The Impact of Physical Activity and Sleep Patterns on Bone Turnover Markers in College Students

by

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ABSTRACT

College students are a niche of young adults, characterized by abnormal sleeping habits and inactive lifestyles. Many students entering college are as young as 18 years old and graduate by 22 years old, a window of time in which their bones are still accruing mineral. The purpose of this cross-sectional study was to determine whether sleep patterns and physical activity observed in college students (N= 52) 18-25 years old at Arizona State University influenced bone biomarkers, osteocalcin (OC) and N-terminal telopeptide of type 1 collagen (NTX-1) concentrations. Students completed various dietary and health history questionnaires including the International Physical Activity Questionnaire short form. Students wore an actigraphy watch for 7 consecutive nights to record sleep events including total sleep time, sleep onset latency and wake after sleep onset. Total sleep time had a significant, negative correlation with OC (r = -0.298, p-value =0.036) while sleep onset latency had a significant, positive correlation with NTX-1 serum concentration (r = 0.293, p-value = 0.037). Despite correlational findings, only sleep percent was found to be significant (beta coefficient = 0.271 p-value = 0.788) among all the sleep components assessed, after adjusting for gender, race, BMI and calcium intake in multivariate regression models. Physical activity alone was not associated with either bone biomarker. Physical activity*sleep onset latency interactions were significantly correlated with osteocalcin (r = 0.308, p-value =0.006) and NTX-1 (r = 0.286, p-value = 0.042) serum concentrations. Sleep percent*physical activity interactions were significantly correlated with osteocalcin (r = 0.280, p-value = 0.049) but not with NTX-1 serum concentrations. Interaction effects were no longer significant after adjusting for covariates in the regression models. While sleep percent was a

significant component in the regression model for NTX-1, it was not clinically significant. Overall, sleep patterns and physical activity did not explain OC and NTX-1 serum concentrations in college students 18-25 years old. Future studies may need to consider objective physical activity devices including accelerometers to measure activity levels. At this time, college students should review sleep and physical activity recommendations to ensure optimal healthy habits are practiced.

DEDICATION

I would like to dedicate my work to my family whom have been there for me through the most stressful days and nights in the Nutrition MS program. I would like to thank my friends who supported me and took an interest in the work I accomplished during my time in the program. I would like to thank fellow lab members Carmen Ortega, Jessie King, Katy Argo, and Kiley Vander Wyst for being there when I needed a lending hand. I would like to give a great thanks to Dr. Corrie Whisner and Dr. Jared Dickinson for being great mentors and helping me grow as a researcher.

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CHAPTER 1

INTRODUCTION

Overview

By 2020, one in two Americans over the age of 50 years will be diagnosed or at risk of osteoporosis.[1,2,3] 33.6 million older American adults are diagnosed with low bone mass which is a risk factor to this disease.[1,2] A total of 1.5 million osteoporotic patients may experience bone fractures in the hip and wrists, costing the US about 10-22 billion dollars from hospitalization to outpatient care.[4] Such fractures are associated with physical limitations and reduced quality of life. However, stronger bones may prevent such fractures.[5] Bone mineral density (BMD) is a clinical measure to assess the strength of one's skeleton.[6] Bone turnover markers (BTMs) including Osteocalcin (OC), a bone mineralization marker and N-terminal telopeptide collagen type 1 (NTX-1), a bone demineralization marker, reflect how bone is remodeled to maintain its strength.[6, 7] Bone has been noted to be influenced by genetic factors including gender and ethnicity.[8] Yet small changes in bone have been associated with lifestyle factors including sleep patterns and physical activity.[9, 10]

Physical activity benefits bone health by enabling bone mineralization in men and women from adolescence to older age. As adults become less active, they were associated with lower bone mineral density (BMD) than those who maintained moderate to vigorously activity. [11, 12, 13]

Resistance and aerobic exercise were observed to increase serum osteocalcin (8.5%) and bone specific alkaline phosphatase (15.8%) concentrations, markers of bone formation, indicating improvement in bone mineralization from these physical

activities.[11] Adults who regularly engaged in physical activity between 18 to 30 years of age had greater bone mineral density than their less active counterparts.[12] While there is much knowledge in the relationship of exercise and bone, there are many gaps and differences within each assessment that need to confirmed and filled to create a full understanding.

Bone mineralization may also be impacted by the amount of sleep an adult obtains at night. Obstructive sleep apnea, a sleep disorder was associated with higher concentrations of bone resorption marker C-terminal telopeptide of type 1 collagen (CTX).[13] This suggested that bone turnover leans towards bone resorption when sleep is disrupted. Adults are recommended to sleep 7-8 hours, but they only sleep on average 6 hours. Women 45 years and older who slept less than 6 hours had lower total BMD than those who slept at least 8 hours.[14,15] Due to their poor quality of sleep, many older adults reported daytime sleepiness and higher frequency of naps compared to those with better sleep quality. Long naps (at least 60 minutes) were associated with lower total BMD in men and women ages 50 years and older.[16,17] Sleep has become an emerging factor in bone health but there is a need to further assess the direct relationship.

Physical activity and sleep seem to be linked with one another; physically active individuals were associated with better sleep quality than their sedentary counterparts.[18] Adults ages 18-65 years who were moderately active, self-reported better sleep and longer total sleep time at night than those who were sedentary.[18,19] Exercise (moderate-to-vigorous) in the evening was associated with excellent sleep efficiency as well as deeper sleep.[20,21] However, the interaction between physical activity and sleep patterns have not been clearly evaluated with regard to bone health.

In the present library of literature, researchers focused on adults 45 years and older when assessing the association between sleep and bone health. This may be due to the risks for osteoporosis increasing by age 50 which coincides with hormonal changes seen at this stage of life.[22] Yet, observing these connections before perimenopause or middle-age has not been done. Young adults between 18-25 years are still undergoing bone mineralization which reaches a peak near age 30.[23] This serves as a crucial window of opportunity for young adults to assure that they reach their genetic potential for peak bone mass and delay the onset of osteoporosis later in life.

College students are observed to be a unique population among young adults, with 64% experiencing abnormal sleep patterns due to shared sleeping areas, shift work and high levels of stress.[24] Lack of physical activity has also been noted in college students as a problem of epidemic proportions.[25] The present study will fill this gap in the literature by examining whether sleep patterns and physical activity interact to predict markers of bone turnover in college students 18-25 years old.

The Study Purpose

The primary aim of this study was to observe the relationship of both physical activity and sleep on bone health marker (OC and NTX-1) serum concentrations in 18-25-year-old, male and female college students.

Research Questions and Hypotheses

Research question 1

Is there a relationship between physical activity and OC serum concentrations in male and female, 18-25-year-old college students?

Hypothesis 1

Physical activity as MET-minutes per week will have a positive relationship with osteocalcin serum concentrations in male and female, 18-25-year-old college students, after adjusting for covariates.

Research question 2

Is there a relationship between sleep patterns and OC serum concentrations in male and female, 18-25-year-old college students?

Hypothesis 2a

Sleep onset latency will have a negative relationship with OC serum concentrations in male and female, 18-25-year-old college students, after adjusting for covariates.

Hypothesis 2b

Total sleep time will have a positive relationship with OC serum concentrations in male and female, 18-25-year-old college students, after adjusting for covariates.

Hypothesis 2c

Wake after sleep onset will have a negative relationship with OC serum concentrations in male and female, 18-25-year-old college students, after adjusting for covariates.

Hypothesis 2d

Sleep percent will have a positive relationship with OC serum concentrations in male and female, 18-25-year-old college students, after adjusting for covariates.

Research question 3

Is there an interaction between physical activity and sleep patterns on OC serum concentrations in male and female, 18-25-year-old college students?

Hypothesis 3a

Physical activity as MET-minutes per week and wake after sleep onset will interact to influence on OC serum concentrations in male and female, 18-25-year-old college students.

Hypothesis 3b

Physical activity as MET-minutes per week and total sleep time will interact to influence on OC serum concentrations in male and female, 18-25-year-old college students.

Hypothesis 3c

Physical activity as MET-minutes per week and sleep onset latency will interact to influence on OC serum concentrations in male and female, 18-25-year-old college students.

Hypothesis 3d

Physical activity as MET-minutes per week and sleep percent will interact to influence on OC serum concentrations in male and female, 18-25-year-old college students.

Research question 4

Is there a relationship between physical activity and NTX-1 serum concentrations in male and female, 18-25-year-old college students?

Hypothesis 4

Physical activity as MET-minutes per week will have a negative relationship with NTX-1 serum concentrations in male and female, 18-25-year-old college students, after adjusting for covariates.

Research question 5

Is there a relationship between sleep patterns and NTX-1 serum concentrations in male and female, 18-25-year-old college students?

Hypothesis 5a

Sleep percent will have a negative relationship with NTX-1 serum concentrations in male and female, 18-25-year-old college students, after adjusting for covariates.

Hypothesis 5b

Total sleep time will have a negative relationship with NTX-1 serum concentrations in male and female, 18-25-year-old college students, after adjusting for covariates.

Hypothesis 5c

Sleep onset latency will have a positive relationship with NTX-1 serum concentrations in male and female, 18-25-year-old college students, after adjusting for covariates.

Hypothesis 5d

Wake after sleep onset will have a positive relationship with NTX-1 serum concentrations in male and female, 18-25-year-old college students, after adjusting for covariates.

Research question 6

Is there an interaction between physical activity and sleep pattern variables on NTX-1 serum concentrations in male and female, 18-25-year-old college students?

Hypothesis 6a

Physical activity as MET-minutes per week and wake after sleep onset will interact to influence on NTX-1 serum concentrations in male and female, 18-25-year-old college students.

Hypothesis 6b

Physical activity as MET-minutes per week and total sleep time will interact to influence on NTX-1 serum concentrations in male and female, 18-25-year-old college students.

Hypothesis 6c

Physical activity as MET-minutes per week and sleep onset latency will interact to influence on NTX-1 serum concentrations in male and female, 18-25-year-old college students.

Hypothesis 6d

Physical activity as MET-minutes per week and sleep percent will interact to influence on NTX-1 serum concentrations in male and female, 18-25-year-old college students.

Definition of terms

Osteoporosis: a skeletal disorder that is characterized by low bone mass and decreased skeletal strength.[26] This disease may increase risks of falling and bone fractures.[26]

Bone mineral density (BMD): measured using a dual x-ray absorptiometry machine, the amount of mineral content (BMC) per square centimeter of bone is calculated to obtain BMD (g/cm^2).

Bone mineral content (BMC): using a dual x-ray absorptiometry machine, the amount of light that passes through a bone is measured in proportion to the amount blocked by mineral structure to calculate mineral content (grams).[27]

Volumetric bone mineral density (vBMD): a dual x-ray absorptiometry is used to scan the width, height and depth of bone in addition to BMD scan to calculate an overall volumetric density of bone (g/cm^3).[27]

Physical limitations: difficulty in performing the following actions; walking a quarter of a mile; walking up 10 steps without rest; standing for more than 2 hours and lifting or carrying an object at least 10 lbs. heavy.[5]

Osteocalcin (OC): calcium binding peptide that is secreted by osteoblasts and later ossifies into the bone matrix. This bone biomarker is used to indicate the level of bone mineralization within the blood.[28,29]

N-telopeptide of type 1 collagen (NTX-1): telopeptides from mature type 1 collagen that are cleaved off during bone resorption. Measurement of these peptides can be used to indicate the level of bone demineralization within the blood.[28,29]

Total sleep time (TST): The time in minutes for which an individual is sleeping at night or during a nap. This is calculated from sleep onset to sleep offset (final awakening).[30]

Sleep percent (SP): the percentage takes into account sleep period, time between sleep onset and offset which is calculated as total sleep time divided by total sleep period multiplied by 100.[31]

Sleep onset latency (SOL): The time in minutes that it takes for an individual to fall asleep. This is calculated from the time the individual turns off the light to go to sleep to the time they fall asleep.[32]

Wake after sleep onset (WASO): The total minutes that an individual is awake after sleep onset. This is the sum of all minutes that an individual is awoken and remains awake during a bout of sleep and before their intended sleep offset.[32]

CHAPTER 2

LITERATURE REVIEW

Osteoporosis is a public health problem in the United States.

One in two women and one in four men will experience a fracture due to osteoporosis in their lifetime.[3] Among the 2010 US population, about 10 million Americans were reported to have osteoporosis.[1,33] It costs the United States $10-22 billion dollars to treat bone fractures caused by osteoporosis and by 2025 it is projected to cost the US $25 billion.[1,33] Medicare patients accrue about $25,906 in expenses to treat their osteoporosis per year.[34] These costs are deemed as an economical burden to the rest of the American population.[33] Osteoporotic patients are at risk of bone fractures, with hip, wrist and pelvic fractures among the most prevalent.[8] Within the older adult US population, white (non-Hispanics) men and women accounted for the most fractures, indicating that race may play a role in bone health.[35] One year after a hip fracture, patients reported not being able to walk without aide and lacked confidence in completing daily tasks.[2,35,36] Such limitations result in osteoporotic patients moving to assisted living housing including nursing homes, which can decrease quality of life.[36] However, osteoporosis is not an inevitable disease and can be prevented through healthy behaviors. The aim of this review is to describe the physiology of bone tissue, external factors that influence bone mass and whether college students are a vulnerable group for behaviors that influence bone health later in life.

Bone physiology

Bone is made up of 30% organic and 70% inorganic material creating a versatile bone matrix within the skeletal system.[37] The organic component includes type 1

collagen, glycoproteins and proteoglycans that contribute to the extracellular matrix.[26,37,38] Inorganic components contribute to the structure of bone tissue as mineral salts for hydroxyapatite crystal formation; this mineral component is crucial to withstand physical force.[37,39]

Within the skeleton, there are two main osseous tissues, cortical and trabecular bone.[23, 37, 38] Cortical bone creates the outer structure of long bones while the trabecular bone makes up inner portions of the bone shaft but primarily acts as a shock absorber at the ends of long bones.[23, 37, 38] Trabecular bone contains a reservoir of calcium and phosphate that can be used for metabolic processes and to maintain optimal serum calcium concentrations that are tightly regulated between 8.9-10.1 mg/dL.[23, 37, 38,40] Cortical bone is built to withstand bending and supports locomotion and is only affected by high deficits in minerals such as calcium and phosphorous.[23, 37, 38] As a person ages, the entire skeleton's mineral content peaks near age 30 and subsequently remodels itself thereafter with age-related declines in mineral mass in response to genetic and external forces.

Bone remodeling is the process by which new bone tissue (organic and inorganic) is built to contribute to an optimal bone mass. Within bone, basic metabolic units (BMUs) are spots in which osteoclasts, osteoblasts, osteocytes and capillary blood are located to achieve bone remodeling.[41] Osteocytes sit within the bone matrix to constantly assess changes in bone tissue and regulate remodeling by either secreting receptor activator of nuclear factor kappa-B ligand (RANKL) or osteoprotegerin (OPG).[7, 41, 42]

The RANK/RANKL system recruits osteoclasts so they can digest the "old" bone tissue.[7, 41, 42] As osteoclasts consume old bone matrix, type 1 collagen fragments as C-

terminal telopeptide of type 1 collagen (CTX) and N-terminal telopeptide of type 1 collagen (NTX) are released into the body and can be assessed as bone resorption markers.[31, 45, 43] Both CTX and NTX are cleared through the renal system and blood.[28, 44-44] Type 1 collagen also contains cross links of Pyridinoline (PYD) and deoxy-pyridinoline (DPD) within its structure, contributing to the mechanical characteristics of bone.[28, 44- 44] PYD and DPD are released by osteoclasts reflecting that collagen cross links were digested during the initiation phase of bone resportion.[28, 44, 45, 44] At the same time, Wnt proteins are secreted by osteocytes which signal to the osteoblasts to increase the secretion of osteoprotegerin (OPG) protein.[28, 44-45] OPG reduces the rate of osteoclasts differentiation, enabling the activated osteoclasts to go through apoptosis (transition phase). [28, 45-44] OPG can act as a decoy receptor for RANKL which prevents it from binding to the RANK receptor on osteoclasts, thereby decreasing bone resorption and allowing osteoblasts to differentiate.[28, 44-44]

Osteoblasts secrete osteoid, new bone matrix, into the newly degraded BMU from which bone mineralization markers are released. [28, 44-44] While osteoclasts degrade type 1 collagen, N-and C-terminal propeptides of type 1 collagen (PINP and PICP) are markers of collagen secretion by osteoblasts.[28, 44-44] This builds the collagen structure within the bone matrix.[28, 44, 45, 44] As the collagen structure is created, bone tissue is calcified with bone specific alkaline phosphatase (BAP) hydrolyzing inorganic phosphate.[28, 44-44] Osteocalcin (OC) ossifies the organic component of the bone matrix further building up bone tissue. Osteoblasts then transform into osteocytes to complete the mineralization process for the newly formed bone tissue.[28, 44-44]

The ratio between RANKL and OPG is crucial for balancing bone metabolism. Higher rates of osteoclast activity through activation of the RANKL/RANK system may induce a catabolic effect on the bone tissue, resulting in low bone mass.[44-47] Increases in RANKL activation resulted in higher trabecular bone loss through assessments of concentrations of bone turnover proteins reflecting both OPG and RANKL systems.[44-47] Cortical bone has also been shown to decrease with the upregulation of RANKL; however, it was observed to be more sensitive to the absence of OPG.[44-47] As the RANKL system takes over, cortical bone begins to become more porous, leading to weaker mineral structures.[44-47] As cortical bone becomes more porous, the greater the risk for bone fracture. Bone formation is also heritable with genetic variation observed among twins for bone specific alkaline phosphatase (BAP), a marker of bone formation.[46] This study suggested that BAP explained 16% of the genetic variation in lumbar spine and 4% of the variation in femoral neck BMD.[49] Genetic variation in RANK receptor and RANK ligand has been shown to increase cortical bone porosity in young men.[47] In peri- and postmenopausal women, variations in the same resorption system were associated with differences in bone mineral density (BMD) of the femoral neck and lumbar spine.[48] Race has also been observed as a genetic factor important for bone health.[35] Young Black adults were observed to have higher BMC and calcium retention compared to their white counterparts.[35,49] This regulation of bone turnover and its effect on bone tissue must be assessed as men and women age to prevent low bone mass and fractures from occurring.

Assessments of bone health.

Adults are advised to have their bone health assessed at 65 years or when women hit postmenopausal status.[50,51] A few factors are often assessed for bone health, including

bone turnover markers, hormone levels and bone mineral density. The most validated tool to measure bone mineral density is the dual x-ray absorptiometry (DXA) machine.[50-53] Alternative tools include quantitative computerized tomography (QCT) and magnetic resonance imaging (MRI) scans.[50-53]The DXA machine integrates both cortical and trabecular bone within sites including femoral neck, and lumbar spine to create a total measurement of bone mineral content (BMC) and bone mineral density (BMD).[50-53] BMD is calculated using BMC and divided by the area of the bone site that is scanned.[29] Due to the two-dimensional nature of DXA measurements, volumetric bone density cannot be determined with this tool.[29] Compared to DXA, QCT can separate cortical and trabecular bone to enable further assessments within a bone site.[50-53] It can also measure volumetric bone density to account for bone size in BMC and BMD values.[50-53]

DXA has been validated across all age groups while QCT has not.[50-53] DXA results are provided as normalized Z-scores (age-specific) and T-scores (relative to a young healthy adult) which are used for clinical and diagnostic comparison.[52, 53] T-scores are calculated in reference to young adult bones without history of fractures for each bone region.[50-54] Z-scores are in reference to age-matched individuals for each bone region. [50-54] Z-scores below 3 standard deviations from the mean is used as a cutoff for low bone mass in adolescents and young adults 18-21 years old. [50-54] T-scores from 1 to 2.5 standard deviations from the mean are considered healthy. [50-54] Osteopenia or low bone mass are diagnosed at a T-score below 1 standard deviation from the normal mean and osteoporosis is diagnosed at a T-score below 2.5 standard deviations.[50-54] BMD changes slowly and takes 6-12 months to detect changes using DXA. This limits the ability to

assess whether treatments are actively improving bone health. [50-54] However, bone turnover markers can explain what is happening to bone remodeling in real-time.

As explained above, bone turnover markers are released into the body as osteoblasts and osteoclasts remodel bone tissue in men and women. Assessing bone markers may trigger red flags in an individual's bone health before decreases in BMD are observed clinically or fractures occur. Bone markers can be clinically measured through urine and blood samples.[6, 7, 29, 30] Urine samples can give concentrations of CTX and NTX while serum samples contain BAP and OC markers.[6, 7, 30, 31] The most common method to measure the concentrations of these markers is through enzyme-linked immunosorbent assays (ELISA).[28,44] Reference ranges are created for each marker by using samples from each age group, menstrual stage and both genders.[7,54,55] These ranges are then used as cutoffs to indicate the ratio of bone resorption and absorption.[7,56,57] Timing during which the sample is collected is important as seen in OC concentrations that are at their highest concentration early in the morning compared to later in the day.[6, 7, 30, 31] Markers including BAP and OC are sensitive to fasting because their concentrations increase after eating a meal.[6, 7, 30, 31] Given the sensitivity of bone turnover markers to fasting, age and gender, the concentrations should be used as a component in the package of tools to diagnose low bone mass and osteoporosis.

Bone health throughout life

Adolescence is a time at which bone develops at a fast rate as well as the start of puberty. Starting at the age of 10 years, girls and boys go through puberty, resulting in changes in sex steroid and growth hormones, impacting bone growth.[56–60] Higher concentrations in bone turnover markers was observed in teen boys and girls compared to

adults, supporting the evidence that adolescence is coupled with drastic changes to bone mineral density.[58-62] During puberty, adolescent boys have a delayed growth spurt but the growth rate is high resulting in higher BMD compared to girls.[58-62] However, this growth is observed to be mainly trabecular bone as teen girls had 3% higher cortical bone mineral content.[58-62] About 10% of bone mass accrual in teen girls is observed at the onset of menarche.[58-62] Timing of puberty including the start of menarche and changes in hormone levels has an inverse relationship to bone mineral density.[58-62]

These sex differences in bone growth are caused by hormonal regulations between boys and girls. Gonadotropin-releasing hormone (GnRH) increases during the pubertal growth spurt, initiating the release of sex steroid hormones.[61, 60] In response, estrogen increases the rate of growth hormone (GH) and growth hormone factor 1 (GHF1). [61, 60] As girls move into their late teens, GH decreases, allowing estrogen to regulate the development of mature bone tissue that would be maintained through adulthood.[60-63]In boys, a combination of androgens and estrogen drive bone growth, resulting in longer bones with higher mineral content.[60-63]Along with their sex hormones, parathyroid hormone (PTH) regulates the rate of bone turnover.[57] High PTH levels in adolescent boys and girls are associated with lower BMD compared to normal levels.[61] By the age of 18, men and women have accrued about 80% of their peak bone mass.

Between ages 18-25 (young adulthood), bone development is still progressing, depending on their genetic variability.[25, 62, 63] Lumbar spine volumetric bone (vBMD) is observed to fully develop by 22 years in men and 29 years in women. [25, 62, 63] Bone turnover markers including NTX and PINP decrease compared to early adolescence suggesting accrual of bone mineral content.[25, 62, 63] This accumulation of BMC

contributes to their total BMD which is maintained throughout adulthood. As young adults go into middle age, BMD decreases in women by37% of their trabecular bone and 6% of their cortical bone while men lose 42% and 15%, respectively, before 50 years.[8, 24, 62]

Women go through several menstrual stages throughout life including premenopausal (start of menarche to 34 years old), perimenopausal (35 to 44 years old), menopausal (45 to 60 years old) and postmenopausal (cessation of menstrual cycle at least 12 consecutive months).[63] As women move from perimenopausal to postmenopausal stage, their estrogen rapidly declines and become deficient.[65] Women going into the perimenopausal stage of life are characterized by higher bone resorption within the overall bone turnover rate compared to premenopausal women.[24, 63] Men start to lose bone between 30-35 years, a stage in life in which testosterone levels steadily decrease .[64] This suggests that sex steroid hormone levels drive bone loss.

Given the relationship between sex hormones and BMD, there are gender differences in osteoporosis risks. Women are diagnosed with osteopenia at 60 years and osteoporosis by 70 years.[8,65] Osteopenia, osteoporosis and general low bone mass all pose higher risks of bone fractures.[8,65] Hip fracture is more prevalent in women around 65 years compared to 75 years in men.[8,26,37] This 10-year delay may be due to their androgen levels, while women at 65 years lack both estrogen and testosterone.[8,26,37] Endogenous factors such as sex hormones during development play a heavy role in bone health, however external factors including diet and exercise may also influence risk for low bone mass.

Diet and Exercise may reduce low bone mass in adults.

Diet has been shown to influence bone health and later risks of osteoporosis. Protein, mineral, and vitamin D intake are crucial components to bone metabolism.[9] Adults are recommended to consume about 46 grams and 56 grams of protein for women and men, respectively.[66] Protein intake from animal and plant-based foods are positively associated with BMD in both middle-aged men and women.[71-73] Low protein intake (less than 46 grams) has been associated with lower femoral neck BMD in women over the age of 50 years.[9,67,68] However, protein was shown to be dependent on calcium intake, with higher intakes of both calcium and protein have been associated with higher BMD compared to high protein only.[69] Calcium alone from different foods was found to influence BMD later in life. Adults (18-30 years old) need about 1000 mg/day of calcium to maintain good bone health.[66] When young women consumed only 300 mg/day for 20 days, a 15% decrease in PINP and 10% decrease in OC was observed.[70] This suggests that calcium may play a role in osteoblast activity in women.

Vitamin D and calcium was associated with higher BMD when consumed together in a whole food or mixed meal.[68,73,74] Vitamin D is added into dairy products, creating an efficient food source for many Americans to consume their recommended 600 IU/day.[9,66] Consuming dairy products including yogurt and milk were shown to increase BMD in adolescences, setting them up for higher BMD at an older age compared to those who did not eat such products.[9,71] Cereal has been a common combination meal in which the fortified grain and milk were associated with reducing low BMD in young adults.[72] Cortical bone decreased in diets low in vitamin D and high in calcium.[73] In the same study, a low calcium/high vitamin D diet did not achieve peak bone mass.[71] This suggests

that calcium and vitamin D work together to properly develop bone. However, phosphorus is another important mineral for bone health that must be consumed. Intake of phosphorus has been associated with higher BMC and BMD in adolescent girls.[74] This association was also found to correlate with reduced risks of osteoporosis in adult years.[72] Adequate nutrient intake plays a role in bone health making it important to assess dietary intake to prevent low bone mass.

In addition to diet, exercise has a great influence on bone health as well. Benefits of exercise on bone health are seen in resistance and endurance types of activities including weight training, jogging and even walking.[75,76] These types of exercises enable bone to sustain certain mechanical loads, increasing the mineralization rate in response to repeated impact, thus increasing strength in bones.[76,77] Whether physical activity was sustained from adolescence or started during middle age, adults were shown to have higher BMD than their sedentary counterparts.[13,] Young adult women who were active during their adolescence were found to have a 10% larger cortical area and 12% greater cortical content of tibial bones.[11] Engaging in a sport or endurance-related activity during adolescence was associated with higher BMD in middle age men.[77] Strength training was observed to increase lumbar spine BMD by 2% and femoral neck by 3.8% in elderly men.[78] Similar findings were found between pre- and postmenopausal women participating in combined resistance and cardio training.[79] While these observations suggest that being active is important to maintain skeletal health, the level of PA is crucial as vigorous to moderate activity has shown more positive results compared to light bouts of exercise.[22] Regardless, the protective effect may be due to the cellular responses within bone tissue. After participating in a weight bearing exercise training for

4 weeks, women in their 40s were observed to have their PINP and OC concentrations increase up to one month after the training.[80] However, findings between habitual physical activity and bone turnover markers have yet to be confirmed.

Sleep pattern may be associated with physical activity and bone health.

Sleep is an essential component to daily living and recently been associated with physical activity and bone health. This emerging association may be due to the secretion pattern of hormones and bone turnover markers that was shown to Different sleep recommendations are given to an individual as sleep needs change throughout the lifecourse.[30,81]

Table 1. Sleep variables to measure sleep pattern from adolescents to older adults.

Sleep Variable	Recommended Duration[1]
Sleep onset latency (minutes)	Teens to older adults: ≤ 15 minutes
Total sleep time (minutes)	Teens (14-17 years): 8-10 hours
	Young adult (18-25) and adults (26-64): 7-9 hours
	Older adults (≥ 65 years): 7-8 hours
Wake after sleep onset (minutes)	Teens to older adults: ≤ 20 minutes
Awakenings (> 5minutes)	Teens to young adults: 0-1 awakenings
	Older adults: 0-2 awakenings
Sleep efficiency (%)	Teens to older adults: ≥ 85%

[1] Hirshkowitz M et al. *SLEH*. 2015;1:233-243.

From young adulthood to the elder years, total sleep time (TST) decreases by up to 12 minutes per decade.[17,82] From middle age to 50 years plus, sleep onset latency becomes delayed, indicating that older adults take longer to fall asleep than their younger counterparts.[17,82] Sleep fragmentation as measured by wake after sleep onset (WASO) has been shown to increase as adults age, increasing 10 minutes per decade from age 30

years.[17,82] Fragmented sleep is associated with daytime sleepiness and poor sleep quality in older adults.[83,84] This causes older adults to take more naps during the day compared to middle aged men and women.[16,17] These changes have been assessed in relation to bone health with mixed results within the adult population.

Many adults reported to have a sleep disorder including sleep apnea, and insomnia.[85,86] Sleep apnea patients who were treated with a continuous positive airway pressure (CPAP) therapy had a significant decrease in CTX urinary marker compared to their baseline results. Correcting the interruption during sleep improved bone turnover rate. Insomnia is characterized by the difficulty of falling and maintaining sleep throughout the night. Adults over the age of 60 years with reported insomnia events were at 49% greater risk of bone fractures.[87] Between men and women of this age, women with insomnia were seen to have a 36% greater risk of bone fracture compared to women who do not have insomnia.[87] In response to the associations above, older adults' sleep patterns were assessed to explore whether habitual sleep pattern may be linked to bone health.

Women 45 years and older were observed to have a positive association between short sleep duration (less than 6 hours) and low BMD.[14] However, a similar finding was seen when middle age and elderly women reported 8 or more hours of sleep per night.[15] Based on current evidence, it has been noted that the relationship between BMD and sleep duration has a U-shape trend.[88] While there is no direct relationship observed between BMD and sleep duration, other factors such as inflammation markers and cortisol levels increase when sleep is interrupted.[87] In young adults, shift work starting at 7 pm were had greater loss in BMD than those whose shifts started in the morning or

afternoon.[89] Young men whom were sleep restricted to 5 hours per night was observed to lower bone formation markers including PINP compared to their older counterparts.[90] Similar sleep restriction was seen with significantly decreased PINP after 1 month of 6 hours of sleep per night and improperly formed new bone after 3 months in adolescent rats.[91] The evidence presented suggests that an interruption of sleep seems to disrupt the circadian rhythm of bone turnover markers, thus influencing overall bone mineral density.

Physical activity seems to have an effect on sleep resulting in longer sleep duration and positive influence on overall sleep quality.[18,20,92,93] The direct link between physical activity and sleep is not known but many suggest that the energy expenditure and muscle damage caused by exercise induces a longer sleep duration to restore tissue and conserve energy.[18,19] Self-reported moderate to vigorous level of activity was positively associated with sleep duration in young men compared to their older counterpart.[93] A similar result was seen in young men and women when they self-reported physical activity and sleep quality.[94] Acute effects of exercise on sleep quality and duration has been the main focus of many studies. Young men whom exercised 2 hours before bedtime were found to have more efficient sleep and overall better sleep quality compared to their baseline results.[92] However, this age group was also observed to have a 14 minute delay in their sleep latency when practicing moderate level exercise 1 hour before sleeping.[95] The same study found decreases in total sleep time, blaming the delay in sleep latency as the cause.[97] There are mixed results within the acute effects of exercise, indicating that many details including timing and type of exercise must be further assessed.[18,96] As adults age, the association between exercising and sleep

duration/quality are more robust. Older adults had a significant association between moderate to vigorous activity and sleep quality.[97] Further assessments must be made on whether it is the residual effect of acute exercise or the long term impact of daily physical activity that causes improved sleep pattern.

College students' behaviors may be related to bone loss later in life.

Traditional college students (18-25 years) are a niche within the young adult population. They are characterized by excessive sedentary behavior, erratic sleep behavior, higher stress levels and unhealthy eating habits compared to those who do not attend college full time.[98,99] Due to high academic demands, fewer than 50% of college students were observed to be inactive, spending 7-8 hours per day sedentary.[25] When asked about their physical inactivity, students reported that they were unable to exercise due to a lack of time and resources/opportunities to be active.[25,100] Yet as stated before, young adults between 18-25 years are nearing the end of the timeframe in which they can accrue more mineral for the attainment of peak bone mass.[101] Poor behaviors, including reduced physical activity, during this vulnerable life stage may increase their likelihood of having low bone mass later in life.

Not only do academic demands reduce college student activity time but also their sleep. College students 18-25 years' experience changes in their sleep behaviors, resulting in wakefulness at night and sleepiness during the day.[102,103] As students moved from freshman to senior year, their sleep duration decreases on the weekdays but increases on the weekends.[104,105] This indicates that students attempt to compensate for their loss of sleep throughout the school week, by sleeping longer on weekends. [104,105] However, this behavior has been negatively associated with overall sleep quality in male

and female students.[106,107] Stress due to academic demands was found to be one main factor that influences sleep quality in students, especially females.[105,106] As students stayed up late to study, the lack of sleep was associated with academic failure.[107] Education on sleep hygiene was noted to provide some benefit to students via better sleep quality.[108,109] Yet, many students fall into the habits of irregular and inadequate sleep habits.

As seen in older adults, sleep seems to be associated with bone health, but this association has not yet been observed in young adults. This could be due to the lack of significant findings among middle aged adults as stated previously. However as discussed, college students are a unique population with opportunities to continue accruing bone mineral. Now, the question is whether the lack of physical activity and erratic sleep patterns are having negative impacts on bone turnover in college-aged students.

CHAPTER 3

METHODS

Study design

This cross-sectional study was conducted at Arizona State University. The primary purpose of the study was to observe the relationships between physical activity, sleep patterns and bone health in 18-25-year-old college students attending Arizona State University. The study was approved by the Institutional Review Board (IRB) at Arizona State University on November 17, 2016.

Participants

Recruitment

Male and female college students were recruited via flyers in the Tempe and Downtown Phoenix Arizona State University (ASU) campuses and online. Recruitment occurred biweekly during each Fall and Spring semester starting in the Fall 2016 semester until the desired sample size was reached. In person recruitment included distributing flyers at Freshman seminar courses, ASU student events and student organization meetings. Online ads were designed on Facebook and purchased to be shown to users who were 18-25 years old and followed any college related topics. Ads were also posted in ASU college-based newsletters that were sent out to students via email. For each recruitment mechanism, students were told that the study was looking for healthy 18-25-year-old male and female college students. Students were also told that they would be asked to complete surveys related to basic demographics, health history and food intake, wear an actigraphy device to record sleep for 7 nights, complete a DXA bone scan and fasted blood draw.

Screening Process

Potential participants were instructed on the flyers to contact a lab member to start the screening process, during which the inclusion and exclusion criteria were assessed. Screening was done in two parts; the first part was a phone/text message/email interview and the other was an in-person interview. During the first part of the screening process, a lab member asked potential participants about their vitamin/mineral/protein supplement use and work schedules. Using the inclusion/exclusion criteria, the potential participant's eligibility was assessed to determine whether they could move on to the in-person interview. The in-person interview was located at the Healthy Lifestyles Research Center on the ASU Downtown Phoenix campus. Potential participants were asked about their health history, medication and dietary supplement use. Their height and weight were measured using a calibrated weight scale and stadiometer to calculate their BMI. Once the potential participant was declared eligible by the lab member, they were immediately asked to go through the consenting process.

Inclusion and Exclusion Criteria

Inclusion criteria for this study included men and women ages 18-25 years, attending ASU at least half time (6 credit hours), with a body mass index (BMI) of 18.5-29.9 kg/m^2. Exclusion criteria for this study included men and women with a history of eating and sleeping disorders, malabsorption and autoimmune diseases, hypertension, and diabetes. Potential participants were excluded if they self-reported taking medications that affect bone metabolism. Potential participants self-reported their employment status by describing their job and regular work schedules. They were excluded if they reported any shift work starting at 7 pm one month prior to or within the same month of

participation. Potential participants were excluded if they self-reported drinking large amounts of caffeine (more than 400 mg/day) and/or alcohol intake (more than 1 drink per day for women and 2 drinks per day for men), at least 3 times per week within the same month of participation. Potential participants also self-reported dietary supplement use. They were excluded if they regularly consumed calcium supplements (containing ≥ 50% of the recommended daily value) as a standalone product, within a multi-vitamin and/or protein powders at least 3 times per day, every week within the same month of participation. Women who planned to become pregnant, were pregnant or breastfeeding were also excluded from the study.

Consent Process

A lab member went over the consent form in detail with each potential participant by explaining each section of the form. Participants were told throughout the consent review that each task was voluntary. They were given chances to ask questions during this review and detailed responses were given. Female participants were told that a pregnancy test was necessary before taking a DXA scan as described in the detailed protocol section. Participants were asked to complete the study tasks immediately after consenting as described in the detailed protocol section.

Study Protocol overview

Consented participants were asked to complete a fasted, venous blood draw to measure bone biomarkers in their serum. Following the blood draw, participants completed a DXA bone scan to obtain bone health measures. Once all clinical assessments were completed, participants were given questionnaires to complete. A health history questionnaire was given to each participant to describe their general health.

The International Physical Activity (IPAQ) short form was used to measure the participants' habitual physical activity. Usual dietary intake was assessed via the dietary screener questionnaire (DSQ).

At the end of the appointment, participants were given a Philips Respironics actiwatch Spectrum Plus watch (Royal Philips, NV) to wear 24/7 for 7 nights. During the 7-night period, the actigraphy device recorded their sleep time during the day and night and sleep variables were extracted from the raw data. On the eighth day, participants met with a lab member to return the watch and completed sleep diary in exchange for their compensation of $20 and a hard copy of their DXA bone scan results. Participants who dropped out of the study did not obtain compensation and were asked to return the actiwatch.

Detailed protocol and measured outcomes

Dietary, health and physical activity surveys

A general health questionnaire was given to participants to fill out and used to assess their age, height, weight, BMI, race, history of illnesses, menstrual cycle (females only), medication use and general dietary habits. The dietary screener questionnaire (DSQ) is a 30-item survey given to each participants to complete in which they were asked how many times per day, week and month they ate certain dairy, protein, fruit and vegetable foods.[110]

The International Physical Activity Questionnaire short form (IPAQ-short form) was administered by a lab member in an interview method to each participant. It was used to examine the participants' usual physical activity levels.[111] IPAQ-short form data

were converted into metabolic equivalent minutes (MET-minutes) for sitting, walking, moderate and vigorous physical activities over the past 7 days.[112]

Venous blood draw

Prior to the blood draw, participants were asked to fast, meaning they could not consume food, drink tea, coffee, or any sweetened drinks for at least ten hours (except water) prior to the study visit. A certified phlebotomist conducted the blood draw on each participant. The venous blood draw was done on an antecubital vein located on the participant's preferred arm or one that was suited for the procedure as determined by the phlebotomist. A total of 15 ml was collected in potassium oxalate/sodium fluoride-treated tubes (grey tops, BD vacutainers). Serum was extracted from the blood samples by centrifugation at speed 10,000 RPM and stored in a -80°C freezer until further biochemical analyses were conducted.

Bone Turnover markers

Enzyme linked immunoassay (ELISA) kits were used to measure OC and NTX-1 concentrations; manufacturer protocols were followed (ALPCO USA and Biomatik Canada, respectively) .[113,114] About 200 ul of serum from each participant was used for each kit. All sample concentrations were quantified using a spectrophotometer (reading at 450 nm, Biotek USA).

Dual-energy x-ray absorptiometry (DXA)

The DXA scan (Lunar iDXA, GE Healthcare, Madison, WI) was conducted by a licensed radiologist. DXA measured bone mineral density of the lumbar spine (L1-L4) and proximal femur.[115] Participants were asked to wear clothing that did not have any metals (i.e. zippers, buttons, snaps, and underwire bra). A urine-based pregnancy test was

completed for each female participant before the scan to confirm they were not pregnant. During the DXA scan, participants were asked to lie still in the supine position as the machine scanned their lower back and hip area. Radiation (1-4 microSieverts) exposure during this procedure was minimal to the participant.[116]

Sleep Pattern Assessments

Each participant was asked to wear a Philips Respironics actiwatch Spectrum Plus watch (Royal Philips, NV) for 7 days and nights to record their sleep.[117] During the 7-day and night period, participants wore the actiwatch 24/7, only taking it off while they showered or swam. The actiwatch recorded 1-minute epochs of activity, light exposure and movement to detect sleep patterns. Total sleep time (TST), wake after sleep onset (WASO), sleep onset latency (SOL) and sleep percent (SP) were calculated from the raw actigraphy data. TST equaled the sum of minutes a person was asleep between sleep onset and offset.[31] WASO was the total minutes in which the person was awake after sleep onset occurs.[118] SOL was the total minutes it took a person to fall asleep from when they were in bed.[32] Sleep period was a duration in minutes calculated from sleep onset to offset, without considering sleep onset latency.[31] SP equaled TST divided by sleep period then multiplied by 100 to obtain a percentage in which the person was asleep within a sleep period.[31]

Statistical Analysis

Variables

OC and NTX-1 serum concentrations were used as the main outcome variables and recorded as continuous variables (ng/ml and nmol/L BCE, respectively). Physical activity was represented as total MET-minutes per week, which included moderate,

vigorous and walking activities. Total MET-minutes per week was used as a continuous variable in statistical analyses. TST, WASO and SOL were recorded in minutes, and sleep percent was calculated percent; all variables were included as continuous variables in models. Race and BMI were used as the main demographic covariates in the regression models. BMI was included as a continuous variable while race was a nominal variable in the statistical analyses. Calcium intake per day (mg/day) was calculated from the DSQ responses and used as a continuous dietary covariate. Bone mineral density (g/cm^2) was used as a descriptive variable to characterize the study sample's bone health.

Statistical methods

SPSS (Statistical Package for the Social Sciences) v. 24 was utilized for all statistical analyses. Due to the sample size (n=52), data were checked for normality using the Kolmogorov-Smirnov Test . Participants were excluded from statistical analyses if they were deemed as an outlier; +/- 3 standard deviations away from the mean. Variables were log-transformed as needed to ensure that assumptions of regression analyses were met, and that normality of model residuals was achieved. Correlation testing was completed using either Spearman or Pearson tests depending on the normality of the tested variables.

Hypotheses 1 and 4

Scatterplots were created between physical activity and serum NTX-1 or OC concentrations to assess the direction of each relationship. Serum OC concentrations were log transformed. Correlation testing (parametric or non-parametric) was performed to assess the strength and significance of each association between physical activity (total MET-minutes per week) and serum NTX-1 or OC concentrations. Separate, multiple

linear regression analyses were performed to assess whether MET-minutes per week predicted NTX-1 or OC serum concentrations (significance, p-value < 0.05). The linear regression models were adjusted for gender, race, BMI and calcium intake/day. Beta coefficients were reported as both raw form and reverse log transformed for models with log OC as an outcome as well as t-statistics. NTX-1 models reported beta coefficients in raw form as well as t-statistics.

Hypotheses 2 and 5 (a-d)

Scatterplots were created between each sleep variable (sleep percent, WASO, TST and SOL) and NTX-1 or OC serum concentrations to assess the direction of each relationship. OC serum concentrations were log transformed. Correlation testing (parametric or non-parametric) was performed to assess the strength and significance of each association between each sleep variable and NTX-1 or osteocalcin serum concentrations. Separate, multiple linear regression analyses were performed to assess whether each sleep variable predicted serum NTX-1 or OC concentrations (significance, p-value < 0.05). The multiple linear regression models were adjusted for gender, race, BMI and calcium intake/day. Beta coefficients were reported as both raw form and reverse log transformed for models with log OC as an outcome as well as t-statistics. NTX-1 models reported beta coefficients in raw form as well as t-statistics.

Hypotheses 3 and 6

Scatterplots and correlation tests between physical activity and each sleep variable were created to assess the direction of the relationship between the two predictor variables. Interaction variables were created by taking the product of physical activity and each of the above sleep variable. Another set of scatterplots and correlation tests

were created between each interaction variable and serum NTX-1 or osteocalcin concentrations to assess the direction of the interaction with the outcome. Separate multiple linear regressions were conducted to assess whether each sleep variable interacted with MET-minutes per week to impact serum NTX-1 or osteocalcin concentrations (significance, p-value < 0.05). The multiple linear regression models were adjusted for gender, race, BMI and calcium intake per day. Physical activity*WASO and physical activity*TST interaction variables were log transformed to ensure normality for multiple linear regression models. Serum OC concentrations were also log transformed. Beta coefficients were reported as both raw form and reverse log transformed for models with log OC as an outcome as well as t-statistics. NTX-1 models reported beta coefficients in raw form as well as t-statistics.

CHAPTER 4

RESULTS

Participant Description

A total of 52 participants were enrolled into the study, including 29 males and 23 females with a mean age of 20 ± 2 years. Among the sample, 63.5% were White (non-Hispanic), 13.5% were Hispanic, 9.6% were Asian, and 13.5% self-reported as mixed race (mixture of Hispanic, Asian, African American and Caucasian). Participants were generally physically active with 55.8% reporting high active, 38.5% reporting moderate active, and 5.8% reporting sedentary behavior. They were also relatively healthy with 67.3% at a normal weight (18.5-24.9 kg/m^2) and 32.7% classifying as overweight (25.0-29.9 kg/m^2). However, 61.6% of the sample size consumed less than the recommended daily allowance (RDA) of calcium within relevant age ranges (1,000 mg/day for 19-30 years and 1,3000 mg/day for 14-18 years old). About 65% of participants were able to fall asleep in less than 15 minutes but 42% were only able to sleep 6 hours per night. **Table 2** shows additional health characteristics of the sample. **Table 3** shows a general description of bone biomarker, sleep and physical activity variables

Table 2. Participant health characteristics.

Variables		Female mean ± SD	Male mean ± SD	Total mean ± SD	Reference values[1]
Body Mass Index (kg/m^2)		22 ± 2.48	24 ± 2.49	23.2 ± 0.38	18.5-24.9 kg/m^2 normal 25-29.9 kg/m^2 overweight ≥ 30 kg/m^2 obese
Calcium intake (mg/day)		909 ± 122	1214 ± 298	1079 ± 280	RDA 1300 mg/day 14-18 years old RDA 1000 mg/day 19-30 years old
Bone Mineral Density (g/cm^2)	Bone Mineral Density L1-L4 (g/cm^2)	1.253 ± 0.15	1.257 ± 0.11	1.255 ± 0.13	male lumbar spine: 16-19 years old 0.813-1.241 g/cm^2 normal male lumbar spine: 20-29 years old 0.884-1.257 g/cm^2 normal female lumbar spine: 16-19 years old 0.832-1.216 g/cm^2 normal female lumbar spine: 20-29 years old 0.884-1.251 g/cm^2 normal
	Bone Mineral Density Femur Neck (g/cm^2)	1.067 ± 0.14	1.215 ± 0.16	1.150 ± 0.17	male femur neck: 16-19 years old 0.758-0.1.260 g/cm^2 normal male femur neck: 20-29 years old 0.748-1.187 g/cm2 normal female femur neck: 16-19 years old 0.686-0.1.121 g/cm^2 normal female femur neck: 20-29 years old 0.711-1.108 g/cm2 normal
	Bone Mineral Density Femur Wards (g/cm^2)	0.953 ± 0.15	1.080 ± 0.18	1.024 ± 0.18	male wards triangle: 16-19 years old 0.622-0.841 g/cm^2 normal male wards triangle: 20-29 years old 0.748-0.949 g/cm2 normal female wards triangle: 16-19 years old 0.637-1.136 g/cm^2 normal female wards triangle: 20-29 years old 0.612-1.107 g/cm2 normal
	Body Mineral Density Femur Trochanter (g/cm^2)	0.833 ± 0.14	0.997 ± 0.14	0.924 ± 0.16	male femur trochanter: 16-19 years old 0.622-1.078 g/cm^2 normal male femur trochanter: 20-29 years old 0.636-1.008 g/cm2 normal female femur trochanter: 16-19 years old 0.562-0.930 g/cm^2 normal female femur trochanter: 20-29 years old 0.558-0.908 g/cm2 normal
	Body Mineral Density Femur Shaft (g/cm^2)	1.247 ± 0.16	1.433 ± 0.19	1.351 ± 0.20	-
	Z-score L1-L4 (g/cm^2)	0.322 ± 0.92	0.421 ± 1.03	0.377 ± 0.97	-
	Z-score Femur Neck (g/cm^2)	0.300 ± 0.96	0.834 ± 1.15	0.598 ± 1.10	-
	Z-score Femur Wards (g/cm^2)	0.143 ± 1.09	0.386 ± 1.13	0.276 ± 1.11	-
	Z-score Femur Trochanter (g/cm^2)	0.022 ± 1.15	0.396 ± 1.09	0.227 ± 1.12	-

[1]Looker, AC Borrud, LG Hughes J. Vital Heal Stat. 2012;11(251).
Body Mass Index was calculated using weight (kg) and height (cm).
Calcium intake: dairy, cereal, fruit and vegetable related DSQ questions from each participant and average of whole sample taken.
RDA: recommended daily allowance, Burwell S, Vilsack T. 2015-2020 Dietary Guidelines - health.gov. Published 2015

Table 3. Description of main variables.

Variables	Female mean ± SD	Male mean ± SD	Total mean ± SD	Reference values[1]
Osteocalcin serum concentrations (ng/ml)	12.1 ± 2.75	21.9 ± 8.5	17.6 ± 8.2	pre-menopausal women: 4.9-30.9 ng/ml men: 3.2-39.6 ng/ml
NTX1 serum concentrations (nmol/L BCE)	18.2 ± 10.8	31.8 ± 12.3	25.8 ± 13.4	normal men: 5.4-24.2 nmol BCE normal women: 6.2-19.0 nmol BCE
Actigraphy TST (minutes)	403 ± 45.3	379 ± 58.3	389 ± 53.9	young adult 18-64 years 420-540 minutes adults: 65 years plus 420-480 minutes not recommended: less than 360 minutes or more than 600 minutes
Actigraphy WASO (minutes)	31 ± 11.6	32 ± 10.9	32 ± 11.1	normal: ≤ 20 minutes
Actigraphy SOL (minutes)	11 ± 10.0	18 ± 17.5	15 ± 14.9	normal: ≤ 15 minutes
Actigraphy Sleep percent (%)	89 ± 8.1	90% ± 4.8	90% ± 6.4	normal ≥ 90%
Physical activity (MET-min/week)	3343 ± 2715.1	4228 ± 2500.7	3837 ± 2609.7	0-599.99 MET-min per week sedentary 600.00-2999.99 MET-min per week moderately active ≥ 3000.00 MET-min per week highly active

[1]Lab Corp. 140830: N-Telopeptide Cross-links (NTx) | LabCorp.

Lab Corp. 010249: Osteocalcin | LabCorp.

Wilson, Sue, Nutt D. Sleep Disorders: Chapter 1 Normal Sleep. 2nd ed. Oxford University Press; 2013.

Schutte-Rodin S, Broch L, Buysse D, Dorsey C, Sateia M, Schutte-Rodin SL. Clinical Guideline for the Evaluation and Management of Chronic Insomnia in Adults. Vol 4.; 2008.

Forde C. Scoring the International Physical Activity Questionnaire (IPAQ).

Correlation and regression analyses on the impact of physical activity in OC and NTX-1 serum concentrations (hypotheses 1 and 4).

Physical activity had a weak, insignificant correlation with serum osteocalcin concentrations (r= 0.274 p-value= 0.052; **Figure 1**). NTX-1 had a similar correlation with physical activity (r= 0.155 p-value = 0.272; **Figure 1**). Physical activity was further assessed to determine whether it predicted serum osteocalcin and NTX-1 concentrations. **Models 1 and 2** were found to be significant after adjusting for covariates in predicting OC and NTX-1 serum concentrations, however physical activity had no impact on the bone markers within each model. After adjusting for covariates, gender was a significant contributor to predicting both osteocalcin (beta coefficient = 3.438 p-value = 0.000) and NTX-1 serum concentrations (beta coefficient = -0.366 p-value = 0.022).

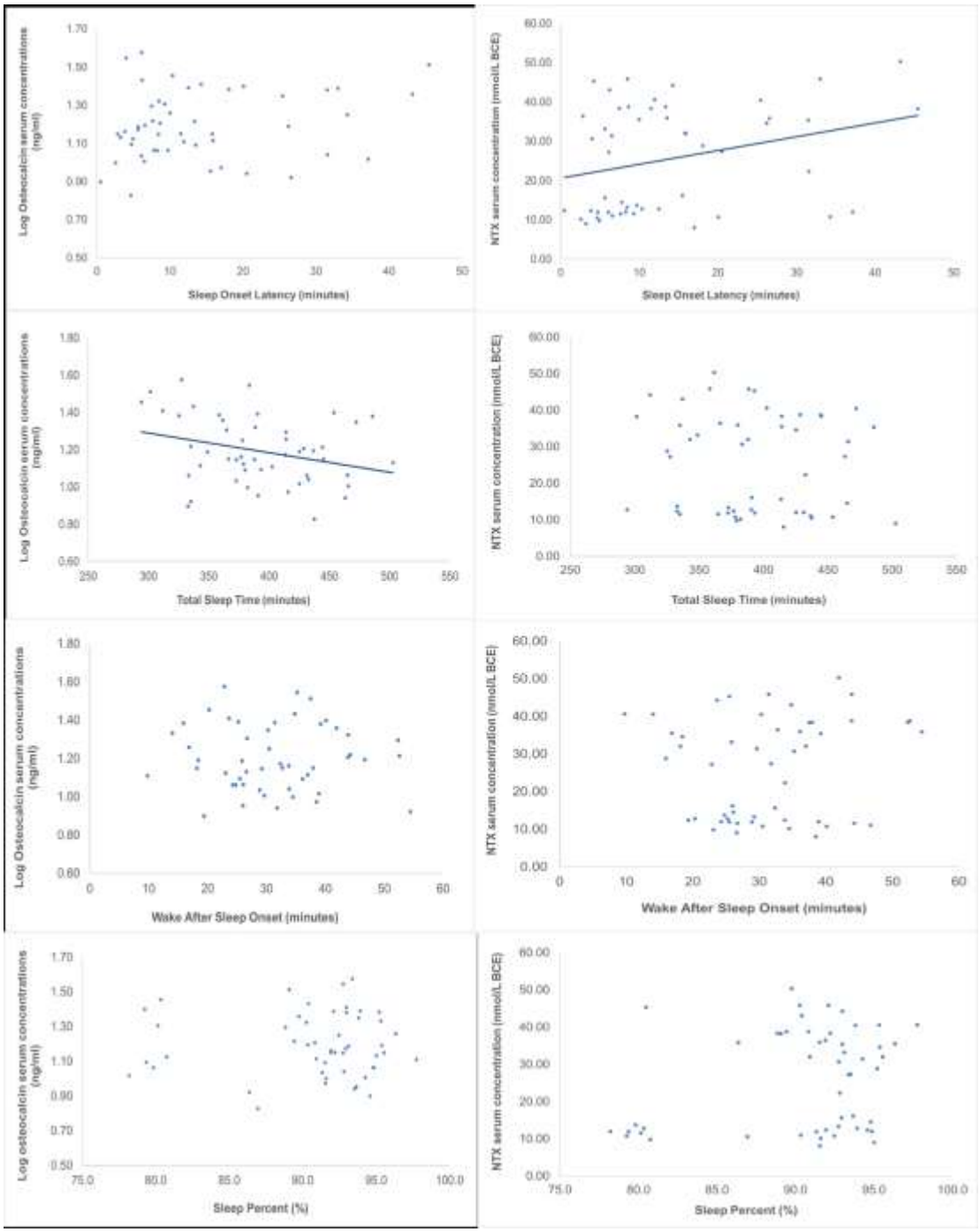

Figure 2. A comparison of associations between each sleep variables (SOL, WASO, TST and SP) and osteocalcin or NTX-1 serum concentrations. Solid line equals significant correlation testing $p < 0.05$.

Model 1. Multiple Linear Regression analysis between osteocalcin and physical activity N = 51.

Model 1[a]												
Variables	Block 1[b]				Block 2[c]				Block 3[d]			
	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value
Gender (female = 1 and male = REF)	-0.668	0.215	-5.823	**0.000**	-0.638	0.230	-4.830	**0.000**	0.536	3.438	-4.730	**0.000**
Race (white = REF and other race = 1)	-0.138	0.728	-1.236	0.222	-0.105	0.785	-0.927	0.359	0.922	8.360	-0.7170	0.477
BMI (kg/m^2)	-	-	-	-	-0.190	0.646	-1.629	0.110	0.818	6.575	-1.7320	0.090
calcium intake (mg/day)	-	-	-	-	0.163	1.455	1.285	0.205	1.154	14.247	1.1310	0.264
Physical activity (MET-min/week)	-	-	-	-	-	-	-	-	1.151	14.172	1.2890	0.204
R-Squared	0.403	-	-	-	0.432	-	-	-	0.440	-	-	-
Change in R-Squared	-	-	-	-	0.050	-	-	-	0.019	-	-	-
R-Square p-value	**0.000**	-	-	-	0.123	-	-	-	0.204	-	-	-
ANOVA p-value	**0.000**	-	-	-	**0.000**	-	-	-	**0.000**	-	-	-

[a]log transformed osteocalcin serum concentrations (ng/ml) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake and physical activity.
[1]Beta coefficient presented in raw form.
[2]Reverse log transformation of raw beta coefficient was calculated.

Model 2. Multiple Linear Regression analysis between N-terminal telopeptide of type 1 collagen and physical activity N = 52.

Model 2[a]									
Variables	Block 1[b]			Block 2[c]			Block 3[d]		
	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value
Gender (female = 1 and male = REF)	-0.496	-3.967	**0.000**	-0.371	-2.422	**0.019**	-0.366	-2.362	**0.022**
Race (white = REF and other race = 1)	0.061	0.489	0.627	0.031	0.239	0.812	0.038	0.286	0.776
BMI (kg/m^2)	-	-	-	0.081	0.601	0.550	0.077	0.568	0.573
calcium intake (mg/day)	-	-	-	0.192	1.308	0.197	0.186	1.250	0.218
Physical activity (MET-min/week)	-	-	-	-	-	-	0.044	0.342	0.734
R-Squared	0.262	-	-	0.292	-	-	0.294	-	-
Change in R-Squared	-	-	-	0.031	-	-	0.002	-	-
R-Square p-value	**0.001**	-	-	0.369	-	-	0.734	-	-
ANOVA p-value	**0.001**	-	-	**0.002**	-	-	**0.005**	-	-

[a]N-terminal telopeptide of type 1 collagen serum concentrations (nmol BCE) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake and physical activity.
[1]Beta coefficient presented in raw form.

Correlation and regression analyses on the impact of each sleep variable (SOL, TST, WASO or SP) in OC and NTX-1 serum concentrations (hypotheses 2 and 5 a-d).

Correlations between the biomarkers and each sleep variable are illustrated in **Figure 2**. SOL had a weak but significant association with NTX-1 serum concentrations ($r = 0.293$ p-value = 0.037), while osteocalcin had a weak but non-significant correlation with SOL ($r = 0.179$ p-value =0.213). TST did not have strong associations with NTX-1 ($r = -0.104$ p-value = 0.469) but had a weak correlation with osteocalcin ($r = -0.298$ p-value =0.036). WASO was not correlated with osteocalcin ($r = -0.021$ p-value = 0.886) or NTX-1 serum concentrations ($r = 0.069$ p-value =0.629). Sleep percent also was not correlated with osteocalcin ($r = -0.019$ p-value = 0.895) and NTX-1 ($r = 0.139$ p-value = 0.331) serum concentrations.

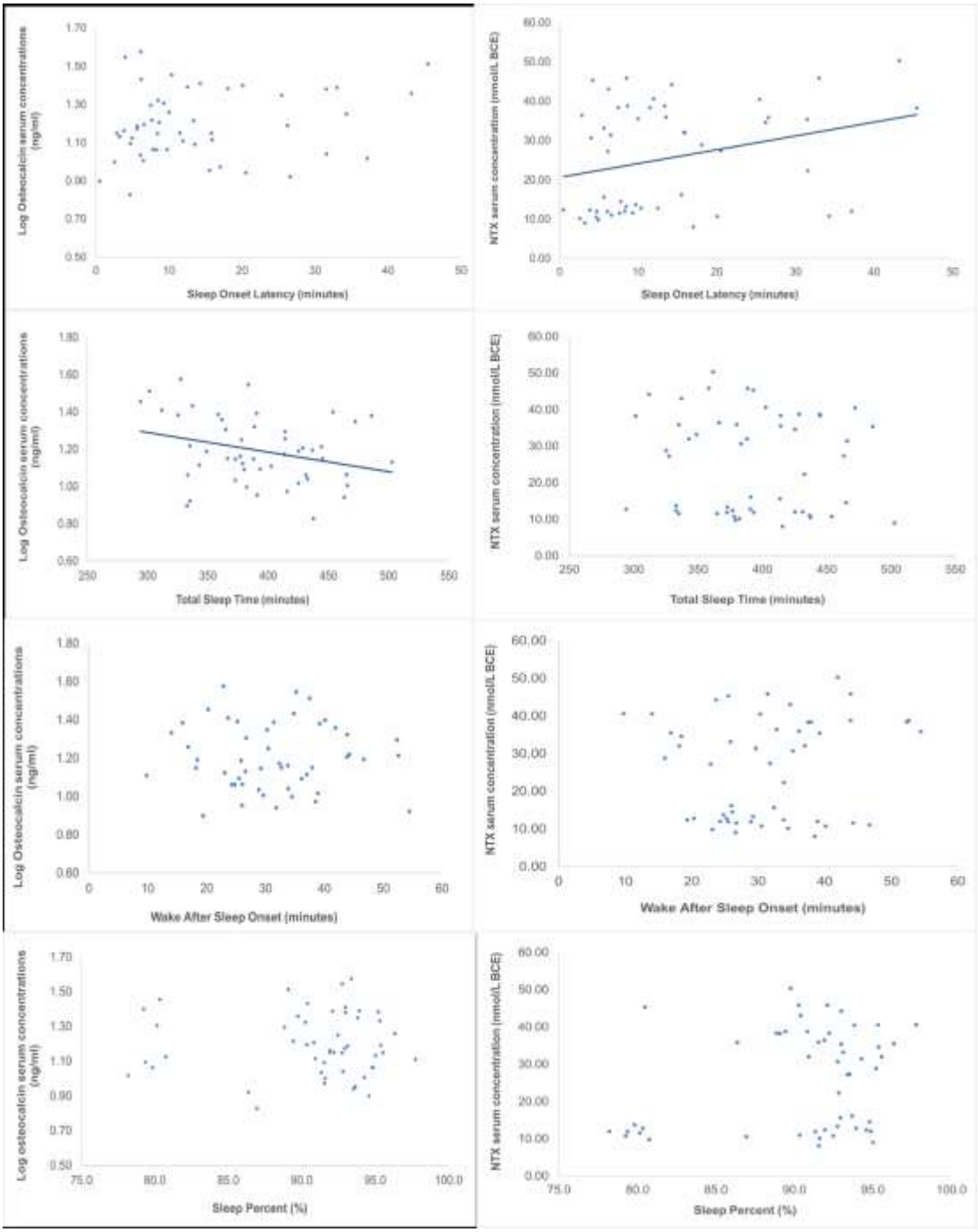

Figure 2. A comparison of associations between each sleep variables (SOL, WASO, TST and SP) and osteocalcin or NTX-1 serum concentrations. Solid line equals significant correlation testing $p < 0.05$.

Further investigation was conducted through regression analyses to determine whether each sleep variable had any influence on predicting osteocalcin and NTX-1 serum concentrations. Models 3-10 were found to be significant when all variables were added but each sleep variable varied in its impact on OC and NTX-1 serum concentrations. **Models 3 and 4** show that SOL was not predictive of osteocalcin (beta coefficient = 1.225 p-value = 0.452) and NTX-1 serum concentrations (beta coefficient = 0.167 p-value = 0.206) after adjusting for covariates. **Models 5 and 6** indicate similar findings for TST associations with osteocalcin (beta coefficient = 0.760 p-value = 0.314) and NTX-1 serum concentrations (beta coefficient = 0.035 p-value= 0.794). WASO did not significantly predict osteocalcin (beta coefficient = 0.769 p-value = 0.319) nor NTX-1 serum concentrations (beta coefficients = -0.006 p-value =0.967) after adjusting for covariates (**Models 7 and 8**).

However, within the TST model, calcium intake had a trending impact on osteocalcin after all other covariates were considered (beta coefficient = 1.758 p-value = 0.067). **Models 9 and 10** show that sleep percent did not predict osteocalcin (beta coefficient = 0.000 p-value = 0.999) but it had a positive association with NTX-1 (beta coefficient = 2.544 p-value = 0.014) serum concentrations after adjusting for all covariates. After covariate adjustment, trending relationships were observed between BMI and osteocalcin (beta coefficient = 0.605 p-value = 0.073) and daily calcium intake and NTX-1 (beta coefficient = 0.253 p-value = 0.082). Across all the models, gender was the most significant covariate in predicting osteocalcin and NTX-1 serum concentrations.

Model 3. Multiple Linear Regression analysis between N-terminal telopeptide of type 1 collagen and sleep onset latency N = 51.

Model 3[a]									
Variables	Block 1[b]			Block 2[c]			Block 3[d]		
	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value
Gender (female = 1 and male = REF)	-0.490	-3.866	**0.000**	-0.333	-2.115	**0.040**	-0.332	-2.121	**0.039**
Race (white = REF and other race = 1)	0.047	0.368	0.714	-0.002	-0.018	0.986	-0.023	-0.176	0.861
BMI (kg/m^2)	-	-	-	0.114	0.825	0.414	0.097	0.707	0.483
calcium intake (mg/day)	-	-	-	0.225	1.495	0.142	0.188	1.237	0.223
Actigraphy Sleep Onset Latency (minutes)	-	-	-	-	-	-	0.167	1.283	0.206
R-Squared	0.251	-	-	0.232	-	-	0.242	-	-
Change in R-Squared	-	-	-	0.043	-	-	0.025	-	-
R-Square p-value	**0.001**	-	-	0.261	-	-	0.206	-	-
ANOVA p-value	**0.001**	-	-	**0.003**	-	-	**0.003**	-	-

[a]N-terminal telopeptide of type 1 collagen serum concentrations (nmol/L BCE) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake, sleep onset latency and physical activity.
[1]Beta coefficient presented in raw form.

Model 4. Multiple Linear Regression analysis between Osteocalcin and sleep onset latency N = 50.

Model 4[a]												
Variables	Block 1[b]				Block 2[c]				Block 3[d]			
	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value
Gender (female = 1 and male = REF)	-0.662	0.218	-5.865	**0.000**	-0.625	0.237	-4.539	**0.000**	-0.623	0.238	-4.539	**0.000**
Race (white = REF and other race = 1)	-0.145	0.716	-1.285	0.205	-0.114	0.769	-0.982	0.332	-0.123	0.753	-1.049	0.300
BMI (kg/m^2)	-	-	-	-	-0.180	0.661	-1.493	0.142	-0.189	0.647	-1.549	0.129
calcium intake (mg/day)	-	-	-	-	0.173	1.489	1.323	0.193	0.151	1.416	1.126	0.266
Actigraphy Sleep Onset Latency (minutes)	-	-	-	-	-	-	-	-	0.088	1.225	0.759	0.452
R-Squared	0.423	-	-	-	0.472	-	-	-	0.479	-	-	-
Change in R-Squared	-	-	-	-	0.050	-	-	-	0.007	-	-	-
R-Square p-value	**0.000**	-	-	-	0.132	-	-	-	0.452	-	-	-
ANOVA p-value	**0.000**	-	-	-	**0.000**	-	-	-	**0.000**	-	-	-

[a]log transformed osteocalcin serum concentrations (ng/ml) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake, sleep onset latency and physical activity.
[1]Beta coefficient presented in raw form.
[2]Reverse log transformation of raw beta coefficient was calculated.

Model 5. Multiple Linear Regression analysis between osteocalcin and total sleep time N = 50.

Model 5[a]												
Variables	Block 1[b]				Block 2[c]				Block 3[d]			
	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value
Gender (female = 1 and male = REF)	-0.662	0.218	-5.865	**0.000**	-0.625	0.237	-4.573	**0.000**	-0.612	0.244	-4.466	**0.000**
Race (white = REF and other race = 1)	-0.145	0.716	-1.285	0.205	-0.114	0.769	-0.982	0.332	-0.115	0.767	-0.996	0.325
BMI (kg/m^2)	-	-	-	-	-0.180	0.661	-1.493	0.142	-0.156	0.698	-1.268	0.212
calcium intake (mg/day)	-	-	-	-	0.173	1.489	1.323	0.193	0.138	1.374	1.021	0.313
Actigraphy Total Sleep Time (minutes)	-	-	-	-	-	-	-	-	-0.119	0.760	-1.019	0.314
R-Squared	0.423	-	-	-	0.472	-	-	-	0.485	-	-	-
Change in R-Squared	-	-	-	-	0.05	-	-	-	0.012	-	-	-
R-Square p-value	**0.000**	-	-	-	0.132	-	-	-	0.314	-	-	-
ANOVA p-value	**0.000**	-	-	-	**0.000**	-	-	-	**0.000**	-	-	-

[a]log transformed osteocalcin serum concentrations (ng/ml) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake, total sleep time and physical activity.
[1]Beta coefficient presented in raw form.
[2]Reverse log transformation of raw beta coefficient was calculated.

Model 6. Multiple Linear Regression analysis between N-terminal telopeptide of type 1 collagen and total sleep time N = 51.

Model 6[a]									
Variables	Block 1[b]			Block 2[c]			Block 3[d]		
	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value
Gender (female = 1 and male = REF)	-0.490	-3.866	**0.000**	-0.333	-2.115	**0.040**	-0.337	-2.108	**0.041**
Race (white = REF and other race = 1)	0.047	0.368	0.714	-0.002	-0.018	0.986	-0.001	-0.011	0.991
BMI (kg/m^2)	-	-	-	0.114	0.825	0.414	0.107	0.751	0.456
calcium intake (mg/day)	-	-	-	0.225	1.495	0.142	0.235	1.499	0.141
Actigraphy Total Sleep Time (minutes)	-	-	-	-	-	-	0.035	0.263	0.794
R-Squared	0.251	-	-	0.293	-	-	0.294	-	-
Change in R-Squared	-	-	-	0.043	-	-	0.001	-	-
R-Square p-value	**0.001**	-	-	0.261	-	-	0.794	-	-
ANOVA p-value	**0.001**	-	-	**0.003**	-	-	**0.006**	-	-

[a]N-terminal telopeptide of type 1 collagen serum concentrations (nmol/L BCE) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake, total sleep time and physical activity.
[1]Beta coefficient presented in raw form.

Model 7. Multiple Linear Regression analysis between N-terminal telopeptide of type 1 collagen and wake after sleep onset. N = 51.

Model 7[a]									
Variables	Block 1[b]			Block 2[c]			Block 3[d]		
	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value
Gender (female = 1 and male = REF)	-0.485	-3.816	**0.000**	-0.334	-2.198	**0.033**	-0.344	-2.171	**0.035**
Race (white = REF and other race = 1)	0.055	0.436	0.665	0.022	0.165	0.870	0.022	0.164	0.870
BMI (kg/m^2)	-	-	-	0.081	0.595	0.555	0.082	0.583	0.563
calcium intake (mg/day)	-	-	-	0.216	1.428	0.160	0.217	1.401	0.168
Actigraphy Wake After Sleep Onset (minutes)	-	-	-	-	-	-	-0.006	-0.042	0.967
R-Squared	0.248	-	-	0.285	-	-	0.285	-	-
Change in R-Squared	-	-	-	0.037	-	-	0.000	-	-
R-Square p-value	**0.001**	-	-	0.317	-	-	0.967	-	-
ANOVA p-value	**0.001**	-	-	**0.003**	-	-	**0.008**	-	-

[a]N-terminal telopeptide of type 1 collagen serum concentrations (nmol/L BCE) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake, wake after sleep onset and physical activity.
[1]Beta coefficient presented in raw form.

Model 8. Multiple Linear Regression analysis between Osteocalcin and wake after sleep onset N = 50.

Model 8[a]												
Variables	Block 1[b]				Block 2[c]				Block 3[d]			
	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value
Gender (female = 1 and male = REF)	-0.660	0.219	-5.833	**0.000**	-0.598	0.252	-4.501	**0.000**	-0.595	0.254	-4.472	**0.000**
Race (white = REF and other race = 1)	-0.161	0.690	-1.425	0.161	-0.133	0.736	-1.178	0.245	-0.131	0.740	-1.160	0.252
BMI (kg/m^2)	-	-	-	-	-0.200	0.631	-1.722	0.092	-0.174	0.670	-1.456	0.152
calcium intake (mg/day)	-	-	-	-	0.221	1.663	1.725	0.091	0.245	1.758	1.879	0.067
Actigraphy Wake After Sleep Onset (minutes)	-	-	-	-	-	-	-	-	-0.114	0.769	-1.008	0.319
R-Squared	0.420	-	-	-	0.489	-	-	-	0.501	-	-	-
Change in R-Squared	-	-	-	-	0.069	-	-	-	0.012	-	-	-
R-Square p-value	**0.000**	-	-	-	0.058	-	-	-	0.319	-	-	-
ANOVA p-value	**0.000**	-	-	-	**0.000**	-	-	-	**0.000**	-	-	-

[a]log transformed osteocalcin serum concentrations (ng/ml) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake, wake after sleep onset and physical activity.
[1]Beta coefficient presented in raw form.
[2]Reverse log transformation of raw beta coefficient was calculated.

Model 9. Multiple Linear Regression analysis between N-terminal telopeptide of type 1 collagen and sleep percent N = 51.

Model 9[a]									
Variables	Block 1[b]			Block 2[c]			Block 3[d]		
	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value
Gender (female = 1 and male = REF)	-0.486	-3.824	**0.000**	-0.352	-2.253	**0.029**	-0.326	-2.204	**0.033**
Race (white = REF and other race = 1)	0.056	0.442	0.660	0.020	0.151	0.881	0.040	0.3180	0.752
BMI (kg/m^2)	-	-	-	0.099	0.714	0.479	0.083	0.63	0.532
Calcium intake (mg/day)	-	-	-	0.196	1.317	0.194	0.253	1.778	0.082
Actigraphy Sleep percent (%)	-	-	-	-	-	-	0.305	2.544	**0.014**
R-Squared	0.249	-	-	0.283	-	-	0.304	-	-
Change in R-Squared	-	-	-	0.034	-	-	0.090	-	-
R-Square p-value	**0.001**	-	-	0.343	-	-	**0.014**	-	-
ANOVA p-value	**0.001**	-	-	**0.004**	-	-	**0.001**	-	-

[a]N-terminal telopeptide of type 1 collagen serum concentrations (nmol BCE) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake, sleep percent and physical activity.
[1]Beta coefficient presented in raw form.

Model 10. Multiple Linear Regression analysis between Osteocalcin and sleep percent N = 50.

Model 10[a]												
Variables	Block 1[b]				Block 2[c]				Block 3[d]			
	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value
Gender (female = 1 and male = REF)	-0.676	0.211	-6.066	**0.000**	-0.661	0.218	-5.028	**0.000**	-0.661	0.218	-4.954	**0.000**
Race (white = REF and other race = 1)	-0.128	0.745	-1.153	0.255	-0.085	0.822	-0.761	0.450	-0.085	0.822	-0.7490	0.458
BMI (kg/m^2)	-	-	-	-	-0.218	0.605	-1.860	0.069	-0.218	0.605	-1.837	0.073
calcium intake (mg/day)	-	-	-	-	0.158	1.439	1.263	0.213	0.158	1.439	1.239	0.222
Actigraphy Sleep percent (%)	-	-	-	-	-	-	-	-	0.000	0.000	-0.002	0.999
R-Squared	0.439	-	-	-	0.497	-	-	-	0.497	-	-	-
Change in R-Squared	-	-	-	-	0.058	-	-	-	0.000	-	-	-
R-Square p-value	**0.000**	-	-	-	0.086	-	-	-	0.999	-	-	-
ANOVA p-value	**0.000**	-	-	-	**0.000**	-	-	-	**0.000**	-	-	-

[a]log transformed osteocalcin serum concentrations (ng/ml) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake and physical activity.
[1]Beta coefficient presented in raw form.
[2]Reverse log transformation of raw beta coefficient was calculated.

Correlation and regression analyses on the interaction effects of PA* SOL, TST, WASO or SP and its impact on OC and NTX-1 serum concentrations (hypotheses 3 and 6 a-d).

Associations between each sleep variable and physical activity are depicted in **Figure 3**. Total sleep time and physical activity had a negative, weak association (r = -0.282 p-value = 0.045). SOL, WASO and SP were not significantly associated with physical activity (r = 0.083 p-value = 0.561; r = 0.162 p-value = 0.255; r = -0.064 p-value = 0.656 respectively). Associations between the bone biomarkers and each interaction variable are illustrated in **Figure 4**. The interaction term PA*SOL had a weak but significant positive association with NTX-1 serum concentration (r = 0.286 p-value = 0.042). Osteocalcin had a moderately strong, and significant positive correlation with SOL x PA (r = 0.308 p-value =0.006). Interaction term TST*PA did not have significant associations with serum NTX-1 (r = 0.107 p-value = 0.457) or osteocalcin (r = 0.231 p-value = 0.106) concentrations. Similar results were found with interaction term PA*WASO, indicating no correlation with osteocalcin (r = 0.205 p-value = 0.153), nor with NTX-1 serum concentrations (r = 0.200 p-value = 0.160). Interaction term SP*PA was not significantly correlated with serum NTX-1 (r = 0.177 p-value = 0.213) but was significantly correlated with serum osteocalcin.

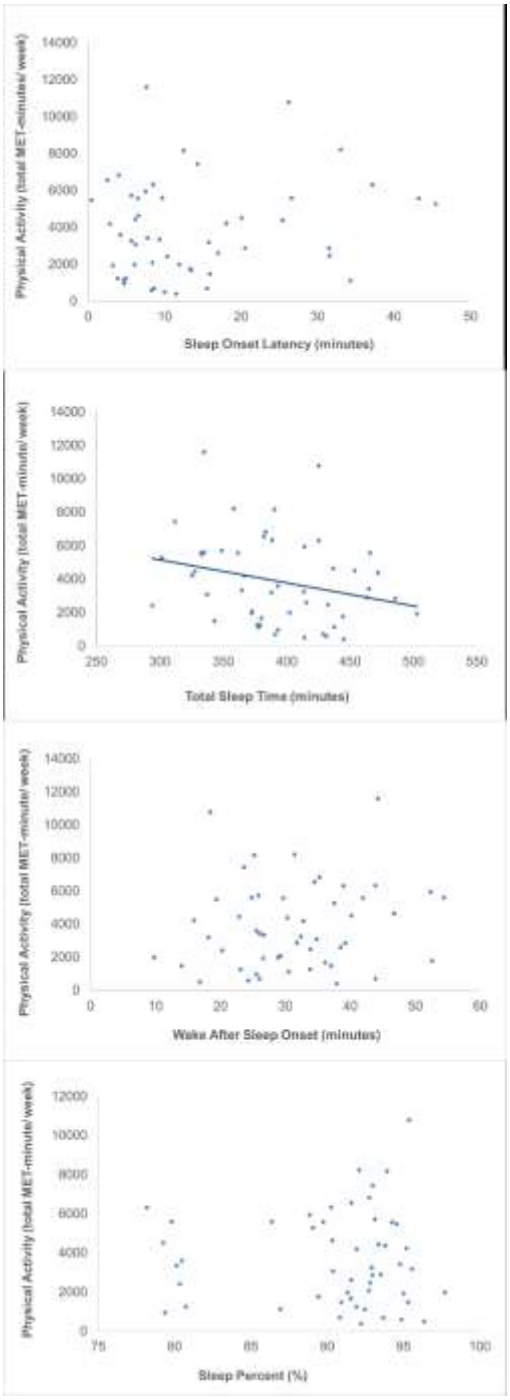

Figure 3. A comparison of associations between each sleep variables TST, WASO, SOL, SP and physical activity. Solid line indicates significance in the correlation test at p-value < 0.05.

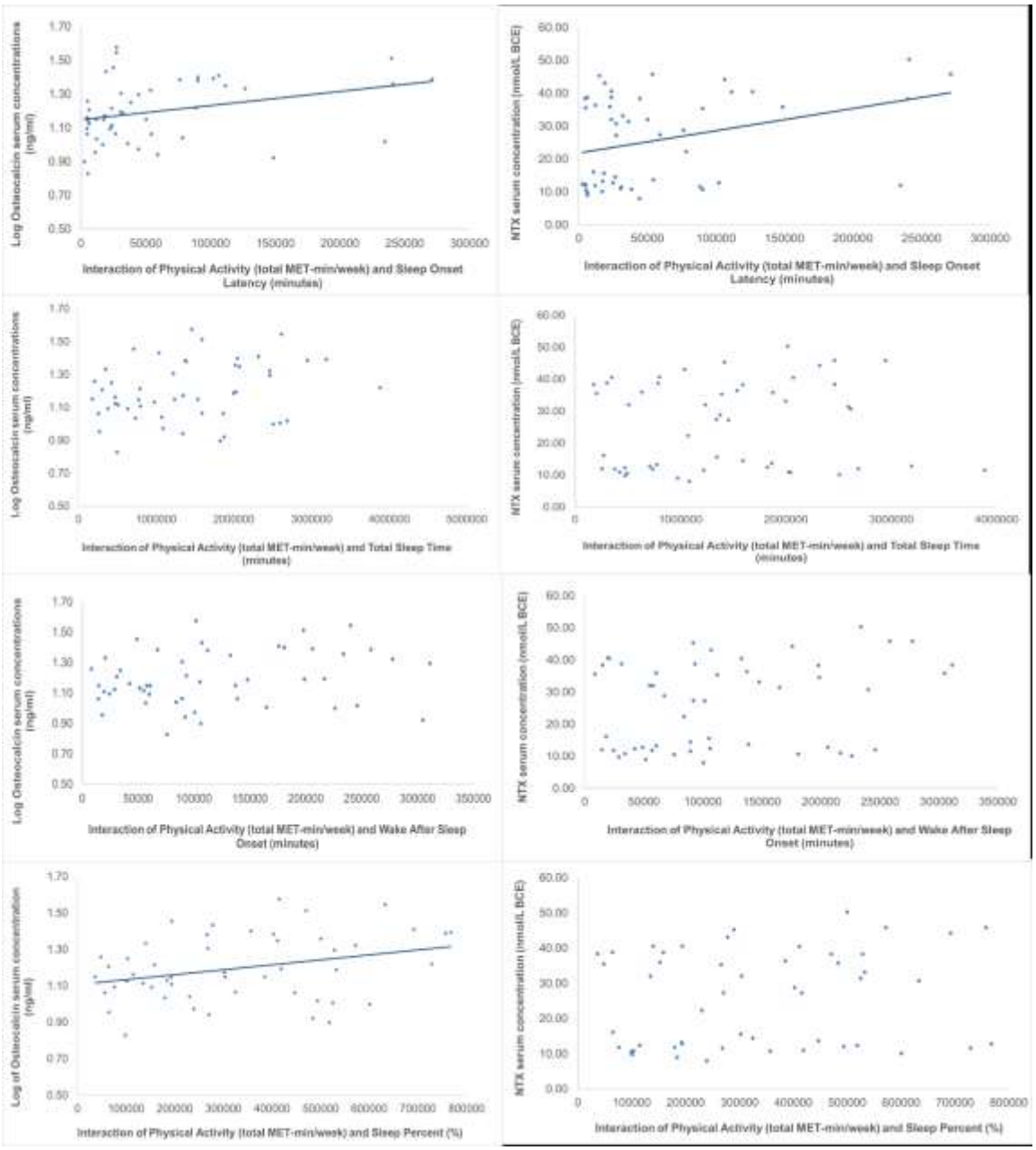

Figure 4. A comparison of associations between interaction variable (physical activity and either TST, WASO, SOL or SP) and osteocalcin or NTX-1 serum concentrations. Solid line indicates significance in the correlation test at p-value < 0.05.

Regression analyses were conducted to determine whether each interaction variable had any influence on predicting serum osteocalcin and NTX-1 concentrations. Models 11-19 were found to be significant when all variables were added as illustrated below. However, interactions term PA*SOL(**Models 11 and 12**), PA*TST (**Models 13 and 14**), PA*WASO(**Models 15 and 16**), and PA*SP (**Models 18 and 19**) were not significantly associated with NTX-1 or osteocalcin after adjustment for covariates. Sleep percent remained significant in the NTX-1 model, before and after its corresponding interaction term was added (beta coefficient = 0.474 p-value = 0.041). Model 16 showed that gender was not significant after PA and WASO were added into the model whereas model 7 sustained that significance with WASO but without PA. In contrast the other models indicated that gender was the most significant factor.

Model 11. Multiple Linear Regression analysis between osteocalcin and interaction of physical activity and sleep onset latency N = 50.

Model 11[a]																
Variables	Block 1[b]				Block 2[c]				Block 3[d]				Block 4[e]			
	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coefficient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coefficient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coefficient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coefficient p-value
Gender (female = 1 and male = REF)	-0.680	0.209	-6.059	**0.000**	-0.646	0.661	-4.672	**0.000**	-0.617	0.242	-4.672	**0.000**	-0.614	0.243	-4.613	**0.000**
Race (white = REF and other race = 1)	-0.153	0.703	-1.36	0.180	-0.118	0.762	-1.029	0.309	-0.124	0.752	-1.066	0.292	-0.111	0.774	-0.932	0.357
BMI (kg/m^2)	-	-	-	-	-0.180	0.661	-1.529	0.133	-0.177	0.665	-1.524	0.135	-0.182	0.658	-1.554	0.128
calcium intake (mg/day)	-	-	-	-	0.158	1.439	1.235	0.223	0.121	1.321	0.958	0.343	0.142	1.387	1.077	0.288
Sleep Onset Latency (minutes)	-	-	-	-	-	-	-	-	0.182	1.521	1.685	0.099	0.252	1.786	1.598	0.118
Physical activity (MET-min/week)	-	-	-	-	-	-	-	-	0.106	1.276	0.954	0.346	0.199	1.581	1.061	0.295
Interaction Physical Activity x Sleep Onset Latency	-	-	-	-	-	-	-	-	-	-	-	-	-0.141	0.723	-0.616	0.514
R-Squared	0.439	-	-	-	0.484	-	-	-	0.525	-	-	-	0.450	-	-	-
Change in R-Squared	-	-	-	-	0.045	-	-	-	0.041	-	-	-	0.541	-	-	-
R-Square p-value	**0.000**	-	-	-	0.153	-	-	-	0.168	-	-	-	0.004	-	-	-
ANOVA p-value	**0.000**	-	-	-	**0.000**	-	-	-	**0.000**	-	-	-	**0.000**	-	-	-

[a]log transformed osteocalcin serum concentrations (ng/ml) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake, sleep onset latency and physical activity.
[e]model adjusted for gender, race, BMI, calcium intake, sleep onset latency, physical activity and interaction of physical activity and sleep onset latency.
[1]Beta coefficient presented in raw form.
[2]Reverse log transformation of raw beta coefficient was calculated.

Model 12. Multiple Linear Regression analysis between N-terminal telopeptide of type 1 collagen and interaction of physical activity and sleep onset latency N = 51.

Model12[a]												
Variables	Block 1[b]			Block 2[c]			Block 3[d]			Block 4[e]		
	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value
Gender (female = 1 and male = REF)	-0.489	-3.841	**0.000**	-0.363	-2.338	**0.024**	-0.324	-2.083	**0.043**	-0.326	-2.071	**0.044**
Race (white = REF and other race = 1)	0.066	0.521	0.605	0.038	0.284	0.778	-0.018	-0.129	0.898	-0.025	-0.176	0.861
BMI (kg/m^2)	-	-	-	0.076	0.556	0.581	0.095	0.698	0.489	0.097	0.708	0.483
calcium intake (mg/day)	-	-	-	0.196	1.313	0.196	0.185	1.239	0.222	0.175	1.12	0.269
SOL (minutes)	-	-	-	-	-	-	0.215	1.645	0.107	0.167	0.752	0.456
Physical activity (MET-min/week)	-	-	-	-	-	-	0.031	0.249	0.804	-0.005	-0.028	0.978
Interaction Physical Activity x Sleep Onset Latency	-	-	-	-	-	-	-	-	-	0.073	0.271	0.788
R-Squared	0.257	-	-	0.288	-	-	0.330	-	-	0.331	-	-
Change in R-Squared	-	-	-	0.031	-	-	0.042	-	-	0.001	-	-
R-Square p-value	**0.001**	-	-	0.374	-	-	0.262	-	-	0.788	-	-
ANOVA p-value	**0.001**	-	-	**0.003**	-	-	**0.005**	-	-	**0.011**	-	-

[a]N-terminal telopeptide of type 1 collagen serum concentrations (nmol BCE) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake and physical activity.
[e]model adjusted for gender, race, BMI, calcium intake, sleep onset latency, physical activity and interaction of physical activity and sleep onset latency.
[1]Beta coefficient presented in raw form.

Model 13. Multiple Linear Regression analysis between osteocalcin and interaction of physical activity and total sleep time N = 50.

Model 13[a]																
Variables	Block 1[b]				Block 2[c]				Block 3[d]				Block 4[e]			
	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coeffiecient p-value
Gender (female = 1 and male = REF)	-0.680	0.209	-6.059	**0.000**	-0.646	0.226	-4.863	**0.000**	-0.619	0.240	-4.648	**0.000**	-0.627	0.236	-4.641	**0.000**
Race (white = REF and other race = 1)	-0.153	0.703	-1.36	0.180	-0.118	0.762	-1.029	0.309	-0.106	0.783	-0.93	0.358	-0.104	0.787	-0.901	0.373
BMI (kg/m^2)	-	-	-	-	-0.180	0.661	-1.529	0.133	0.114	1.300	0.889	0.379	-0.166	0.682	-1.367	0.179
calcium intake (mg/day)	-	-	-	-	0.158	1.439	1.235	0.223	0.164	1.459	1.477	0.147	0.119	1.315	0.919	0.364
Physical activity (MET-min/week)	-	-	-	-	-	-	-	-	-0.082	0.828	-0.698	0.489	0.302	2.004	1.079	0.287
Total Sleep Total (minutes)	-	-	-	-	-	-	-	-	-	-	-	-	-0.051	0.889	-0.392	0.697
Interaction Physical Activity x Total Sleep Time	-	-	-	-	-	-	-	-	-	-	-	-	-0.148	0.711	-0.539	0.593
R-Squared	0.439	-	-	-	0.484	-	-	-	0.520	-	-	-	0.523	-	-	-
Change in R-Squared	-	-	-	-	0.045	-	-	-	0.036	-	-	-	0.003	-	-	-
R-Square p-value	**0.000**	-	-	-	0.153	-	-	-	0.207	-	-	-	0.593	-	-	-
ANOVA p-value	**0.000**	-	-	-	**0.000**	-	-	-	**0.000**	-	-	-	**0.000**	-	-	-

[a]log transformed osteocalcin serum concentrations (ng/ml) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake, total sleep time and physical activity.
[e]model adjusted for gender, race, BMI, calcium intake, total sleep time, physical activity and interaction of physical activity and total sleep time.
[1]Beta coefficient presented in raw form.
[2]Reverse log transformation of raw beta coefficient was calculated.

Model 14. Multiple Linear Regression analysis between N-terminal telopeptide of type 1 collagen and interaction of physical activity and total sleep time N = 51.

Model 14[a]												
Variables	Block 1[b]			Block 2[c]			Block 3[d]			Block 4[e]		
	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value
Gender (female = 1 and male = REF)	-0.489	-3.841	**0.000**	-0.363	-2.338	**0.024**	-0.357	-2.215	**0.032**	-0.373	-2.216	**0.032**
Race (white = REF and other race = 1)	0.066	0.521	0.605	0.038	0.284	0.778	0.038	0.279	0.781	0.041	0.294	0.770
BMI (kg/m^2)	-	-	-	0.076	0.556	0.581	0.080	0.556	0.581	0.069	0.466	0.644
calcium intake (mg/day)	-	-	-	0.196	1.313	0.196	0.188	1.210	0.233	0.180	1.141	0.260
Physical activity (MET-min/week)	-	-	-	-	-	-	0.026	0.197	0.844	0.456	0.392	0.697
Total Sleep Time (minutes)	-	-	-	-	-	-	-0.022	-0.154	0.878	0.067	0.241	0.811
Interaction Physical Activity x Total Sleep Time	-	-	-	-	-	-	-	-	-	-0.420	-0.372	0.712
R-Squared	0.257	-	-	0.288	-	-	0.289	-	-	0.292	-	-
Change in R-Squared	-	-	-	0.031	-	-	0.001	-	-	0.002	-	-
R-Square p-value	**0.001**	-	-	0.374	-	-	0.961	-	-	0.712	-	-
ANOVA p-value	**0.001**	-	-	**0.003**	-	-	**0.016**	-	-	**0.029**	-	-

[a]N-terminal telopeptide of type 1 collagen serum concentrations (nmol BCE) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake, total sleep time and physical activity.
[e]model adjusted for gender, race, BMI, calcium intake, total sleep time, physical activity and interaction of physical activity and total sleep time.
[1]Beta coefficient presented in raw form.

Model 15. Multiple Linear Regression analysis between osteocalcin and interaction of physical activity and wake after sleep onset N = 50.

Model 15[a]																
Variables	Block 1[b]				Block 2[c]				Block 3[d]				Block 4[e]			
	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coefficient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coefficient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coefficient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coefficient p-value
Gender (female = 1 and male = REF)	-0.676	0.211	-6.066	**0.000**	-0.661	0.218	-5.028	**0.000**	-0.619	0.240	-4.783	**0.000**	-0.622	0.239	-4.778	**0.000**
Race (white = REF and other race = 1)	-0.128	0.745	-1.153	0.255	-0.085	0.822	-0.761	0.450	-0.078	0.836	-0.719	0.476	-0.085	0.822	-0.769	0.446
BMI (kg/m^2)	-	-	-	-	-0.218	0.605	-1.860	0.069	-0.176	0.667	-1.529	0.134	-0.182	0.658	-1.570	0.124
calcium intake (mg/day)	-	-	-	-	0.158	1.439	1.263	0.213	0.211	1.626	1.681	0.100	0.206	1.607	1.630	0.111
Physical activity (MET-min/week)	-	-	-	-	-	-	-	-	0.075	1.189	0.698	0.489	-0.115	0.767	-0.431	0.669
Wake After Sleep Onset (minutes)	-	-	-	-	-	-	-	-	-0.230	0.589	-2.148	0.037	-0.323	0.475	-2.016	0.050
Interaction Physical Activity x Wake After Sleep Onset	-	-	-	-	-	-	-	-	-	-	-	-	0.228	1.690	0.780	0.440
R-Squared	0.439	-	-	-	0.497	-	-	-	0.552	-	-	-	0.558	-	-	-
Change in R-Squared	-	-	-	-	0.058	-	-	-	0.055	-	-	-	0.006	-	-	-
R-Square p-value	**0.000**	-	-	-	0.086	-	-	-	0.084	-	-	-	0.440	-	-	-
ANOVA p-value	**0.000**	-	-	-	**0.000**	-	-	-	**0.000**	-	-	-	**0.000**	-	-	-

[a]log transformed osteocalcin serum concentrations (ng/ml) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake, wake after sleep onset, and physical activity.
[e]model adjusted for gender, race, BMI, calcium intake, wake after sleep onset, physical activity, and interaction of wake after sleep onset and physical activity.
[1]Beta coefficient presented in raw form.
[2]Reverse log transformation of raw beta coefficient was calculated.

Model 16. Multiple Linear Regression analysis between N-terminal telopeptide of type 1 collagen and interaction of physical activity and wake after sleep onset N = 51.

Model 16[a]												
Variables	Block 1[b]			Block 2[c]			Block 3[d]			Block 4[e]		
	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value
Gender (female = 1 and male = REF)	-0.486	-3.824	**0.000**	-0.352	-2.253	**0.029**	-0.327	-2.019	0.050	-0.327	-2.002	0.052
Race (white = REF and other race = 1)	0.056	0.442	0.660	0.020	0.151	0.881	0.030	0.225	0.823	0.019	0.139	0.890
BMI (kg/m^2)	-	-	-	0.099	0.714	0.479	0.107	0.750	0.457	0.107	0.742	0.462
Calcium intake (mg/day)	-	-	-	0.196	1.317	0.194	0.194	1.245	0.220	0.190	1.203	0.235
Physical activity (MET-min/week)	-	-	-	-	-	-	0.708	0.483	0.907	-0.068	-0.180	0.858
Wake After Sleep Onset (minutes)	-	-	-	-	-	-	-0.040	-0.302	0.764	-0.121	-0.548	0.587
Interaction Physical Activity x Wake After Sleep Onset	-	-	-	-	-	-	-	-	-	0.192	0.460	0.648
R-Squared	0.249	-	-	0.283	-	-	0.293	-	-	0.297	-	-
Change in R-Squared	-	-	-	0.034	-	-	0.010	-	-	0.003	-	-
R-Square p-value	**0.001**	-	-	0.343	-	-	0.739	-	-	0.648	-	-
ANOVA p-value	**0.001**	-	-	**0.004**	-	-	**0.014**	-	-	**0.025**	-	-

[a]N-terminal telopeptide of type 1 collagen serum concentrations (nmol BCE) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake, wake after sleep onset, and physical activity.
[e]model adjusted for gender, race, BMI, calcium intake, wake after sleep onset, physical activity, and interaction of wake after sleep onset and physical activity.
[1]Beta coefficient presented in raw form.

Model 17. Multiple Linear Regression analysis between osteocalcin and interaction of physical activity and sleep percent N = 50.

Model 17[a]																
Variables	Block 1[b]				Block 2[c]				Block 3[d]				Block 4[e]			
	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coefficient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coefficient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coefficient p-value	Beta Coefficient[1]	Beta coefficient[2]	T-statistic	coefficient p-value
Gender (female = 1 and male = REF)	-0.680	0.209	-6.059	**0.000**	-0.646	0.226	-4.863	**0.000**	-0.640	0.229	-4.776	**0.000**	-0.647	0.225	-4.726	**0.000**
Race (white = REF and other race = 1)	-0.153	0.703	-1.360	**0.000**	-0.118	0.762	-1.029	0.309	-0.097	0.800	-0.855	0.397	-0.097	0.800	-0.839	0.406
BMI (kg/m^2)	-	-	-	-	-0.180	0.661	-1.529	0.133	-0.189	0.647	-1.614	0.114	-0.197	0.635	-1.632	0.110
calcium intake (mg/day)	-	-	-	-	0.158	1.439	1.235	0.223	0.125	1.334	0.974	0.336	0.132	1.355	1.005	0.321
Physical activity (MET-min/week)	-	-	-	-	-	-	-	-	0.173	1.489	1.495	0.142	0.489	3.083	0.515	0.609
Sleep Percent (%)	-	-	-	-	-	-	-	-	-0.022	0.951	-0.188	0.851	0.034	1.081	0.169	0.867
Interaction Physical Activity x Sleep Percent	-	-	-	-	-	-	-	-	-	-	-	-	-0.306	0.494	-0.335	0.739
R-Squared	0.439	-	-	-	0.484	-	-	-	0.515	-	-	-	0.516	-	-	-
Change in R-Squared	-	-	-	-	0.045	-	-	-	0.031	-	-	-	0.001	-	-	-
R-Square p-value	**0.000**	-	-	-	0.153	-	-	-	0.259	-	-	-	0.739	-	-	-
ANOVA p-value	**0.000**	-	-	-	**0.000**	-	-	-	**0.000**	-	-	-	**0.000**	-	-	-

[a]log transformed osteocalcin serum concentrations (ng/ml) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake, sleep percent and physical activity.
[e]model adjusted for gender, race, BMI, calcium intake, sleep percent, physical activity and interaction of physical activity and sleep percent.
[1]Beta coefficient presented in raw form.
[2]Reverse log transformation of raw beta coefficient was calculated.

Model 18. Multiple Linear Regression analysis between N-terminal telopeptide of type 1 collagen and interaction of physical activity and sleep percent N = 51.

Model 18[a]												
Variables	Block 1[b]			Block 2[c]			Block 3[d]			Block 4[e]		
	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value	Beta Coefficient[1]	T-statistic	coeffiecient p-value
Gender (female = 1 and male = REF)	-0.489	-3.841	**0.000**	-0.363	-2.338	**0.024**	-0.295	-1.975	0.055	-0.312	-2.054	**0.046**
Race (white = REF and other race = 1)	0.066	0.521	0.605	0.038	0.284	0.778	0.038	0.299	0.766	0.038	0.303	0.764
BMI (kg/m^2)	-	-	-	0.076	0.556	0.581	0.101	0.776	0.442	0.081	0.606	0.548
calcium intake (mg/day)	-	-	-	0.196	1.313	0.196	0.226	1.577	0.122	0.245	1.674	0.101
Physical activity (MET-min/week)	-	-	-	-	-	-	0.140	1.097	0.279	0.910	0.873	0.387
Sleep Percent (%)	-	-	-	-	-	-	0.336	2.654	0.011	0.474	2.109	0.041
Interaction Physical Activity x Sleep Percent	-	-	-	-	-	-	-	-	-	-0.748	-0.744	0.461
R-Squared	0.257	-	-	0.288	-	-	0.387	-	-	0.395	-	-
Change in R-Squared	-	-	-	0.031	-	-	0.099	-	-	0.008	-	-
R-Square p-value	**0.001**	-	-	0.374	-	-	0.037	-	-	0.461	-	-
ANOVA p-value	**0.001**	-	-	0.003	-	-	**0.001**	-	-	**0.002**	-	-

[a]N-terminal telopeptide of type 1 collagen serum concentrations (nmol/L BCE) is the dependent variable.
[b]model adjusted for gender, and race.
[c] model adjusted for gender, race, BMI and calcium intake.
[d]model adjusted for gender, race, BMI, calcium intake, sleep percent and physical activity.
[e]model adjusted for gender, race, BMI, calcium intake, sleep percent, physical activity and interaction of physical activity and sleep percent.
[1]Beta coefficient presented in raw form.

CHAPTER 5

DISCUSSION

Summary

The purpose of the study was to observe whether physical activity and sleep patterns predicted osteocalcin (OC) and N-terminal telopeptide of type 1 collagen (NTX-1) serum concentrations in college students 18-25 years old. Physical activity did not correlate nor predict OC (r = 0.274 p-value = 0.052; beta coefficient = 14.172 p-value = 0.204) and NTX-1 serum concentrations (r = 0.155 p-value = 0.272; beta coefficient = 0.044 p-value = 0.734). Only sleep percent was able to predict NTX-1 serum concentration (beta coefficient = 0.305 p-value = 0.014). Total sleep time had a significant correlation with OC serum concentration (r = -0.298 p-value = 0.036). Sleep onset latency had a significant correlation with NTX-1 serum concentration (r = 0.293 p-value = 0.037).

Physical Activity had no impact on OC and NTX-1 serum concentrations.

Physical activity was neither associated nor did it contribute to the variance of OC and NTX-1 serum concentrations. In the present study, the IPAQ short form questionnaire was used to assess habitual exercise. Unfortunately, this instrument does not capture the specific types of activity that are being performed. Therefore, results from this study are contradictory to the beneficial effects seen on BMD with weight bearing exercise and vigorous physical activity.[79,119,120] Further, athletic young adults were shown to have higher rates of bone formation compared to their non-athletic peers.[121] The timing of physical activity across the life course may also impact the ability to see effects on bone, as boys who exercised more than three hours per week as early as 15 years old had higher BMD at 30 years of age, compared to sedentary counterparts.[12] In the present

study, male and female college students reported higher activity levels (3837 MET-minute/week) than collegiate athletes in a recent study (1115 MET-minute/week).[79] The lack of an association between physical activity and bone biomarkers observed in this study suggests that over reporting might have occurred among our participants or that the timeframe during which physical activity was measured (7 days) was not adequate to evaluate changes in bone biomarkers.

In response to the benefits of physical activity on BMD, related bone turnover markers were assessed in the present study. Young men in an 8 week anaerobic exercise training program had a mean serum OC concentration of 10.1 ug/L after the program, while the present study observed a mean concentration of 21.9 ng/ml among male students.[80] Women in their 20s were observed to have OC serum concentrations of 12 ng/ml after an 8-week resistance and aerobic combined exercise program.[11] The current study observed 12.1 ng/ml of OC concentration among female students. Despite these observations in bone formation serum concentrations, physical activity did not predict bone formation in the present study. This could be due to the fact that these other studies centered around exercise interventions while the current study aimed to capture habitual behavior while also accounting for multiple covariates. Exercise is a potent stimulator of bone turnover, as exercise has repeatedly been shown to induce anabolic effects to bone.[26,43,122,123] However, the present study demonstrated that habitual physical activity among young adults may not have the same degree of impact on bone turnover rate compared to other lifestyle and genetic factors.

Sleep patterns did not predict OC and NTX-1 serum concentrations.

Overall, sleep patterns were not predictive of OC and NTX-1 serum concentrations, except for a positive association between sleep percent and NTX-1 (after adjustment for covariates). Sleep percent relates most to sleep quality as it assesses the proportion of actual sleep from sleep onset to offset.[31] Sleep quality has been positively associated with BMD and a reduced risk of osteoporosis among older men and women.[126,128] The current study indicated that male students obtained a 90% and female students obtained an 89% sleep percent, indicating that they obtained higher quality sleep. This contrasts with the overwhelming report of poor-quality sleep in college students.[106, 129] While sleep percent is not a widely used variable, these data suggest that sleep quality may also be an important factor for bone health.

Total sleep time (TST) has been negatively associated with bone mineral density in both middle aged and older adults (45-65 years old).[15,87,124] Further, sleep restriction to 5 hours per night for 3 weeks, lowered N-terminal propeptide of type 1 collagen (PINP) by 28% in young men (20-27 years old) compared to men 55-65 years old.[90] In the current study, a negative, significant correlation was observed between total sleep time and osteocalcin ($r = -0.298$ p-value = 0.036); however, this relationship disappeared after adjusting for covariates. Despite observations of linear relationships, U-shaped relationships may also exist between sleep and bone turnover makers, where those receiving less than 6 or greater than 10 hours of sleep had lower BMD.[88] Students in the current study obtained about 6 hours of sleep per night. This is not uncommon among college students as they have been reported to sleep about 6-8 hours per night, depending

on their year in college.[106] Further work is needed to explore impacts of varying sleep amounts on bone biomarkers.

Sleep onset latency (SOL) had a positive correlation with NTX-1 in the present study ($r = 0.293$ p-value = 0.037) but this sleep variable did not predict OC and NTX-1 serum concentrations in models including covariates. In elderly osteoporotic patients, a sleep onset latency of more than 30 minutes was associated with lower BMD, after adjusting for gender, age and BMI.[125] A similar observation was found among men and women 55 years old in which their sleep latency was associated with a greater risk for osteopenia.[126] The present study found a significant correlation between NTX-1 and SOL, supporting that delayed sleep onset may play a role in bone resorption. The mechanism of this association could be that longer SOL results in shorter sleep time.[24] This shortened sleep time may inhibit bone formation, thereby allowing bone resorption rates to increase.[91] Young men and women in the present study were found to take 15 minutes to fall asleep (SOL). This is slightly shorter when compared to the 17-30 minutes observed in other college students.[105, 106] The lack of delayed sleep onset in our cohort may explain why we did not find an association between bone turnover and sleep onset latency..

Wake after sleep onset (WASO) did not correlate nor predict OC and NTX-1 serum concentrations. Frequency of sleep awakenings are indicative of sleep fragmentation. College students were seen to have at least 2 awakenings per night across a 7-night observation.[24,105] Sleep fragmentation is often seen in insomniac patients.[87,127] Patients with insomnia have more fragmented sleep which has been associated with low BMD.[87.127] While frequency of sleep fragmentation was not used in the present study, WASO (total minutes of night awakenings) is a similar measure. Students in the present

study had a total of 32 minutes of WASO, indicating they spent some of their sleep time awake in the middle of the night. Given the short WASO time observed in this cohort, it is possible that the present study cohort did not reach a WASO threshold capable of influencing bone turnover biomarkers.

Interaction terms of physical activity and sleep patterns did not predict OC and NTX-1 serum concentrations.

Physical activity has been noted as an antidote to troubled sleeping. [18,19,93] Specifically, exercise regimens of at least 4 weeks have been shown to decrease SOL and improve self-reported sleep quality in adults 18-65 years.[19] High intensity aerobic exercise 2 hours before bed has also been shown to increase sleep efficiency as well as reduce WASO in young men.[92] However, total sleep time had mixed results ranging from no association with physical activity in young adults to moderate associations in older adults.[18,96] In the present study, total sleep time had a significant, negative correlation with physical activity ($r = -0.282$ p-value = 0.045), suggesting that that physical activity may shorten sleep time. Some data are supportive of this finding, as exercising 1 hour prior to bedtime was show to decrease total sleep by 14 minutes in young men compared to a no exercise night.[130] Such findings may indicate that physical activity causes excitement and reduces the ability for a person to fall asleep.

Interaction terms of PA*SOL had a significant, positive association with OC and NTX-1 serum concentrations ($r = 0.308$ p-value = 0.006; $r = 0.286$ p-value = 0.042 respectively). These interactions were not maintained after the inclusion of covariates in multivariate models. The correlations seen in the current study could be due to SOL having a catabolic effect on bone while physical activity has an anabolic effect. Rather

than favoring either bone resorption or absorption, PA*SOL may increase both metabolic pathways of bone remodeling. The interaction term PA*SP had a significant, positive association with OC serum concentration. While sleep percent has not been directly assessed in relation to bone health, it is indicative of sleep quality which has been positively associated with BMD in previous studies.[131]

Gender played a significant role in bone remodeling.

The current study showed that gender was a significant factor among race, BMI, calcium intake, physical activity and each sleep variable assessed. Gender has long been noted as a significant factor for bone accrual during adolescence and bone loss in older age (50 years +).[38,56] Therefore, it was expected to see differences in OC (12.1 ng/ml women; 21.9 ng/ml men) and NTX-1 serum concentrations (18.2 nmol/L BCE women; 31.8 nmol/L BCE men) in the present study. Given the gender differences in bone turnover observed in this study, gender/sex should be considered in future studies attempting to evaluate associations of sleep patterns, physical activity and bone health.

Public health implications on college students.

Sleep patterns may have been insignificantly associated with bone remodeling in the present study, but the benefits of good sleep including improved memory and mood make it an important component of wellbeing.[24,103,132] The present study reported 6 hours of TST and 32 minutes of WASO indicating that sleep is not only short but also disrupted. Educating students on the importance of sleep has resulted in positive health outcomes in other studies. Through online educational modules, college students awareness of sleep pattern status were able to improve their sleep quality over an 8-week period.[133] Total sleep time also increased in high school students after they learned about the importance

of sleep in four 50-minute sessions in the classroom.[134] College students should be aware of the sleep recommendations in order to improve their stress levels and ensure academic success.

At the same time college students should make an effort to obtain the recommended amount of physical activity (30 min/day).[12] The current study did not find an association between physical activity and bone remodeling in students. Regardless, being physically active is a vital component to healthy living. Engaging in physical activity has been shown to improve stress levels, depressive symptoms and decrease BMI in college students.[98,135] However, students have reported that their sedentary behavior is caused by the lack of time to exercise due to academic demands as well as the lack of resources.[99] Universities should consider promoting recreational facilities and activities to ensure that students enhance their wellbeing through physical activity.

Strengths and limitations of the study.

The current study had strengths and limitations in its design that may have influenced the results. The current study was a cross sectional design, only allowing a single assessment of bone turnover markers, physical activity and sleep patterns. Surveys and devices used in the present study were validated in similar populations, confirming the accuracy of data obtained from participants. Including both genders in the study allowed comparisons and better generalizability to all college students.

There were strengths and limitations to sleep assessment in the present study that may have resulted in the insignificance observed. Measuring sleep patterns for 7 consecutive nights was determined to be an effective method when using an actigraphy watch, a device second best to the polysomnogram test conducted in a lab.[136,137]

However, college students practice highly irregular sleep patterns in response to academic demands, gaining less sleep during mid-term or finals weeks.[138] Variability in sleep from academic events may have contributed to the lack of findings regarding bone markers.

The IPAQ short form has been deemed as a reliable tool to measure physical fitness in all age groups of various health status but in the present study over reporting may have resulted in the insignificant findings.[139] Further, the IPAQ short form has been critiqued to be more accurate when used among larger cohorts;[140] our sample was smaller than recommended sample sizes for this instrument. The present study had 52 college students making over reporting moderate to vigorous activity more profound. In order to gain accurate responses from participants, clarification on the specific activities in which students are engaging at moderate and vigorous levels may be needed. Accelerometers may also provide less biased reports of sedentary and active time.

Osteocalcin and NTX-1 may not have been enough to understand the connection between physical activity and sleep patterns on bone health. Osteocalcin and NTX-1 were determined to be closely associated with bone mineral accrual in older men and women, indicating that the markers are highly relevant to osteoporosis risk.[29,55] However, bone biomarkers are highly variable, with highest concentrations in the morning, when obtained in a fasting state.[29,141] While the protocol in the current study obtained fasting bloods to measure osteocalcin and NTX-1, comparing concentrations from different commercial assays has been shown to be variable.[29] This would make comparing results from one study to another difficult depending on the analysis methods implemented.

CHAPTER 6

CONCLUSION

Sleep patterns and self-reported physical activity did not predict bone turnover in college students (18-25 years). The present study found a positive association between total sleep time and osteocalcin and sleep onset latency with NTX. This may suggest that sleep duration may be important for bone mineral accrual while delayed onset of sleep may increase bone resorption. Despite initial correlations, these associations were not sustained after adjusting for covariates. Overall, gender seemed to be the strongest predictor of each bone biomarker, suggesting that genetics drives a large portion of bone turnover. Regardless of the lack of significant findings in this study, college students remain a high-risk population as they frequently experience reductions in physical activity and sleep duration and quality. Future research is needed to better understand the impact of sleep pattern and physical activity on bone health. Studies may need to consider more objective measures in physical activity to accurately gain moderate to vigorous activity. Gender should be further assessed through each association to better understand the relationship of sleep pattern, physical activity and bone health.

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APPENDIX A

CONSENT FORM

CONSENT FORM

Effects of sleep on bone mineral density in college-aged males and females.

INTRODUCTION
This form is meant to provide you with information that may affect whether you want to participate in this research study. This form also serves to record your consent if you agree to join the study.

RESEARCHERS
Professors Corrie Whisner, PhD and Megan Petrov, PhD, along with undergraduate students Patty Esch and Leslie Hammon at Arizona State University will oversee this research study.

STUDY PURPOSE
Our primary aim is to see if sleep patterns impact bone health.

DESCRIPTION OF RESEARCH STUDY
If you decide to join we will assess your sleep habits and measure the strength of your skeleton.

You are being asked to take part in this study because you are between the ages of 18-25 years, of a healthy weight, and are in general good health. You will be asked to complete a diary to record your sleep habits for seven nights while wearing a wrist-worn sensor called an ActiWatch to monitor your sleep. You also will be asked to schedule and complete a Dual-energy X-ray Absorptiometry (DEXA) scan to measure the strength of your bones.

Visit 1:
You will visit the ABC-1 Building on the Downtown Phoenix campus of Arizona State University and complete the following:

1. Surveys about your sleep, physical activity, dietary, and medical history.
2. Height and weight will be taken.
3. Wear a small movement sensor for 7 days to measure your sleep.
4. Fill out a 7-night sleep journal.
5. Schedule a DEXA scan and fasting blood draw.

Total time for visit 1 is approximately 2 hours.

Visit 2 and/or 3:

1. If you were not able to complete your DEXA scan earlier in the week, then it can be done at this visit.
2. Complete fasting blood draw.
3. Turn in sleep journal and ActiWatch.

Total time for visit 2 is approximately 1 hour.

The **total time commitment** to finish all study tasks is about 3 hours.

Testing:

Sleep Patterns
Your sleep will be measured in two ways. First, you will wear a small movement sensor (like a watch) called an ActiWatch for 7 days in a row. This device is worn on your wrist at all times of the day, unless showering or swimming. Second, you will fill out a 7-day sleep diary.

Bone Mineral Density
Bone mineral density will be assessed via DEXA scan (Lunar iDXA, GE Healthcare, Madison, WI). You will be asked to lie flat on your back, face up, on a padded table. This will take about 20 minutes. The scanner arm of the DEXA machine passes over your entire body, from head to foot. The scanner will not enclose you nor will the scanner arm touch you. You can wear regular clothing (no metal allowed). A licensed radiologic

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technologist will perform all DEXA scans at the ABC-1 building. All female participants will be asked to complete a urine pregnancy test to confirm that they are not pregnant before performing the DEXA.

Blood Biomarkers
We will draw 15ml of fasting (you have not eaten for 10 hours) blood to measure compounds that tell us about your bone health. Blood draws will be performed by a highly trained nurse and/or certified phlebotomist. This procedure will take about 15 minutes.

RISKS
The risks for this study will be no more than that incurred in daily life. You may incur very small doses of ionizing radiation from the DEXA scan. Anytime you are exposed to radiation there are potential risks. For this particular exam, you will be exposed to minimal radiation (1-4 microSieverts). This amount lies within an acceptable range as provided by the US FDA. For comparison, you would receive radiation exposure of about:

- 80 mS on a transatlantic airline flight of 8 hours
- 50 mS living in Denver, Colorado, at an elevation of 5,000 feet for 4 weeks, or
- 30-40 mS during a typical chest x-ray.

If there is ANY chance of a participant being pregnant then they will not undergo DEXA scanning. With this in mind, all female participants of child bearing age will be asked to take a urine pregnancy test immediately prior to each DEXA scan. If a participant becomes pregnant during the course of the study, she must immediately inform the staff, and withdraw from the DEXA portion of the study.

The blood draw involves a needle puncture which may cause some discomfort and bruising or risk of infection. Blood draws will be carried out by experienced medical staff who will clean the insertion site and use sterile tools. Other possible risks of a blood draw include dizziness, fainting and nausea. All blood draws will be conducted while the participant is seated to ensure safety in case any of these possible side effects occur.

As with any research, there is a chance that you may be subject to risks that have not yet been identified.

BENEFITS
There are no direct benefits to you for joining this study. If you would like to receive a copy of your study results you can contact study staff to obtain a copy of your DEXA scan. Please email whisnerlabasu@gmail.com for this purpose.

NEW INFORMATION
If the researchers find new information during the study that would change your decision about participating, they will provide this information to you immediately.

CONFIDENTIALITY
All information obtained in this study is strictly confidential unless required by law. The results of this research study may be used in reports, presentations, and publications, but your name or identity will never be revealed. In order to maintain confidentiality of your records, study staff will use subject codes on all data collected. Names will be kept on a master list separate and secure from all data collected. Access to this file will be limited to study investigators.

WITHDRAWAL PRIVILEGE
It is ok for you to say no to participating in this study. Even if you say yes now, you are free to say no later. You may withdraw from the study at any time. Your decision will not affect your relationship with ASU or cause a loss of benefits to which you might otherwise be entitled.

Your participation is voluntary and if you decide not to participate or decide to withdraw from the study it will not affect your course grades, treatment, care, and/or employment status while at ASU.

_____
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COSTS AND PAYMENTS
All study procedures will be provided to you at no cost. If selected to participate you will be paid $20 (cash) for completion of the study. Study completion includes all testing visits and questionnaire completion. No money will be awarded until your ActiWatch has been returned to study staff.

COMPENSATION FOR ILLNESS AND INJURY
If you agree to participate in the study, your consent will not waive any of your legal rights. However, no funds have been set aside to compensate you in the event of injury. In the event of a medical emergency first aid will be administered and if necessary, 911 will be called.

VOLUNTARY CONSENT
Any questions you have concerning the research study or your participation will be answered by study staff at whisnerlabasu@asu.edu or 602-827-2261.

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at 480-965-6788.

This form explains the nature, demands, benefits and risks of the project. By signing this form, you agree to assume any risks involved. Your participation is voluntary. You may choose not to participate or to withdraw at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you.

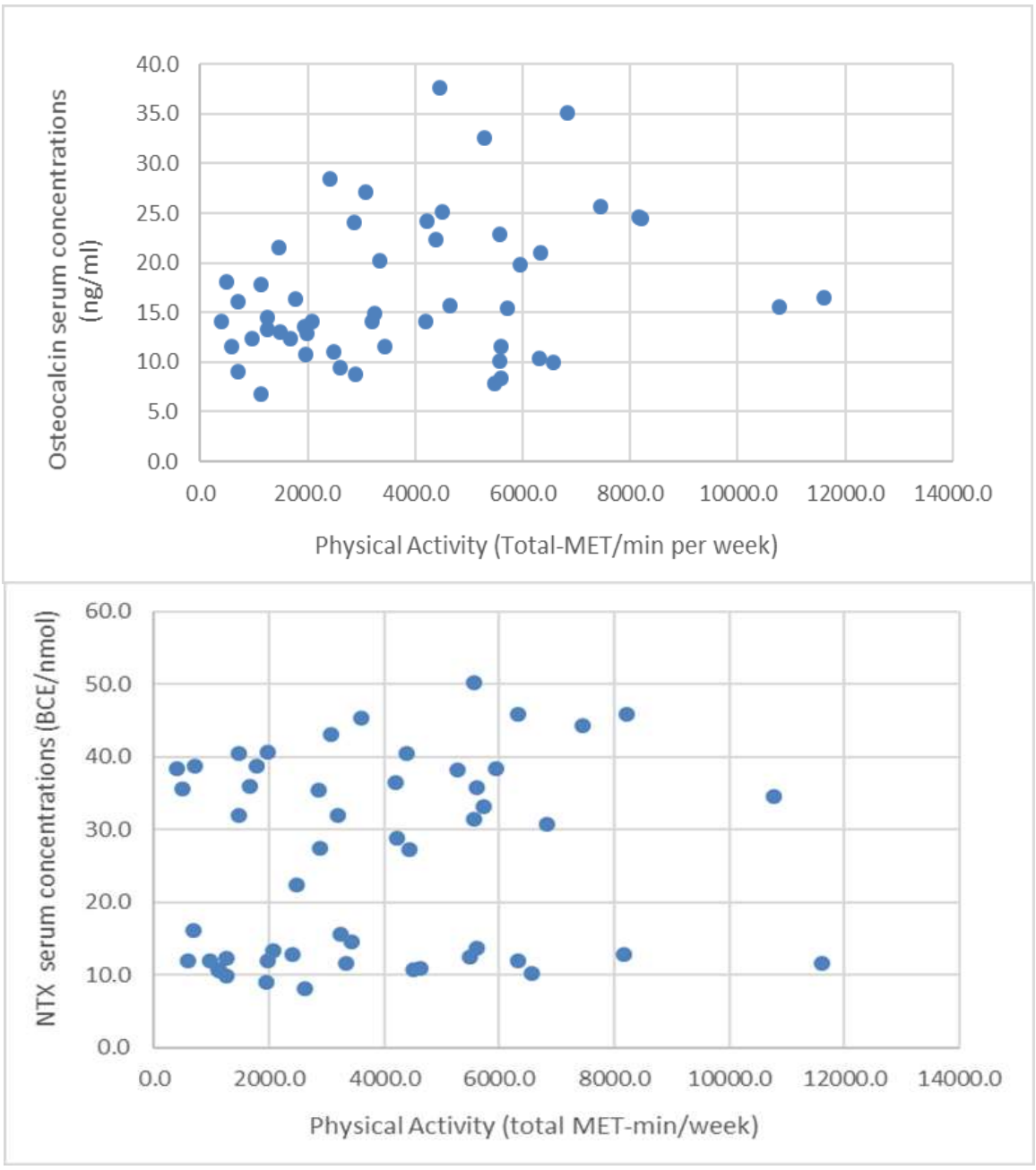
Signing below shows that you consent to join in the above study

Subject's Signature	Printed Name	Date

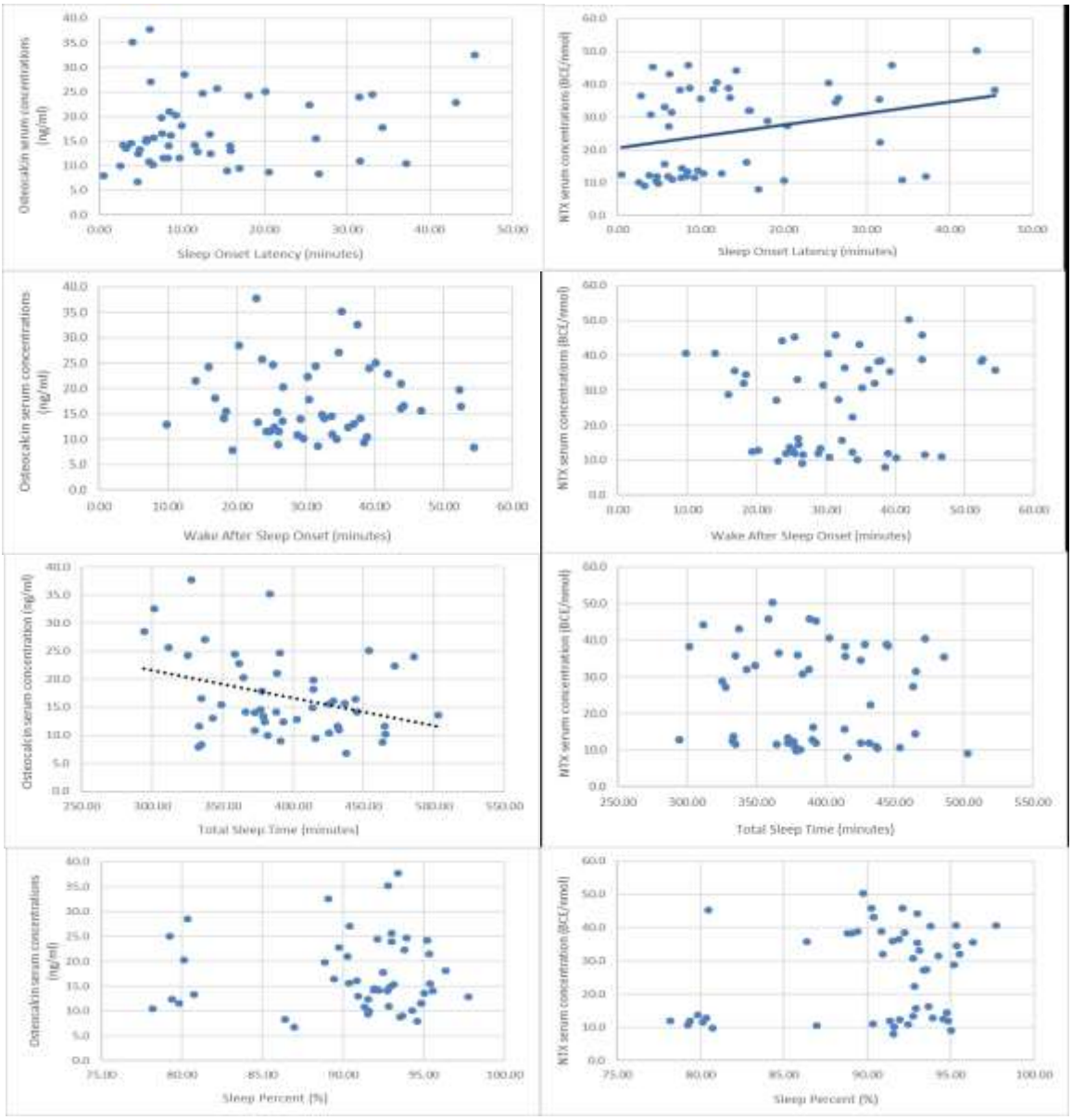
Contact phone number	E-mail

APPENDIX B

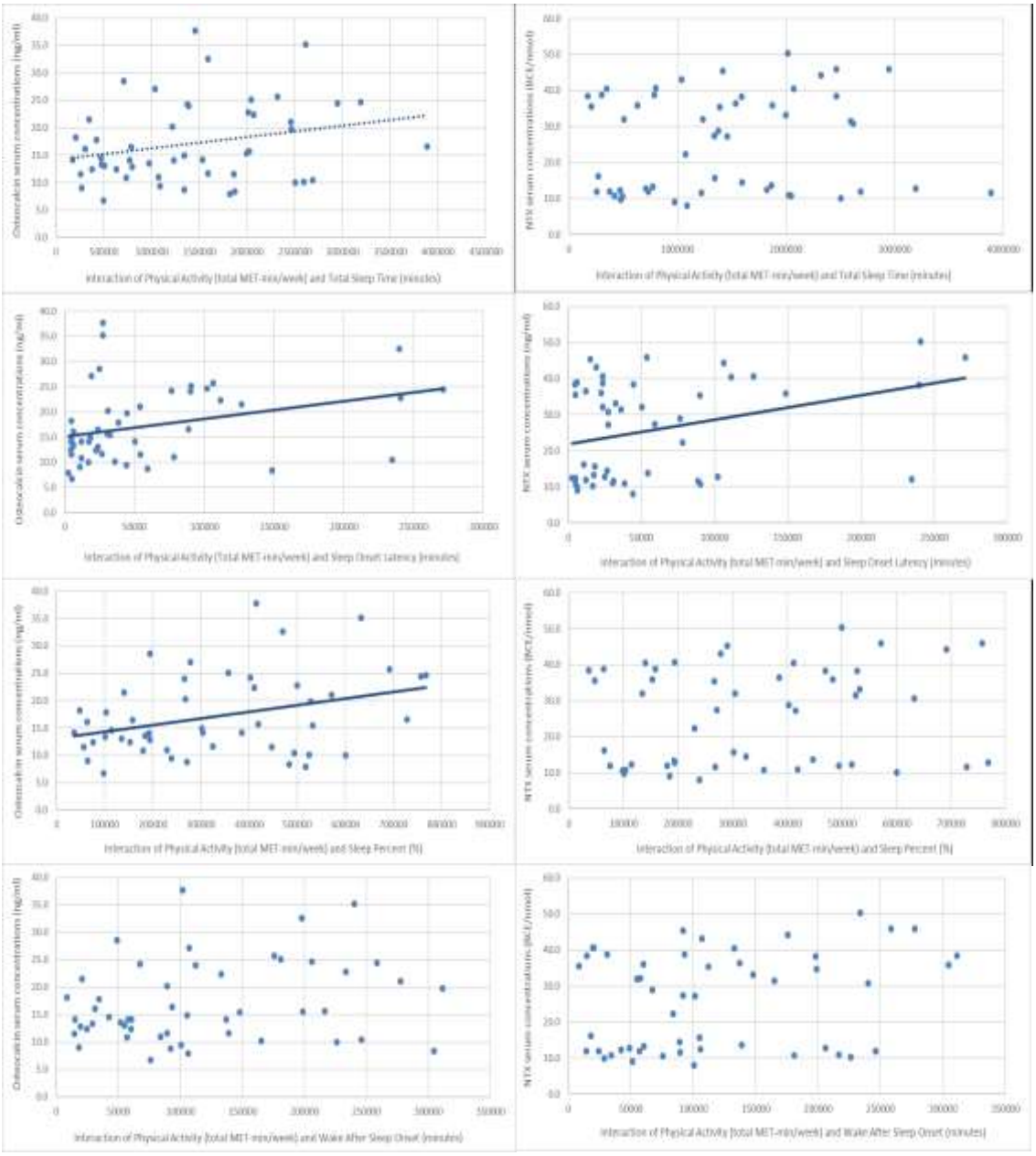
SUPPLEMENTAL GRAPHS



Supplemental figure 1. A comparison of associations between physical activity and osteocalcin (raw form) or NTX-1 serum concentrations



Supplemental figure 2. A comparison of associations between each sleep variables (SOL, WASO, TST and SP) and osteocalcin (raw form) or NTX-1 serum concentrations. Solid line = significant correlations. Solid line = significant correlations. Dotted line = trending significance.



Supplemental figure 3. A comparison of associations between interaction variable (physical activity and either TST, WASO, SOL or SP) and osteocalcin (raw form) or NTX-1 serum concentrations. Solid line = significant correlations. Dotted line = trending significance.