter 3: Methodology

This chapter includes the search methods for the relevant literature, and discuss the process used to gather information leading to these objectives.

The search for articles to support this study was primarily accomplished through the online research services of the Yo San University library, as well as through the UCLA Medical Library in Los Angeles, California, USA.

The objectives of this study were three: to a) examine the quantitative data at the cellular and molecular levels and weigh the evidence for chronic insomnia and the experimentally induced sleep deprivation as a risk factor for pathogenesis of chronic diseases, b) examine the current evidence for the effectieness of acupuncture in alleviating chronic insomnia and co-morbidities and improving sleep of persons with chronic insomnia and chronic co-morbidities with varied conditions, and c) examine the nature of the relation between chronic insomnia and aging.

Research Design

The research synthesis data was compiled through the online search of 420 articles in medical journals found on Pubmed and Google Scholar, and articles accessed through the Yo San University Library using the following key word: acupuncture and chronic insomnia, chronic insomnia and autoimmune diseases and acupuncture, aging, chronic insomnia and acupuncture, chronic insomnia and chronic co-morbidities in epidemiological studies, sleep deprivation studies and acupuncture treatment for cardiovascular conditions, daytime consequences of insomnia and chronic insomnia, and and acupuncture treatment, chronic insomnia and schizophrenia, chronic insomnia and depression, chronic insomnia and anxiety, acupuncture mechanism, acupuncture and Parkinson’s Disease, chronic insomnia and

Alzheimer's Disease, Molecular clocks, Free radicals, antioxidants and oxidative stress and chronic disease.

Instruments and Procedures

The research synthesis data were collected and analyzed using the article abstraction forms created by the researcher starting on June 1, 2014 and ended on December 1, 2015. Data collected from each article were then organized and used to chart the findings. A summary of findings was generated to show the effects of acupuncture on sleep/sleeplessness and chronic insomnia, and on chronic co-morbidities such as cardiovascular diseases and heart failure, hypertension, stroke, neurodegenerative disease such as Alzheimer's and Parkinson's disease, major depression, major anxiety, Schizophrenia, Bi-polar disease, psychosis, autoimmune diseases including rheumatoid arthritis and fibromyalgia, diabetes, digestive tract disorders, and other disorders that were found to be associated with chronic insomnia.

Point combinations, sham control treatment and other control methods used in studies of acupuncture, and other TCM modalities such as acupressure or cupping, and exercise, nutrition, and life-style affects on sleeplessness were also categorized to enable the author to obtain an overview of the research methodologies used. The literature search was conducted at the Yo San Library and at the UCLA Medical Library.

Inclusion and Exclusion Criteria

This study was based on studies published in English, in peer-reviewed journals. Studies had to properly document their instruments and tests used to diagnose insomnia, as well as methodology for recruiting their participants. Their control groups and statistical tests used to analyze their data had to be clearly specified and appropriate. Only chronic insomnia and associated chronic diseases were included, relying on the APA definition of chronic insomnia.

Chapter 4: Results

Acupuncture for Treating Insomnia and Co-morbidities

Study 1

Jialing, S., Sung, M., Huang, M. et al., 2010. Effectiveness of Acupressure for Residents of Long-Term Care Facilities with Insomnia: A Randomized Controlled Trial

Ss were 50 Patients (Px) with insomnia in long-term care facilities. 25 Px in experimental group, in which Px received 5-weeks of HT-7 bilateral acupressure. The Control group of 25 Px received a light touch on HT-7.

Insomnia was measured by the Athens Insomnia Scale (AIS) and PSQI (both subjective outcome measures). A randomized and controlled trial (RCT) to test effectiveness of bilateral HT-7 acupressure for insomnia in long-term hospital residents with insomnia Tx. They tested how long its effect could last, and found that the beneficial effects of acupressure on HT-7 may improves insomnia for up to two weeks after the treatment.

Results

a. HT-7 Acupressure was ($p<0.05$) => effective for Insomnia in long-term residents.

b. This Tx effect lasted 2 wks ($p<0.05$)

Conclusions

1. Offering Acupressure on a regular basis could improve insomnia in long-term care facilities.
2. Acupressure on HT-7 may improve insomnia for up to 2 weeks after Tx.
3. This is a safe and effective insomnia Tx.

Study 2

Cristian, A., Katz, M., Cutrone, E., and Walker, RH. (2005). Evaluation of Acupuncture in Tx of Parkinson's disease: A Double-Blind Pilot study. Movement Disorders, 20(9): 1185-1188.

a. 14 stage II or III PD Px with a stable PD medication for at least 30 days before and a stable medical condition, received Acupuncture Tx.

b. 14 male veterans => Experimentally Received 5 sessions of Electro-acupuncture over 2-wk period – as part of Tx protocol for chronic non-Acupuncture points on arms, legs and Scalp in areas with no known Acupuncture points. Needles were inserted just under the skin.

Measures

a. Compare effectiveness of EA (electroacupuncture) and Western Medications w/non-acupuncture points for PD Px.

b. Measured improvement on UPDRS motor score, by a blinded rater.

c. Measured PDQ-39 and PDQ-8.

d. Geriatric Depression Scale.

Results

On all measures - No significant results ($p > 0.08$) bet groups compared to baseline.

Conclusions

a. Findings that there was a subjective improvement in some Ss, such as tremors, handwriting, difficulty in walking, and in sleeping.

b. Improved disease progression, the right dose of medications required, and in medication's side-effects

c. Improvement in auditory evoked brainstem potentials

d. There appeared to be a trend for improved motor scores on the unknown acupuncture UPDRS

e. Decreased body discomfort

f. Improved quality of life (PDQ-8)

g. Decreased nausea

h. Decreased sleep problems

i. A significant challenge to acupuncture trials is the lack of appropriate control group, including in this study

Study 3

Sixel-Döring , Schweitzer, Mollenhauser and Trenkwalder C. (2011). Intraindividual Variability of REM sleep Behavioral Disorder in Parkinson's Disease: A Comparative Assessment Using a New REM Sleep Behavior Disorder Severity Scale (RBDSS) for Clinical Routine. J Clin Sleep Med, 7(1):75-80.

a. 20 PD Px w/RBD were investigated w/ PSG (Polysomnography)

b. 73 motor behavior events during REM sleep were graded both visually and polysomnographically.

c. Rating was conducted by two blind raters

d. Final RBD severity was determined by the highest score given

Testing a new RBDSS for clinical routine use on 20 PD Px identified with REM Sleep Behavior Disorder (RBD).

Results

a. Inter-rater reliability of the scale was 0.8 for movement data, and 0.89 for vocalization data.

b. All Px had severely disturbed sleep w/ reduced sleep efficiency, loss of slow wave sleep (SWS), sleep fragmentation, and an increased periodic limb movement (PLM).

c. 40% of Px showed violent behavior, but only on one night of the 2 PSG nights.

d. Final RBD severity score differed in 60% of Px between first and second nights.

Conclusions

1. The new RBDSS (RBD severity Test) was found to be a reliable, easy to use, tool for assessing motor events during REM sleep with PSG.

2. Severity and Phenomenology of RBD show a significant variability in the individual PD patient.

Study 4

Yeung, Chung, Zhang, Yap, and Law, 2009. Electroacupuncture for Primary Insomnia: a Randomized Controlled Trial. Sleep, 32:1039-1047.

a. A randomized, single-blinded, placebo-controlled parallel-groups design – at a university-based sleep clinic – designed to compare the effects of EA (electro-acupuncture) *versus* placebo-controlled acupuncture.

b. Major assessments were at baseline and one week after treatment.

c. Authors followed the Consort and Stricta recommendations in designing and reporting the controlled trial.

d. Ss = 60 Chinese adult volunteers who reported having insomnia three or more nights per week for at least three months with symptoms that meet the DSM-IV criteria for primary insomnia.

e. Their Insomnia Severity Score (ISC) was at least 15.

f. Ss were recruited by advertisements in local newspapers.

g. The 60 Ss were selected from the initial pool.

1. Ss were screened by polysomnography and by a structured clinical interview for the DSM-IV prior to randomization.

2. Inclusion criteria: (a) Ethnic Chinese, (b) Aged 18-65 years old, (c) Insomnia complaints for three or more nights per week, for at least three months, (d) Diagnosis of primary insomnia according to DSM-IV, (e) total score of ISI, the primary outcome measure, was at least 15, indicating insomnia of moderate severity.

3. Placebo blunt needle at the same acupoints as the experimental group, but the needle tip only touched the skin and gave a pricking sensation, but the needle was not fixed inside the copper handle, so that the needle moved inside the handle and appeared to be shortened. The needles were held by a surgical tape or by hair pins, as in the EA group, and was connected to the same electric stimulator, but with zero frequency and zero amplitude (the Streitberger and Kleinhenz placebo needle).

4. All Ss were told to report to the acupuncturist if they felt the impulse was too strong. The setting, acupuncturist, treatment frequency, and duration were the same as for the AE group.

5. Eligible Ss completed a sleep diary for one week and a three-day actigraphy recording in the week prior to a scheduled baseline visit. At the baseline visit Ss returned the completed sleep records and were asked to fill out a set of self-reported questionnaires. They were then randomly assigned to either EA or to the placebo group by an independent administrator using a computer-generated list of random numbers, and received their first treatment on the same day. In the first-week after treatment the Ss returned their first-week's sleep diary and three-day actigraphy recording for the previous week, and completed the same set of questionnaires as before. It was not possible to blind the only acupuncturist in this study. The questionnaires sleep diaries, and actigraphy results were analyzed by independent research assistants, who were blind to the group allocation,

Results

a. There was no significant between-group difference in ISI total score, the primary outcome measure, nor for the total scores of PSQI, or the sleep-diary derived SOI, TST, WAS, nor for sleep quality At week-1 after Tx.

b. The only significant difference between groups ($P < 0.002$) was in sleep-diary derived SE (Sleep Efficiency), with greater improvement in the EA group from baseline to post-treatment.

c. There was also a significant difference between groups in actigraphy-derived SE at post-treatment ($P < 0.04$), with greater improvement in the EA group from the baseline to one week post-treatment.

d. All the adverse effects were mild.

Conclusions

1. To the best of the authors' knowledge, this is the first study using acupuncture for treating of primary insomnia, using a well documented screening process, randomization, placebo acupuncture needles, validated subjective scales, and objective measures.
2. Compared to the non-invasive placebo needling, EA significantly improved both subjective and objective measures of SE.
3. The proportions of Ss achieving sleep-diary derived WASO of 30 minutes or less and a SE of at least 85% after treatment were significantly higher in the EA group.
4. The data suggest that EA can be considered as a safe, well-tolerated and potentially useful non-pharmacological intervention for primary insomnia.
5. Improved efficacy might be achieved by increasing session frequency and having individually tailored acupuncture regimens.
6. It would be worthwhile to explore whether combination treatment strategies, such as acupuncture and cognitive behavioral therapy, could produce synergistic therapeutic effects.
7. Despite its methodological limitations, the present study provides important data on primary insomnia treatment by acupuncture. Some advantage was found for EA over noninvasive placebo acupuncture.

Study 5

Yeung, Chung, Tso, Zhang, Zhang, Ho. (2011). Electroacupuncture for Residual Insomnia Associated with a Major Depressive Disorder: A Randomized Controlled Trial.

Study objective: to evaluate efficacy and safety of electroacupuncture as an additional treatment for residual insomnia associated with major depressive disorder (MDD).

Study design: Randomized Placebo-controlled.

Setting: A psychiatric outpatient clinic

Ss: A Community sample of 60 Chinese adult volunteers who reported having had insomnia three or more nights per week, with symptoms that meet the DSM-IV criteria for primary insomnia - insomnia for at least 3 months woth ISI total score of at least 15.

a. Ss were screened using polysomnography and the Structured Clinical Review for DSM-IV prior to randomization.

b. EA Tx at E-Yintang, Du-20, bilateral ear Shenmen, E-SSC, E-Anmian three times a week for 3 wks *versus*. placebo acupuncture (Streitberger needles) at same points.

c. Self-reported questionnaires, one-week Sleep Diaries, and 3-day actigraphy – that were collected at baseline and one wk after treatment of.insomnia severity Index (ISI) is the primary outcome measure.

Results

a. Both groups had significant improvement compared w/ pretreatment baseline: a significantly greater improvement in sleep and sleep efficiency measured by Sleep Diaries and actigraphy in the EA group. But not significant.

b. No significant between-group difference was found in ISI and other outcome measures.

c. Higher proportions of Ss had less than 30 minutes of wake time after sleep onset and sleep efficiency of at least 85% at the post-treatment visit.

d. All adverse events were mild

Conclusions

1. Researchers found a slight advantage for EA over placebo acupuncture in this short-term treatment of primary insomnia.
2. The researchers concluded that further studies are needed to verify the effectiveness of acupuncture for insomnia.

Study 6

Lundeberg, T. and Lund, I. (2007). Did 'The Princess on the Pea' Suffer from Fibromyalgia Syndrome? The Influence on Sleep and the Effects of Acupuncture.

A Qualitative Review

a. REM *versus* NREM stages

b. Sleep Regulation

c. Sleep and pain

d. Sleep disturbance in FMS (Fibromyalgia Syndrome)

e. Acu, Sleep disturbance and FMS

f. Sleep and memory processing

g. Memory processing, FMS and Acupuncture

Results

Acupuncture Tx sign improved pain, sleep and cognitive fns in some FMS Px

Study 7

Zhang, Y., Ren, G., and Zhang, X. (2010). Acupuncture plus Cupping for Treating Insomnia in College Students. Journal of Traditional Chinese Medicine, 30(3): 185-189.

Study Design

Groups: Treating insomnia – treating syndromes of other general issues.

Ss: 92 Students w/insomnia randomly divided:

Treatment: Treatment group (52 Ss) *versus* Control (40 Ss)

Experimental Treatment => Received Acupuncture and Cupping

Control => 'No specific Intervention' – only general Acupuncture

Inclusion Criteria: SRSS >22 (insomnia score), no severe systemic disease, no alcohol- nor drug-dependence

Treatment group: Acupuncture on Du-20, Du-23, E-Yintang, PC-6, HT-7, ST-36, SP-6, LIV-3 (Specifics of needling).

Tx: Six treatments per week, 30 minutes of needling and three cupping Tx per week of Bilateral moving up and down along the spine.

Control groups: Ss differentiated into four syndromes:

1. **HT and SP Deficiency**: acupuncture on UB-20, UB-15, HT-7, SP-6

2. **Fire Hyperactivity** due to **Yin Deficiency**:Acupuncture on PC-7, KD-3, HT-7, LIV-3

3. **ST Disturbance**: Ren-12, ST-40, ST-45, SP-1.

4. **LIV Fire Disturbing Upwards**: LIV-2, GB-44, GB-20, HT-7.

Each group received cupping on the same acupoints as group's treatment main points.

Results

a. Therapeutic Effect: **Mild Insomnia** – no difference between groups. Both groups

showed a significantly faster recovery than those with moderate insomnia ($P<0.01$)

b. Severe Insomnia – same tendency: Tx group > Control

c. SRSS was reduced in both groups, significantly greater decrease in the Tx group ($P < 0.05$)

d. In both groups, Post-Tx > Pre-Tx: Acupuncture and cupping (Tx group) > Control: ($P<0.01$) for Tx group, ($P < 0.05$) for control group.

Conclusions

1. Most Ss had moderate insomnia
2. Researchers obtained differences between the Tx and Control groups, with a stronger therapeutic effect of the treatment in the Tx group.

Study 8

Li, L. and Lu, J. (2010). Clinical Observation on Acupuncture Treatment of Intractable Insomnia.

Inclusion criteria: Px with 2 of the following Sx (symptoms) for at least one year:

a. Difficulty falling asleep

b. Unsound sleep with frequent awakenings (fragmented sleep)

c. Short sleep with waking up early and unable to resume sleep

d. Dream-disturbed sleep all night

e. No refreshed sensation after sleep

Exclusion criteria: Px with a somatic illness or mental disorder.

(1) **Tx group**: main acupoints: Du-20 through E-SSC, PC-6, ST-36, SP-6, KD-3, etc.

a. Needles retained 60 minutes on 10 consecutive days, then three-day interval and two more such Tx courses.

b. Optimal Tx time was 4 pm

c. Ear points: Shenmen, HT, SP, KD, Sympathetic, Subcortex – treated with seeds of Vaccariae. 2-3 ear points were chosen on one side each time, and pressed several times two hours before bedtime.

d. Indirect moxibustion with drug cake was applied at: UB-15, 20, 23 for Px with poor constitution.

(2) **Control group**:

a. Acupoints: Du-20 thru E-SSC only.

b. No ear acupuncture, nor back acupuncture.

c. Control Px were treated with needles retained for 30 minutes

d. Tx arranged in AM or PM, with no special requirements

(3) **Criteria for Therapeutic effects:**

Cured: Sleep returned to normal with daily sleeping of about seven hours and all accompanying Sx gone.

Markedly Relieved: All accompanying Sx markedly relieved and daily sleeping time was over five hours.

Improved: Sleep was improved, all accompanying Sx relieved to varied degrees.

Failed: No improvement after treatment.

Results

a. Total effective treatment rate was 98.0% in Tx group *versus* 77.5% in control group.

b. Therapeutic effect in Tx group > in control group ($P < 0.01$).

Conclusions

Body acupuncture plus auricular therapy may show better effect for intractable insomnia if accompanied by moxibustion on the Back-Shu points.

Study 9

Chen, J., Chao, Y., Lu, S., Shiung, T. and Chao, Y. (2012). The effectiveness of Valerian acupressure on the sleep of ICU Patients: A Randomized Clinical trial.

Objectives

a. Severely ill Px often have sleep difficulties, which are exacerbated for ICU (Intensive Care Unit) Patients.

b. Acupressure and/or Valerian aromatherapy were reported as promoting sleep.

c. The purpose of this study was to explore the effectiveness of Valerian acupressure on the sleep of ICU patients.

Background

Severely ill hospitalized patients frequently experience sleep difficulties. The noise of physiological monitoring systems, warning alerts, lights, and frequent or complex treatments disrupt patients' night sleep, often resulting in sleep deprivation and fragmentation that negatively affect patient recovery. Celik et al. (2005) and Tamburri et al. (2004) found that in ICUs, nurses and medical staff visit Px room over 40 times every night.

When critically ill patients are deprived of sleep, their immune systems are weakened, decreasing their ability to recover. Steroid body secretions increase and interfere with regular healing properties and resistance to disease. Additionally, respiratory muscles may become flaccid, affecting breathing and resulting in high carbon dioxide and low oxygen levels in the blood, which prolongs Px's dependency on respirators and other medical equipment. Taken together, all of these make the Px's sleep quality of the severely ill a serious issue for medical and nursing stuff.

(1) This study is a case study of a randomized experimental design.

(2) Ss were recruited from ICU of a medical center – 41 Ss (30 men and 11 women) in the acupressure group, and 44 Ss (35 men and 9 women) in the control group.

a. **Inclusion Criteria**: Ss were conscious, literate, communicable, agreed to participate in this study, insomnia score was less than 15, i.e. ICU Px is stable and in a less critical condition.

b. **Exclusion Criteria**: Hand/foot amputees, Dx of bilateral paralysis or convulsions, using sedatives, or had been taking sleeping pills for over a month.

(3) **Intervention**: 2.5% valerian essential oils were applied to acupoints *Shenmen* (HT-7), *Neiguan* (PC-6) and *Yongquan* (KD-1), before acupressure treatment was applied on the same acupoints by researchers who attended acupressure courses, under the supervision of professional TCM practitioners, to ensure accurate point location,

using the thumb to apply the acupressure at a 90° vertical angle. Pressure was applied for five seconds, then released for one second. Pressure began light and was progressively increased to 3-5 kg until Px indicated they felt pain, numbness, or swelling. Pressure was

continuously applied to each acupoint for three minutes (Ma, 2005; Xu et al., 2006). Total time for one acupressure session on the three points bilaterally was 18 minutes.

Before performing the acupressure, the researchers employed a scale to assess the degree of pressure applied under observation of colleagues for 10 seconds, to ensure the right pressure to be applied and maintained within the range of 3-5 kg.

Conclusions

1. The results indicate that valerian acupressure on acupoints *Shenmen* (HT-7), *Neiguan* (PC-6) and *Yongquan* (KD-1) can improve ICU patients' sleep duration and quality. Relaxation responses experienced immediately after applying valerian

acupressure can be observed using a HR variability analyzer, an objective outcome.

2. In critically ill patients with sleep difficulties, acupressure on acupoints *Shenmen*

(HT-7), *Neiguan*, (PC-6) and *Yongquan* (KD-1) acupoints can be an effective

alternative, or reduce the use of sedatives and promote better sleep quality.

Study 10

Bosch, van Luijtelaar, van den Noort, et al., 2013. Sleep Ameliorating Effects of Acupuncture in a Psychiatric Population.

This interesting pilot study has a 2x3 factorial design, with six groups with a total of 40 participants. The three groups of Px were healthy, depressed, or schizophrenic patients. They were randomly assigned to the experimental group (a three-month acupuncture treatment), or to the control group (waiting list).

From a TCM point of view, insomnia often occurs in patients that later develop schizophrenia and other mental disorders. "Sleeping is critical to mental health patients," said Sun Si Miao, a great Chinese medical doctor from the Tang dynasty, who was the first to employ

traditional Chinese sleeping therapy for treating mental disorders. Nigel Wiseman (editor), "Soothing the Troubled Mind," p. 96, writes that "insomnia is common to all kinds of mental disorders, and is the most commonly seen symptom, and is a symptom from the earliest stages of schizophrenia", and "preventing insomnia is significant for maintaining health and protecting the strength of the intellect". Dr. Maoshing Ni writes, "Insomnia is a major contributor to the aging process and the breakdown of the immune system" (*Secrets of Longevity*, p. 248).

Rational

Among psychiatric patients, the two large groups that need long-term treatment are those suffering from depression and schizophrenia. In both groups of patients, their disorders are characterized by marked sleep disturbances. Despite the clinical success of both anti-depressants and anti-psychotic Western drugs, these tend to further aggravate their condition by causing drowsiness.

Px are therefore often advised to take them at night, which causes sleeping problems such as excessive dreaming and increased TST, even though taking these drugs at night minimizes their daytime drowsiness.

Tiredness, drowsiness and poor sleep interfere with these patients' ability to engage with therapeutic services due to being too tired and unmotivated if they do not see the point or do not want to take medicines that worsen their insomnia. Presumably these are not the only factors that are of importance, but they seem highly relevant in Px groups that suffer from depression as well as for those suffering from schizophrenia, since both diseases cause insomnia. Previous studies show that adherence to pharmaceutical Tx is negatively correlated with sleep disturbance and depression.

This pilot study compares the effects of acupuncture on the subjective quality of sleep in long-term Px of schizophrenia and Px of depression with healthy participants. Patients continued their regular psychiatric Tx, including medications while being also treated with acupuncture. Participants included:

1) 16 schizophrenia Px, 10 women and 6 men, average age 44.25 year old, with average illness duration of 13.56 ±1.59 years.

2) 16 depression Px, 12 women and 4 man, of average age 50.94 year old, with average illness duration of 5.94 ± 1.05 years.

3) 8 healthy participants.

Mental health Px were identified and approached by their therapist, who offered them to volunteer if they wished to try this new Tx. Px who agreed to participate did so voluntarily, and signed an informed consent form, and their therapist signed for their mental ability to understand the form.

Instruments to Assess Depression, Schizophrenia and Insomnia

1) Beck's Depression Inventory II served as the inclusion criteria for depressed Px for this study.

2) Positive and Negative Symptom Scale (PANSS) for schizophrenia served as the inclusion criteria for this study for schizophrenia Px for this study.

3) PSQI (Pittsburgh Sleep Quality index) (German version) pre-Tx and post-Tx of acupuncture, to assess Ss's subjective quality of sleep.

The PSQI questionnaire consists of 18 items, divided into seven components, that can each be scored from 0 to 3. PSQI total score results in the sum of the component scores, and can be any score between 0-21. The higher the score, the worse is their sleep quality. Ss with a score

below 5 have a good sleep quality. There is, however, a tendency to use 6 as a cutoff point, to be more selective.

The validity of this test was found to be good in Px of primary insomnia, since a high correlation was found between PSQI scores and the sleep diary as well as a significant correlation with polysomnographic measurements (Tsai, Wang, Wang et al., 2005).

PSQI has a high sensitivity and specificity for patients with insomnia and patients with depression and schizophrenia. Cronbach's alpha for the total score was 0.77. In the USA it is considered one of the two top insomnia scales, together with the ISI (Insomnia Severity Index).

Acupuncture Needles Used

0.25 x 25mm, or 0.20 x 15mm, stainless steel single use (AcuPro C, Wujiang City Cloud and Dragon Medical Device Co., Ltd., China).

Experimental Intervention

Tx group participants received acupuncture treatments (Tx) once a week for 12 consecutive weeks. Individualized acupuncture was applied according to TCM principles after a careful individual diagnosis (Dx) by a licensed medical practitioner with more than five years experience.

This Tx took place in a lighted room with very soft background music (Enya) playing. This soft music played along with the acupuncture was done in compliance with the ethics committee demands, in order to reduce as much as possible anxiety in Px with schizophrenia and to make them feel comfortable. The soft background music was kept constant for all the participants during all the sessions.

Participants were seated as a group in 12 “relax” chairs, the back of which could be adjusted to put up the feet, to sit in an near-lying position. Each Px could decide whether to sit upright or recline almost lying down.

Sitting up or reclining face up limited access to acupoints on the back. Patients came into the room in intervals, so as to reduce the waiting time. Needles were retained for one hour, after which the needles were removed. The group treatment setting made sure practitioners were directly at hand in case anxiety arose; this was one of the important demands made by the ethics committee.

If individuals had personal questions or sensitive matters to be discussed prior to the Tx, there was an empty adjacent room next to the Tx room where confidentiality could be assured. As there were two acupuncturists present, the other Px (patients) would not be left alone in the mean time.

Procedure

All participants were tested in an experimental testing room in the clinic, by apprentices who were blind to group or time of testing. The healthy control group was tested at T1 (pretest) only, whereas the participants with schizophrenia and depression were tested at both T1 and T2 (post-test). Next, Ss were randomly assigned to a Tx or to waiting list condition. The entire study lasted 13 weeks: 12 weekly acupuncture sessions, pre-testing and post-testing.

At the end of the study, all participants received a debriefing and were individually informed about of their own test results. Px on waiting list were given an opportunity to attend an acupuncture Tx after T2 if they wanted to, to provide an equal Tx opportunity. The current study stopped at T2, after which there was no further testing, so that any acupuncture provided after T2 became part of their regular Tx.

A one-way ANOVA (Analysis of Variance) and post hoc (Bonferroni) T-tests whenever appropriate, were the statistical tools to assess the significance of outcome measures.

Results

The depression acupuncture group showed a significantly reduced PSQI total score ($P<0.003$). For the schizophrenia acupuncture group, significant reductions were found in PSQI total score ($P=0.048$), sleep latency ($P<0.038$), sleep disorders ($P<0.033$), and for decreased need of pharmaceutical medications ($P<0.033$).

For both depression and schizophrenia waiting-list conditions, no significant differences were found between the PSQI pre- and post-test outcomes.

Side Effects

Two Px reported bruising as side effects after one of the acupuncture Tx sessions. One Px reported feeling extremely tired after the first session. No other side effects were reported.

Conclusions

1. All patients were chronically ill. Significant improvements were found on the PSQI Total Score for both Tx groups, indicating that Px slept better after 12 acupuncture treatments. The waiting-list condition groups showed no significant improvements.
As suggested by Hametner et al. (2012), a cut-off score of 6 was used to clinically differentiate between Px with sleep problems as opposed to Px with good sleep.

2. The schizophrenia Px group falls below the PSQI Total Score 6, the clinically relevant score after Tx. By contrast, the depression Px group has improved, and although the differences might not seem large, they are borderline clinically relevant.

3. Three subscales (PSQI Latency, PSQI Medication, and PSQI Disorders) showed significant improvements in the schizophrenia group, but not in the depression group. This

indicates that Px with schizophrenia benefited more from the acupuncture Tx than the Px with depression. Noticeably, these schizophrenia patients fell asleep faster and even approached normal levels on the PSQI Latency subtest, reaching levels that are commonly found in healthy controls. They also used less sleeping medication, and reached normative levels on the PSQI Disorders subtest.

4. Five of the schizophrenia Tx group stopped using sleeping medication during the acupuncture project, whereas one waiting-list schizophrenia Px who had not used sleep medication beforehand, started using sleep medication.

5. As a result of acupuncture, some participants reduced their sleep medications in consultation with their psychiatrist. These participants saw this as a benefit of the acupuncture Tx. Six of the depression group (two in the Tx and four in the waiting list condition) stopped using their sleep medication, while another depression Px in the waiting list started to use sleep medication.

6. Medication reduction is usually seen by patients as positive, and is felt as an improvement or achievement. It may be that the promise of medication reduction through acupuncture may be a motivating factor for attending the acupuncture Tx sessions. However, there are possible pitfalls in reducing medication because such Px might become more vulnerable to breakdown. Further research is needed to confirm this cited study's subjective comments, to investigate the true impact of acupuncture.

7. There were no dropouts from this project, as opposed to 30-40% dropouts from other therapeutic projects in this clinic. The participants reported feeling less tired, more relaxed, better able to sleep and were satisfied with the Tx.

8. Both acupuncture treatment groups demonstrated significantly lower post-Tx PSQI scores, but acupuncture seemed more effective for this study's schizophrenia patients than for its depression Px. Acupuncture seems capable of improving sleep in both types of patients with long-lasting psychiatric problems.

Limitations

1. Since the study involves acupuncture, the problem of no suitable control group or placebo needs to be addressed (MacPharson, White, Cummings et al., 2010).

2. The use of a standardized protocol for acupuncture is unheard of in clinical TCM practice except for detoxification protocols (NADA) used for addiction and trauma. Using such a standardized Tx would not shed any light on the possible effect of acupuncture as an add-on Tx. In this study, a pragmatic randomized controlled study (RCT) was used, an approach that attempts to answer "real world" question whether
acupuncture as an add-on Tx improves sleep more than without it. These researchers goal was to deliver better Tx to Px, which implies the need to evaluate what can be done in daily practice. Is acupuncture better Tx than what is currently offered to these Px?

3. There is anxiety about giving acupuncture to people with schizophrenia in Europe, since it is not normally practiced and people in psychiatric hospitals are not normally left alone with needles or with other dangerous objects. Moreover, anxiety exists that the needles might become part of hallucinations or psychotic thoughts. The present study further proves that people with schizophrenia can be safely treated by acupuncture and that the use of these needles did not evoke negative emotional reactions. It is important to realize that in this pilot study, positive results were obtained in Px with schizophrenia who were ill for over 10 years.

4. Acupuncture may be a suitable and cost-effective add-on treatment for these two groups of patients, particularly when conducted in a group mode. It is obvious that the positive outcomes of this pilot study warrant further and larger-scale research, but the tentative conclusion is that the present study shows that acupuncture appears to influence sleep in a positive way in sleep-disturbed patients and seems a suitable add-on Tx in psychiatry, even in Px with long-term depression or schizophrenia.

Study 11

Lee, Baek, Park, et al. (2009). Intradermal Acupuncture on Shen-Men and Nei Kuan Acupoints Improves Insomnia in Stroke Patients by Reducing the Sympathetic Nervous Activity: A Randomized Clinical Trial.

Study Rational

50-60% of post-stroke Px suffer from post-stroke insomnia, caused by anxiety resulting from hyperactivity of the SNS (Sympathetic Nervous System).

Based on the hypothesis that acupuncture can control the ANS, a RCT (Randomized Controlled Trial) was conducted by these researchers in a previous study, and based on those results. The results in that report were attributed to the inhibitory effects of Shen-Men (HT-7) and Nei Kuan (PC-6) on sympathetic activity; however, the direct variables related to the Ss's autonomic nervous function were not assessed.

This study was therefore conducted to confirm the effectiveness of intradermal acupuncture on a larger sample of Px with post-stroke insomnia, to evaluate the effects of the Tx on the ANS function via 24 hours of monitoring the Ss's ambulatory BP (blood pressure) and HR (heart rate) variability.

Study Design: A double-blind randomized controlled trial exploring the effect of Intradermal Acupuncture on post-stroke insomnia.

Ss: Hospitalized stroke Px w/insomnia were enrolled in the study, randomly assigned to a real intra-dermal acupuncture group (RA group), or a sham acupuncture group (SA group).

Needles: Dong bang sterile disposable intra-dermal acupuncture needles, 0.18 x 6mm.

RA group: Received intradermal acupuncture on Shen-Men (HT-7) and Nei Guan (PC-6) for three days. n=27 (randomly assigned). Experimental group.

SA group: Received sham acupuncture on the same points, n=25 (randomly assigned). The Control Group.

Study's Objectives:

The effectiveness of the acupuncture Tx on insomnia was measured using the ISI (Insomnia Severity Index) and AIS (Athens Insomnia Scale). These scales were examined by an independent blind neurologist at 8:00 am before the treatment, and three days after the Tx (Bastien et al., 2001). This study is a complementary study to the researchers' previous study (Kim et al., 2004).

Instruments employed for outcome measures:

ISI (Insomnia Severity Index) measures 5 factors:

a. Assessed the severity of Px's insomnia problem.

b. The degree of Px's discontent with their current sleep pattern.

c. The extent to which Px's sleep problems interfer with daily functions.

d. How noticeable the Px thought their sleeping problem was by others in terms of impairing the quality of their life.

e. How worried Px was about his current sleep problem. Each question is scored 0-4 (0= not at all, 4= very much)

AIS (Athens Insomnia Scale) measures 8 factors:

a. Sleep onset

b. Awakenings during night

c. Final awakening

d. Total sleep duration

e. Sleep quality

f. Day-time well being

g. Day-time functioning capacity

h. Day-time sleepiness

Each factor is scored 0 (no problem) to 3 (very much).

BP, HR and EKG were measured by 24-hour ambulatory monitors. Each Ss' BP was automatically measured from 6:00 am to 10:00 pm, and on the following day every two hours from 10:00 pm to 6:00 am. Daytime and nighttime BPs were calculated from the average values in the awake period between 6:00 am to 10:00 pm and in the sleeping period between 10:00 pm to 6:00 am, respectively. Ss were automatically examined by their ambulatory blood pressure monitor at baseline and again after three days of the timed intervention. Ss were assessed while adhering to their usual diurnal activity with minimal restrictions.

Stroke Diagnosis

Stroke was diagnosed when neurological deficits were accompanied by the corresponding abnormal CT (computed tomography) or MRI (Magnetic Resonance Imaging) findings in the brain.

Exclusion Criteria

a. Px treated with sedative, antidepressant tranquillizer, narcotic analgesics, antihistamine, or amphetamine containing drugs.

b. Patients who had disorientation, dysphasia, or nocturnal voiding frequency.

Intervention

One acupuncture needle was inserted bilaterally into each of the two points, and a piece of skin tape (1 x 1cm) was placed on each needle to firmly hold it in place for three days.

During the 24 hours that their BP was monitored, each participant wore the ambulatory ECG recorder in addition to the ABPM (ambulatory BP recorder), to assess their HR variability.

The parameters recorded of the HR variability were RR intervals (milliseconds) and SDNN (standard deviation of NN interval) in time domain, and LF (low frequency). HF (high frequency), and LF/HF ratio in the frequency domain.

Statistical Tests

Chi-square test => used for categorical variables – including gender, frequency of medical history, and diurnal variation of blood pressure pattern.

T-Test (independent) => used for continuous variables such as age, insomnia-related scales, the mean BP to compare both groups RA (Experimental group) and SA (Sham control group).

Results

The final analyses were performed on 52 Ss (27 in RA group, 25 in SA group). No significant differences were found between the two groups in their baseline features: age, gender, medical history, HTN, or severity of insomnia.

60 Px were enrolled in this study, but eight Ss dropped out before completing the study when they were discharged from the hospital.

This was a complementary study to these researchers' previous study (Kim, Lee, Jung et al., 2004). Two new measures were added to evaluate the effect of acupuncture Tx on the function of the ANS (autonomic nervous system): 24-hour ambulatory recorders of BP and of ECG (HR variability).

1) Insomnia

Three days after the acupuncture Tx => insomnia significantly improved in the RA group, as indicated by both ISI and AIS scores ($P<0.001$), compared with the controls (SA group). These results coincided with these researchers' previous study (Kim et al., 2004).

2) Blood Pressure

A significant improvement was found for the diurnal variation of BP, less Px showed non-dipping of their BP in the RA group compared with controls ($P<0.05$). Acupuncture Tx did not significantly lower BP.

3) Heart Rate

Acupuncture Tx significantly lowered the LF/HF ratio in the RA group compared to controls. In normal healthy people, nocturnal BP falls to about 10-20% below their daytime BP, which is termed the "dipper" pattern. "Non-dipper" refers to a diurnal change in BP in which there is no nocturnal fall in BP, or the fall is attenuated so there is little or no difference in nocturnal BP compared with the diurnal BP. Based on Ss's data, non-dippers, generally resulting from sympathetic activity, were defined as Ss whose systolic BP and/or diastolic BP nocturnal decrease was 10% lower than their daytime BP.

The underlying mechanisms of the nocturnal BP decline are still unclear, but many studies have reported that non-dipper patterns are associated with an elevated risk of severe internal organ damage, especially to the heart, such as left ventricular hypertrophy and myocardial infarct, brain (stroke), and kidneys. The non-dipper phenomenon is strongly associated with abnormal ANS, especially with sympathetic hyperactivity.

In this study, 82.7% of the insomnia subjects were non-dippers, which points to a major role played by sympathetic hyperactivity in their post-stroke sleep disorder. In the RA group the number of non-dippers decreased from 22 (81.5%) to 17 (63%), while no such decrease was found in the control group.

A higher SDNN indicates characterizes a healthy heart, whereas a lower SDNN measure suggests possible heart disease. LF (0.04-0.15 Hz) and HF (0.15-0.40 Hz) represent the activity of the sympathetic nervous system (SNS) and para-sympathetic nervous system (PSNS), respectively. At the baseline assessment, the mean LF/HF ratio of all Ss was higher than the normal value, indicating that the post-stroke insomnia may have resulted from their imbalanced ANS function.

Considering that a higher ratio of LF/HF reflects an imbalanced ANS function mainly caused by sympathetic hyperactivities. These results indicate that hyperactivities were stabilized in the RA group. These results coincide with findings of many studies reporting the inhibitory effects of Shen-Men and Nei Guan on sympathetic activity.

Intra-dermal acupuncture treatment on HT-7 (*Shen-Men*) and PC-6 (*Nei-Guan*) significantly improved insomnia on the insomnia-related scales, both of which had been confirmed to have high consistency, reliability and validity. These results show a stable effectiveness of Intra-dermal acupuncture treatment for post-stroke insomnia.

Conclusion

It can therefore be concluded that intradermal acupuncture on HT-7 (*Shen-Men*) and PC-6 (*Nei-Guan*) is a useful therapeutic method for post-stroke onset of insomnia, since it reduces sympathetic hyperactivities.

12. Huang, W., Kutner, N. & Bliwise, D. (2011). Autonomic Activation in Insomnia: The Case for Acupuncture.

Acupuncture represent a unique type of treatment for poor sleep due to its direct effects on peripheral nerves and muscles, which in turn modulate the autonomic nervous system's (ANS) tone and the CNS activation. Current belief is that the biological basis of insomnia typically involves the arousal of both the CNS and the ANS.

Acupuncture exerts profound influences through a wide range of potential neural and/or hormonal mechanisms that participate in modulating the sleep-wakefulness cycle.

These researchers describe three case studies of otherwise intractable insomnia, that brings a successful application of TCM to treating poor sleep. In the current work only one of the three is described. They suggest that further research of the relationship between acupuncture and insomnia and the autonomic regulation to help guide better selective applications of acupuncture for insomnia.

Case Study

A Caucasian woman 55 years old, presented with sleep difficulties 6 night per week for one year. She used melatonin, lorazepam (2-4 mg) and/or acetaminophen pm nightly to help her sleep. She reported significant trouble falling asleep and staying asleep. Patient (Px) worked as a magazine editor, often read and wrote in bed, had a very irregular sleeping hours, and

significant stress, with sometimes knee pain as well. She never underwent polysomnography (PSG), and it was unclear if she also had obstructive sleep apnea (OSA).

Her past medical history was significant for ovarian cancer, and status post chemotherapy 5.5 years earlier, bilateral foot neuropathy that resulted from the chemotherapy, bilateral knee osteoarthritis, hypertension (HTN), and border-line diabetes.

She had sulfa allergy, and took regular medications for HTN (ramipril and hydrochlorothiazede), depression and neuropathy (duloxetine), and hypercholesterolemia (atorvastatin). She lived with her husband and denied use of tobacco, alcohol, ot illicit drugs. Her body mass index (BMI) was 29.8.

Her PSQI score was 17, indicating very poor subjective sleep. Sjhe had 2 nights of sleep monitored with sleep diary and wrist band actigraph before starting the acupuncture therapy. She recorded 6 hours for the first night and 4 hours for the second night, and used 4 mg lorazepam the first night and 2 mg for the second night. Wrist actigraph recorded sleep efficiency (SE) of 60.5%. Averaged recorded sleep time (TST) was 315 minutes (5 hours and 15 minutes) with 58.5 minutes wake time after sleep onset (WASO) averaged over the two nights, which are about four hours sleep per night.

Her depression score was 24 on the Geriatric Depression Scale (GDS) (Yesavage, Brink, Rose et al.,1982), suggesting a substantially depressive mood.

She then received acupuncture treatments on acupoints Yin Tang (Ex-HN-3), An Mian (Ex-HN-22), HT-3 (Shenmen), KD-3 (Taixi), and LIV-3 (Taichong). Each session lasted 45-60 minutes of total interaction with the acupuncturist, including a brief history and exam and the acupuncture treatment. Sterile, single-use disposable metal acupuncture needles of 0.25 mm in diameter and 40 mm in length were used. The areas of acupoints were sterilized with alcohol

swabs before inserting the needles, which were inserted into the appropriate depth of each acupoint, but De Qi was not necessarily elicited.

A total of 12 sessions were conducted twice a week for 4 weeks, followed by once a week for 4 more weeks. Needles stayed in without further manipulation for 30 minuts before being removed. Five days after starting acupuncture therapy Px spontaneously reported that she sleeps much more (7.5-8 h on sleeo diary) than normal, and stayes asleep after she fell asleep. One night she she did not eed medication.

At the end of the 8-week treatment course, Px continued to report she was falling asleep easier and stayed asleep longer. She had another 2 days of post treatment actigraphy monitoring, when she chose not to use medication. On sleep diary she reported the first night to be fragmented due to "great deal of pain in my knees", and the second night still characterized by "some knee pain". Her average TST for these two nights was estimated at 7 hours.

Her PSQI score decreased to 8, and her GDS dexreased to 19. Wrist actigraph recorded an average two night SE of 76.7%, TST 490 minutes, and WASO 136 minutes. Her health-related quality of life scale (HRQoL) showed a most substantial improvement in the subscales of role limitations due to physical health and social functioning, also improved vitality, amotional well-being, and general health. She reported she has decreased the use of lorazepam from daily use to once a week over the following 12 weeks.

Zhao, K. (2013). Acupuncture for the Treatment of Insomnia (Review).

While the following section is based on Zhao's review (2013), his bibliographical citations are not included.

In our modern times insomnia seems to rapidly spread worldwide, and more so in the last decade, which lowers the quality of life and impairs peoples' functional ability, even to the point

of causing fatal accidents. On the other hand, although pharmacological treatment is effective and many times gives the expected "quick fix", it frequently results in significant, even severe short- and/or long-term side effects.

Many studies have been recently published on clinical applications of acupuncture in treating insomnia and the mechanisms underlying acupuncture effects on health. This chapter attempts to provide a systematic review on these research findings.

Recent clinical studies, mostly the randomized controlled trials (RCT), demonstrated positive effects of acupuncture treatment for insomnia. Some of the studies demonstrated that acupuncture improved sleep better than Western pharmaceutical drugs; however, acupuncture has certain inherent methodological problems since it does not easily lend itself to the scientific requirements of Western medicine standards. The clinical efficacy of acupuncture for insomnia is evidenced by basic scientific neuroendocrinological studies, which demonstrate that acupuncture is capable of modulating a wide range of neuroendocrinological factors.

Acupuncture as a major modality of traditional Chinese medicine (TCM), is based on the meridian-collateral theory of TCM. It involves stimulating selected acupoints by using sterilized thin solid needles inserted into acupoints. Acupuncture has been widely used to treat a variety of clinical conditions, in particular those involving pathological changes in neuroendocrinology and in the ANS (autonomic nervous system), such as depression, insomnia and difficult menopause.

Insomnia is a common sleep disorder. According to the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV, 2000), primary insomnia is a clinical condition with mainly complaints of difficulty initiating or maintaining sleep, or a nonrestorative sleep for at least one month. The sleep disturbance and associated daytime fatigue cause clinically significant distress and/or impairment of social, occupational, or other important areas of functioning (American

Psychiatric Association, 2000).

The Great British Sleep Survey of 2012 found that out of its 11,129 participants, 5,083 reported possibly having an insomnia disorder. The average age was 39. Similarly, according to NIH Consensus and State-of-the-Science Statements, multiple millions of individuals have been affected by insomnia each year in the US (NIH, 2005).

Another investigation crossing multiple countries in Asia and Africa, showed that the prevalence of insomnia in these countries ranged between 3.9% to 40% (Stranges, Tigbe, Gòmez-Olive, Thorogood, and Kandala, 2012).

The economic impact of insomnia is high, so treating it effectively is in everyone's interest. For treating insomnia, cognitive and behavioral treatments are helpful, yet are insufficient for chronic insomnia, where a pharmacological treatment may be required.

In the UK, short-acting benzodiazepines or Z-medicines, such as zopiclone, or melatonin may be prescribed. The medicines may, however, have significant side effects, such as feeling hungover and drowsy during the day. Since these medicines lose their clinical efficacy with long-term use, they may be unsuitable for chronic insomnia. Due to such limitations as clinical efficacy and concerns of side effects, more and more insomnia patients seek alternative treatments.

For such patients acupuncture was found to be a popular choice. With the widespread use of acupuncture for treating insomnia, many basic and clinical studies have been conducted, attempting to examine the clinical efficacy and the action mechanisms underlying the acupuncture treatment.

Chinese medicine is a medical system with its own unique theories about physiology and pathology of the human body. The theories are mainly based on long-term holistic clinical

observations, rather than on the body anatomy and biochemical principles. Hence, interpretations of the clinical condition, diagnosis and treatment are very different in TCM compared with Western medicine.

These great differences in the treatment approaches of TCM and acupuncture make it very difficult to abide by the Western experimental requirements of double blinded and randomized clinical trials, making it difficult to compare and evaluate acupuncture clinical efficacy compared to the Western pharmaceutical treatment.

For instance, TCM treats patients with individualized treatment plans, whereas clinical trials according to Western medicine require standardized interventions. Finding a suitable control group is another common difficulty for clinical trials of acupuncture.

In Chinese medicine, insomnia is believed to be a pathological hyper-arousal or restless condition, related to the functions of the heart and brain. Additionally, different causes could lead to insomnia. Acupuncture is capable of regulating the function of the heart and brain through stimulating certain acupoints on the body. Many clinical studies, including RCTs, have been reported on acupuncture treatment of insomnia, with the large majority of them demonstrating positive clinical effects of acupuncture treatment for insomnia.

In clinical studies of acupuncture treatment for insomnia, the most frequently used outcome measurements include the PSQI (Pittsburgh Sleep Quality Index) and the ISI (Insomnia Severity Index), both subjective measurements, yet with substantial validation and reliability.

Actigraphy and PSG have also been frequently used in clinical studies of acupuncture treatments for insomnia, as objective measurements. Many clinical studies reported that the total scores of ISI and PSQI, as well as the separate scores for each of the items, were significantly reduced following acupuncture treatment of insomnia patients.

Significant differences were also found between acupuncture treatment and no-treatment control groups (e.g., the 'waiting list' condition). The decreased scores on ISI and/and PSQI was accompanied by a higher therapeutic effective rate of improving insomnia. Acupuncture treatment also reduced the sleep-onset latency and increased sleep duration and sleep efficiency.

Recent findings additionally suggest that for sleep quality and daytime functioning, acupuncture treatment can be as effective or superior to conventional Western medicine, such as oral trazodone, zolpidem, or estazolam. Consistent with the subjective outcome measurements, results of PSG showed that compared with sham acupuncture, the real acupuncture treatment significantly increased the percentage of SWS sleep, and effectively improved sleep quality in postmenopausal women with insomnia (Hachul et al., 2013).

Although the majority of the many clinical trials published in recent years showed significant effectiveness of acupuncture treatments for insomnia, conclusions from most of these systematic reviews as to acupuncture's efficacy in healing insomnia remained inconclusive.

The Placebo Control Problem

While most clinical studies reported positive results of acupuncture treatment for insomnia, there were also some puzzling conflicting findings, such as a clearly significant improvement in subjective sleep measures such as PQSI, yet failure to demonstrate any difference for the objective actigraphy measures – when compared to noninvasive placebo acupuncture.

In one recent RCT study, Yeung et al. (2011) found no differences between electro-acupuncture and minimal acupuncture as objective measurements (superficial needle- touch of nonacupuncture points), although both groups indicated significant improvement of insomnia that was significantly different from the noninvasive placebo acupuncture group.

This has been a common problem plaguing clinical trials of acupuncture treatment. Similar problems were found in RCT studies of acupuncture treatment for headache (Melchart et al., 2005). These findings have led some researchers to suggest that the effects shown in clinical trials could be purely due to a needling nonspecific stimulation that is unrelated to TCM theory (Yeung et al., 2011).

However, the true argument here is whether sham or minimum acupuncture are at all appropriate controls. First, regardless of inserting the needles superficially or deeper, the needles produced stimulations that were still effective enough to trigger physical responses. Secondly, in acupuncture practice, it is common to use a combination of multiple acupoints for the treatment of various clinical conditions at the same time.

It is common knowledge that different acupoints may have similar or equal actions, especially acupoints located on the same meridian. Even non-acupoints may still evoke certain kinds of actions, depending on their locations. The extent or reasons of overlap in acupoint needling actions among different points on the body is far from clear.

In addition, according to Chinese medicine theories, acupuncture meridians are not merely isolated lines on the body, but are functional pathways, which include related skin areas, as well as muscles and tendons, and specifically related organs. All these have made it very difficult to designate appropriate placebo points as controls in clinical studies of acupuncture treatment.

Methods Used for Stimulating Acupoints

There are two aspects to an acupuncture treatment: the stimulation method, and the acupoints' selection. Various methods have been used as means of stimulation in clinical practice. The most commonly used stimulation method is a manual insertion of the needle into

relevant acupoints.

Other stimulation methods include electrical acupuncture (Yeung et al., 2011), acupressure, catgut embedding, penetrating acupoints, auricular acupuncture, and moxibustion. Clinical trials or observations have shown that using any of these different stimulation methods results in significant effectiveness for improving insomnia, although there may be some differences in the extent of therapeutic efficiency.

For instance, studies have shown that penetrating needling has better results in improvement of insomnia, compared to conventional acupuncture. However, the available evidence indicates that stimulation mode is not the key issue for achieving clinically positive results with acupuncture for insomnia.

Acupoint Selection

Studies have revealed a wide range of acupoints chosen to treat insomnia. According to Chinese medicine and acupuncture theories, patients are treated individually depending on each patient's individual conditions, so a different combination of acupoints is selected for each patient. The variation in acupoint selection is problematic for comparing different clinical trials.

In 20 clinical trials for acupuncture treatment of insomnia conducted in 2007–2013, a total of 30 points were used. Among these 30 acupoints, acupoints used for insomnia over 20% of the time are Baihui (Du-20), Sishencong (EX-HN1), Shenmen (HT-7), Shenting (Du-24), and Sanyinjiao (SP-6). Acupoints used in these clinical studies ranged from one single point to a combination of over eight points, and all the acupoints were used effectively in treating insomnia.

Mechanisms Underlying the Acupuncture Treatment

Insomnia was found to be associated with a number of neuroendocrinological disorders.

It has been suggested that both CNS and ANS are involved in the pathology of insomnia, and substantial evidence indicates that multiple neurotransmitters may be involved in the pathophysiology of insomnia, including the SNS, GABA, opiate, and melatonin.

Although the underlying acting mechanism of acupuncture treatment of insomnia is far from clear, many studies suggested that acupuncture may improve insomnia by acting on the nervous system to modulate the activities of neurotransmitters.

SNS and the Hypothalamic–Pituitary–Adrenal (HPA) Axis

Evidence demonstrates that insomnia is accompanied by sympathetic hyperactivity, and that insomnia patients have an increased metabolic activation, with elevated heart rate and sympathetic nervous system activation during sleep.

Studies have also demonstrated that norepinephrine induces wakefulness in the CNS via both alpha and beta-receptors, and that suppression of CNS norepinephrine release results in a profound sedation. A recent study reported treating insomnia using auricular acupuncture, and found that improved insomnia (as indicated by PQSI) was related to an increased cardiac parasympathetic activity and a decreased sympathetic activity.

A double-blind RCT has shown intradermal acupuncture on Shenmen (HT-7) and Neiguan (PC-6) to stabilize the sympathetic hyperactivity in insomnia patients, as indicated by a decreased ratio of the low-frequency power/high-frequency power in the heart rate variability analysis, a change that was closely related to the significant improvement of insomnia, as indicated by ISI.

Other acupoints that appear to have similar actions are Sishencong (EX-HN1) and Xinshu (BL-15). Another recent study investigated the sedative effect of acupuncture in healthy volunteers by monitoring the bispectral index and heart rate. However, no differences were

observed between the true acupuncture and the sham control. This result may suggest that the sedative action of acupuncture can only be seen in anxious or insomnia patients.

Other studies have demonstrated that electroacupuncture of Neiguan (PC-6) and Xinshu (BL-15), or Shenmen (HT-7), or Quchi (L1-11) and Zusanli (ST-36) have increased the hypothalamic norepinephrine (NE), dopamine (DA), and serotonin (5-HT). These results have been controversial.

The HPA axis seems to be an important neuroendocrine system involved in the regulation of sleep. Activating the HPA system may lead to insomnia, since stress stimulates HPA system and very commonly induces insomnia. Animal studies found that electroacupuncture on Baihui (GV-20) and Yintang (EX-HN3) can effectively reduce adrenal cortisol content and downregulate hippocampal expression of the glucocorticoid receptor mRNA.

Another study in rats indicated that acupuncture on Shenmen (HT-7) has reduced plasma corticosterone and adrenocorticotropin hormone (ACTH) levels. These findings lend further support to the clinical use of these acupoints for treating insomnia.

GABA

GABA was found to be an important neuromediator for regulating sleep. GABA usually subdues physiological activities in the brain, exerting its activities by binding to the GABA(A) receptor. Research has found that average brain GABA levels may drop 30% below normal controls in primary insomnia patients. Brain GABA levels were shown to be negatively correlated with waking after sleep onset, as recorded by PSG in insomnia patients.

Some medications currently used in clinical practice for the treatment of insomnia, such as benzodiazepine, zolpidem, and eszopiclone, are based on their positive modulating activities of the GABA(A) receptor. These medicines relieve insomnia by potentiating brain GABA

action, which in turn exerts an inhibitory effect on sympathetic NE transmission, thus reducing hyperarousal. Direct evidence of NE release has been shown in animal studies with midazolam.

Recent evidence showed that acupuncture for insomnia significantly increased GABA level and upregulated the expression of GABA(A) receptors in hypothalamic neurons of the rat. The positive response acupoints included Sanyinjiao (SP-6), Neiguan (PC-6), Zusanli (ST-36), Shenmen (HT-7), and the combination of Shenmai (BL-62) and Zhaohai (KI-6). This finding may explaine the insomnia relieving effect of these acupoints in clinical acupuncture practice. Among these acupoints, Shenmen (HT-7) and the combination of Shenmai–Zhaohai (BL-62–KI-6) were found to be the most effective, with significantly superior upregulating results. Additional studies provide further evidence that acupuncture on Shenmen (HT-7) stimulates GABA neurotransmission.

Acupuncture on Baihui (GV-20) showed a protective effect against impairment of cortical GABAergic neurons in an ischemic stroke animal model. Another study with a Parkinsonian rat model demonstrated that electroacupuncture on Baihui (GV-20) induced an increase of brain GABA levels. Increased GABA activities in the brain are expected to produce sedative effects, contributing to insomnia relief.

Melatonin

Melatonin is secreted by the pineal gland of the brain, and is important in helping to regulate the body's circadian sleep cycle, and therefore plays an important role in maintaining normal sleep. Normally, melatonin is secreted during the night, and a certain level of melatonin in the blood is essential for normal sleep. It has been found that nocturnal melatonin production was reduced in elderly insomnia patients, suggesting a possible association between this hormone and primary insomnia.

Melatonin has been used for treating insomnia, and results from clinical trials have demonstrated the efficacy and safety of extended melatonin release for treating primary insomnia. A meta-analysis has shown that melatonin decreases sleep onset latency, increases total sleep time, and improves overall sleep quality.

A clinical trial demonstrated that the endogenous nocturnal melatonin secretion of anxious patients was significantly increased following five weeks of acupuncture treatment. The increase was associated with significant improvements in sleep onset latency, total sleep time, and sleep efficiency as measured by PSG. Patients' anxiety conditions were significantly reduced as well. Acupressure on Shenmen (HT-7) may help normalize the nocturnal secretion of melatonin in insomnia patients, which may improve sleep quality at the same time.

Other Neuroendocrinological Factors

Studies have also suggested some other neuroendocrinological factors that may mediate the effects of acupuncture in the treatment of insomnia. Opioidergic neuro-transmissions in the brain have been found to be involved in the regulation of sleep–wake rhythm, and endogenous opioids may play a role in maintaining normal sleep.

Electroacupuncture on Anmian (EX-17) seems to increase NREM sleep, an effect that seemed to be mediated by a pathway involving cholinergic activation, stimulation of opioidergic neurons to secrete beta-endorphin in both the brainstem and hippocampus, and regulate sleep via m-opioid receptors. EA (Electroacupuncture) on Zusanli (ST-36) and Sanyinjiao (SP-6) significantly increased NREM sleep, REM sleep, and total sleep time during an acute morphine withdrawal in the rat model, an action presumed to be mediated by EA- facilitated release of endogenous opioids.

Discussion and Conclusions

Insomnia is a common disease affecting great numbers of the population. Although there are effective pharmacological medications available, significant side effects have limited their clinical applications and long-term use.

Acupuncture has long been used successfully to treat insomnia, to find out why and how this traditional modality works, many more clinical and experimental studies will need to be conducted.

As described earlier, there are still no clear conclusions about the effectiveness of acupuncture treatment on insomnia, largely due to the quality of methodologies used in the previous clinical studies. There are several weaknesses that exist in the methodology of previous research studies, including a small sample size, questionable placebo control or sham control, arguable use of standardized intervention, and a variety of acupoint selections.

Some of these difficulties are caused by the conflict between the Western scientific "golden standards" required for RCT and the TCM clinical practice of acupuncture. For example, the individualized and wholistic treatment principle in Chinese medicine negates the RCT requirement for a standardized intervention. For future studies, a solution has to be found to overcome these difficulties.

A placebo control is important for a proper Western clinical trial, but it is frequently problematic for clinical research in TCM and acupuncture. Acupuncture is a physical intervention, and it is known that physical interventions are more likely to produce a placebo effect in clinical trials. As mentioned earlier in this chapter, sham needling may not be an appropriate placebo control for acupuncture clinical trials. It is believed that a noninvasive placebo intervention would be more suitable for acupuncture clinical studies.

Subjective outcome measurements have been used in most of the previous clinical studies, and it appears that subjective measurements are more susceptible to placebo effects. Introducing more objective outcome measurements could be helpful in reducing placebo effects. In the case of insomnia, actigraphy and PSG (polysomnography) are useful objective measurements for clinical studies.

As mentioned above, a very wide range of acupoint selections have been used in the clinical trials, in addition to a variety of stimulation or manipulation methods, causing difficulties in interpreting and comparing the efficacy of acupuncture insomnia trials to Western clinical trials.

At present, evidence from the available studies is incapable of providing a clear and conclusive answer as to the relative effectiveness of acupuncture treatments for insomnia.

Similar questions have been raised in a recently published literature review on acupuncture effect and central autonomic regulation. In most studies, the acupoint selections were based on TCM theories. The variations in acupoint selections may be due to different acupuncture styles such as body acupuncture, abdominal acupuncture, or scalp acupuncture.

Additionally, TMC treats patients individually according to the specific Chinese medicine syndrome differentiations of each patient. It is always a debatable issue for RCT (Randomized Controlled Trial) since it uses standardized interventions, which makes it impossible for RCT to represent the individualized acupuncture practice.

To solve this problem, it might be helpful to recruit clinical trial participants by using combined diagnosis standards from both Chinese medicine and Western medicine, for instance, insomnia patients with a further differentiation using Chinese medicine diagnostic principles. In this case, acupoint selection can be standardized according to the Western medicine diagnosis.

In fact, a clinical study has been conducted with participants who have insomnia due to deficiency of both the heart and spleen. In this article, the neuroendocrinological activities of the acupoints commonly used for treating insomnia have been reviewed. Compared with the results of clinical trials, it can be seen that the most frequently used acupoints also demonstrate certain types of neuroendocrinological actions.

Acupuncture can cause responses from a wide range of neuroendocrinological pathways, even with a single acupoint such as Shenmen (HT-7). With a combination of different acupoints, the induced neuroendocrinological reactions would be more complicated. At present, the full picture of the specifics of all the involved acupoints is still far from clear. There are also some confusing findings that need to be resolved in the future.

The efficacy of acupuncture when employing the most commonly used acupoints for insomnia is further supported by evidence coming from neuroendocrinological actions of these acupoints. Despite the difficulties, further investigation into the efficacy of acupuncture treatment for insomnia is warranted. It would be beneficial for future studies to apply more integrated approaches to explore both clinical effectiveness and the underlying neurophysiological mechanism for acupuncture treatment of insomnia.

Almeida, Guerra, Oliveira, et al. (2014). A Hypothesis for the Anti-inflammatory and Mechano-transduction Molecular Mechanisms Underlying Acupuncture Tendon Healing

In China, acupuncture has been used to treat a range of diseases for at least 2000 years. Acupuncture is an ancient healing art that evolved in China and is currently flourishing in the USA and Europe, as both primary and adjunct therapy for a variety of chronic conditions.

Stimulation of the acupuncture needles is believed to elicit profound psychophysical responses by harmonizing or balancing the Qi (vital energy), as well as the blood flow to the entire body.

The clinical practice of acupuncture is growing in popularity worldwide and is the most popular complementary and alternative treatment in use today. The WHO (World Health Organization) recommends acupuncture to treat over 40 diseases. The USA National Institutes of Health (NIH) list several diseases that can be treated with acupuncture, including adult postoperative and chemotherapy-related nausea/vomiting, postoperative dental pain, addiction, stroke rehabilitation, headache, menstrual cramps, tennis elbow, fibromyalgia, myofascial pain, osteoarthritis, low back pain, carpal tunnel syndrome and asthma.

Clinical studies have demonstrated therapeutic effects of acupuncture on tendinopathy, but well controlled randomized trials (CRT) are needed to confirm a causal relationship. In general, these preliminary studies have shown that acupuncture helps with both pain and functional activity in the study participants.

Research shows that EA (electrical acupuncture) at ST36 (Zusanli) and BL57 (Chengshan) increased the concentration and organization of collagen during the proliferative phase of the Achilles tendon healing in rats (12). This study is unique in indicating that acupuncture has the potential to improve biochemical and morphological characteristics of tendons during healing.

However, the molecular pathways underlying these effects are unknown. Accumulating evidence indicates that EA or manual acupuncture at ST36 stimulates anti-inflammatory (AI) processes. Several studies have shown that pro-inflammatory molecules are involved in doing just the reverse as they decrease collagen synthesis.

Previous studies indicate that insertion and manipulation of acupuncture needles activate cytoskeletal remodeling by fibroblasts in subcutaneous connective tissue. Downstream effects of cytoskeletal remodeling may include secretion and modification of extracellular matrix components.

Recent studies show that mechanical needling stimulation increases production of type I collagen by fibroblasts. Based on these and other studies, the researchers of this report discuss the potential AI (anti-inflammatory) and MT (mechano-transduction) of molecular mechanisms of acupuncture on collagen synthesis during the tendon healing process in the rat animal model.

The Hypothesis

The researchers' hypothesis is that acupuncture increases type I collagen synthesis and subsequent reorganization during tendon healing, through co-stimulation of acupupoints with AI effects, as well as acupoints located at anatomical sites connected with the injury site (i.e., acupuncture points with a MT-effect). Stimulating such acupoints potentially activates AI mechanisms in inflammatory cells and MT mechanisms in tenoblasts (fibroblasts in tendons).

Foundations of the Hypothesis

Following tendon injury, acute inflammation sets in for 3-7 days. The inflammatory process begins with platelet activation and the formation of hematoma, followed by erythrocytes and inflammatory cells (mainly neutrophils) infiltration into the injury site. During the first 24 hours, monocytes and macrophages are the predominant cell types at the injury site, to phagocyte necrotic material and attract other inflammatory cells from surrounding tissues by releasing vasoactive and chemotactic factors, such as vasodilators and pro-inflammatory molecules.

During the inflammatory phase the cytokines tumor necrosis factor α (TNFα) and interleukins IL-1β, IL-6 and IL-8 are known to have pro-inflammatory properties. TNFα causes

tenocytes to decrease type I collagen deposition, and induces production of IL-1β, IL-6, IL-8, IL-10 and prostaglandin E2 (PGE2). IL-1β is an important pro-inflammatory mediator, which promotes prostaglandin synthesis. In damaged tissue, PGE2 functions to promote vasodilation and pain hypersensitivity. PGE2, like TNFα and interferon γ (IFNγ), decreases collagen synthesis.

Acupuncture induces AI properties during the inflammatory phase, whereas production of pro-inflammatory molecules reduces type I collagen deposition. Healthy tendon tissue is primarily composed of type I collagen (approximately 95% of total collagen), which provides it with strength and elasticity. Therefore, reducing pro-inflammatory molecule synthesis could increase type I collagen levels.

The researchers hypothesize that acupuncture increases type I collagen in the tendon during healing, through inhibition of the anti-inflammatory pathway of inflammatory cells, and activation of MT (mechano-transduction) pathways in the tenoblasts. AP-1, activator protein-1; COX1/2, cyclo-oxygenase 1 and 2; IFNγ, interferon γ; IL-1β, interleukin 1β; IL-6, interleukin (6); MAPKs, mitogen-activated protein kinases; NF-κB, nuclear factor-κB; PGE2, prostaglandin E2; TLR2/4, toll-like receptor 2 and 4; TNFα, tumour necrosis factor α, synthesis of type I collagen and improve the biomechanical properties of the tendons.

ST36 is a key point on the stomach channel commonly used for treating gastric symptoms such as nausea and vomiting. In addition, several studies indicated that acupuncture on ST36 exerts AI effects through inhibition of TNFα, IL-1β, IL-6, IFNγ, and PGE2 synthesis.

Cytokine IL-10 has also been implicated in acupuncture AI effects when SP6 (Sanyin-jiao) is used in a mouse model of peritonitis. Inhibition of Toll-like receptors (TLRs) is one potential molecular mechanism responsible for the observed acupuncture AI effects. LRs, which

are evolutionarily conserved proteins that recognize microbial molecules, initiate the innate immune response and modulate the adaptive immune system.

Recent evidence suggests that in addition to TLR's function as sensors of exogenous or foreign pathogen-associated molecular patterns (PAMPs), TLRs can recognize and mediate responses to endogenous stimuli. Heat shock protein, a protein released by cells undergoing necrotic cell death, may activate innate immune cells through a TLR4-dependent mechanism.

Moreover, necrotic cells were recently shown to activate the nuclear factor-κB pathway (NF-κB) and inflammatory gene production in a TLR2-dependent manner. At the onset of trauma, TLRs detect the release of endogenous ligands, contributing to the pro-inflammatory response to injury. A recent study has shown that TLR2, TLR4 and TLR9 play different selective roles in both the initial proinflammatory response and the post-trauma adaptive immune response.

At the downstream effectors of TLR signalling, proinflammatory cytokines are known to rise in the circulation during a variety of stress responses. Specifically, cytokines IL-1β, TNFα and IL-6 were previously suggested as proximal mediators during the early stages of inflammation. Inhibition of the TLR-NF-κB pathway and downstream effectors may therefore have an AI effect.

Recent studies demonstrate that acupuncture inhibits the TLR2/4-NF-κB pathway, as well as the production of the downstream cytokines TNFα, IL-1β and IL-6 (13,14). This is probably one of the molecular mechanisms responsible for acupuncture's AI effect and consequent increase in synthesis of type I collagen during the healing process.

Acupuncture and Mechano Transmission (MT)

MT could be defined as the cells' ability to transform mechanical stimuli into bio-chemical changes. Previous studies indicated that insertion and manipulation of an acupuncture needle result in a mechanical connection of the needle to connective tissue, winding of tissue around the needle, thus generating a mechanical signal by pulling on collagen fibers during needle manipulation, mechanically trasmitting the signal into cells. The acupuncture mechanical signal is delivered into cells through extracellular matrix tension, which results in cytoskeletal remodelling and increased cell body's cross-sectional area.

Cytoskeletal remodeling occurs through both genes Rho and Rac signaling, as well as actomyosin interactions. Rho and Rac regulate the assembly and organization of filamentous actin (F-actin) in response to extracellular cues. F-actin is part of the cytoskeleton, which seems to be the key structural element allowing transmission of externally applied mechanical forces to the cell, and converting these forces into biochemical responses. Type I collagen secretion is a potential downstream response to such mechanical signal.

Studies show that mechanical stimuli can promote the synthesis of type I collagen through the MT pathway, which is composed of mitogen-activated protein kinases (MAPKs), the most prominent kinases activated by mechanical stimuli.

The MAPKs are involved in mechanical force transduction, comprising three different pathways: (a) extracellular signal regulated-kinase 1/2 (ERK1/2), (b) c-Jun N-terminal kinase, and (c) p38 kinase. These three pathways regulate gene expression through activation of transcription factors, such as activator protein-1 (AP-1).

The MT effects on tissue repair are best characterised in the field of physiotherapy through the application of exercises (mechanotherapy). Recent studies have shown that

mechanical stimulation increases the production of type I collagen through activating the ERK-AP-1 pathway in fibroblasts.

These MT data suggest that acupuncture mechanical stimuli have the potential to increase type I collagen synthesis through activating MAPKs-AP-1, as well as Rho/Rac-F-actin in tenoblasts, close to the needle's stimulation site.

A previous study by these researchers showed that EA in rats increases the concentration and reorganization of collagen during the tendon healing proliferative phase by needling ST36 and BL57.

Their hypothesis states that ST36 inhibited inflammation and BL57, which is located at the transition of the triceps surae into the Achilles tendon (an anatomical site connected with the site of the injury), activated the MT pathway in tenoblasts. According to traditional theory, BL57 is used as a local acupuncture point to relax the muscles and tendons of the lower leg. In this model, both acupoints work together to increase collagen concentration and reorganization.

Conclusions

The extracellular matrix of healthy tendon tissue is primarily composed of type I collagen (approximately 95% of the total collagen), which provides strength and elasticity. Following injury, type III collagen increases (from 1–3% to 20–30% of the total collagen), compared with uninjured tendon (1–3% of total collagen III).

Type III collagen tends to produce smaller, less organized fibrils resulting in an increased risk of tendon re-rupture. Despite post-injury remodelling, there is a consensus in the literature that the composition, structure and biomechanical properties of the scar tissue of injured tendons never return to the quality of uninjured tendons. The main goal of regenerative therapies is

therefore to improve the scar tissue quality. Understanding the mechanisms underlying the effects of these therapies is fundamental for developing them.

The purpose of this report was to present the possible molecular mechanisms underlying the tendon healing process. The researchers' hypothesis is that acupuncture modulates systemic AI, and the local MT molecular pathways lead to the synthesis of type I collagen. For activating the MT pathway, acupoints' selection must follow the criterion of at least one acupoint being located at an anatomical point that is connected with the site of injury.

To support this criterion for acupoints' selection, a recent study indicated that treating tendinopathy with dry needling at the pathological tissue decreased the pain, but did not renew its physical activity, which can be achieved by collagen-I synthesis during the healing process. This leads to the recovery of the biomechanical and functional properties of these structures. Increasing the blood flow is another effect of local needling, and has been suggested as possibly playing an important role in treating tendinopathy.

If this hypothesis is confirmed, new protocols for treating other injuries including skin, muscle, ligament, joint capsule and nerve, could be developed based on the principles of systemic AI and local MT effects of acupuncture. In addition to its collagen I synthesis effect, ST36 has an analgesic effect which decreases the necessity of analgesic drugs during the inflammatory phase of the tissue healing.

Additonally, acupuncture may be a viable alternative for such patients who are intolerant of certain drug classes (e.g., AI drugs) due to gastrointestinal, liver or kidney issues. This hypothesis is therefore important to stimulate research in the fields of tissue regeneration and to unravel the acupuncture mechanism of action.

Future studies using tendon healing models to discern the molecular pathways in collagen synthesis and reorganization will enable us to understand the influence of acupuncture therapy on tendon and other tissue healing. This line of study could potentially lead to a novel therapeutic alternative for treating soft tissue injuries.

TCM Treatments of Insomnia with Nutrition

Chinese medicated diet is not a simple combination of food and Chinese medicinal herbs, but a rather special highly elaborated diet made of Chinese medicinal herbal, food and condiments, under the theoretical guidance of diet preparation based on differentiation of TCM symptoms and signs. In addition to having the efficiency of medicinal Chinese herbs, it is also attentive to the delicacy of food. Nutrition has been used to prevent and cure diseases, build up one's health and prolong one's life (from the course introduction, Special Course on TCM Nutrition, Barcelona, Spain, 2000).

Three Traditional Chinese Recepies for Insomnia

(1) Drink of Longan and wild Jujube seeds (Longan Zao Ren Yin)

Ingredients:

Long Yan Rou (Arillus Longan) – 10g

Suan Zao Ren (Wild Jujube Seed - Semen Ziziphi Spinosa - roasted) – 10g

Qian Shi (Gordon euryale seed - Sem. Euryales) – 12g

Processing:

a. Pound the roasted wild jujube seeds into pieces and put them in a gauze bag.

b. Put Gordon euryale seeds into 500ml water, boil them for about 30 minutes.

c. Add the Longan aril and wild jujube seeds and boil them for another 30 minutes.

d. Remove the wild jujube seeds and add just the right amount of white sugar. Filter to get the extract.

Instructions: Drink it any time and eat the longan aril and gordon euryale seeds.

Efficacy: Nourishes the Blood, Tranquilizing the mind, tonifies the KD and Controls nocturnal emissions.

Indications: This kind of drink can be taken by those who suffer from palpitations, insomnia, forgetfulness, listlessness and nocturnal emission and other symptoms resulting from HT-Yin Deficiency and HT-Blood insufficiency which is unable to benefit the KD-Yin in te Lower Jiao d/t internal disturbance by Deficiency Fire.

(2) Goat (or sheep) Liver Soup (Si Wu Gan Pian Tang)

Ingredients:

Goat (or sheep) liver – 200g

Shu Di Huang (Prepared rehmannia root - Rx. Rehmannia preparata) – 10g

Chuan Xiong (Rhizoma Chuanxiong) – 3g

Dang Gui (Chinese Angelica root - Rx. Anelica Sinensis) – 6g

Bai Shao (White peony root - Rx. Paeonia Alba) – 8g

Gou Qi Zi (Wolfberry fruit - Fr. Lycii) – 10g

Mo Han Lian (Eclipta - Hb. Ecliptae) – 6g

Suan Zao Ren (Parched wild jujube seed - Semen Ziziphi Spinosa) – 6g

Powdered (White pepper powder - Pulvis Fr. Piperis Albi) -1g

Gourmet powder – 2g

Soaked edible fungus (Auricularia) – 20g

Cooking wine – 2g

(Day Lily - Flos Hemerocallis Immaturus) – 10g

Wet starch – 20g

Chicken broth – 400g

Refined salt – 6g

Soy sauce – 3g

Processing:

a. Wash the above medicinal herbs clean, and put them in an earthware pot; add water to decoct them and let the decoction settle to remove the sediment.

b. Wash the goat liver clean, and cut it into thin slices, and put it into a bowl.

c. Add the refined salt, soy sauce, cooking wine and wet starch, and mix thoroughly.

d. Put the earthware pot with the decoction over a strong fire, add the chicken broth, edible fungus and day Lily.

e. When the decoction boils, remove the edible fungus and day Lily, and put them in a soup bowl.

f. Spread the liver slices into the pot; skim off the froth when it boils again; add salt, the pepper powder, the prepared lard and gourmet powder when the liver is done. Then put it into the soup bowl to eat.

Instructions: It can be taken with bread (or cooked rice).

Efficacy: Nourishes the liver, replenishes the blood, improves vision acuity, and calms the mind.

Indications: Night blindness, dim eyesight and optic atrophy caused by liver-blood Deficiency, palpitation, insomnia, forgetfulness. And irregular menstruation in women, as well as other symptoms due to heart-blood deficiency.

(3) Cooked rice with Dang Shen and Chinese Dates

Ingredients:

Dang Shen (Rx Codonopsis Pilosulae) - 10g

(Chinese date - Fr Ziziphi Jujubae) – 20g

(Polished long grain of glutinous rice - Sem Oryzae glutinousae) – 250g

White sugar – 50g

Processing:

a. Put dangshen and Chinese dates in an earthen ware pot, add water to soak to let them expand. Decoct them in water for about 30 minutes, then remove the dangshen and Chinese dates.

b. Wash the glutinous rice clean, put in in a big porceline bowl with just the right amount of water, and steam until the rice is done. Turn the bowl upside down to put the cooked rice on a plate. Put the dangshen and Chinese dates on the rice. Add white sugar into the prepared extract of dangshen and Chinese dates, and concentrate it. Then pour it on the cooked Chinese date rice.

Instructions: may be eaten for breakfast.

Efficacy: strengthens the spleen and nourishes Qi

Indications: Qi deficiency due to weak constitution, manifested as lassitude, palpitation, insomnia, loss of appetite, loose stool, dropsy, etc.

Dr. Maoshing Ni's Food Recommendations for Treating Insomnia

As many current sleep studies confirm, it has been Dr. Ni's understanding based on his family's long and rich TCM medical heritage, that sleep is restorative to our mind and vitality. Based on these studies as well as on Dr. Ni's TCM heritage, he perceives sleep as critical for our organs to function well, since our tissues mostly detoxify at night during sleep.

Recent studies show that when sleep-deprived for a mere 72 hours, human subjects manifest a great decline in white blood cells production and activity, which is an indication for the attenuated function of the immune system, which results in greater vulnerability viral, bacterial and fungi infections, which then enable the appearance of mood disorders, such as anxiety and major depression, in addition to digestive ailments, including elevated cholesterol and blood pressure (Ni, 2006, p. 202).

The following are a few of Dr. Maoshing Ni's recommendations for trearing insomnia, as cited from three of his published books.

Chinese Tradional Nutrition for Treating Insomnia

(1) **Jujube Seed** is a powerful remedy for both insomnia and fatigue (*Second Spring*, p. 189, 330): Seeds of the Jujube date are a traditional sedative, which promote a good sleep. When taken as supplement, the recommended dosage is up to 500mg/day.

According to TCM, the heart is said to house the spirit. When the heart is weak, the spirit grows restless and cannot properly rest at night, so we experience insomnia or else sleep poorly and wake up unrefreshed. Jujube calms the spirit, strengthens the heart, and facilitates a good sleep.

Modern research shows that jujube seeds are rich in saponines, which reduce irritability, anxiety, and fatigue, while promoting relaxation and sleep. A great advantage of this seed is that it does not make you tired when you take it during daytime, in fact, it seems to promote clarity and decreases fatigue-related nervousness.

(2) **Passiflora (passion flower) for a peaceful sleep**: steep 1 to 2 heaping tablespoons of the dried herb in a cup of hot water (*Second Spring*, p. 190, 330), drink just before bedtime. The typical dosage for the supplement form is 200 mg per night.

Passiflora is found in various species worldwide, and its medicinal properties are valued everywhere. Passion flower is a fruit that makes delicious juice; its fresh or dried leaves are traditionally used as a therapeutic tea to treat insomnia, hot flashes, muscle spasms, and nervous digestive disorders. It is found in many calmness-inducing preparations.

Passiflora contains harmala alkaloids, which prevent the breakdown of neurotransmitters such as serotonin and dopamine, thus improving mood and promoting restful sleep. Passion flower is also known for its pain-relieving properties and could act to fight H. Pylori, the bacteria that causes ulcers (2nd Spring, p 190).

TCM recognizes that the yin, which is the female essence, slowly becomes depleted during menopause. Yin is a calming essence needed for balancing the fiery yang, so when we are low on yin, a major symptom is insomnia, manifested as difficulty falling asleep or experiencing episodes of waking up late at night and being unable to resume sleep. Hot flashes, night sweats, irritability, and agitation are all related to this root condition.

To ensure a good night sleep, take a calming tea before bedtime. It is recommended to use one of the following traditional Chinese herbs: Ziziphus seed, bamboo shavings, and oyster shell, all of which sooth the mind and the spirit.

(3) **5-HTP Supplement (5-Hydroxytriptamine)**. It is the precursor of Serotonin and Melatonin, which converts into serotonin in the brain, and has a tranquilizing effect. The recommended dosage of this supplement is 200 mg per night, taken just before bedtime (*Second Spring*, p. 191, 330).

(4) **Longan** (**Long Yan Rou - Arillus Euphoriae Longanae - dragon's eye fruit)**. Both its taste and shape resemble lychee and has many therapeutic qualities. Longan calms the spirit and mitigates insomnia, nourishes the blood, promotes good vision, and strengthens the bladder to stop incontinence. Recommended for irritability during pre-menopause, and for insomnia.

Longan fruit contains substantial amounts of vitamins A and C, and minerals such as iron, magnesium, phosphorus, and potassium, which are much needed during pre-menopause. Longan's use as a tonic herb in Chinese medicine may be due to its phenolic acids, which protect the liver and act as antioxidants.

The dried fruit makes a wonderful snack to add to a trail mix. For a peaceful night's sleep, it is recommended to eat a small handful every day. It can be found as fresh or dried longan at Asian markets, in select health food stores, and online (*Second Spring*, p.188).

(5) **Starflower (borage)**: its leaves are used in salads and soups, and its beautiful star-shaped flower has a sweet, honey-like taste that is used in desserts, as a calming tea, and for ornamentation. The seed produces borage oil, the best-known plant-based source of gamma-linolenic acid (GLA). This omega-6 fatty acid reduces inflammation, thus helping to combat rheumatoid arthritis, nerve damage, and memory-loss as occurs in Alzheimer's disease.

Borage also contains oleic and palmitic acids, two fatty acids with known cholesterol-lowering properties. Starflower's ability to mitigate hot flashes during menopause is its best

quality. You can make tea infusions of starflower or take the borage oil in a supplement form, available from health food stores and Chinese herbalists (*Second Spring*, p 203).

(6) **Wild Yam [Shan Yao] Rz Dioscorrea Oppositae**: Premenopause's erratic and disruptive hot flashes are a direct result of the hormonal imbalance. Wild Yam harmonizes the body's hormonal system, and is used not only to calm hot flashes but also to alleviate other menopause symptoms such as night sweats, joint pain, and insomnia.

Wild Yam strengthens the kidneys, liver, spleen and pancreas. Studies in China have found that the fiber in Chinese wild yams help combat hyperlipidemia, a fatal dsease found worldwide, by reducing high levels of fatty lipid molecules in the bloodstream.

The tuber-root also acts to decrease insulin resistance in the body, making it an ideal food or supplement for people with a tendency for diabetes. When using a whole yam, soak it before cooking. Yam extract supplement is also available in health food stores (*Second Spring*, p. 204).

(7) **Pearl Powder (Zhen Zhu Mu – Margaritifera**): The medicinal use of the crushed and powdered natural pearl in Chinese medicine is a very ancient practice. Prized by the Chinese royalty for its famous anti-aging properties, pearl powder has been traditionally used in herbal remedies and in ointments to be massaged into the skin to prevent premature skin aging, clear surface inflammation and acne, improve vision, and calm the mind and spirit. Rich in minerals that benefit the skin, natural pearl has much more to offer than just its beauty as an adorment (*Secrets of Longevity*, p. 70).

(8) **Avoid Coffee:** When you experience stress, anxiety, a racing mind, or insomnia, it is strongly recommended to cut out caffeine. It is a CNS stimulator that works against our attempts to relax the body and calm the mind. If you still enjoy the taste of coffee, you might turn to the decaffeinated version, but beware: many commercial coffees are decaffeinated using methylene

chloride, a chemical that interferes with the blood's ability to deliver oxygen. This causes the heart to work harder in an attempt to supply the needs of all the body cells.

In case the person has angina pectoris, and has switched to decaffeinated coffee to avoid triggering symptoms, he/she will want to make sure that his/her brew has been swept clean of caffeine (97% is considered clean), using a water process. It is recommended to check the label when buying coffee beans at the health food store, or ask the coffee server at the café (*Secrets of Longevity*, p. 50).

(9) Folk remedies for Insomnia – drink tea made with celery and beet tops in the evening, two hours before bedtime (*The Tao of Nutrition*, p. 31).

(10) Amaranth - for Anxiety or Insomnia toast ¼ cup amaranth in the oven until slightly brown, remove and steep in an amaranth cup of hot water for 5 minutes and sip for immediate relief of anxiety, or for insomnia. Take just before bedtime (*The Tao of Nutrition*, p. 71).

(11) Wheat - for insomnia, menopause, restlessness. Make tea from one cup of wheat, 12g licorice, and 15g Chinese black dates. Drink one cup three times daily (the Tao of Nutrition, p. 79).

(12) Lima beans - for insomnia. Eat soup for dinner made of lima beans with other calming foods like turkey and sage. Lima beans strengthens the Qi, nourishes blood, clears heat, resolves dampness, promotes bowel movement, fortifies the lungs, and calms the spirit (*The Tao of Nutrition*, p. 83).

Exercise Recommended to Improve Sleep

As recent studies confirm, Dr. Ni identified insomnia as a major contributor to the breakdown of the immune system, which accelerates the aging process.

Dr. Ni cites a famous Taoist physician, Ge Hong, of the Han dynasty in the third century, who promoted four sets of exercises to treat and prevent insomnia (Ni, 2006, pp. 248-9). Modern Chinese studies indicate that when performed nightly for 2-4 weeks, they can dramatically improve sleep quality in individuals suffering from chronic insomnia. Dr. Ni calls them "the four moves to checkmate insomnia". All four exercises are performed just before bedtime, while lying down mainly on your back:

Exercise 1

1. Lie on your back with bent knees.

2. Pull your knees up towards your chest and breath naturally

3. Hold this position for one minute, then relax and straighten your legs, rest your arms and hands at your sides.

Exercise 2

1. Still on your back, stretch both arms upward above your head while inhaling.

2. Bring your hands down while exhaling, and massage your body from your chest to your abdomen, then rest your hands at your sides.

3. Repeat with every breath for about one minute.

Exercise 3

1. Stay lying on your back, and make fists with both hands, then place your fists under your back as high as possible to your shoulder blades, on either side of the spine.

2. Take three complete breaths.

3. Re-position your fists one notch downward, and repeat.

4. Move downward every third breath, until your fists reach your waist level.

5. Take five breaths, then position your fists on either side of your tailbone.

6. Take five more breaths.

Exercise 4

1. Turn over and lie face down.

2. Place your hands under your abdomen. Slowly inhale, filling your abdomen and chest, and feel the energy permeate your entire body.

3. Slowly exhale and visualize negativity leaving your body.

4. Pause after each exhalation, and relax every muscle. Do this for one minute

(Ni, 2006, pp 248-9).

After completing the above cited four anti-insomnia moves, Dr. Ge Hong suggests to adapt the "deer sleep posture", imitating a sleeping deer in the wild (Ni, 2006, pp. 250):

1. Lie down on your right side.

2. Bend your right elbow and locate the palm facing up in front of your face.

3. Rest your left arm and elbow on your hip, hand dropped down in front of your abdomen.

4. Your right leg is naturally straight, while your left knee is bent and resting on the mattress in front of your right thigh (Ni, 2006, p 250).

A breathing exercise to experience deep relaxation and improve sleep (Ni, 2009, p 192):

Inhaling is a yang action that brings oxygenated air into our body, and in Dr. Ni's view it is the secret to good breathing. It nourishes the body cells with oxygen. Exhaling is a yin action,

that moves out of our body deoxygenated air together with toxins, which results in deflation and loosening up.

When inhaling, we should fill up our abdomen first, watch it rise, then fill up our chest, so that our ribs expand.

On exhaling, we should let the air out of the ribs, then out of the abdomen, compressing our belly to make sure that all the air is out. When we don't think we can exhale any more air, we aught to push out some more air, then more, until there is nothing left to expel. We should then repeat the process slowly and deliberately. By the end of the fifth exhalation, we will find ourselves gloriously calm, even dreamy (Ni, 2009, p192).

Overcoming Sleep Apnea (Ni, 2009, p 193):

If one feels sleepy all the time, mentally foggy, and has trouble staying awake to get through the day, the person may be suffering from sleep apnea, a common condition that ofen goes undiagnosed.

People with sleep apnea stop breathing multiple times during the night, and wake up gasping for air, or do not wake up al all. Sleep apnea causes nearly 40,000 deaths a year.

Simple natural solutions that could help:

a) If you are overweight, trim down. Excess fatty tissue in the back of the throat can obstruct the upper airway, causing you to stop breathing. Many people overcome apnea by simply losing weight.

b) Always sleep on your side, never on your back.

c) Don't get overly tired Take a little nap during the day, and pace yourself to avoid overwork, and establish a regular sleep pattern.

If none of this works, see your doctor and get a breathing device called CPAP that delivers oxygen at night.

Forming Healthy Habits to Improve Sleep

A common symptom of aging is cognitive decline, manifesting as loss of memory and concentration. Interestingly, these same symptoms are considered the main "side effects" of insomnia.

(1) To treat or prevent these symptoms, Dr. Ni suggests acupressure massage on GB-20 (Ni, 2006, p 252): Stimulate GB-20, two easy-to-find acupressure points on the back of the upper neck at the base of the skull.

a. Cross your fingers of both hands behind your upper neck with your palms cradling the back of your head, with both thumbs in the grooves on each side of your neck, and your index fingers crossing one another below your skull, just above the thumbs.

b. Simply sit on a chair, lean your head back, and let it rest against the pressure of your thumbs and index fingers.

c. Inhale through the nose and exhale through the mouth, slowly and deeply, and try to relax your entire body.

d. Do this for 2-5 minutes.

You will increase blood flow to the brain and at the same time relax the neck muscles, which often tense up due to stress and constrict blood vessels in the area.

(2) Form your own routines and rituals that help you go to sleep and stay asleep (Ni, 2006, p 204). From Dr. Ni's extensive interviews with over 100 centenarians, some useful

suggestions for facilitating going to sleep are: Hot baths, foot massage, journaling, meditation, aromatherapy, relaxing music, reading spiritual books, praying, and taking an evening stroll.

These and other rituals could help you calm your mind and feel peaceful within. Once you find an activity that works for you, be sure to practice it consistently, to program your body for achieving peaceful and refreshing sleep.

In Eastern medicine it has long been believed that respect for nature's cyclical changes brings health, while violating its rhythms leads to disease (Ni, 2006, p 205). Biochemical changes occur when humans transgress the natural behavior patterns associated with the division of night and day.

Modern recent studies show that shift workers on night duty and those with unpredictable working hours have a 30% higher risk of heart attack than day workers with set hours. Mice forced to live on a night-shift schedule had a lifespan 11% shorter than normal.

(3) Dwindling memory, decreasing concentration and a slowed response-time associated with aging are largely caused by reduced blood flow to the brain and loss of brain cells. In addition to proper nutrition and exercise, mental fitness boosting activities are imperative to prevent age-related cognitive decline (Ni, 2006, p 206).

Read and learn new things, find new hobbies, do crossword puzzles, add up your bill in your head while shopping to stimulate brain cell activity and in some cases the growth of new pathways.

(4) A popular practice among centenarians is body brushing, using a dry brush with natural bristles to sweep the surface of the entire body (Ni, 2006, p 207). Besides eliminating dead skin cells and improving skin hygiene, body brushing can also increase blood circulation in small capillaries in the skin, to promote vibrant skin tone and boost skin immunity against

infection. An alternative to brushing the body is body scrubbing: use a dry cloth or a moist rag, to vigorously scrub your body from head to toe.

(5) Soaking your feet in a hot bath before you sleep will produce a healthy relaxation response (after taking a calming tea or supplement, such as passiflora (see the Diet and Nutrition).

(6) Three-scent sleep remedy (Ni, 2009, p 194): After a stressful day, unwind and clear your mind by relaxing in a comfortable chair, putting on some soothing sounds, and reading something light and entertaining are all good methods to get ready for some restful sleep. But as you ease your senses, don't forget your sense of smell. Research has found that certain aromas can fill you with feelings of tranquility. Lavender, vanilla, and green apple are among the best smells to help lower anxiety and induce sleep. You can use essential oils of these scents and apply them to the back of your neck, or to the inside of your wrists. Even better, indulge in a warm bath with those oils dissolved in the water. Before bed, you might enjoy a glass of hot milk with natural vanilla flavoring for a calming effect inside and out (Ni, 2009, p 194).

(7) Stop restless leg syndrome (RLS) with Epson salts (Ni, 2009, p 195): RLS disrupts your sleep and never allows you to reach the deep state you need in order to wake up refreshed in the morning. When you have this condition, your nervous system sends random impulses to your legs at night, causing involuntary jerks and shudders. Of course, Western science offers medications for this condition, but why not avoid their potential side effects and take a drug-free approach?

Try soaking your feet in a hot bath with Epson salts and massaging them. This calms the entire nervous system, especially your legs, as your body absorbs the magnesium sulfate that is

in the salt. You can also try taking 500 mg magnesium in a supplement form before bedtime for a similar effect, calming the legs and promoting a good night's sleep.

(8) Take a Nap to Prevent a Heart Attack (Ni, 2006, p 216): One of the best ways to lower stress on your heart is to take a nap during the middle of the day. TCM has observed that in the body's circadian rhythms, noontime is the peak hour for the heart. Therefore, Chinese doctors advise calming activities and rest at this time of the day to maintain the health of the cardiovascular system. Researchers have found that individuals who napped at least 30 minutes a day were 30% less likely to develop heart disease than those who didn't nap. A siesta is a sign of wisdom, not laziness!

(9) Get in line with Earth's Energy (Ni, 2006, p 163): Feng Shui is the study of energy meridians that crisscross the Earth and the practice of aligning with them. The planet is like a large, magnetized ball with positive and negative charges circulating up and down its longitudes, and the electromagnetic impact on its inhabitants is subtle yet profound.

Arranging your surroundings in harmony with the earth's energy meridians will bring health, while violating this energy web can result in imbalance and illness. Whenever possible, your sleep position should be on a north-south longitude line. Some people find that once their sleep position is aligned, they have never slept better. Taking advantage of nature's gift is the ultimate purpose of Feng Shui.

(10) Your bedroom is your cocoon (Ni, 2006, p 164): Since you spend almost a third of your life sleeping, the bedroom is the most important room in your home. Ideally, your bedroom should be located away from the entrance and from the street, in the quietest area of the house. The décor should be minimal, not busy or distracting, and in soothing colors like shades of blue, green, or gray. Lighting should be dim, and music tranquil. If you live in a noisy neighborhood,

take soundproofing measures to achieve a quiet atmosphere. The overall feel should be cozy, safe, and cocoon-like.

Televisions and computers should not be placed in the bedroom, because they generate electro-magnetic fields and positive ions that can induce irritability and agitation. You should not have plants in your bedroom, since at night they give off carbon dioxide and deplete the oxygen in the air you breath. Work to create the best atmosphere possible in your bedroom to promote restful sleep, which is fundamental to good health and long life.

(11) Form your own routines and rituals that help you go to sleep and stay asleep (Ni, 2006, p. 204). From Dr. Ni's extensive interviews with over 100 centenarians, some useful suggestions for facilitating going to sleep are: hot baths, foot massages, journaling, meditation, aromatherapy, relaxing music, reading spiritual books, praying, and taking an evening stroll.

These and other rituals could help you calm your mind and feel peaceful within. Once you find an activity that works for you, be sure to practice it consistently, to program your body for achieving peaceful and refreshing sleep.

In Eastern medicine it has long been believed that respect for nature's cyclical changes brings health, while violating its rhythms leads to disease (Ni, 2006, p 205). Biochemical changes occur when humans transgress the natural behavior patterns associated with the division of night and day. Modern recent studies show that shift workers on night duty and those with unpredictable working hours have a 30% higher risk of heart attack than day workers with set hours. Mice forced to live on a night-shift schedule had a lifespan 11% shorter than normal.

(12) Easy awakening – to ward off stroke (Ni, 2006, p 212): Strokes and heart attacks occur most commonly between 6 am and noon. This is because when people arise from sleep and plunge into the activities of the day, their bodies experience a sudden increase in blood

pressure, temperature, and heart rate. This jolt taxes the system and creates strain on weak artery walls. Avoid literally jumping out of bed. It's better to gradually wake up with soft music, stretches, and self-massage before getting into the shower or driving.

The Chinese Taoists have passed down a morning ritual that eases the transition between sleep and wakefulness in a gently stimulating way. As soon as you wake up, massage your sensory organs: eyes, nose lips, and ears. Gently tap and brush your scalp with your fingertips. Massage the rest of your body with a stroking action from your neck down to your shoulders, elbows, hands, chest and abdomen, hips, knees, and feet.

Finally, massage your lower back with your palms. Inhale through your nose and exhale through your mouth three times to push out toxins. Then take three deep inhalations of fresh air to fill your cells with vital oxygen.

13) Take a tip from the Tortoise (Ni, 2006, p 213): Observe that in nature animals with a high metabolism die early and those who burn energy more slowly can live for many years. Take the example of the hummingbird: its fast metabolism burns out the organism within two summers, whereas a giant tortoise can live past 100 years.

Burning fuel to keep up a faster metabolic rate generates free radicals, which damages cellular DNA and produces a cascading chain of degeneration. Pace your daily life so that activities are punctuated with rest and restoration, avoid consuming stimulants, and reduce stress.

Eat appropriately for your circumstances: a light vegetarian diet for those with sedentary habits, and a higher-protein diet for a physically taxing lifestyle. Don't "live fast and die young" – instead be like the tortoise and get to the finish line!

TCM Recommended Lifestyle Changes

(1) Get at least seven to eight hours of sleep (Ni, 2006, p 203). Uninterrupted sleep is essential for maintaining good health and a long life. Chronic sleep deprivation can hasten memory loss onset and causes diabetes and high blood pressure. Eastern medicine has long known that adequate nighttime sleep helps restore the yin (substance) and keep the yang (function) in check. The conditions characterizing Yin depletion and Yang excess are exactly the symptoms manifested in Western studies.

The ideal sleep time recommended by ancient Chinese medical texts is eight hours. Average adults sleep eight hours and fifteen minutes when allowed to sleep as much as possible.

(2) Acupuncture can balance your body's chemicals (Ni, 2009, p 171): acupuncture helps to stimulate the body's physiological production of neurotransmittors (such as serotonin, norepinephrine and dopamine), so it is often used as a complementary treatment to conventional psycho-pharmacology and psychotherapy for mood and sleep problems, especially for perimenopausal women, to regain a stable emotional footing.

Typical treatments in Westerm medicine involve drug therapy with antidepressants that are selective serotonin uptake inhibitors (SSRIs), along with sleeping pills, both of which can be helpful for cases of serious clinical depression.

(3) To sleep like a baby without taking drugs, try the stress release meditation: breathe consciously, relax, and with each exhale focus on relaxing each area of your body in sequence, starting from the top of your head and moving down to your toes.

Regular meditation and relaxation exercises lower adrenaline, normalize cortisol levels, and promote abundant Dehydroepiandrosterone (DHEH), a precursor hormone that can convert to estrogen, progesterone and testosterone, all essential for our body's anti-aging defenses to

work. As we age, the body levels of DHEH drop precipitously. An undersupply of DHEH can lead to muscle weakness, joint pain and depression.

Our Adrenal Glands secrete three important hormones which help us deal with stress:

a. Epinephrine, helps drive blood into or heart and muscles. Most people overuse this hormone due to stress, which results in exhaustion and malaise.

b. Cortisol, a steroid that helps reduce inflammation and increases energy and appetite in traumatic and stressful situations. Too much of it can promote bone loss, kidney damage, weight gain, immune system disorders, and even cancer.

c. DHEH (Dehydroepiandrosterone**),** a hormone precursor, remains latent until it converts into the hormone needed by the body.

(4) **Acupuncture Lowers Cholesterol** (Ni, 2009, p 208): As you get further into menopause, the hormones estrogen, progesterone, and testosterone get out of balance. Estrogen builds up the lining of the uterus and is also secreted during ovulation.

Progesterone causes the lining to be eliminated during menstruation. Testosterone boosts muscle strength and libido. All three are synthesized from cholesterol.

Many menopausal women see a rise in their cholesterol level that has nothing to do with diet. The body is trying to manufacture hormones as glands decline in this function. The natural way to correct high cholesterol during menopause is to stimulate glands to again produce normal levels of hormones.

Chinese medicine relies on acupuncture and acupressure to restore healthy functioning to unbalanced organ systems. Before you take harsh cholesterol-lowering drugs and risk their side

effects, see an acupuncturist. In particular, you want to work on the liver and spleen networks by stimulating the points SP-10, SP-6, and LIV-3.

(5) **Daily rituals for women in midlife** (Ni, 2009, p 172): When you have symptoms of menopause, it means that your body is essentially trying to produce more estrogen. The pituitary gland, sensing a lack of estrogen attempts to stimulate the ovaries to produce more. Estrogen levels fluctuate drastically in response, which results in hot flashes, emotional swings, and agitation.

To counteract the chaotic adjustment going on in your body, establish a series of rituals that will stabilize your system:

a. In the morning start the day with meditation. Spend 10-15 minutes in meditative relaxation. You can play calming music if you desire.

b. Choose a particular time during daylight hours to exercise. This will balance your chemistry.

c. Take a bath with Epson salts, scent the air with lavender, and write in a journal to get thoughts out of your mind and down on paper. This will help your mind and body to calm down.

d. One hour before bedtime, reprogram your brain by dimming the lights. This helps the brain and body to prepare for sleep.

Chapter 5: Discussion

Summary of Findings

This study had three objectives:

(a) Examine the current evidence for a causal relationship between chronic insomnia or experimentally-induced sleep-loss and chronic co-morbidities. The current work investigator hypothesized that chronic sleep-loss is the root cause of chronic co-morbidities that were found to be associated with chronic insomnia/sleep deprivation.

(b) Research the effects of sleep and sleeplessness on aging.

(c) Study the effectiveness of TCM treatment of chronic insomnia and its co-morbidities, including treatments of acupuncture, acupressure, cupping, and nutrition. This investigator describes 12 studies of acupuncture and other TCM treatments, which successfully brought relief to very sick patients with very mild or no side effects.

For example, acupuncture proved to be quite effective in long-term institutionalized Schizophrenia patients and depression patients, who suffered from insomnia for long years, in whom acupuncture treatment improved sleep and reduced their reliance on western sleep medications that caused them considerable day-time impairment as well as losing their effectiveness with long-term use. The acupuncture treatment had practically no side effects or very few light ones. The other 11 studies of other TCM treatments demonstrate good to very good effectiveness as well.

The reasons for conducting this research were several: epidemiological or other retrospective studies do not seem to give clear answers as to the causes or consequences of insomnia. Hence, this investigator was not able to assess the strength nor the causal relations between the chronic insomnia and the associated chronic co-morbidities studied.

If we look at the first epidemiological study discussed here (Budhuraja et al., 2011), the researchers' findings clearly indicate significant associations between chronic insomnia and serious chronic medical disorders. The odds of suffering from chronic insomnia were 2.1 for people with stomach ulcers ($P<0.001$); 2.0 fold in people with neurological problems ($P<0.001$); 1.9 fold in people with chronic obstructive pulmonary disease (COPD, $P<0.001$); 1.8 higher in people with arthritis or migraine ($P<0.001$); 1.7 fold in women suffering from menstrual problems ($P<0.001$); 1.6 fold in persons with asthma ($P<0.05$); 1.6 higher in individuals who had heart disease ($P<0.005$), 1.5 higher in persons having hypertension ($P<0.001$) or diabetes ($P<0.001$), as compared with people that did not have these disorders.

Additionally, chronic insomnia frequency was found to increase with the number of medical disorders a person had. These are very high odds and indicate the strong association between these chronic diseses and chronic insomnia, yet it is not possible to discern which came first, the chronic disease or the chronic insomnia.

Taylor et al. (2005) studied the relation between insomnia, anxiety and depression, where they found that people with chronic insomnia had a 9.82 fold likelihood to suffer of a clinically significant depression, and a 17.35 fold likelihood of having a clinically significant anxiety compared with people who did not have insomnia. These odds were considerably greater in African Americans than in Caucasians.

Women had significantly higher levels of depression than men. Additonally, participants in this study who had an increased number of awakenings had higher levels of depression, as did participants with a combined insomnia, with both a delayed sleep onset and difficulty remaining asleep.

Compared with the above reviewed first study (Budhiraja et al., 2011), these are compellingly stronger associations between chronic insomnia, anxiety and depression. Does this mean that insomnia has caused by chronic anxiety? Or does insomnia trigger depression, or maybe both, anxiety and depression?

We cannot conclude which came first and caused any of the other two disorders to draw such causal conclusions. Hence, there is not enough evidence for a causal relationship in either direction in these retrospective epidemiological studies.

Prospective studies, such as the study conducted by Buysse, Angst, Gamma, et al. (p. 40), to study the "prevalence, course, and co-morbidity of insomnia and depression in young adults" in a Swiss community.

591 participants were recruited as young healthy adults. No treatment was given, only an infrequent assessment, to study young adults with a primary insomnia, and trace the co-morbidities that develop and their time of onset. Ss were evaluated by physical and psychiatric exams, as well as reported sleep symptoms in six interviews over twenty years.

The following categories of insomnia emerged from this study:

a) The annual prevalence of the one-month insomnia gradually increased over time, with a cumulative prevalence of 20% and greater than two-fold a risk among women.

b) In 40% of the Ss, insomnia changed into more chronic forms of insomnia over time.

c) Insomnia, either with or without co-morbid depression, proved highly persistent.

d) Insomnia lasting two-three weeks predicted major depressive episodes and a major depressive disorder in subsequent interviews (17% to 50% of these participants developed a major depressive episode in a later interview).

e) “Pure” insomnia and “pure” depression were not longitudinally related to one another, whereas insomnia co-morbid with depression were longitudinally related.

Conclusions

a) This longitudinal study confirmed the persistent nature of insomnia and the increased risk of developing a subsequent depression among individuals with insomnia.

b) The data support a spectrum of insomnia (defined by duration and frequency) co- morbid with, rather than secondary, to depression.

The investigator of this work reasons differently: since insomnia chronologically preceded the depression, it would be reasonable to assume that insomnia might have caused the depression, and not the reverse, although there might have been a third factor unrelated to insomnia that has caused depression. Yet a major depression does not suddenly occur without any etiology, there usually are at least tendencies which depend on genetic or environmental or life-style-factors which trigger depression. It is ntuitively reasonable to believe that a long history of poor sleep might result in a major depression – but there are no warranties.

That was the reason for the current work investigator to look specifically for cellular and/or molecular studies, to provide quantitative evidence at the most basic levels, and possibly decifer the mechanisms of action at the molecular and cellular levels, to enable a reliable predictor of future development of a major chronic medical condition, especially when facing great physical/psychological stress.

Sleep Deprivation as a Risk Factor for Pathogenesis

In their three recent studies chosen for this review (Everson et al., 2005, 2008; Everson & Szabo, 2011), Everson et al. induced extended and severe chronic sleep deprivation (SD) in rats, employing a sophisticated computerized method to induce 10% of sleep in "totally sleep deprived" (TSD) rats, then used the main quantitative measures of cellular and/or molecular stress, to clearly prove the resulting great oxidative stress shown to be specifically caused by severe sleep deprivation.

Dr. Everson et al. (2011) then allowed these rats almost four months of sleep *ad libitum* to trace the extent of systemic recovery from the previous extreme sleep loss that greatly affected these animals' bodies.

Even though the prolonged sleep recovery appeared to almost reverse all the symptoms of this severe sleep deprivation, including the greatly negative energy balance, the serious loss of body weight despite the animals' greatly increased hyperphagia, and their enlarged and modified internal vital organs relative to the rats' body weight, skin and paws' injuries, great loss of and remodeling of adipose tissue, as well as systemic infections. This severe oxidative stress seemed to have left permanent systemic effects in these animals.

From their previous studies, the findings of Everson et al. (1989, 2009) showed that even the strongest among these rats would have died in two to three weeks' time if they were not given the recovery sleep of 48 hours, and the lengthy sleep recovery of nearly four months after the 72 days of SD (Everson & Szabo, 2011).

The equivalent results of noxious stimuli that the researchers bring for comparison clearly indicate the great negative impact of chronic sleep deprivation on these animals' health.

In their study in human Ss, Thompson et al. (2011) found that shorter sleep duration (but not sleep quality) was associated with an increased risk of colorectal adenomas in patients undergoing a routine screening colonoscopies. Individuals sleeping less than six hours per night had an almost 50% increased risk of developing colorectal adenomas, compared to Ss sleeping more than seven hours. This association was independent of central obesity and insulin resistance.

The studies cited in Everson's studies suggest that this magnitude of increased risk that they observed in their study, was comparable to the increased risk of colorectal cancer associated with having a first-degree relative affected by this disease, or with consuming large amounts of red meat. Their data seem therefore to suggest that even a modest increase in sleep duration could have a substantial effect at the population level.

Valko et al' findings (2007) are also discussed here, of the literature on cellular and molecular imbalance resulting in oxidative stress, and the mechanisms causing the pathogenesis of three medical conditions plus aging: ischemia/reperfusion, rheumatoid arthritis, and Parkinson's disease.

Implications for Theory

Naidoo (2009) in his review of Alzheimer's disease, actually shows us the cellular processes that seem to be responsible for pathogenesis and aging in general. The principal mechanism found to act in response to cellular stress in UPR (unfolded protein response) involves three phases, depending of the severity of the cellular stress:

1. Adaptation – occurs during moderate stress, when UPR is healthy, and adaptive, at which point it is cytoprotective.

2. Alarm – takes place during greater stress, when adaptation fails, which then activates genes that mediate defense host mechanisms that lead to activating an inflammatory response.

3. Apoptosis – occurs during persistent extended stress conditions, when ER (endoplasmic reticulum) stress burden becomes prolonged and too great, which then activates the executioner pro-apoptotoic signaling pathways that leads to cell death.

Naidoo (2009) states that all the components of the UPR pathway (which is related to the process of "ER Stress") occur in chronic sleep deprivation**.** Naidoo characterized aging as "tipping the scale" towards apoptosis, especially in an organism that is depleted of antioxidants and energy (ATP), which is what happens in chronic sleep deprivation, as clearly shown by Everson et al. (2005, 2008, 2011).

Furthermore, Naidoo (2009) writes that both sleep deprivation and the cyclical intermittent hypoxia occurring in sleep apnea lead to ER stress, during which the protein production machinery is compromised and protein misfolding takes place. The ER stress responds to this stress by upregulating a series of coordinated cellular protective signaling pathways called UPR, a response that protects the cell against the deleterious effects of misfolded proteins, which can form toxic protein aggregates.

This response is highly conserved in all the species studied so far, including fruit flies, birds and rodents.

This protective response can be overwhelmed by additional stress, and if the cumulative burden is too great, then proapoptotic signaling is activated, which stops protein synthesis, among other actions. This takes place in older animals with short-term sleep deprivation, with cyclical intermittent hypoxia in certain motor neurons, and even in young animals that are undergoing a prolonged sleep deprivation.

Aging seems to be a central factor in the individual's response to all and any forms of stress. Klerman et al. (2013) show in their innovative study that the aging-related sleep decline occurs only during the NREM sleep phase, which has been shown to be the restorative stage, when the organism replenishes its depleted substrates for rebuilding its resources, such as ATP and antioxidants (Everson et al., 2011; Valko et al., 2007).

In their research, Klerman et al. (2013) found that the probability of awakening from sleep during NREM sleep was considerably greater (x6) in older persons than in young adults. Independent of bout length (the length of time within each sleep phase), the number of transitions between NREM and REM sleep stages, relative to the number of transitions to wake, was approximately 6 times higher in young persons as compared with older individuals, which tend to transition from NREM into wakefulness, resulting in sleep fragmentation, which explains older people's difficulty of maintaining sleep. The researchers suggest that treatment to improve age-related sleep should thus target this change in awakenings.

The research conducted by Rytkönen et al. (2010) in young, middle-aged and old male rats, proved that there was a clear age-dependent impairment in the molecular mechanism underlying homeostatic sleep regulation, mediated by nitric oxide release in the basal forebrain.

Normally, sleep deprivation invariably leads to increased NREM sleep intensity during the recovery sleep period. In this study, the NREM sleep intensity response was clearly attenuared in old animals and even in middle-aged individuals, as a result of adenosine receptors' loss of sensitivity. Since adenosine plays a major mediating role in this process of recovery sleep, this loss of sensitivity disrupts the homeostatic sleep regulation, a finding consistent with previous studies in both animals and humans.

The researchers employed an *in vivo* microdialysis in the basal forebrain, and tested whether these impairments in sleep homeostasis of aged rats could be changed by infusing the inducible nitric oxide synthase and nitric oxide into the basal forebrain, a mechanism shown to be critical in homeostatic sleep regulation.

Conclusions

a) Aging attenuates the sleep deprivation-induced iNOS, resulting in attenuated nitric oxide production in the basal forebrain, which results in a reduced recovery sleep response. This fails to induce the increased NREM sleep intensity in response to "Sleep Debt" due to sleep-loss. An infusion of nitric oxide-donor into the basal forebrain normally increases all these factors, to induce an increased NREM sleep intensity and a reduced sleep onset latency, suggesting an age-related decreased sensitivity of this area to nitric oxide (NO). Old rats also showed impaired adenosine accumulation in sleep deprivation.

b) Aging-related disturbances begin gradually in middle-age.

c) Taken together, the inability to produce nitric oxide during a prolonged waking period (chronic SD), and the aging-dependent insensitivity of the BF to the sleep-loss and to the promoting effects of NO, led to a diminished homeostatic sleep response in aging individuals.

d) Sleep has to be recognized as the critically essential basic need that it is, just like oxygen, warmth, food and water (Everson et al, 2005, 2008; Everson & Szabo, 2011; Luyster et al., 2012). Chronic sleep-loss greatly accelerates aging to shorten the lifespan.

Implications for Practice

Since these aging-related sleep disturbances begin during middle-age, it would be advisable to make some important life-style and diet changes starting at 35-40 years of age, to develop a routine of good habits in order to avoid the development of the various sleep impairments that we have seen above as a result of aging.

The suggestions brought by Dr. Maoshing Ni for treating insomnia (pp. 186-200 below) could be very helpful, as well as acupuncture treatment and exercise could help prevent insomnia and enjoy restful sleep. These recommended changes along with avoiding toxic drugs that cause dependency or addiction and have serious side effects, can greatly help to avoid depleting our energy and substrates.

Limitations of the Current Study

Only English publications have been used for this review, and because this is a doctoral capstone, the scope, time and space that can be dedicated to a subject matter are always limited.

In studies that involve acupuncture, the problem of no suitable control group or placebo control needs to be addressed in an integrated way, that will take into account both the TCM as well as the Western Medicine approach to therapy.

Recommendations for Future Research

Studying the mechanisms of acupuncture treatment for insomnia, and other TCM modalities for treating insomnia, especially in old age, could be very helpful.

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Appendix A: IRB Letter

YO SAN UNIVERSITY
OF TRADITIONAL CHINESE MEDICINE

October 8th, 2014

Hannah Goode
5870 Melrose Ave. Ste 3373
Los Angeles, CA 90038

Dear Hannah,

Your research proposal has been approved, with no additional recommendations effective through March 31, 2016.

Should there be any significant changes that need to be made which would alter the research procedures that you have explained in your proposal, please consult with the IRB coordinator prior to making those changes.

Sincerely,

P.P.

Penny Weinraub, L.Ac.
IRB Coordinator

13315 W Washington Blvd, Los Angeles 90066

Appendix B: Abbreviations

ACTH = adreno-corticotropic hormone
AD = Alzheimer's Disease
ADH = anti-diuretic hormone
AIS = Athens Insomnia Scale – a subjective outcome measurement.
ALS = Amyotrophic Lateral Sclerosis
ALT = Alanine Amino-Transferase
ANS = autonomic nervous system
AP-1 = activator protein-1
ASK 1 = kinase 1, that is an apoptosis signal-regulating gene
AST = Aspartate Amino-Transferase
BDI = Beck Depression Inventory = one of the most commonly used depression measures, with extensive reliability and validity data to back it up.
BL = baseline
BMI = body mass index
BP = blood pressure
CAM = complementary and alternative medicine
Cdk-5 = Cd Kinase 5
CHD = coronary heart disease
CI = confidence interval (of statistical tests - usually 95%).
CNS = central nervous system
COPD = chronic obstructive pulmonary disease
CSD = chronic sleep deprivation, or experimentally-induced prolonged sleep deprivation
CSF = Cerebro-Spinal Fluid; aCSF = artificial cerebrospinal fluid.
CT = Computed Tomography
CVD = cardiovascular disease
CX = brain cortex
DETA –NO = a synthetic NO donor
Dioxygen = O2, Molecular oxygen
DMN = Default Mode Network = the resting state neuronal network, which is active during sleep or during the brain's resting mode, which is synaptically and metabolically more active than other brain regions
Dx = diagnosis
EA = electro-acupuncture
EEG = electroencephalography, an objective measure of brain wave-form activity during sleep as recorded in polysomnography sessions.
EKG = ECG = electrocardiogram, electrocardiography
EMG = Electromyography, used to record any of the movements of skeletal chin/neck muscles, or leg muscles during polysomnography sessions.
EOG = Electrooculography – used for recording an electrooculogram of eye-moving muscles during polysomnography sessions
FDG = fluoro-deoxy-glucose
FMS = Fibromyalgia Syndrome
fMRI = functional MRI
GABA = Gamma-aminobutyric acid

GC = glucocorticoid (a steroid hormone produced and secreted by the adrenal gland).
GGT= Gamma Glutamamyl-Transferase
G-6-PD = glucose-6-phosphate dihydrogenase
6-GPD = 6-phospho-gluconate-dihydrogenase
GPX = glutathione peroxidase
GSSG = glutathione disulfide (oxidized glutathione)
GSSG-R = glutathione reductase (catalizes the rection of reducing GSSG back to GSH)
GSH = glutathione
HD = Huntington's disease
HDL = high-density lipoprotein
HF = high frequency
HIS48 = an antibody for assessing type of antigen-source (e.g. if serum contains neutrophils in case of HIS48 positive labeling)
HPA = the Hypothalamic–Pituitary–Adrenal axis
HR = heart rate
HRV = heart rate variability
HTN = hypertension
ICU = intensive care unit
iNOS = an inducible nitric oxide synthase – an enzyme that induces nitric oxide production
ISC = Insomnia Severity Score
ISF = fnterstitial fluid
ISI = Insomnia Severity Index – one of the two most frequently used questionnaires for self-reporting sleep-difficulties and sleep-loss. Like PSQI, it is considered a subjective outcome measurement.
LC = locus coeruleus – a brain region
LD cycle = light-dark cycle
LDL = low-density lipoprotein
LF = low frequency
LRs = evolutionarily conserved proteins which recognize microbial molecules, initiate the innate immune response and modulate the adaptive immune system.
LSR = lengthy (extended) sleep recovery
LTD = latero-dorsal tegmentum - a brain region
MAPKs = mitogen-activated protein kinases
MDD = major depressive disorder
mM = milli-Molar (a standard solution concentration measure)
MPO brain = medial preoptic area - a brain region
MPO = myelo-peroxidase, an enzyme constituent of neutrophils
MRI = Magnetic Resonance Imaging [fMRI = functional MRI]
MT = mechano-transduction
NADP = Nicotin Amide Adenine Dinucleotide Phosphatase = a coenzyme of numerous dehydrogenases (enzymes), such as the one acting on glucose-6-phosphate, that occurs especially in red blood cells and plays a role in intermediary metabolism similar to NAD.
NADPH = the reduced form of NADP
nNOS = neuronal nitric oxide synthase
NOS = nitric oxide synthase (enzyme that regulates NO synthesis)

eNOS = nitric oxide synthase, which is constitutively expressed endothelial (the nNOS isoform Nitric oxide synthase.
iNOS = inducible NOS
NO2 = nitrite
NO3 = nitrate
NOx = nitrite and nitrate collectively
NREM = Non-REM sleep = non-rapid eye movement sleep
NRT = number reduction task
P = probability, a statistical concept for expressing the significance of the results.
PAMPs = pathogen-associated molecular patterns (of the immune response)
PD = Parkinson's disease
PET = Positron Emission tomography
PLM = periodic limb movement
PSD = partial sleep deprivation
PSG = polysomnography
PSNS = para-sympathetic nervous system
PSQI = Pittsburgh Sleep Quality Index - The most frequently used questionnaires for self-reporting sleep-difficulties and sleep-loss (considered a subjective outcome measurement).
RBD = REM sleep breathing difficulty
RBDSS = RBD Severity Scale
RCT = Randomized Controlled Trial
RHT = retino-hypothalamic tract
RLS = restless leg syndrome
REM = rapid eye movements sleep = paradoxical sleep = dream sleep
RNS = reactive nitrogen species of free radicals in the organism
ROS = reactive oxygen species of free radicals in the organism
RRC = intervals on a cardiogram - an ECG recording of the time domain of the HR – in milliseconds (ms)
RR = relative risks
SD = sleep deprivation, sleep-deprived/sleep loss
SDB = sleep-disturbed breathing – augmented with aging
SDNN = standard deviation of NN interval - of the time domain of the HR
SE = sleep efficiency - declines with aging
SDB = sleep-disturbed breathing
SNS = sympathetic nervous system
SOD = superoxide dismutase
SR = sleep recovery
Ss = subject(s) = study participants
STAI = State Trait Anxiety Inventory = one of the most frequently used self-reported anxiety measures, considered a subjective outcome measure
SWS = Slow Wave Sleep in NREM sleep = the fundamental cellular process that organizes brain waveforms seen on EEG during sleep => Sleep spindles and slow waves => declines with Aging
SWA - slow wave activity – also called delta activity, 0.5-4 Hz EEG, in NREM sleep.
Sx = symptoms

TCM = Traditional Chinese Medicine
TLRs = toll-like receptors (in tendon healing, they detect the release of endogenous ligands, contributing to the pro-inflammatory response to the injury).
LRs = evolutionarily conserved proteins which recognize microbial molecules, initiate the innate immune response and modulate the adaptive immune system.
TSD = total sleep deprivation
TSH = thyroid stimulating hormone
TST = total sleep time
Tx = treatment
WHR = waist-to-hip ratio (for obesity assesment)
WASO = wake after sleep onset (increase with aging)

Appendix C: Everson - Tables

Everson et al., 2005

Study objective:

To trace the oxidative stress and the antioxidant response of three peripheral tissues (Liver, Heart, Lung) during sleep deprivation (SD) of 5 and 10 days and in a post-SD stage of SR (sleep recovery) of 48 hours.

Experimental Design:

	TSD	PSD	Controls	SR 48h
5 days			Baseline	No
10 days			Baseline	Yes

52 animals, 26 experiments (each with 2 animals => 2 controls or 1TSD and 1PSD),

	Sleep obtained - % of Baseline TST		
	TST	NREM	REM
Baseline Conditions (Control):	54%	48%	6%
PSD (Partially Sleep Deprived):		38%	3%
TSD (Totally Sleep Deprived):	5.4%	4.5%	1%

- Animals were kept under constant light and 28°C (rats thermo-neutral zone)
- Animals' sleep obtained by both TSD and PSD groups was reduced in amount, and highly fragmented, mainly composed of transitional sleep, and fragmented high-amplitude NREM sleep.
- The percentage of time the platform rotates is 18-22%, and does not increase across experimental days.

Initial Procedures:

1) Surgery to implant electrodes into cranium and temporalis muscle => for EEG
2) At least seven days recovery from surgery
3) Baseline conditions => Sleep *ad libitum* and housing platform rotation 6 seconds hourly.

Assays => of indices of oxidative stress resulting from SD/SR

1) Glutathione (GSH) content => in liver, Lung, Heart tissues (GSH is the major marker for antioxidant depletion or repair).
2) Glutathione Peroxidase (GPX) activity } Two major enzymatic antioxidant and
3) Catalase Activity } indices of GSH recycling. Normally both are under tight enzymatic regulation to maintain homeostasis.

4) Plasma amino-transferases => ALT (Alanine Aminotransferase)
AST (Aspartate Aminotransferase)
GGT (Gamma **Abbreviations** -Glutamyltrasferase)
Indices of peripheral cell-membrane damage and leakage of cell content
5) G-6-PD => First enzyme in the oxidative pentose phosphate pathway
6) 6-PGD => Second enzyme in the oxidative pentose phosphate pathway
7) GSSG-R activity => determined by the) GSSG-R recycling assay, a kinetic method of absorbance spectrophotometry (Griffith, 1980).
8) Body weight
9) Food intake

Everson 2008

Study objective: Determine whether increased phagocytes in the circulation indicates a migratory traffic into tissues, which would indicate an inflammatory response to cellular stress.

Experimental Design:

SD duration	TSD	PSD	Controls	SR 48h
5 days			Baseline conditions	No
10 days			Baseline conditions	Yes

44 live animals, 22 experiments (each with 2 animals => 2 controls or 1TSD and 1PSD)

	Sleep obtained - % of Baseline TST		
	TST	NREM	REM
Baseline Conditions (Control):	54%	48%	6%
PSD (Partially Sleep Deprived):		38%	3%
TSD (Totally Sleep Deprived):	<5.4%	4.5%	1%

- Animals were kept under constant light and 28°C (rats thermo-neutral zone)
- Animals' sleep obtained by both TSD and PSD groups was reduced in amount, and highly fragmented, mainly composed of transitional sleep and a fragmented high-amplitude NREM sleep.
- The percentage of time the platform rotates was 18-22%, and did not increase across experimental days.

Initial Procedures:

1) Surgery to implant electrodes into cranium and temporalis muscle => for EEG
2) At least seven days recovery from surgery
3) Baseline conditions => Sleep *ad libitum* and housing platform rotation six seconds every hour.

Assays
Of indices of phagocytes' migration => to determine if there is an inflammatory process:

1) Immunohistochesmistry => Determine leukocytes' location => use counts of HIS 48, the antigen found in all granulocytes => if positive labeling (compared to positive labeling of CD 45, the leukocytes' common antigen), indicates granulocyte migration traffic in the blood circulation.
2) Heme oxygenase-1 (an inducible **heat shock protein-32**) => marker of cellular stress => shows increased response to inflammation and to oxidative stress.
3) MPO (myelo-peroxidase enzyme) activity => a practically exclusive enzyme to neutrophils => a constituent enzyme of neutrophils => indicating neutrophil migration into peripheral tissues in an ongoing inflammation process.
4) Plasma corticosterone => A marker of cellular stress. When plasma levels are too low it indicates corticosterone depletion => in response to the previous SD.

Everson 2011

Study objective: To broaden our understanding of the physiological characteristics that arise from chronic sleep deficiency and to determine if residual consequences remain afterrecovering the lost sleep over an extended *ad-libitum* sleep..

Experimental Design:

TSD	PSD	Controls	SR (48hrs)	LSR (17-18wks)
72 days and 6 x 48 h		Ambulation Baseline	Yes	Yes
72 days and 6 x 48 h (tissues)		Ambulation Baseline	Yes	No

16 live animals, 22 experiments with 2 animals each => 2 controls **or** 1TSD and 1PSD
20 animals' preserved tissues from a previous study (Everson and Szabo, 2009).

	Sleep obtained as a % of Baseline TST			
	TST	NREM	REM	SR REM
Ambulatory Control:	55%	48%	7.3%	
PSD (Partially Sleep Deprived):		46-48%	7.3%	
TSD (Totally Sleep Deprived):		34-40%	2.4-3.4%	11-13%

- Animals were kept under constant light and 28°C (rats thermo-neutral zone)
- Animals' sleep obtained by both TSD and PSD groups was reduced in amount, and highly fragmented, mainly composed of transitional sleep, and fragmented high-

amplitude NREM sleep.
- During SR => REM sleep rebounded => 50-67% more than during SD period => 11-13%, while NREM sleep was not significantly different from SD period.
- The percentage of time the platform rotated was in this study 26%, and did not increase across experimental days.

Initial Procedures:
1) Surgery to implant electrodes into cranium and temporalis muscle => for EEG
2) At least seven days recovery from surgery
3) Baseline conditions => Sleep *ad libitum* and Housing platform rotation for 6 seconds every hour => also during RS and LRS periods.

SD in Forced Ambulation Control Animals
The control forced ambulation group (controls) was subjected to conditions that were matched to the experimental group, including surgery, daily measurement procedures, and equal total duration of ambulation requirements, except that for control animals the platform rotations described above were consolidated, to permit a lengthy uninterrupted sleep, with the rotation schedule consisting of a 90 minutes with consolidated 150 seconds rotation, then stationary for 30 seconds, followed by a 198 min with no rotations. This schedule was repeated five times per day.

In control animals this schedule produced a reduced NREM sleep, from 54% during baseline to 46-48% of sleeping time during any given 10-day ambulation period. REM sleep did not differ from baseline. During the 48-hour SR stage, the average percentage of TST (total sleep time) spent in either NREM or REM sleep did not statistically differ from baseline (Everson and Szabo, 2009).

Measurements /Observation
1) During SD period => daily food and water intake, daily body weight (b.wt)
2) In the middle of cycles 5 and 6 => collected food and fecal waste => to measure caloric values (to rule out mal-absorption and feeder waste, to explain animal b.wt loss)
3) During LRS period => daily food and water intake, daily body weight (b.wt) during LRS first month, then every 48 hours up to day 115 in TSD and PSD animals.
4) Observed skin or other external pathology => Dermatosis on paws and fur
5) Measured intestinal length in ambulatory controls and SD animals
6) Weighed internal organs mass => liver, heart, kidneys, spleen in ambulatory controls and SD animals (with or without LSR).
7) Weighed organs' lipid, protein, water, ash => liver, heart, kidneys, spleen in ambulatory controls and SD animals, with and without LSR
8) Weighed organs' lipid, protein, water and ash => of intestine and skeletal muscle in ambulatory controls and SD animals, with or without LSR
9) Weighed adipocytes (white, brown) in the omentum, epididymus, and in the surrounding mesenteric lymphnodes.

Assays:
1) Plasma osmolality
2) Plasma insulin
3) Plasma leptin
4) Plasma cholesterol
5) Plasma LDL (low-density lipoprotein)
6) Plasma HDL (high-density lipoprotein), glucose, triglycerides, phosphorus, creatinine, urea nitrogen, plasma total protein.

Description of the Bergmann-Rechtschaffen Disk Method
(Everson and Szabo, 2011) Designed and tested by Bergmann et al. in 1989, and underwent minor modifications to validate the method, which can be reliably programmed to produce the desired amount of SD in laboratory animals, specifically designed for rats as animal models.

"Rats were housed and observed in this apparatus two animals at a time. The apparatus consists of a large round platform of 46 centimeter in diameter, divided by a Plexiglas wall into two equal parts, within an open-air enclosure. A rat is housed on each side of this wall, with both rats being studied at the same time. The long electric cables attached to the electrodes on their head, go up through the upper air opening to connect with the computerized polysomnographic equipments, thus enabling the two rats to move freely.

The floor area on each side of the platform is sufficient to permit the rat to eat, groom, explore, and lie down fully to sleep. Beneath each side of the platform there is a pan of shallow water less than an inch deep, which encourages the rat to stay on the platform. The divided platform and pans are enclosed by high Plexiglas walls, and the entire apparatus is open at the top, permitting the operation of swivels and counterbalanced boom assemblies mounted outside the enclosures, to connect the implanted electrodes to a computer – yet allowing the two enclosed animals free movement.

Each six-second rotation of the platform requires a short walk, since the rat is displaced from a typically comfortable spot at the widest radius of the platform to a narrow spot that induces the rat to move in order to avoid stepping off the platform and into the shallow water.

This apparatus has been used with minor changes since 1989 in the first author's laboratory, and has been validated for its high selectivity under freely moving conditions. It reliably produces sleep reduction to only 10% of baseline total sleep, mostly consisting of a transitional and highly fragmented NREM sleep.

The apparatuses were cleaned at least every other day, and specially designed food tubes that allowed each rat to gnaw on a pellet while the crumbs fell into a catching receptacle enabled dependable measurement of food intake.
This housing platform was attached to a computer, so that when the SD rat was awake, the housing platform was kept stationary and did not interrupt other behaviors. For the TSD group, sleep onset directly triggered the platform rotation for six seconds, so that the animal had to walk in order to remain comfortably on the platform. On the other hand, for the PSD group the

platform’s rotation was not associated with their sleep onset, so they could sleep at the time their paired TSD rat was not trying to sleep” (Everson and Szabo, 2011).