The Effects of Sleep on Bone Mineral Density in College Aged Males and Females

by

Patricia Rose Esch

Abstract

Sleep is imperative for health and wellness with direct impacts on brain function, physiology, emotional well-being, performance and safety when compromised. Many physical, physiological, and psychological functions are affected by lack of sleep. Prolonged insufficiency in sleep has been linked to chronic health conditions including obesity, heart disease, high blood pressure, diabetes and stroke[i]. While there is still much to be learned from sleep habits and the ripple effect that sleep has on many facets of our lives. One thing we do understand is how important sleep is how valuable sleep is to activating our pathways of growth, regeneration, and restoration. In recent research, sleep has been increasingly linked to bone metabolism. Adolescents and young adults are increasingly affected by factors affecting the maintenance of regular sleep schedules. College and university students are a potentially vulnerable population to sleep deprivation and sleep insufficiency. Possible factors that could contribute to poor sleep hygiene include, but are not limited to, academic pressures, social activities, and increased screen time. Arguably, students are still experiencing bone mineralization, until the age of 30 or even 40 years old, which makes it more important to understand the effects that altered sleep patterns could have on continued development of bone health. The connection between sleep and bone regeneration is mostly researched in elderly or post-menopausal populations. It is our understanding that to date, studies assessing the risk of sleep insufficiency on bone mineral density in college students have not been conducted. We propose a proof of concept study in which college students will complete cross sectional assessments on sleep duration, variability, and environment. We hypothesized that college-aged students, between the ages of 18-25 years, with shorter sleep durations, greater sleep schedule variability, and poorer sleep environments will have significantly lower bone mineral density. ActiGraph monitoring, via a wrist ActiWatch (Actiwatch Spectrum Plus, Philips Respironics, Inc., Bend, OR), was used to quantitatively measure sleep habits for up to 7 consecutive days. During the week-long study participants also captured their self-reported sleep data through the use of a sleep diary (Consensus Sleep Diary-Core). Participants were measured one time within the study for bone mineral density of the lumbar spine and total hip through a dual energy x-ray absorptiometry (Lunar iDXA, GE Healthcare, Madison, WI). This was a preliminary analysis of a larger cross-sectional analysis which will ultimately include 50 participants. This study subset looked at 17 participants, of which there were 14 females and 3 males, (n=5, 1 and 11 Hispanic, Black and White, respectively). The mean age of participants was 20.8±1.7 y with an average BMI of 22.9±3.2 kg/m^2. ActiWatch measurement data showed a mean daily sleep duration of participants to be 437.5 ± 43.1 (372.5 – 509.4) minutes. Mean sleep efficiency (minutes of sleep divided by minutes of time in bed) and mean number of awakenings were 87.4±4.3 (75.4-93.4) minutes and 32.1±6.4 (22.3-42.7) awakenings, respectively. The median time for wake after sleep onset (WASO) was 34.5±10.5 (18.3-67.4) minutes. The mean bone mineral density (BMD) for the hips was 1.06±0.14 (0.81-1.28) g/cm^2 with a mean BMD of the lumbar spine being 1.24±0.12 (0.92-1.43) g/cm^2. Age-matched Z-scores of the hips was 0.31±0.96 (-1.6-2.1) and lumbar spine was 0.53 (IQR: 0.13, 0.98; -2.25-1.55). Neither sleep duration nor sleep efficiency was significantly correlated to BMD of either locations. While WASO was positively associated with hip and spine BMD, this value was not statistically significant in this population. Overall, associations between sleep and BMD of the femur and spine were not seen in this cohort. Further work utilizing a larger cohort will allow for control of covariates while looking for potential associations between bone health, sleep duration and efficiency.

Introduction

Populations around the world are demonstrating increasingly worsened sleep habits[ii] which, arguably has been sparking conversations regarding public health concerns in relation to the longitudinal effects of these negative sleep habits. Where we once did not fully grasp the importance of sleep and thought it to be generally invaluable; we now know that rather than switching off during sleep, many body functions activate pathways of growth, regeneration, and restoration. Bone regeneration is one pathway that sleep is speculated to have a role in activation and regulation. To date, the majority of the research conducted surrounding the impact of sleep on bone mineral density (BMD), a measure of bone strength which oftentimes is used to assess the risk of fracture, is focused on elderly and post-menopausal populations.[iii,iv,v,vi] This data shows sleep duration, sleep sufficiency, and sleep disorders impact bone health in various ways. In one study[vii], a population of post-menopausal women were more likely to have conditions of weakened bones, such as osteopenia which can later develop into osteoporosis, if their average bedtime was after midnight. Delayed bedtimes is hypothesized to affect circadian rhythm, subsequently enacting a change in many metabolic processes affecting bone regeneration. Similarly, it has been demonstrated that sleeping too long can inversely affect bone mineral density (BMD) in post-menopausal women.[viii] A study of Korean citizens over the age of 60 showed that BMD in women, but not men, at total hip and femur neck were lower in the women who slept the longer than 75% of the study participants, suggesting that too much sleep can increase risk for osteopenia and osteoporosis. Conversely, it was found that women over 45 years of age who had decreased average sleep duration (<6 hours of sleep per night) demonstrated lower total BMD when compared to women aged 18-45 years[v]. In post-menopausal women, the risk of osteopenia and osteoporosis is correlated to insufficient sleep duration and delayed bedtimes. While all this data highlights important findings, studies correlating sleep habits and BMD are still vastly limited and require continued rigorous research to determine the true relationship of these variables.

Current research and literature surrounding the effects of sleep on BMD of young adults is minimal, at best. This population is important to focus on because adolescents are increasingly affected by factors that decrease sleep time and sleep efficiency. While, it is a common misconception that as we age we require much less sleep than we did as adolescent, recent studies have uncovered that our biological sleep requirements are not dramatically altered with increased age. Rather, it is an increase in societal pressures which cause the largest change in sleep patterns[iii]. It is a combination of biochemical, sociological, and psychological pressures that stem from societal expectations, which ultimately shorten sleep duration and weaken overall sleep quality for young-adults and adults.

As students get further into their education, bedtimes are consistently pushed later into the evening with earlier morning wake times[ix]. The transition from middle school to high school has been shown to impact the sleep schedule of many students around the globe[x]. Changes in sleep patterns and sleep quality among college students are consistent with these results. A well-accepted social stereotype is that of the average college student being overwhelmingly busy and stressed; emotionally and academically. The change from students' usual home environments and the level of academic rigor that is required in college are oftentimes drastic differences from high school. These factors can have been further categorized as: bedtime autonomy, academic pressure, screen time and social networking[x]. Arguably, there is no greater amount of bedtime autonomy afforded to a young adult than that afforded to college students. For most students, college is the first time in their life when they will live with someone who has historically had a

very different sleep schedules than them. This new environment for many, but not all students, is just one way that sleep time is challenged in college. Smart phone use, which can simultaneously account for social networking and screen time, has negative impacts on sleep quality[xi]. Academic and social pressures can account for delayed bedtimes, but more importantly they are responsible for earlier awakenings.[xi] Adding these factors together—an overall later bedtime with earlier awakenings—equates to a grave decrease in sleep sufficiency. College students regularly report high levels of academic and social stress plaguing their busy lives[xii]. This stress, in turn, decreases the amount of sleep they will receive. Lack of sleep has been proven to manifest downstream effects; those effects that are suspected to relate to bone growth and regeneration will be discussed later.

In a 2010 study,[xiii] 1,105 students at a large, private university in the Midwest demonstrated the alarming prevalence of insufficient sleep habits of students enrolled in university. The study reported a majority of the population experiencing, "chronically restricted sleep,[xiv] with the total population averaging only 7.02 hours of total sleep time per night. The National Sleep Foundation (NSF) recommends that students receive 7-9 hours of sleep every night[xvi]. This is alarming since it brings to light the fact that the majority of students are bordering and below the bottommost limits of recommended amounts of sleep. Only 29.4% of the population reported receiving 8 or more hours of sleep every night. Not only are students experiencing less time in bed, but they are also exhibiting poor sleep quality. A similar study was implemented in a different university population, but still showed similar trends. Conducted at a small, rural university on a group of 191 students, the study found only 11% of the population was receiving consistently good sleep[xiv]. Across the board, college students seem to be suffering from sleep difficulties; whether that be falling asleep, staying asleep, or demands interfering with sleep. These conclusions provoke questions and warrant investigations into what this endemic sleep insufficiency can affect. There are many aspects of health and daily life where sleep is known to play a pivotal role.

It has been established that sleep is imperative for health and wellbeing. In fact, sleep deprivation can have a grave impact on physical, physiological, psychological, and social functions. Even further, many chronic health conditions plaguing the United States are extensively linked to habitual sleep insufficiency[i]. Our current scientific understanding of sleep represents only the tip of the iceberg. Sleep is an important regulator of hormone synthesis and metabolism, both of which affect BMD. Research continues to demonstrate the connections of sleep on our molecular pathways for growth, regeneration, and restoration. During sleep you allow your body to build muscle and repair tissue[xv]. In order to better understand the impact that sleep might specifically have on bone regeneration, it is first necessary to understand the processes involved in bone regeneration and how these might overlap with the biochemical processes that are activated or inhibited during sleep.

Beginning at conception and continuing until death, bone will develop and consistently remodel itself. Bone, being dynamic tissue that it is, serves as a reservoir for vital minerals, most notably Ca^{2+}, as well as biologically active molecules such as insulin like growth factors (IGF-1)[xix]. The three major cellular players involved in bone growth and regeneration are osteoblasts, osteoclasts, and osteocytes. Active osteoblasts create collagen, the major organic component of bone. These cells become embedded in the matrix and then further differentiate into what are known as osteocytes, or bone cells. Osteoclasts are multinucleated cells that are derived from monocytes, which function to break down bone in order to release various biologically relevant minerals and molecules, like Ca^{2+} and IGF-1, for the body to use. These three cell types are

integral to understanding bone biology, and how bone can have such defined periods of growth and regeneration throughout one's lifespan.

During adolescence, drastic changes in bone development occur. Specifically, puberty can result in a doubling of bone mass[xvi]. In adolescent boys, increases in bone mass are generally most significant between the ages of 13-17; whereas, in females this increase is most prevalent between the ages of 11-14.[xvii,xviii] It is important to note here that it is bone mass, not bone mass density which doubles during puberty. Puberty affects the size of bones much more than it does the volumetric density. Bone will continue to accumulate density and by the age of 18 or 20 for girls and boys, respectively and up to 90% of bone mass will have been gained. However, bone material continues to be accrued until reaching peak bone mass (PBM) which can be up until approximately 30 years of age, sometimes even until 40 years of age[xix]. Peak bone mass is the maximum strength and density that bony tissue will reach in one's lifetime. In adulthood, PBM is an important predictor of osteoporosis[xix]. In fact, it has been stated that optimizing one's potential for PBM early in life may be more effective for osteoporosis prevention than attempts at minimizing bone loss later in life[xix]. This means that adolescence and young adulthood is a critical time to focus on bone health. Investing in the bone health of growing, young adults could potentially lead to less fractures in late adulthood.

During puberty the lumbar spine and femoral neck have a dramatic rate of increase, growing by four- to six-fold in just 3-4 years.[xix] In a longitudinal study conducted to study accumulation of bone mass over time, it was determined that for girls by 16 years of age there was a marked reduction in BMD gains in lumbar spine and femoral neck and between the ages of 17-20 years old BMD gain completely halted. For boys, BMD accrual did not begin to decline until 17-20 years of age and even then there was still significant bone growth at the aforementioned sites[xx]. Lumbar spine, femoral neck, and hip are areas of the skeleton which are critical for mobility and are most affected by osteopenia and osteoporosis. Osteoporosis is generally described as osteoclasts breaking down bone in a disproportionate ratio to the amount of additional bone that osteoblasts are remodeling, which ultimately decreases BMD. Weakened BMD poses a high risk because it increases the likelihood of bone fracture. Many studies focus on these sites—lumbar spine, femoral neck, and total hip—for the reasons mentioned above, and also because these areas are well-validated sites, which are easily assessed by dual-energy X-ray absorptiometry (DXA) scans.

Gonadal Steroids, Growth Hormone (GH), and IGF-1

While it has been established that by 17 or 18 years of age, the age of college matriculation, students will have completed the most significant portion of their bone growth[xix]; it is also important to note that bone growth does not definitively halt at a specific age in all people. Students that are 25 years of age and younger could still have varied growth patterns, this was discussed previously. The main hormonal contributors of bone development in adolescence are gonadal steroids, growth hormone (GH), and IGF-I[xxi]. During the pubertal growth spurt there is an increase in circulating IGF-1 levels, which directly regulate bone growth and density[xxii]. Factors such as IGF-1 relate to BMD through various intermediates, which are most often derived from diet. Dietary calcium, phosphorus, magnesium, and protein are just a few of the necessary components to facilitate strong, healthy bone growth. As demonstrated by the pathway highlighted below (Fig) these nutrients are vital ingredients, especially in adolescents and young adults who are still developing bone mineral. Calcium (Ca^{2+}), inorganic phosphorus (P_i), and protein are metabolized into essential amino acids (EAA) that are circulated into the

bloodstream. These EAAs then influence the production of GH in the liver, which stimulates the production and secretion of hepatic IGF-1 into the bloodstream[xxiii]. This endocrine pathway has a positive feedback system that will continue to propagate bone growth as long as the body is supplied with ample amounts of dietary calcium, phosphorus, magnesium, and protein. This biochemical pathway supports the major claims of diet playing a pivotal role in the healthy development of children and adolescents. Other factors, such as stress hormones also play a large role in bone growth[xix].

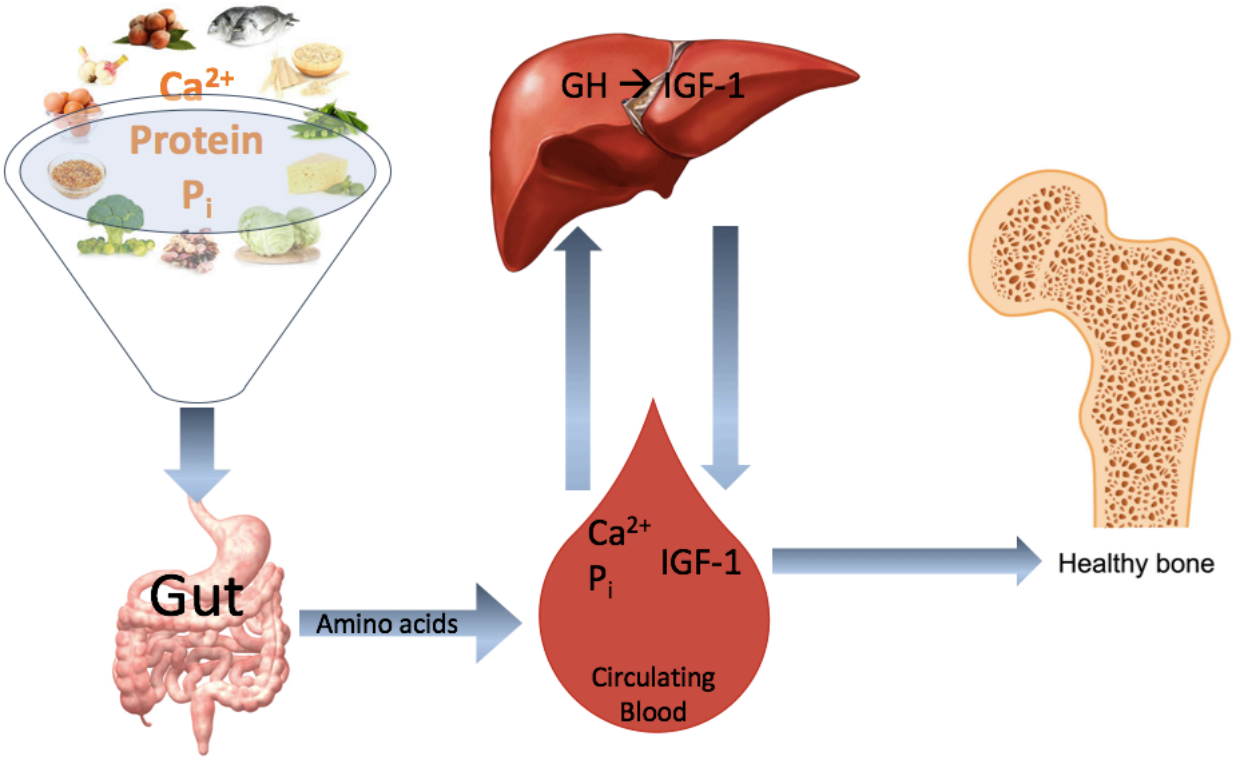


Figure 1. Adapted from a figure in reference xxiv*. During growth, specifically in puberty, insulin like growth factor (IGF-1) is important to developing healthy bone density. Dietary nutrients and minerals are metabolized by the gut to then be converted to IGF-1 in the liver. This hormone then is transported to bone where it works in conjunction with other molecules to initiate osteoblasts. Remaining, or unused, IGF-1 is integrated into the bone matrix where it can later be released through osteoclast activity and subsequently increase osteoblast activity. Therefore, negating the pathways that usually cause one-sided bone loss.*

Serotonin and Melatonin

Several neurochemicals – neurotransmitters, neuropeptides, and neurohormones – are important to a consistent sleep/wake cycles. Of those, serotonin is one of the most well associated neurotransmitters with wake schedules. The biochemical processes of serotonin signaling are not fully understood, and in fact, it is quite confusing to researchers even today. However, what is known is that a high level of circulating serotonin is associated with wakefulness and low levels with sleepiness[xxiv]. Melatonin, the product of serotonin conversion, is often thought to induce sleepiness. This makes sense with what is currently understood about serotonin. Serotonin is converted into melatonin through the addition of an acetyl and methyl group (Figure 1). It is these structural conversions that allow for these two hormones to have such different roles in wake/sleep cycles.

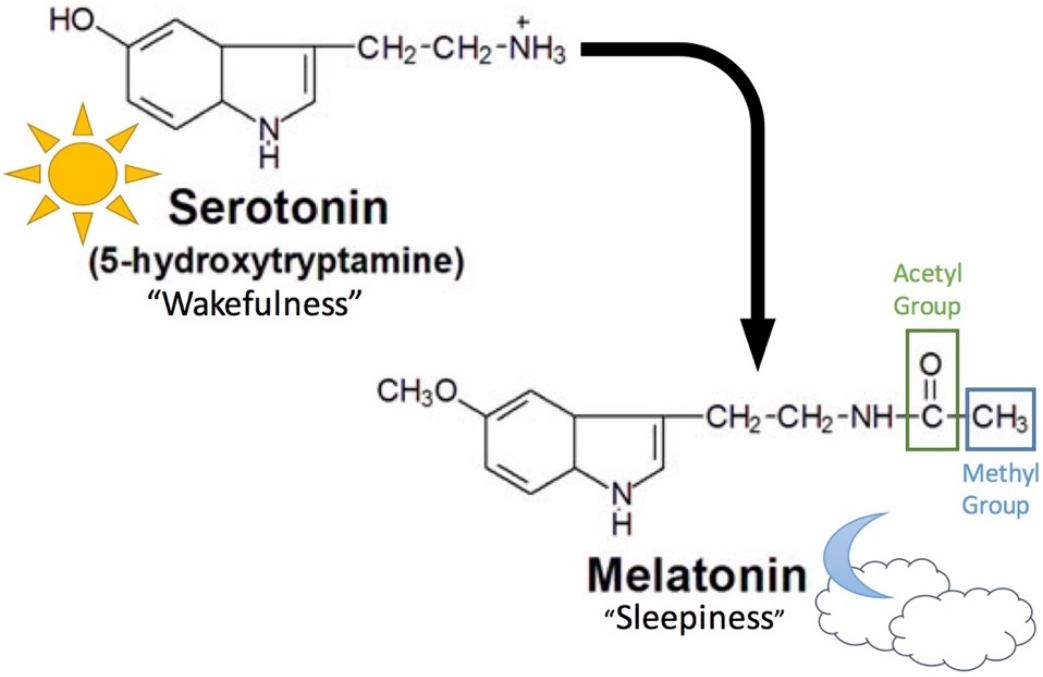


Figure 2. The conversion from serotonin to melatonin. There circadian rhythm is regulated, in part, by the conversion of these molecules. In the evening certain cells, osteocytes included, increase their receptors for melatonin. Therefore, the conversion of serotonin to melatonin is vital for processes within theses cells to be activated.

Serotonin has historically been believed to have a regulatory effect on bone mineral density. Serotonin receptors, which bind specifically to serotonin (5-hydroxytryptamine), have been linked to osteoclast, osteoblast, and osteocyte activity[xxvii]. Recently the mechanisms by which serotonin works to regulate bone metabolism, specifically in bone-resorption pathways, has been mapped in mouse models.[xxv] After eating a meal, signals from the stomach increase the Tryptophan hydroxylase-1 (TPH-1) activity in the small intestine. This increase in activity affects specialized neuroendocrine enterrocromaffin cells in the duodenum. The duodenum is the first and shortest part of the small intestine, which receives partially digested food and prepares it for absorption in the small intestine. It also induces serotonin production and releases it into the blood system. This system is therefore completely separate from the serotonin producing system in the brain. This gut-derived serotonin (GDS) is what inhibits bone formation. Osteoclast activity is still activated, and therefore bone is disproportionally resorbed without formation compensation. While the effect of serotonin on bone resorption is not yet completely understood, it has already started to bring clarity to the link between bone loss and clinical depression.

People experiencing symptoms of clinical depression also are at risk for decreased BMD.[xxvi] Selective Serotonin Reuptake Inhibitors (SSRIs) are commonly prescribed for psychiatric conditions, such as depression. The biochemical purpose of SSRIs is to inhibit 5-HTT receptors and block serotonin reuptake, thus causing an increase circulating serotonin. While being a very effective anti-depressant, this increase in serotonin contributes to lower BMD, a pathophysiological response that is most greatly affecting women.[xxvii] Therefore, bone loss is increasingly important to prevent in populations affected by depression and anxiety, and especially those which are already vulnerable—i.e. women. As mentioned earlier, college

students are experiencing increasing pressure both emotionally and academically. Depression and anxiety rates in college students are on the rise, as more students are seeking the services of university counseling centers.[xxviii] Understand the long-term impact that depression and prescription SSRIs can have on bone health and osteoporotic risk must become a greater priority.

Many factors influence bone mineralization, most notably genetics, hormone profiles, nutritional intake, physical activity level, and body composition. Some of these factors were demonstrated above. Most of the abovementioned factors have the potential to both, be changed by and enact change on sleep activity. Sleep and bone mineral content illustrate a complex web of interconnectivity. Therefore, it is necessary to understand how changes in sleep patterns can impact bone health. This is especially important in college students who are experiencing increased sleep insufficiency at an age where proper bone mineralization is a key determinant for PBM. To our knowledge, this study is one of the first to study effects of sleep on bone mineral density in college students. Therefore, we aim to determine whether shorter sleep duration (< 7 hours of sleep) and decreased sleep efficiency (< 85%) were indicative of lower BMD among college students between the ages of 18-25 years.

Materials & Methods

Study Sample

This study is a preliminary subset of a larger cross-sectional study which will ultimately include 50 college males and females, ages 18-25 years, living in and around the greater Phoenix metro area. Enrollment to date is 17 participants (14 females, 3 males; n=5, 1 and 11 Hispanic, Black and White, respectively) with a mean age of 20.8 ±1.7 years with a mean BMI of 22.9 ±3.2 kg/m^2.

Inclusion and Exclusion Criteria

To have been included in this study students should be English-speaking, attending a university during the semester of participation, between the ages 18-25, with Body Mass Index (BMI) within the normal to overweight range (18.5-29.9). will be eligible to participate in this study. Exclusion criteria included any history of eating and sleep disorders, malabsorption and autoimmune diseases, movement disorders (such as Restless Leg Syndrome), current high blood pressure and diabetes. Participants that did shift work during the month prior to and/or the month of the study were excluded. Participants were excluded if they had been taking any over-the-counter or prescribed sleep medications. Exclusion extended to participants who were taking medications known to affect bone such as diuretics, glucocorticoids, blood pressure medication, etc. However, even though recent research indicates that SSRIs affect bone mineral density, there are not yet validated enough trials to determine the extent of the impact. Therefore, participants that reported taking an SSRI (n=1) was not excluded from the study. Criteria also excluded any participants drinking excessive caffeine (>400mg per day[xxix]), or alcohol (women = 1 drink per day, men = 2 drinks per day[xxx]). Participants using calcium supplements, including calcium from multi-vitamins, were excluded from participating due to the important relationship between calcium and bone mineral density. If participants were pregnant, trying to become pregnant or lactating they were excluded according to DXA scan safety regulations.

Study Design

Participants were scheduled to meet with researchers at a clinical research facility in Downtown Phoenix, Arizona where they were screened for aforementioned inclusion/exclusion

criteria prior to the consenting process. This was completed individually and facilitated by study personnel who verbally asked participants to report their status on the criteria necessary to be included in the study. Following approval of eligibility, written informed consent was obtained in-person. To assure that participants understood the study procedures, they were asked to summarize the study in their own words prior to signing the consent document. Before signing, study personnel answered all remaining participant questions to assure that they were fully informed of study procedures. Once all of the participants' questions were answered, the participants signed the consent form. Each participant was provided with a copy of their consent form for their records.

The participants were asked to complete all study tasks within approximately one week's time. Participants were asked to complete a health history questionnaire as well as questionnaires discussing their dietary intake patterns, food-based calcium consumption, perceived stress levels, physical activity, and depression symptoms. During the study period, each participant had a DXA scan and a fasted blood draw (8.5 mL). The blood draw was stored for later analysis of biochemical markers of bone turnover. ActiWatch Spectrum Plus (Philips Respironics, Bend, OR) monitors were worn for seven consecutive days along with the completion of a seven-day Consensus Sleep Diary (CSD)[xxxi].

Demographics, Health Behaviors/History and Anthropometrics

Subjects' weight was measured on a standard scale to the nearest tenth of a kilogram. Height was assessed using a stadiometer and recorded to the nearest tenth of a centimeter. Body mass index (BMI) was then calculated as kg/m^2. Sociodemographic and health history data were self-reported on the health history questionnaire and included race, ethnicity, birth date (for calculating age at time of study participation), year in school, family health history and current medication and supplement use. Questions from the short international physical activity questionnaire (IPAQ) and perceived stress scale (PSS)were included in the health history questions to adjust statistical models for stress and regular physical activity which may influence sleep. Additionally, depressive symptoms and dietary intake patterns were assessed using the Center for Epidemiologic Studies Depression Scale and the 26-item Dietary Screener Questionnaire, respectively. Since calcium is known to have such a dramatic role on bone development, students also were asked to discuss their habitual dietary calcium consumption in the Calcium Intake questionnaire.

Bone Mineral Density Testing

Bone mineral density was assessed via Dual-energy X-ray Absorptiometry (DXA) (Lunar iDXA, GE Healthcare, Madison, WI). DXA is considered by many experts to be the practical gold standard and criterion method for measuring body composition[xxxii]. DXA is used instead of air displacement plethysmography (BODPOD) for a higher degree of accuracy for predicting bone mineral density and Lunar iDXA's ability to accurately measure and track changes in bone density[xxxiii]. Tracking changes in bone composition is a main outcome measure, therefore the use of a device with high precision (DXA) is essential to assess these changes.

Before completing the DXA female participants were asked to confirm their pregnancy status by taking a urine pregnancy test. Once confirmed that the participant was not pregnant the lab technician proceeded with the DXA. During the DXA participants were asked to lie flat on their back, face up, on a padded table for at least 7 minutes. The scanner arm of the DXA machine passed over the entire body, from head to foot. The scanner did not enclose the

participant nor did the scanner arm touch them. Participants could wear regular clothing (no metal allowed). If any of their clothing contained metal, they were given paper medical gowns to wear during the scan. A licensed radiologic technologist performed all DXA scans at the clinical research facility.

Sleep Testing

Participants were orientated on how to complete a seven-night sleep diary while simultaneously wearing a wrist-worn sensor to track sleep and activity levels. The Consensus Sleep Diary (CSD)[xxvii] used in this study was the shortened version, that also assessed naps. The wrist worn sensor that was used was the ActiWatch Spectrum from Phillips Respironics Inc (Bend, OR). Data gathered via ActiWatch and CSD were used to determine sleep duration (<7 hours, 7-8 hours, 8-9 hours, or >9 hours) and sleep efficiency (<85% or >85%+), the other sleep variables included were wake after sleep onset (WASO), sleep onset latency, and number of awakenings.

Data Analysis

Data analyses involved the assessment of BMD distributions and sleep habits (sleep duration, sleep efficiency, wake after sleep onset, sleep onset latency, and average number of awakenings) among college-aged males and females. Bone mineral density and content measures of the lumbar spine, and total hip was evaluated relative to self-reported sleep duration data collected via the CES-D. Wilcoxon rank-sum, regression, and correlation analyses were used to evaluate different aspects of these relationships while allowing for covariate adjustments (e.g. demographics, weight status, stress, physical activity and dietary intake variables). All analyses were performed using IBM SPSS Statistic 24 software provided by Arizona State University.

Statistical Power

The proposed study design was adequately powered to see connections between sleep patterns and bone mineral density. For this preliminary look, 16 participants were screened and included in the study. The amount of participants did not provide enough degrees of freedom to test all possible covariates against. In this small dataset potential non-linear relationships were not yet retrievable. Larger cohorts are necessary to characterize potential associations between bone health, sleep duration, and sleep efficiency while controlling for covariates such as calcium intake, gender, race, and clinical depression.

Data Management and Confidentiality

To maintain subject confidentiality, all participants were assigned a random number or letter sequence which was used for study identification. Questionnaire data generated was de-identified using the subject identification codes as soon as they were received from participants. All blood sample containers were labeled with the subject identifier before being distributed so that names or personal information was not needed on containers. Once the data was received in the lab, only the code numbers were used to refer to subject information. Consent forms and any identifying information, including paper copies, were kept in a separate file. All de-identified data—including ActiWatch, DXA, sleep diary, diet and demographic data used for statistical analyses—were saved on a secure ASU server, within a shared collaborator folder so that researchers could share access to the data as needed. Data will be retained for 10 years and all paper forms will be shredded and digital files erased.

Results

Characteristics of Participants

A total of 16 participants that completed the study in full were included in this analysis, of those there were 14 female and 3 male participants. The mean age of the participant sample was 20.8 ±1.7 years of age, the youngest participant was 18 years and the oldest was 24 years old. The mean height of the sample size was 166.48 cm ±6.7 and the average weight was 63.60 kg ±11.5. These participants had a mean body mass index (BMI), which was derived from height (cm) and weight (kg) of participants, of 22.9 ±3.2 kg/m^2.

Descriptive Statistics	Minimum	Maximum	Mean ±SD
Age (years)	18	24	20.8±1.7
Height (cm)	156.00	180.20	166.5±6.7
Weight (kg)	53.50	87.80	63.6±11.5
Calculated Body Mass Index (kg/m^2)	19.30	32.80	22.9±3.2

Table 1. *Descriptive statistics of participant cohort. The table shows that the average students fell within the inclusion criteria intended for this study; demonstrating a cohort of students between the ages of 18-25 years and having a body mass index measurement between the limits of 18.5-29.9 kg/m^2.*

Sleep patterns of the patterns for participants were analyzed in accordance with NSF[xvi] suggested healthy sleep duration range for 18-25 year olds; 7-9 hours of sleep per night (420-540 minutes). Minimum total sleep range 87.3 – 453.3 minutes, bordering and extending well below the recommended minimum. Maximum total sleep range 433.3 – 693.3 minutes. Average daily sleep time was 437.5 ± 43.1 minutes, and the daily sleep time range among participants was 372.5 – 509.4 minutes. Appropriate sleep efficiency is defined as being >85% sleep efficiency(cite). The participant cohort demonstrated a mean sleep efficiency (Eq. 1) was 87.4% ±4.3 minutes, with a total range of 75.4-93.4% efficiency. Sleep efficiency was not correlated to sleep duration by any statistical significance (Graph 1).

SLEEP MEASUREMENTS (mean values ±SD)	All	Male	Female
ActiGraph Data. Minimum Total Sleep Time (minutes)	334.32±81.6	356.67±43.0	329.53±88.1
ActiGraph Data. Maximum Total Sleep Time (minutes)	553.10±73.7	545.67±65.4	554.69±77.5
ActiGraph Data. Average Total Sleep Time (minutes)	437.49±43.1	431.68±42.2	438.73±44.7
ActiGraph Data. Minimum Sleep Efficiency (%)	79.31±14.4	82.37±2.5	78.65±15.9
ActiGraph Data. Maximum Sleep Efficiency (%)	92.36±2.9	92.99±3.8	92.22±2.8
ActiGraph Data. Average Sleep Efficiency (%)	87.38±4.3	87.71±3.0	87.30±4.6
ActiGraph Data. Average Wake After Sleep Onset (minutes)*	34.53±10.5	30.82±11.3	35.33±10.6
ActiGraph Data. Average Number of Awakenings	32.13±6.4	32.00±8.5	32.16±6.2
ActiGraph Data. Average Sleep Onset Latency (minutes)*	16.73±12.3	18.00±3.4	16.46±13.5

Table 2. *Characteristics of sleep in study cohort. The average variables measured in this study were total sleep time, sleep efficiency, wake after sleep onset, number of awakenings, and sleep onset latency. These variables were further separated into male and female categories, to better understand the differences that sex can have on sleep variables. The categories labeled * indicate there was non-normal distribution of these variables. Therefore, median values were taken in table 3.*

SLEEP MEASUREMENTS (median values)*	All	Male	Female
ActiGraph Data. Average Wake After Sleep Onset (minutes)	33.57	32.58	34.00

ActiGraph Data. Average Sleep Onset Latency (minutes)	14.13	19.82	11.75

Table 3. *The measurement of average wake after sleep onset and average sleep onset latency were found to have non-normal distribution. Therefore, they were measured through their median values to accurately represent their distribution.*

Bone mineral density was measured at both hips (total hip) and the lumbar spine (T1-T4), and was subsequently characterized with an age matched Z-score. The mean BMD for the hips was 1.06 ±0.14 g/cm^2, with a range of 0.81-1.28 g/cm^2. The age-matched Z-score mean for total hip density was 0.31 ±0.96, with a range of -1.6-2.1. (IQR: 0.13, 0.98). The mean BMD for lumbar spine was 1.24 ±0.12 with a range of 0.92-1.43 g/cm^2. The age-matched Z-score mean was 0.53 (IQR: -2.25-1.55).

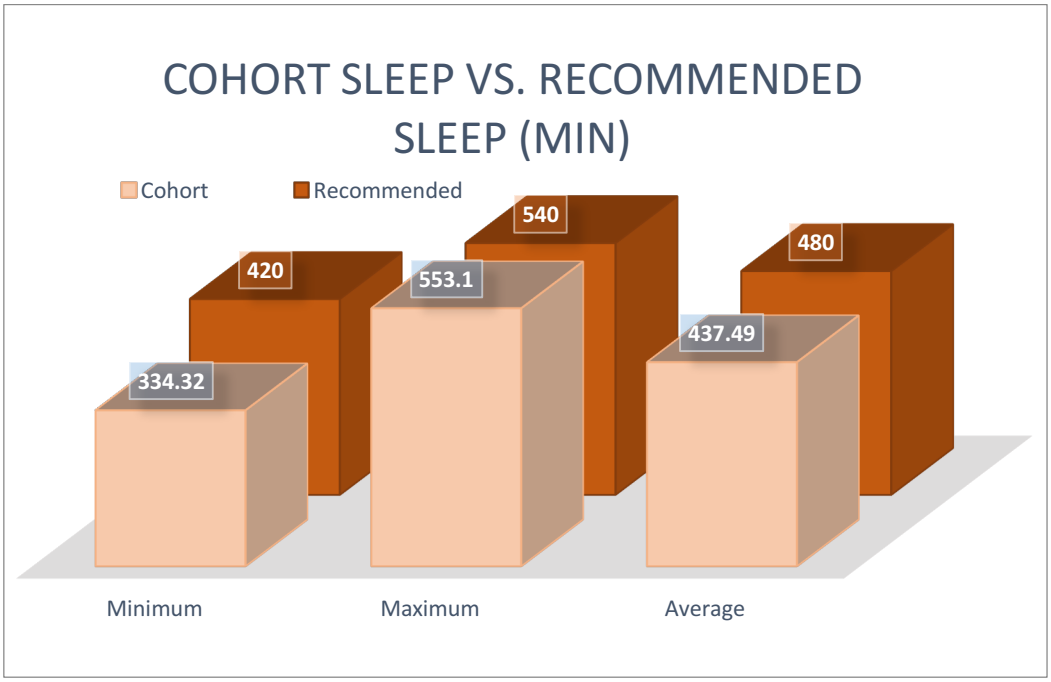
Upon further analysis, the measurements for BMD and corresponding Z-scores were separated between male and female cohorts. The mean BMD for the total hips in female participants (n=14) was 1.04±0.13 g/cm^2. The age-matched Z-Score was 0.267. The female cohorts demonstrated a mean lumbar spine BMD of 1.22±0.12 g/cm^2, the age-matched Z-Score for the spine was 0.34 (IQR: 0.100, 1.000).

For male participants (n=3) the BMD of the total hip was 1.18±0.11 g/cm^2, and a Z-Score at the hip of 0.77. The male sample size was not large enough to determine interquartile ranges (IGR). The mean BMD of the spine in the males was 1.31±0.12 g/cm^2. The age-matched Z-score of the lumbar spine was 0.80.

Calcium Intake (Quality Food Score)	Poor	14
	Fair	2
	Good	1
	Excellent	0
CESD. Depression Score Total	Depressed	3
	Not depressed	14

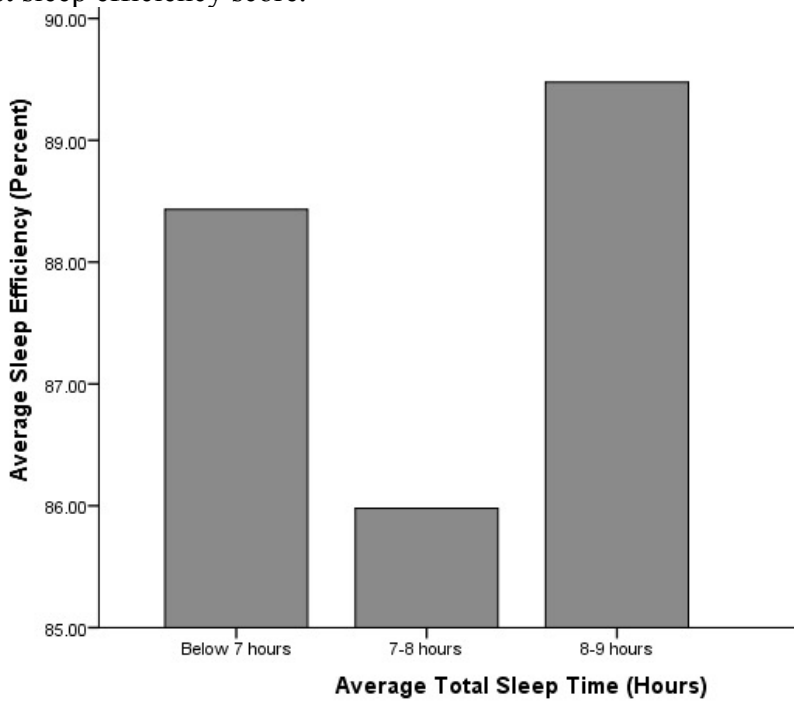
Table 4. *Characterization of confounding variables in study cohort. Measured through calcium intake survey and depression score survey, respectively. These variables indicate the prevalence of calcium intake and depression in the study population.*

When comparing the total average sleep of these participants with that of the previously mentioned recommended average (7-9 hours), it can be seen that the average minimum sleep duration falls below the lower limit of recommended sleep, and the average maximum sleep duration lies above the upper limit of the recommended duration. However, these variables are best demonstrated by the median value of total sleep. The average sleep duration of participants fell just barely above the lower limit of recommended sleep (437.49 minutes, 420 minutes; respectively). The average sleep duration, is therefore, unsettlingly low among the population studied.



Graph 1. *The average amount of sleep that students received was compared the National Sleep Foundation's recommended amount for young adults (ages 18-25). The average duration of sleep of the study population lies on the lower end of the accepted range.*

Further analysis of sleep duration and sleep efficiency shows that the two variables may not be intimately linked to one another. Sleep data was groups that slept below 7 hours, 7-8 hours, and above 8 hours per night. This data demonstrated that all three groups were averaging above 85% sleep efficiency per night. However, it was the group that slept 7-8 hours that received the lowest sleep efficiency score.



Graph 2. *Average total sleep time (hours) demonstrated no correlation to average sleep efficiency (percent) in the study cohort. The participants that slept 8-9 hours on average had the highest overall sleep efficiency, while those that slept between 7-8 hours had the lowest sleep efficiency.*

Discussion

This study was designed as a cross-sectional analysis assessing the potential association between sleep duration and sleep efficiency on the density of bone in college aged males and females. The aims of looking at this cohort, which will ultimately be expanded to a larger study group, were to determine if any associations are detectable within this small dataset. Early detection of BMD loss can lead to early prevention methods in individual cases and population wide public health efforts.

In regards to the study hypothesis, no significant correlations were found between sleep duration and BMD of the total hip or lumbar spine, nor were there significant correlations between sleep efficiency and BMD at the measured sites. When other measured sleep variables were taken into account, wake after sleep onset (WASO) was found to have a positive association with BMD at both sites. However, these findings were not statistically significant.

When separating the cohort into male and female participants, and assessing the measured sleep and bone variables there were not many differences to be extrapolated on. Interesting to note was that females had a longer sleep duration (438.73±44.7) than males (431.68±42.2), but had lower percentage sleep efficiency (87.71 ±3.0; 87.30 ±4.6, respectively). While the cohort numbers are not balanced enough to definitively make claims about the difference in male and female sleep patterns, it will be something important to note when assessing the larger study. As previously mentioned, women have much higher risk of bone fracture in older age and historically higher rates of osteopenia and osteoporosis. Therefore, this disconnect between the relationship between sleep duration and sleep efficiency in women could lead to interesting further studies.

Sleep efficiency increases by 2 and 3.5% in participants who sleep less than 7 hours and over 8 hours, respectively. The lowest average sleep efficiency was seen in the participants who slept between 7-8 hours on average. The connection between sleep efficiency and sleep duration is a critical component of understanding how college students sleep. If they are sleeping within the recommended hours (7-9 hours) but most of that time is spent restless, awake, or otherwise then sleep efficiency may be a more accurate sleep variable to measure. The NSF does a quarterly analysis of how well Americans are sleeping and splits it into four categories. The numbers between quarterly reports seems to be generally consistent, but there is a big gap sleep duration and sleep quality. While 77% of people (on average per year) are receiving appropriate amounts of sleep as recommended by the NSF, only about 67% of those people are receiving good quality sleep (above 85%).

Almost 99% of calcium in the body is stored in bones[xxxiv]. This bountiful mineral, in fact, it is the most abundant mineral found in the human body, is necessary for many functions not the least of which is bone strength. When dietary calcium intake is insufficiently low, which can sometimes result from poor absorption, the body then uses the reserves of Ca^{2+} in bones to continue metabolic function within the body. This has continually been demonstrated and validated in populations of all ages. The cohort in this study reported underwhelming low calcium intake. Surveys show that 82% of the population was receiving poor amounts of calcium intake, with only 2 and 1 participants receiving fair and good amounts of calcium, respectively. Improper dietary calcium intake has been at the forefront of public health efforts for many years,

and it seems this study provides resounding evidence to continue to push for increased calcium intake in college students.

Conclusion

While SBMD and sleep weren't correlated there were still markers in this cohort of students which indicate insufficient sleep, which validates the data previously found discussing the poor sleep habits demonstrated in college students. There were also strong indications of low calcium intake in the diets of these students, suggesting a potential impact in long-term bone health. Although there was no correlation found between these two factors, the impacts of these variables independent of one another still demonstrate a potential public health risk. As these people age they become more vulnerable to loss of bone density and chronic diseases, which are correlated to BMD and sleep sufficiency, respectively.

Potential interventions for both of these variables, sleep and bone health, could be beneficial for universities to explore. Offering sleep hygiene classes, starting classes later in the day, giving students the resources to enact pre-sleep rituals, and encouraging students to us blue light blocking apps on their electric devices could help to increase the efficiency and duration that students are sleeping. Dining halls and on-campus advertisements could bring awareness to their calcium intake needs. Specifically, it could be important to educate students on the fact that even as 18-25 year old, their bones are still growing and regenerating. Therefore, they can still benefit from increasing their calcium intake. There are a lot of important impacts that such interventions could lead to as the current and future generations of college students grow older.

This small dataset, while not suggestive of associations between BMD of total hip and spine with measurements of sleep, still has the potential to show significant findings with a larger cohort. Further work is needed in to characterize potential associations between bone health and sleep duration and efficiency in larger participant cohorts. This small subset did not provide enough degrees of freedom to assess these associations while controlling for covariates. Potential non-linear relationships were not yet detectable in this small dataset. The larger study will allow for these relationships to be accounted for and their significance better understood. If this data remains insignificant even within the larger cohort, a longitudinal study might be more beneficial to understand when exactly sleep does become significant to bone mineralization. It also could be beneficial to assess the inclusion and exclusion criteria of this study more critically. When recruiting a population of students, it may be necessary to look at students that may not have good bone health. Measuring their sleep habits over time, could lead us to better understand the impacts that sleep may have on their bone health.

---

[i] Institute of Medicine (US) Committee on Sleep Medicine and Research; Colten HR, Altevogt BM, editors. *Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem.* Washington (DC): National Academies Press. 2006. 3. Available from: https://www.ncbi.nlm.nih.gov/books/NBK19961/

[ii] Walch, O., Cochran, A., Forger, D. A global quantification of "normal" sleep schedules using smartphone data. Science Advances. 2016.

[iii] Kim, N., Choi, H.-R., Kim, S.-W., Kim, B.-S., Won, C.-W., & Kim, S.-Y. (2014). Association between Bone Mineral Density and Sleep Duration in the Korean Elderly Population. *Korean Journal of Family Medicine*, *35*(2), 90–97. http://doi.org.ezproxy1.lib.asu.edu/10.4082/kjfm.2014.35.2.90

[iv] Fu, Xiaohua; Zhao, Xinya; Lu, Han; et al. Association between sleep duration and bone mineral density in Chinese women. Bone 2011. 49:1062-1066. Retrieved from: http://dx.doi.org/10.1016/j.bone.2011.08.008

[v] Sforza, E., Thomas, T., Barthélémy, J.-C., Collet, P., & Roche, F. (2013). Obstructive Sleep Apnea is Associated with Preserved Bone Mineral Density in Healthy Elderly Subjects. *Sleep*, *36*(10), 1509–1515. http://doi.org.ezproxy1.lib.asu.edu/10.5665/sleep.3046

[vii] Wang, K., Wu, Y., Yang, Y. et al. Endocrine (2015) 49: 538. doi:10.1007/s12020-014-0493-6
[viii] Kobayashi, D., Takahashi, O., Deshpande, G.A. et al. Association between osteoporosis and sleep duration in healthy middle-aged and elderly adults: a large-scale, cross-sectional study in Japan. *Sleeping and Breathing.* (2012) 16: 579. doi:10.1007/s11325-011-0545-6
[ix] Carskadon, Mary A. Sleep in Adolescents: The Perfect Storm. *Pediatric clinics of North America*. 2011. 58(3): 637–647.
[x] Carskadon, M., Wolfson, A., et al. Adolescent Sleep Patterns, Circadian Timing, and Sleepiness at a Transition to Early School Days. *Sleep.* 1998. 21(8):871-881.
[xi] White, A., Buboltz, W., Igou, F. Mobile Phone Use and Sleep Quality and Length in College Students. *International Journal for Humanities and Social Science.* 1(18): 51-58.
[xii] Misra, R., McKean, M. College students' academic stress and its relation to their anxiety, time management, and leisure satisfaction. American Journal of Health Studies. 2000. 16(1):41-51.
[xiii] Lund, H.; Reider, B.; Whiting, A.; Prichard R. Patterns and Predictors of Disturbed Sleep in a Large Population of College Students. *Journal of Adolescent Health*. 2010. 46: 124-132
[xiv] Buboltz, W., Brown, F., Soper, B. Sleep Habits and Patterns of College Students: A Preliminary Study. 2001. *Journal of Ameican College Health.* 50(3): 131-135.
[xv] "Excessive Sleepiness" *The National Sleep Foundation.* 2017.
[xvi] Saggese, G., Baroncelli, GI., Bertelloni, S. Puberty and Bone Development. *Best Pract Res Clin Endocrinol Metab.* 2002 Mar;16(1):53-64.
[xvii] Kroger, H., Kotaniemi, A., Kroger, L., Alhava, E. Development of bone mass and bone density of the spine and femoral neck—a prospective study of 65 children and adolescents. *Bone Miner.* 1993 Dec;23(3)171-82.
[xviii] *Pediatric Bone: Biology & Diseases (Second Edition). 2011.*
[xix] Burckhardt, P., Michel, C. The peak bone mass concept. *Clin Rheumatol*. 1982. 8(2):16-21.
[xx] Theintz, G., Buchs, B., et al. Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. *J Clin Endocrinol Metab.* 1992. 75(4): 1060-5.
[xxi] Saggese, G., Baroncelli, GI., Bertelloni, S. Puberty and Bone Development. *Best Pract Res Clin Endocrinol Metab.* 2002 Mar;16(1):53-64.
[xxii] Yakar, S., Rosen, C. J., Beamer, W. G., Ackert-Bicknell, C. L., Wu, Y., Liu, J.-L., et al. (2002). Circulating levels of IGF-1 directly regulate bone growth and density. *The Journal of Clinical Investigation*, *110*(6), 771–781. http://doi.org/10.1172/JCI15463
[xxiii] Bonjour, J.P., Chevalley, T., et al. Importance and relevance of peak bone mass in the prevalence of osteoporosis. Salud Publica Mex. 2009. 51:Suppl 1:S5-17.
[xxiv] Portas, C., Bjorvatn, B., Ursin, R. Serotonin and the sleep/wake cycle: special emphasis on microdialysis studies. *Progress in Neurobiology*. 1998. 60(2000): 13-35.
[xxv] Yadav, V., et al. Pharmacological inhibition of gut-derived serotonin synthesis is a potential bone anabolic treatment for osteoporosis. *Nature Medicine.* 2010. 16: 308-312.
[xxvi] Mezuk, B., Eaton, W., Golden S., Lee, H. Depression, antidepressants, and bone mineral density in population-based cohort. *J Gerontol A Biol Med Sci.* 2008. 63(12): 1410-15.
[xxvii] Sansone, R., Sansone, L. SSRIs: Bad to the Bone. *Innov Clin Neurosci*. 2012. 9(7-8): 42-47.
[xxviii] Novotney, A. Students Under Pressure. *American Physiological Association.* 2014. 45(8): 36.
[xxix] Caffeine: EFSA consults on draft assessment. *European Food Safety Authority.* January 15, 2015. Retrieved October 17, 2016, from http://www.efsa.europa.eu/en/press/news/150115
[xxx] Alcohol and Public Health. *Center for Disease Control and Prevention.* August 6, 2016. Retrieved October 17, 2016, from http://www.cdc.gov/alcohol/faqs.htm
[xxxi] Carney, C., Buysse, D., Ancoli-Israel, S., et al. The Consensus Sleep Diary: Standardizing Prospective Sleep Self-Monitoring. *Sleep.* 2012 Feb 1. 35(2): 287-302.
[xxxii] St-Onge, MP, Wang, J, Shen, W et al. Dual-energy x-ray absorbtiometry-measured lean soft tissue mass: differing relation to body cell mass across the adult life span. *J Gerontol A Biol Sci Med Sci*. 2004. 59(8): 796-800.
[xxxiii] Micklesfield, LK et al. Dual-energy X-ray performs as well as clinical CT for the measurement of visceral fat. *Obesity (Silver Spring)*. 2012: 20(5):1109-1114.

[xxxiv] Office of Dietary Supplements - Dietary Supplement Fact Sheet: Calcium. 2016, Nov. from https://ods.od.nih.gov/factsheets/Calcium-HealthProfessional/