January 2017

Does Dietary Melatonin Play a Role in Bone Mineralization?

Martha Renee Wasserbauer
Syracuse University

ABSTRACT

Introduction: Melatonin is generated as a product of normal circadian rhythm and is also is thought to play an important role in maintaining bone mineral density (BMD) by reducing chronic inflammation. Postmenopausal women are at an elevated risk of BMD loss due to declining estrogen and a natural decrease in melatonin synthesis with increasing age. Endogenous melatonin production is largely influenced by exposure to external light cues, but recent research has indicated that serum melatonin may be increased by the consumption of melatonin-rich foods. The purpose of this study was to quantify dietary-derived melatonin and examine its effects on inflammation, BMD, and sleep in a sample of postmenopausal women.

Methods: Cross-sectional analysis of data from the National Health and Nutrition Examination Survey (NHANES) was conducted to examine differences in melatonin consumption, BMD, and sleep in postmenopausal women with chronic and low-level inflammation indicated by level of C-reactive protein (CRP). Data from the years 2005-2010 was included in this study.

Results: 110 postmenopausal women were included in the analysis with 55 subjects included in each inflammatory group. Individuals with normal CRP had a significantly greater intake of dietary melatonin ($p=0.03$) and higher BMD ($p<0.05$) than individuals with chronic inflammation. Normal inflammatory subjects also had a significantly higher intake of folate ($p<0.0001$), vitamin B6 ($p=0.0005$), and magnesium ($p=0.0005$) than subjects with chronic inflammation. Hours of sleep did not differ significantly with CRP level ($p=1.0$). Individuals with chronic inflammation exhibited a negative correlation between BMD and CRP ($r= -0.27$, $p=0.04$).

Conclusions: The results suggest that dietary-derived melatonin may play an underlying role in mitigating inflammation and increasing BMD in postmenopausal women by reducing inflammation and oxidative stress. Melatonin may enhance the effects of other antioxidants and anti-inflammatory nutrients, and is part of dietary patterns rich in plant foods. Foods with melatonin also contain a variety of nutrients that act as coenzymes and cofactors for synthesis of endogenous melatonin.

DOES DIETARY MELATONIN PLAY A ROLE IN BONE MINERALIZATION?

BY

Martha R. Wasserbauer

B.S., St. Lawrence University, 2014

Master's Thesis

Submitted in partial fulfillment of the requirements for the degree of

Master of Science in Nutrition Science

Syracuse University

June 2017

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Margaret Voss, for providing me with continuous support and guidance throughout the process of writing this thesis. Her inspiration, expertise, and confidence in my ability to succeed have made this a rewarding and fulfilling journey. Thank you for helping me recognize my strengths and discover my passion for research.

I would also like to thank Dr. Lynn Brann and Dr. Sudha Raj for serving on my proposal and defense committees and for providing me with valuable feedback. Thank you to Dr. Rick Welsh for serving as my oral defense chair.

Thank you to all of my friends and classmates. I could always count on them for much needed support and laughter throughout the past two years to get through the stressful times.

I would like to thank my family for providing me with never-ending love, support, and encouragement throughout my education. Thank you for teaching me to be a lifelong learner, to take on new challenges with enthusiasm and confidence, and to aim high by following my passions.

Finally, I would like to thank Eric for his unparalleled love and support, for believing in my ability to succeed, and for being there for me every step of the way. I would not have been able to get through this challenging process without him, and am grateful that I was able to share this experience with him.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

Part I: Literature Review and Proposed Study Design

Introduction

Inflammation and Bone Mineral Density

Chronic inflammation is thought to play a role in reducing bone mineral density (BMD) through a number of mechanisms. Pro-inflammatory cytokines, including c-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), interleukin (IL) 1, and immune cells are responsible for disrupting normal bone metabolism and preventing growth of new bone, or osteogenesis, from occurring.[1,2,3] CRP is an acute phase reactant marker of chronic, low-grade inflammation. A better understanding of why blood CRP levels change may help clarify the way in which chronic inflammation alters BMD over time.[2] The relationship between CRP and BMD has been previously evaluated using data obtained from the National Health and Nutrition Examination Survey (NHANES). This analysis found that CRP concentration was significantly associated with BMD in a dose-dependent relationship in both men and women.[1] Specifically, there was an inverse correlation between CRP concentration and BMD in the lumbar spine independent of potential confounding variables.[1] Forty-seven percent (47%) of the study participants were postmenopausal women. Within this subset of participants, those with high CRP levels were also more likely to have a low BMD, osteopenia, or osteoporosis in the femur.[1] High CRP has also been shown to be a good predictor of fractures in individuals with osteoporosis.[2]

To understand the link between CRP and BMD, it is necessary to consider several cell signaling pathways commonly upregulated by inflammation. The receptor activator of nuclear factor-κβ is the protein RANKL, a pro-inflammatory cytokine that binds to the RANK receptor

of osteoclasts to promote resorption, which is the breakdown of bone.[2] Osteoprotegerin (OPG) is an antagonist to RANKL that prevents RANKL from binding to the RANK receptor, therefore preventing bone breakdown.[2] The ratio of RANKL/OPG is increased by a number of factors, including low estrogen and glucocorticoids, and plays a role in regulating T cells and B cells and the production of inflammatory cytokines.[2] In addition, TNF-α has been shown to directly increase the amount of RANKL, allowing osteoclast precursors to become mature osteoclasts.[2] Therefore, bone formation and resorption may be related to CRP concentration, as CRP is produced by the liver in response to TNF-α, IL-1, and IL-6 secretion.[2]

TNF-α is also known to promote the production of osteoclasts, while inhibiting the development of mesenchymal stem cells (MSC) to osteoblasts, thereby increasing bone resorption.[3] TNF-α may exert its effects on BMD by way of the bone morphogenetic protein (BMP) pathway, which is responsible for regulating osteoblasts and osteoclasts.[3] BMP promotes osteogenesis by binding to and activating the BMP receptors of bone, resulting in the phosphorylation of receptor-regulated R-SMAD proteins.[3] R-SMADs then form complexes with other SMAD proteins, which act as transcription factors to up regulate genes involved in osteogenic differentiation and production of new bone.[3] However, SMAD ubiquitination regulatory factor 1 (SMURF1) causes the degradation of SMAD proteins, which turns off SMAD signaling and osteogenesis.[3] In vitro studies have shown that TNF-α not only up regulates SMURF1 but also inhibits osteoblasts.[3] Furthermore, TNF transgenic mice had SMAD protein levels and BMDs that were significantly lower than levels found in wild-type mice.[3] This demonstrates an important link between the inflammatory response of the immune system and bone formation, and that chronic inflammation may play a role in reducing BMD in postmenopausal women.

In summary, chronic inflammation from pro-inflammatory cytokines and acute phase proteins, including CRP, TNF-α, and IL-1, stimulates the cell signaling molecules RANKL and SMURF1 that in turn stimulate bone resorption. Other signaling molecules, including OPG and BMP, antagonize the effects of RANKL and SMURF1, which promote growth of new bone and prevent bone loss. Therefore, it is important to prevent excessive production of pro-inflammatory molecules to maintain the integrity of bone health.

Melatonin's Influence on BMD and Inflammation

Melatonin is a hormone that is important for maintaining adequate BMD. Endogenous melatonin is synthesized in the pineal gland when light is transferred through the suprachiasmatic nuclei, retinohypothalamic tract, and the sympathetic nervous system.[4] At the molecular level, melatonin is formed when tryptophan is hydroxylated by tryptophan hydroxylase (TPH) to form 5-hydroxytryptophan (5-HTP), which is then decarboxylated by aromatic amino acid decarboxylase (AADC) to form 5-hydroxytryptamine (5-HT/serotonin) (Figure 1).[5,6] Serotonin is then converted to N-acetylserotonin using arylalkylamine-N-acetyltransferase (AANAT), which is converted to melatonin with hydroxyindole-O-methyltransferase (HIOMT) (Figure 1).[5] Light inhibits the synthesis of melatonin whereas darkness activates melatonin production, which is why melatonin promotes sleep at night.[4,7]

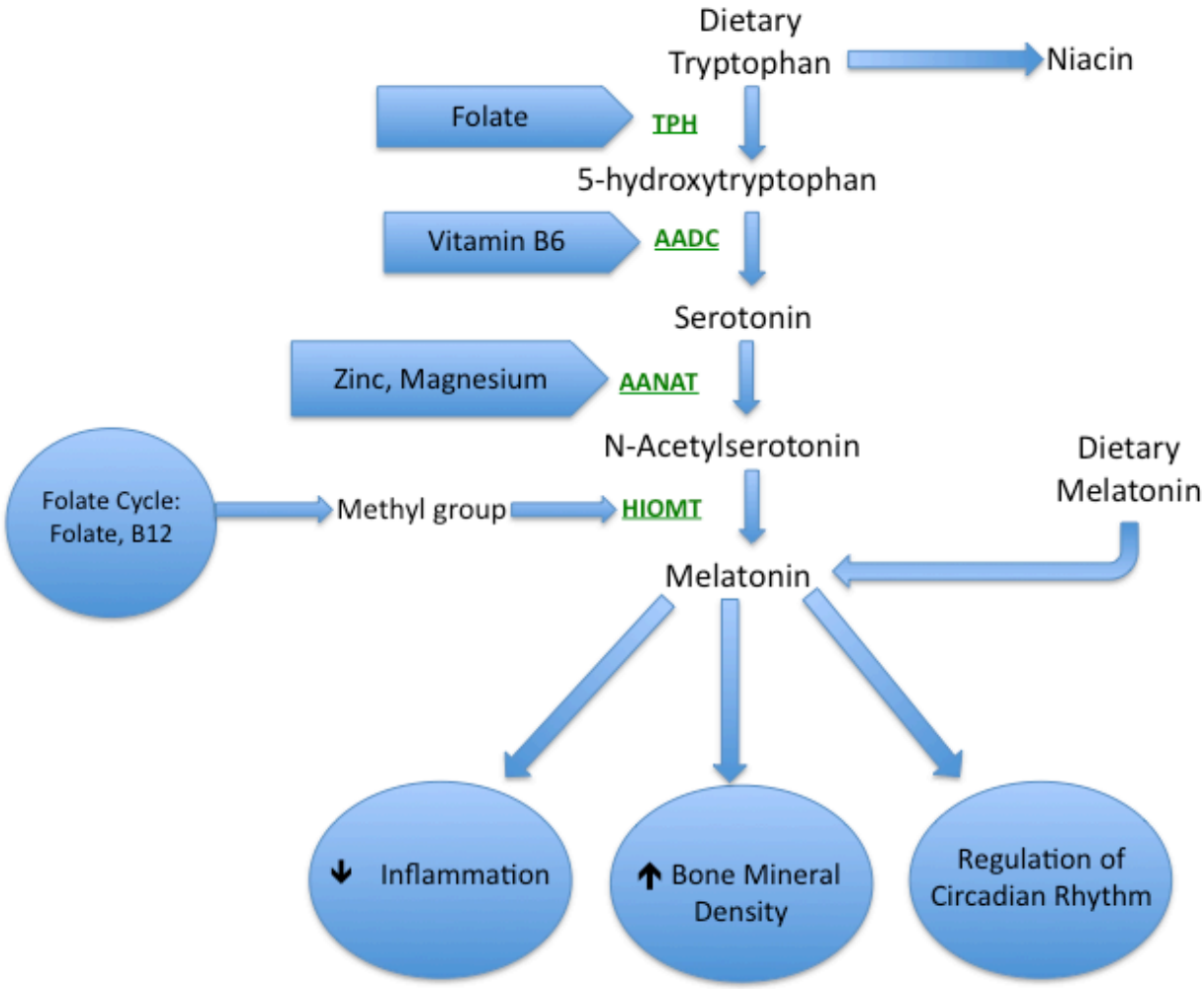


Figure 1. Pathway of melatonin synthesis, nutrient influences on pathway enzymes, and proposed actions of melatonin in the body. Adapted from Peuhkuri K, Sihvola N, Korpela R. Dietary factors and fluctuating levels of melatonin. *Food & Nutrition Research*. 2012;56.

When melatonin is released into circulation in the blood, a portion will be metabolized in the liver by cytochrome P450, where it is converted to 6-sulfatoxymelatonin (6-SMT/aMT6S) to be excreted in urine (Figure 2).[8] However, approximately 30% of circulating endogenous melatonin bypasses metabolism in the liver and is taken up by other tissues.[8] In all types of cells, melatonin may be non-enzymatically metabolized to 3-hydroxymelatonin, which is used to scavenge free radicals. Alternatively, melatonin may be converted to kynuramine derivatives that function as anti-inflammatory molecules and antioxidants (Figure 2).[8] Important kynuramine derivatives include N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) and N^1-acetyl-5-methoxykynuramine (AMK) (Figure 2).[8] Synthesis of AFMK and AMK is thought to occur in extrahepatic tissues because these tissues have reduced cytochrome P450 activity, which prevents melatonin from being converted to 6-SMT/aMT6S.[8]

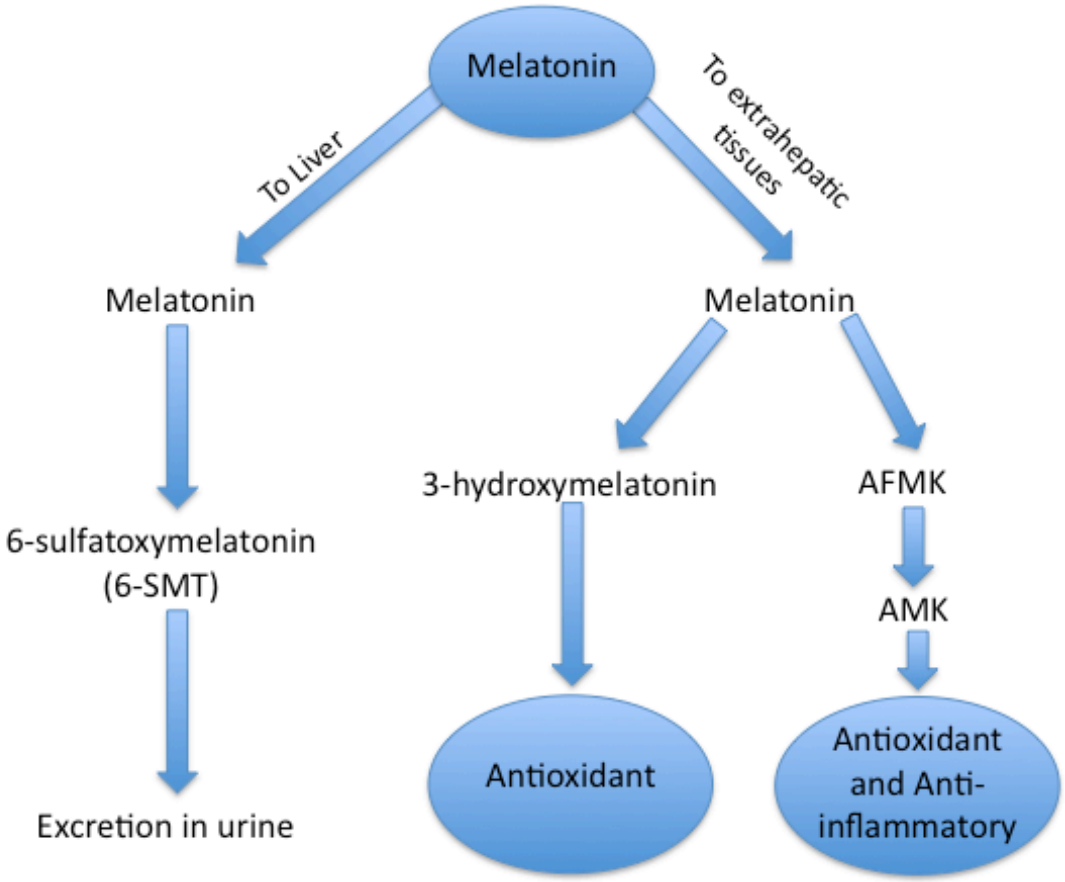


Figure 2. Metabolism of melatonin in the liver and extrahepatic tissues. Adapted from Pandi-Perumal S, Srinivasan V, Maestroni G, Cardinali D, Poeggeler B, Hardeland R. Melatonin. *FEBS Journal*. 2006;273(13):2813-2838.

Melatonin may promote increased BMD in a number of ways. While melatonin is primarily synthesized in the pineal gland, there is evidence that bone marrow may also make melatonin. The membrane-bound melatonin G protein-coupled receptors known as MT1 and MT2 are present in both osteoclasts and osteoblasts.[4,9] It is thus possible that bone cells take up circulating melatonin or generate melatonin locally and convert it to 3-hydroxymelatonin, AFMK, and AMK to maintain bone integrity. In vitro studies have demonstrated that rat bone cells exposed

to melatonin had elevated uptake of calcium, cell differentiation of pre-osteoblasts, and increased expression of bone marker proteins, including matrix sialoprotein, osteopontin, osteocalcin, and alkaline phosphatase.[8]

Melatonin is also known to increase BMD by preventing oxidative stress in bone cells.[4,8] Studies with melatonin in vitro and in vivo have demonstrated that melatonin is superior to glutathione at scavenging hydroxyl radicals, and more effective at neutralizing peroxyl radicals than vitamin E, C, and β-carotene.[10,11] Research has shown that melatonin has the ability to scavenge peroxynitrite anions and hydrogen peroxide, as well as limiting the production of superoxide radicals at the electron transport chain.[12] Specifically, melatonin may prevent leakage of electrons at the electron transport chain and increase transfer of single electrons between components of the chain.[8] Melatonin may also be converted to a melatonyl cation radical at cytochrome *c,* which allows melatonin to act as a final electron acceptor to reduce excess O_2^- production that could lead to bone damage.[8] Melatonin is also known to increase γ-glutamylcysteine synthetase, resulting in elevated glutathione production and increased expression of superoxide dismutase, both of which are endogenous antioxidants that scavenge reactive oxygen species (ROS).[11] Oxidative stress increases the production of RANKL, thereby allowing osteoclasts to differentiate and result in bone resorption.[4] Melatonin combats bone resorption caused by oxidative stress by acting as an antioxidant to scavenge ROS.[4,8] Further, one molecule of AFMK, which is a product of melatonin metabolism in extrahepatic tissues, has the ability to scavenge four free radicals, which may also protect BMD and prevent bone resorption.[8] It has also been suggested that melatonin has the ability to promote osteogenesis and increase BMD by inhibiting peroxisome proliferator-activated receptor gamma (PPAR-γ).[4] By

inhibiting PPAR-γ, MSCs would be directed to mature into osteoblasts instead of osteoclasts and bone marrow adipogenesis would be reduced, therefore increasing BMD.[4]

Melatonin is also responsible for regulating the immune system and lymphoid tissue differentiation by reducing the inflammatory state.[6,7] As previously discussed, pro-inflammatory cytokines reduce BMD via activation of osteoclasts and inhibition of osteoblasts. Melatonin has the ability to inhibit nuclear factor-κβ, TNF-α, and RANKL and prevent these inflammatory cytokines from disrupting osteogenesis.[8,7] Within mouse osteoblasts, melatonin was able to reduce RANKL mRNA in a dose-dependent manner and increase the expression of OPG mRNA by up to 550%.[4] Other studies have demonstrated that melatonin can prevent osteoclast differentiation by reducing the expression of TNF-α and IL-1 mRNA.[9] TNF-α is involved in the upregulation of SMURF1, which inhibits osteoblast activity, but melatonin's ability to inhibit the production of TNF-α mRNA and its subsequent actions on SMURF1 activation and SMAD protein degradation enables MSC to promote bone formation.[3] Melatonin is also responsible for reducing nuclear factor-κβ activation and preventing the subsequent activation of osteoclasts and bone resorption.[8] Since the secretion of melatonin and its impact on the immune system are closely linked, it is clear that both factors play a combined role in determining bone metabolism.

Overall, there is evidence that melatonin is intimately tied to BMD, as bone cells contain melatonin receptors and may have the ability to synthesize melatonin locally. Oxidative stress negatively affects osteogenesis, but melatonin acts as a potent antioxidant by directly scavenging free radicals and increasing the production of other endogenous antioxidants. Melatonin also inhibits the production of pro-inflammatory cytokines and prevents these molecules from activating osteoclasts. Therefore, melatonin may be an important molecule to stop bone loss and

increase the formation of new bone in individuals with elevated inflammation and oxidative stress.

The Effects of Circadian Rhythm, Disruption of Melatonin Synthesis, and Inflammation on BMD

Darkness is one of the main signals that stimulates the production of melatonin and allows for quality sleep and regulation of circadian rhythm.[4,7] Therefore, one must be exposed to enough darkness at night and experience a normal circadian rhythm in order for adequate endogenous melatonin production to occur. However, individuals with sleep disorders, those who are awake during the normal sleep period, and individuals exposed to excessive light before sleep experience circadian disruption that may compromise melatonin metabolism.[7] Circadian rhythm dysfunction and subsequent inhibition of melatonin production may cause problems with transcriptional feedback loops, which cause cell permeability to increase, the immune system to over activate, and pro-inflammatory cytokine production to increase.[7] With the presence of an inflammatory state and a reduction in serum melatonin, it is possible that BMD may be negatively affected by circadian rhythm disruption. In fact, studies have shown that night workers exhibit both lower than normal levels of melatonin and a greater risk of fractures from osteoporosis, suggesting that the inhibition of melatonin plays a role in BMD.[4] This is significant in that approximately 70 million individuals meet the criteria for a sleep disorder.[7]

Melatonin production may also be inhibited when inflammatory cytokines, including TNF-α, IL-1β, and IL-6, prevent the pineal gland from properly metabolizing melatonin.[6] Since the pineal gland is not protected by the blood brain barrier, pro-inflammatory cytokines are able to alter its function by reducing melatonin production and increasing synthesis of inflammatory

cytokines.[6] There are a number of physiological mechanisms by which pro-inflammatory cytokines may prevent the pineal gland from synthesizing adequate melatonin. IL-1β injections in the intracerebroventricular region of ewes were shown to reduce the expression of TPH at the gene level, as well as decrease the amount of 5-HTP in the pineal gland.[6] That study also found that the concentration of AANAT and its gene expression was decreased with the addition of IL-1β.[6] This suggests that IL-1β may reduce pineal gland production of melatonin primarily through reduced gene expression of TPH and AANAT.[6]

Additional studies have shown that elevated TNF-α significantly reduces the amount of melatonin produced at night in women experiencing acute inflammation.[6] When TNF-α levels dropped to a normal level, nighttime melatonin production was restored, which demonstrates that pro-inflammatory cytokines have the ability to disrupt melatonin metabolism.[6] When considering the important role that melatonin plays in promoting proper BMD, it is reasonable to suggest that inflammatory-mediated inhibition of melatonin production could negatively impact osteogenesis. Additional studies are needed in this area to adequately evaluate the effects of chronic inflammation on melatonin synthesis and the implications for bone metabolism.

Food as a Source of Melatonin

A large number of foods contain melatonin. The ability of humans to obtain melatonin from exogenous sources raises the question of whether dietary melatonin could influence important body functions, including bone metabolism and inflammatory pathways.[5,13,14] Plants are known to contain significantly higher levels of melatonin than animals. Melatonin has been found in many plant parts, including leaves, flowers, seeds, roots, and bulbs.[14] Plant melatonin

concentration varies depending on environmental conditions, genotype, and stage of development.[13,14,15] Melatonin is thought to act as a defense mechanism for plants to enable them to survive harsh conditions, since they cannot physically move to avoid exposure.[13] Since animals are mobile and can avoid these conditions, they may have less of a need to produce high levels of endogenous melatonin.[13] For example, genes that up regulate melatonin production were found to be elevated in plants under heat stress and increased melatonin concentrations were present in plants exposed to high UV radiation.[14] Plant foods with notable levels of melatonin include pistachios, walnuts, tart cherries, grapes, corn, rice, barley, and herbs, such as St. John's wort (Table 1).[5,13-30] In addition, significant levels of melatonin have been found in milk, coffee, beer, wine, and other products that are fermented with yeast.[5,23,17] Since a wide variety of plant-based foods contain melatonin concentrations that may be clinically relevant, it can be hypothesized that ingesting these foods might increase serum melatonin and contribute to health benefits, such as increased BMD and a reduced inflammatory response.

Recent studies have also demonstrated that other animal products, including eggs, salmon, and meats contain moderate amounts of melatonin (Table 1).[24] Since animal products constitute a large part of the diet of industrialized countries, it is important to analyze the potential contribution of these foods to blood melatonin concentration.[24] It has been suggested that melatonin might be produced in other tissues besides the pineal gland, such as the skin and the gut.[24] Extrapineal melatonin may be used as an antioxidant and to reduce inflammation locally, rather than being released systemically to contribute to overall blood melatonin concentration; this is why melatonin could be present in animal tissues typically consumed by humans.[24] Of the available research, eggs seem to contain the highest concentration of melatonin and the levels in meats compare to those found in some varieties of seeds.[24,14] Cow's and goat's milk have also

been identified as having low concentrations of melatonin.[25] Although melatonin in meats and fish may help preserve shelf life and flavor by acting as an antioxidant to prevent oxidation, it is possible that the finite amount of melatonin in a piece of meat would eventually be degraded to combat oxidation.[24] Therefore, the age of an animal product, as well as how an animal was raised and what it was fed, would make it difficult to define the exact melatonin content of an animal product. Based on currently available research, it is unclear whether meats and other animal products make significant contributions to blood melatonin concentration.

Table 1. Examples of plant and animal foods containing melatonin. Melatonin concentrations were averaged if multiple values were reported in the literature.[5,13-30]

Name of Food	**Melatonin Concentration (ng/g)**
Vegetables	
Asparagus	0.01
Beet	0.01
Black olive	0.31
Cabbage	0.2
Carrot	0.28
Cauliflower	0.82
Celery	7
Chilies (dried)	1.35
Corn	378.31
Cucumber	0.44

Garlic	0.58
Ginger	1.01
Hunag-qin root	7110
Kohlrabi	7.8
Lupin root	55.6
Onion	0.16
Radish	0.68
Rutabaga	5.6
Taro	0.05
Tomato	0.48
Tomatoes (dried)	128.75
Turnip	0.6
Fruits	
Apple	0.11
Banana	0.29
Grapes (flesh)	0.005
Grapes (skin)	62
Kiwi	0.02
Mango	0.7
Mango juice	0.7
Orange	0.15

Orange juice	0.15
Papaya	0.24
Papaya juice	0.241
Pineapple	0.21
Pineapple juice	0.30
Pomegranate	0.17
Strawberry	5.17
Sweet cherry	20.09
Tart cherry	13.95
Animal Foods	
Beef (raw)	2.1
Chicken (meat and skin, raw)	2.3
Cow's milk	0.014
Dried egg solids	6.1
Lamb (raw)	1.6
Pork (raw)	2.5
Salmon (raw)	3.7
Yogurt	0.13
Grains (cooked)	
Barley	15.11

Bread (Yeast-leavened)	1.17
Oat	13.92
Rice	31.09
Rye	0.05
Wheat	25.01
Seeds	
Black mustard	129
Coriander seed	7
Flax seed	12
Green cardamom	15
Sunflower seed	29
White mustard	189
Nuts and Oils	
Almonds	39
Olive oil	0.079
Pistachios	230,000
Walnut	1.21
Sprouts	
Alfalfa sprouts	0.65
Broccoli sprouts	0.44

Lentil sprouts	0.22
Mung bean sprouts	0.17
Onion sprouts	0.30
Radish sprouts	0.54
Red cabbage sprouts	0.86
Sunflower sprout	0.77
Herbs	
Feverfew, gold	1920
Feverfew, green	2450
Morning glory leaves	0.0005
Morning glory shoots	0.004
Red pigwid (shoots)	0.2
St. John's wort flowers	4390
St. John's wort leaves	1750

Recent studies have attempted to evaluate the effects of melatonin-rich foods on melatonin concentrations in humans, primarily by measuring plasma melatonin levels.[5] Humans produce between 10-80 μg of melatonin during the night, which subsequently declines during the day.[5] Studies in healthy females have illustrated that plasma melatonin ranges from 19.0-197 pg/mL in full darkness at 3:00 AM, and that levels significantly decrease upon exposure to light at 8:00 AM to a range of 3.3-93.2 pg/mL.[31] A different research laboratory considers normal serum melatonin ranges during the day and night to be 2.0-80 pg/mL and 18.5 -180 pg/mL

respectively.[32] Current lab reference ranges for salivary melatonin, which is 40% of that found in plasma, are less than 10.5 pg/ml from 7am-9am, less than 0.88 pg/ml from 3pm-5pm, and 2.53-30.67 pg/ml from 2:30am-3:30am.[33,5] Observations in mammals show that tissue and blood levels of melatonin consistently stay within specific ranges during the daytime and increase to set ranges at night.[13] It is also important to measure the deactivated metabolite of melatonin, 6-SMT/aMT6S, which is excreted in the urine and is an accurate indicator of the concentration of melatonin in the blood, as melatonin is quickly put into circulation and metabolized.[5]

There is promising research that suggests a healthful diet containing melatonin-rich plant foods might have a positive effect on plasma melatonin concentration. Given the important role of melatonin in the body, it is plausible that melatonin consumption could reduce inflammation and increase BMD. Evidence also suggests that melatonin from food has the ability to increase serum antioxidant capacity and reduce oxidative stress, which could improve BMD.[12] Consumption of walnuts by rats was shown to significantly increase serum melatonin levels in comparison to rats that did not receive walnuts.[28] A study examining vegetable intake in women found a 16% higher excretion of 6-SMT/aMT6S for women consuming an amount of vegetables in the highest quartile compared to participants in the lowest quartile.[34] A similar study found that study participants consuming an average of 1288 ng of melatonin per day from vegetables significantly increased 6-SMT/aMT6S excretion, illustrating that plasma melatonin concentration and breakdown of melatonin likely increased.[35] Additional studies have shown that consumption of juice from oranges, pineapples, and bananas increased serum melatonin concentration and melatonin metabolism, as measured by 6-SMT/aMT6S, and elevated serum antioxidant capacity, as measured by increased single electron and hydrogen atom transfer at the electron transport chain.[36,29]

It is, however, unknown whether melatonin in food directly influences plasma concentration or whether other compounds in food, such as vitamins, minerals, and tryptophan, increase melatonin production in the pineal gland.[5] It is currently known that consuming an adequate amount of dietary tryptophan promotes the synthesis of melatonin, as tryptophan is the starting molecule for endogenous melatonin production.[5] Indeed, tryptophan restriction has been shown to reduce melatonin production.[5] Consumption of tryptophan-rich foods has the ability to improve sleep time and quality, as well as increase excretion of 6-SMT/aMT6S.[37] Other vitamins and minerals may also increase endogenous melatonin production. For example, it is known that folate and vitamin B6 are used as coenzymes for TPH and AADC to convert tryptophan to serotonin.[5] AANAT converts serotonin to N-acetylserotonin and may require zinc and magnesium to function properly.[5] In addition, it has been suggested that n-3 polyunsaturated fatty acids (PUFA) are needed for melatonin synthesis, as rats consuming a diet deficient in n-3 PUFA had inadequate secretion of melatonin at night and possible disruption of AANAT.[5] While some studies have shown positive effects of nutrients in food on plasma melatonin concentration and secretion of 6-SMT/aMT6S, other studies have found no association between the aforementioned nutrients and melatonin status.[5] Furthermore, individuals with a limited caloric intake may have reduced melatonin synthesis since the pineal gland requires glucose to synthesize melatonin.[5] Studies have shown that individuals consuming less than 300 kcal per day for a period of 2-7 days had a 20% decrease in blood melatonin concentration.[5]

The research presented here is designed to further explore the links between dietary sources of melatonin and inflammation and bone mineral density. Recent analysis shows that commonly eaten foods contain significant levels of melatonin that may directly contribute to plasma melatonin concentration and metabolism of melatonin to 6-SMT/aMT6S. Given the important

role of melatonin as an antioxidant and regulator of sleep and inflammation, melatonin may be an under-recognized nutrient that positively contributes to osteogenesis and increased BMD.

Study Population: Postmenopausal Women

This research project will focus on postmenopausal women. Women are at a higher risk of bone loss, osteoporosis, and fractures after menopause due to declining estrogen levels, which reduces the ability of calcium to be incorporated into bone.[38] Of the 45 million adults in America over age 50 who have osteopenia and osteoporosis, 80% of these individuals are women.[39] Current recommendations for preventing bone loss include consumption of adequate calcium and vitamin D, engaging in weight-bearing exercise, monitoring bone health, and receiving medication to prevent further bone loss.[39] However, it is known that inflammatory markers, including CRP, are elevated in women with osteoporosis and that there is a shift to higher serum CRP, TNF-α, IL-1α, and IL-6 in postmenopausal women.[40,41,42] Additional studies have demonstrated that there is a link between CRP concentration and fracture risk from osteoporosis, and that it is important to develop a greater understanding of the mechanism between the immune system and bone metabolism.[2] Identifying additional dietary and lifestyle factors that affect systemic inflammation may be a key consideration in better understanding the pathogenesis of osteoporosis in women. Research exploring the relationship between these factors in postmenopausal women is currently scarce.[2]

While it is understood that melatonin confers anti-inflammatory benefits and promotes proper bone metabolism, it is not completely clear how the age-related decline of melatonin secretion could affect these systems in postmenopausal women.[9,4] Since melatonin secretion

naturally declines with age, it has been suggested that this may contribute to the manifestation of sleep disorders, as well as problems with other body systems that require melatonin to properly function.[43] Reduced melatonin production is known to affect the normal 12 hour light/12 hour dark cycle of the circadian rhythm, which disrupts sleep.[8] Studies have found that postmenopausal women with sleep disorders had significantly lower production of melatonin at night than women without sleep problems.[43] Thus, there is reason to suspect that abnormal sleep patterns may reduce melatonin release, subsequently increasing chronic low grade inflammation and oxidative stress, and reducing BMD.

Supplemental melatonin has the ability to increase BMD in postmenopausal women by potentially reducing the inhibition of osteoblasts by oxidative stress.[44] Specifically, low-dose melatonin supplements provided to postmenopausal women at 1 mg/day for one year was able to significantly increase femoral neck BMD compared to controls and a dose of 3 mg/day significantly increased spine BMD.[44] Further, providing a dose of melatonin from 0.5-6.0 mg/day to individuals with an age-related decline in melatonin synthesis may promote a normal sleep/wake cycle.[8] Most drugs that are currently used in individuals with osteoporosis are expensive and may have detrimental side effects, but melatonin has been shown to be both effective and safe at promoting osteogenesis and preventing bone loss.[9,44]

There is limited knowledge on whether melatonin from food can provide similar anti-inflammatory benefits and promote osteogenesis in humans. Exploring how a diet containing melatonin-rich foods affects inflammation, circadian rhythm, and BMD would provide a better understanding of how dietary-derived melatonin, as well as nutrients that enhance endogenous melatonin production, could improve BMD in postmenopausal women. Current dietary recommendations for preventing bone loss in this life stage focus on adequate calcium and

vitamin D intake, but it is possible that endogenous and exogenous sources of melatonin may play an equally important role in promoting bone health.[39] Knowing whether there are additional therapeutic foods that could be recommended to postmenopausal women to prevent inflammation, increase serum melatonin, and prevent loss of BMD could be an additional strategy to mitigate osteopenia and osteoporosis. Therefore, researching the individual and combined effects of inflammation, sleep, and dietary-derived melatonin on BMD in postmenopausal women would provide valuable insight into this relatively unexplored field and in a population at a higher risk for bone loss.

Interactions Between Estrogen and Melatonin and Effects of Age on Melatonin Synthesis

Melatonin production naturally decreases with age and therefore may play a role in the reduced bone health that many older adults experience.[9] This holds true for menopausal women and thus may be important in the manifestation of osteoporosis.[4] Studies examining the effects of melatonin on osteoblasts both in vitro and in vivo with rats have shown that, along with decreased secretion of melatonin, MT1 receptors for melatonin on osteoblasts also reduce in number with age.[9,4] Since MT1 receptors of bone are thought to allow melatonin to exert beneficial effects on BMD, it is suggested that an age-related decline in MT1 may cause osteoblasts to lose their sensitivity for the limited amount of melatonin available.[4]

Further, melatonin may interact with estrogen to produce beneficial effects on BMD. Studies in rats have demonstrated that estrogen and melatonin work in a synergistic relationship to improve bone health.[4] In ovariectomized animals receiving 25 μg/ml of melatonin per day, bone alkaline phosphatase levels were elevated compared to control animals, and their BMD and bone

mineral content (BMC) declined significantly after 60 days without estrogen.[4] However, the ovariectomized animals that did not receive a melatonin supplement had significantly increased levels of urinary deoxypyridinoline, which is a marker for bone resorption.[4] In a similar study, it was found that ovariectomized rats receiving both melatonin and estrogen had higher BMD of the tibia and whole skeleton than rats receiving melatonin alone.[4] While these studies were performed in animal models, it is plausible that reduced estrogen availability during postmenopause may decrease the effectiveness of dietary and supplemental melatonin to increase BMD.[4] Therefore, a combination of declining estrogen that occurs during and after menopause, as well as the age-related reduction in melatonin secretion, may negatively affect BMD, leading to osteopenia and osteoporosis. However, providing postmenopausal women with adequate melatonin from food and supplements and ensuring that estrogen levels are adequate may help prevent age related loss of BMD.[8]

Study Objectives

This study was conducted to evaluate the relationship between dietary melatonin, CRP, sleep, and BMD in postmenopausal women. The effect of nutrients on CRP, BMD, and melatonin intake were evaluated, including calories, protein, vitamin B6, vitamin B12, folate, vitamin C, magnesium, and zinc.

Methods

Study Design

We conducted a cross-sectional study consisting of a secondary data analysis of diet and biomarkers of inflammation in postmenopausal women. We used data from the National Health and Nutrition Examination Survey (NHANES) collected between 2005-2010. The NHANES survey contains data on a sample of participants who are representative of the general population of the United States.[45] NHANES conducts physiological and laboratory tests, as well as dietary and health surveys, to assess nutrition status, disease risk, and disease prevalence in study participants.[45] The NHANES variables of interest for this study included blood CRP concentration, femur BMD, sleep patterns, two-day diet records, total nutrient intake, physical activity, blood HDL cholesterol level, and standard biochemistry profile.

We used CRP as a measure of inflammation, as it is a sensitive, nonspecific indicator of chronic inflammation and was successfully used for development of the Dietary Inflammatory Index (DII).[46,47] CRP is released within 6-10 hours following an inflammatory event, and has a short half life of 5-7 hours, which means that the influence of diet and lifestyle on serum CRP concentration can be observed quickly.[47] CRP is an important inflammatory marker for this study, as elevated levels are associated with reduced BMD, and because melatonin has the ability to reduce serum CRP by acting as an anti-inflammatory molecule.[1,2,48] Medical nutrition therapy guidelines define a low inflammatory-risk serum CRP concentration as below 1.0 mg/L, a moderate risk from 1.0-2.9 mg/L, and a high risk as more than 3.0 mg/L.[47] Concentrations equal to or greater than 10.0 mg/L are likely due to an acute inflammatory response, rather than chronic inflammation.[47]

Female participants were included in our study if their last menstrual period occurred more than 12 months before the time of data collection due to menopause. Subjects were included if they had data collected for our variables of interest, including a DXA scan of the femur, 2 days of 24-hour dietary recalls, CRP data, HDL cholesterol measurement, standard biochemistry profile, sleep data/sleep disorder information, and physical activity data. Subjects were also included if they had a CRP of < 10.0 mg/dL. Participants were excluded if they had not gone through menopause, if they did not have data for our primary variables of interest, including femur BMD, 24-hour dietary recalls, and sleep disorders information, and if they had a CRP of > 9.9 mg/dL. Once study participants were selected, they were separated into two groups according to their level of inflammation with a CRP level of 2.0 – 9.9 mg/dL indicating chronic inflammation and a CRP of less than 2.0 mg/dL indicating low/normal inflammation. We evaluated serum HDL cholesterol, as this is an additional indicator of chronic inflammation that may be used to validate CRP concentration.[47] Alkaline phosphatase was also included in our analysis, as multiple studies have shown that it is a marker for bone turnover and bone loss, which could be used to assess degree of inflammation in our study sample.[49,50,51]

Our data included information about ethnicity, education level, age, socioeconomic status, smoking, alcohol, and medications. Although these variables provide an opportunity to statistically control for these factors in analysis, we were unable to include them in our analysis. The large number of variables of interest and the limited number of participants that met the inclusion criteria made it necessary to limit the number of environmental and socioeconomic covariates controlled for in the statistical models. We therefore limited our analysis to relevant dietary components and major biomarkers of inflammation. Increasing the number of covariates

would have increased the dimensionality of the dataset to a degree that would have reduced our ability to assess the relationships between these variables and dietary melatonin.

Data Collection

DXA Scan: BMD was collected from study participants using a DXA scan of the femur.[45] The Hologic QDR 4500A fan beam X-ray body densitometer was used to evaluate participant BMD and BMC.[45]

CRP Concentration: Latex-enhanced nephelometry and a Behring Nephelometer were performed to assess participant CRP levels.[45]

Sleep Disorders: A Computer-Assisted Personal Interviewing system was used to administer a questionnaire to study participants to evaluate sleeping habits.[45]

Physical Activity: A Computer-Assisted Personal Interviewing system was administered to collect information on participant physical activities and sedentary activities.

Diet and Supplements: Two, 24-hour dietary recalls were performed by trained interviewers to assess participant food and nutrient intake. The first interview was conducted in person at the NHANES Mobile Examination Center and a variety of measuring and reference tools were used to help participants estimate food portion sizes.[45] The second interview was conducted via telephone 3-10 days after the first recall, and participants were provided with measuring tools and portion guides to estimate amounts of food consumed.[45]

Standard Biochemistry Profile: The NHANES Laboratory/Medical Technologists Procedures Manual (LPM) was used to collect and process participant samples using the Beckman Synchron

LX20.[45] Each sample was frozen and sent to the National Center for Environmental Health for analysis.[45] Alkaline phosphatase was measured using a 2-amino-2-methyl-1-propanol buffer.[45]

HDL Cholesterol: The LPM was used to collect and process blood samples and an HDL cholesterol direct immunoassay method was used to quantify HDL.[45]

Assessment of Dietary Melatonin

We quantified food melatonin content using the melatonin concentration (ng/g) of individual foods that have been reported in the literature. If multiple melatonin concentrations were reported for a particular food, the values were averaged to derive an approximate melatonin concentration. The individual food serving sizes collected by NHANES in the two, 24-hour dietary recalls were reported in grams, which allowed us to quantify an approximate number of nanograms of melatonin consumed for each serving of food.

We included the melatonin concentrations of raw, unprocessed fruits, vegetables, and juices, but excluded produce that had been cooked or processed, as studies have shown that melatonin is degraded at high temperatures.[23] Additional literature reports that heat processing and chemical treatment of olive oils may alter melatonin concentration.[17] Based on this observation, it is plausible that other foods exposed to excessive processing or heat would also have a limited melatonin content. Meats and eggs were excluded from our analysis, as there is insufficient evidence available to show the degree to which melatonin is degraded in animal products after cooking. Yeast-leavened breads were included, as evidence shows that fermentation enables a significant amount of melatonin to remain intact in the crumb of bread after baking.[23] Cooked and processed foods containing wheat, barley, rye, oats, and rice were included if specified as

whole grain, as available literature has reported that these grains contain melatonin after exposure to hydrothermal processing of 200°C.[30] Combination foods containing multiple ingredients were excluded since we could not determine the number of grams of each individual ingredient. We also included herbs and supplements, such as St. John's wort, that would significantly contribute to total melatonin intake. While we originally intended to include melatonin supplements in participants' dietary melatonin consumption, the single individual who reported taking a pure melatonin supplement was identified as an outlier when conducting our tests for normality. Since melatonin supplements contribute a significantly greater amount of melatonin than that obtained from whole foods, we decided to exclude the single individual taking a melatonin supplement from our analysis. Therefore, we only considered melatonin derived from foods in our analysis.

In addition, while both coffee and wine contain high concentrations of melatonin, caffeine and ethanol may interfere with melatonin metabolism.[5] Caffeine blocks adenosine receptors that enable AANAT production, which inhibits endogenous melatonin synthesis.[5] Caffeine also prevents melatonin breakdown by competing with melatonin for binding with the cytochrome P450 detoxification enzyme in the liver, which results in elevated blood melatonin levels.[5] Studies have obtained mixed results on alcoholic beverages containing melatonin and have shown that ethanol reduces 6-SMT/aMT6S output by 9% after two drinks while some have shown that alcohol elevates serum melatonin.[5] However, it is suggested that ethanol disturbs AANAT gene expression and may impair normal sleep patterns.[5] Therefore, we excluded coffee, beverages containing similar amounts of caffeine, and alcoholic beverages from the dietary melatonin score.

We assume that exogenous preformed melatonin would bypass the pathway where melatonin is synthesized from tryptophan, and have the ability to directly enter systemic circulation or enter tissues where needed (Figure 1). While we acknowledge that a proportion of dietary tryptophan is used for endogenous melatonin synthesis, dietary averages and serum concentrations of tryptophan were not available for our population of interest, thus preventing us from including tryptophan in the dietary melatonin score.

Statistical Analysis

Our analysis was conducted in SAS statistical software (SAS 9.4, English, Cary, NC) and SPSS statistical software (IBM SPSS Statistics 24, Armonk, NY). Prior to conducting all analyses, we checked for the normality of all variables and made logarithmic and inverse transformations as necessary. The combined dataset with the two groups of study participants with chronic inflammation (CRP 2.0-9.9 mg/dL) and low inflammation (CRP < 2.0 mg/dL) contained a large number of variables that required us to reduce the dimensionality of the dataset. We used factor analysis to reduce the number of variables included in our analysis, and to identify the most relevant variables contributing to differences observed between the two groups. In addition, we used a discriminant function analysis to create a further refined predictive model to assess which predictor variables provided the maximum separation between the chronic and low inflammatory groups. After conducting factor analysis and discriminant function analysis and identifying the dietary, biochemical, BMD, and lifestyle factors explaining the majority of the variance between the chronic inflammatory and normal inflammatory groups, we focused on examining the relationships between these variables and CRP level for the remainder of our analysis.

Means tests were used to compare variables of interest between the chronic inflammatory and low inflammatory groups. Simple linear regression was used to examine the relationship between variables of interest and CRP level between each group. We also conducted multiple regression analysis within each inflammatory group to identify possible interactions between dietary, biochemical, BMD, and lifestyle variables. As a supplement to the linear regression, a path analysis of significant and borderline significant relationships between variables was performed within each inflammatory group, which enabled us to graphically represent variable relationships. Finally, we evaluated dietary sources of melatonin within the chronic and normal inflammatory groups to quantify the percent contribution of each food group to total melatonin intake. These food groups included fruits, vegetables, grains, dairy, and nuts and seeds.

Part II: Thesis Manuscript

Introduction

Melatonin plays an important role in a number of biological processes, including regulation of inflammation and oxidative stress, promoting BMD, and regulating circadian rhythm. It has been suggested that melatonin produced endogenously in the pineal gland, as well as dietary-derived melatonin, are converted to metabolites that are taken up by extrapineal tissues to reduce inflammation and scavenge free radicals.[8] Melatonin may also be taken up by bone via melatonin receptors and bone may have the ability to produce melatonin locally, as in vitro studies suggest that melatonin increases the activity of osteoblasts and bone growth.[4,8,9]

Inflammation and oxidative stress lead to degradation of bone, through activation of RANKL, pro-inflammatory cytokine-mediated activation of osteoclasts, and increased SMURF1 activity.[1,2,3,4] However, melatonin has been shown to reduce inflammation and bone loss by inhibiting the production of TNF-α and IL-1 mRNA, and preventing RANKL and SMURF1 from activating.[6,7,8,9] Melatonin may also reduce reactive oxygen species and subsequent damage to bone by scavenging free radicals and increasing the activity of the endogenous antioxidants glutathione and superoxide dismutase.[4,8,11] Having adequate circulating melatonin is also necessary for regulating a normal sleep cycle, which may be negatively affected by chronic inflammation and could have a detrimental impact on BMD.[4,6,7]

While further research is needed to understand the exact mechanisms by which melatonin affects these processes, what is known is that many foods contain varying concentrations of melatonin and that consuming melatonin from whole foods increases plasma concentration of melatonin. A wide variety of plants, fermented products, and animal-sourced foods contain

melatonin and studies have found that consumption of vegetables and fruits containing melatonin increase excretion of melatonin metabolites and may increase serum antioxidant capacity.[28,29,35,36] However, it is not known whether consuming melatonin from foods increases an individual's capacity to reduce inflammation and oxidative stress, and increase BMD.

Our target population consists of postmenopausal women, as women are at a higher risk of osteopenia, osteoporosis, and fractures after menopause due to declining estrogen levels.[38,39] Current nutritional recommendations for osteoporosis focus on consuming adequate calcium, vitamin D, and engaging in weight-bearing exercise to prevent bone loss.[39] However, these recommendations do not necessarily promote the growth of new bone in women who already have osteopenia or osteoporosis. Further, postmenopausal women are at a higher risk of having elevated inflammatory markers, reduced synthesis of endogenous melatonin, and disrupted circadian rhythm, which could increase the risk of bone loss in this lifestage group.[40,41,42,43,2,4,9] Recent studies have indicated that melatonin supplementation has the potential to increase BMD in postmenopausal women without detrimental side effects.[8,9,44] This led us to focus on whether consumption of melatonin from whole foods could be a potential therapeutic mechanism for reducing inflammation, regulating sleep cycles, and promoting healthy BMD in postmenopausal women. The goal of the current research was to conduct a cross-sectional analysis of NHANES data to investigate whether dietary-derived melatonin is associated with inflammatory markers, femur BMD, and hours of sleep.

Data Reduction and Statistical Analysis

Our total sample size was 110 with 55 subjects in each inflammatory group. We conducted a factor analysis in SPSS to reduce the dimensionality of the dataset and to identify key variables responsible for the majority of the variation observed between the chronic inflammatory and low/normal inflammatory groups. One round of factor analysis was completed. The analysis included all dietary, biochemical, BMD, and lifestyle factors of interest (Appendix A). We chose to retain four factors within our analysis, each of which had an Eigenvalue greater than 1.0 and together accounted for 56% of the variance in the data (Appendix B). After evaluating each factor analysis plot, we determined that factors 1, 2 and 3 best distinguished the variation between each inflammatory group because this model provided the best visual separation between members of each group.

Positive and negative factor loadings of greater than 0.50, regardless of direction, were used to select variables most strongly associated with factors 1, 2 and 3. However, we also considered variables with a factor loading of greater than 0.30 to account for biological significance. Factor 1 explained 27.9% of the variance between the chronic and low inflammatory groups, which was strongly correlated with magnesium, vitamin B6, protein, folate, calories, zinc, vitamin C, melatonin, vitamin B12, and HDL cholesterol, with a weak correlation with CRP (Appendix B). Factor 2 explained 10.7% of the variance between each group and the variables HDL cholesterol, alkaline phosphatase, vitamin C, melatonin, zinc, calories, protein, and CRP exhibited the strongest correlation (Appendix B). Factor 3 explained 9.3% of the variance with CRP, hours of sleep, vitamin C, femur BMD, and vitamin B12 being strongly correlated (Appendix B).

After assessing the results of the factor analysis, we conducted a discriminant function analysis in SPSS to create an additional predictive model to further separate the chronic and low

inflammatory groups (Appendix C). CRP was removed from this analysis as it was originally used to separate study participants into the chronic and low inflammatory groups. This made the variable collinear with other variables in such a way that it violated the assumptions of multivariate analysis. This rationale also validated the removal of CRP from the discriminant function analysis, which enabled us to better assess the contribution of the other dietary, biochemical, and lifestyle variables to differences between each inflammatory group. In addition, we removed sedentary time from the dataset, as 33 individuals in the dataset had missing values for this variable. Removing this variable allowed us to include the majority of study participants in our analysis.

We conducted means tests in SAS on the significant variables identified from factor analysis and discriminant function analysis to analyze the differences between the chronic inflammatory and low inflammatory groups (Appendix D). Several variables had non-normal distributions. Melatonin, HDL cholesterol, vitamin C, magnesium, and zinc were $\log_{10}$-transformed and alkaline phosphatase was 1/x-transformed to obtain a normal distribution. We attempted to normalize CRP but were unable to accomplish this due to the highly skewed distribution of the data. Therefore, a non-parametric ANOVA was used for CRP, as this test is robust at assessing statistical significance in a skewed dataset. In addition, a non-parametric ANOVA was performed on hours of sleep, as this data also followed a non-normal distribution. T-tests were used to evaluate the means of femur BMD, caloric intake, and vitamin C. Non-parametric ANOVAs were used to assess the means of alkaline phosphatase, HDL cholesterol, melatonin, protein, folate, vitamin B12, vitamin B6, magnesium, zinc, sedentary time, and hours of sleep to account for differences in sample size between the two inflammatory groups. The sample size differences were due to missing data in the original dataset or from outliers that were removed

during analysis. Outliers were excluded if their values were less than or greater than two standard deviations from the mean. Variables with excluded outliers included melatonin, alkaline phosphatase, HDL cholesterol, calories, protein, folate, vitamin B12, vitamin B6, magnesium, zinc, sedentary time, and sleep.

We next ran a series of simple linear regressions between CRP and the selected dietary, biochemical, BMD, and lifestyle variables (Appendix E). By plotting the regressions between each group, we were able to characterize the relationship of each variable to level of inflammation and how variables responded differently within each inflammatory group. Multiple linear regression was also performed using SAS on all variables, including the variables excluded through factor analysis and discriminant function analysis, to explore the interactions between inflammatory markers, dietary components, BMD, and lifestyle factors (Appendix F).

Based on the results from the multiple linear regression, we created path analysis plots in the AMOS (Analysis of a Moment Structures) module of SPSS for both the chronic and normal inflammatory groups (Appendix G). AMOS is used for Structural Equation Modeling (SEM), path analysis, analysis of covariance, and confirmatory factor analysis. After identifying significant and borderline significant relationships in the multiple linear regression output, we created equations within each inflammatory group to represent the variables that would explain the variance for the variables melatonin, CRP, alkaline phosphatase, HDL cholesterol, femur BMD, protein, and sleep. For example, within the chronic inflammatory group, the variables significantly correlated with melatonin included folate, protein, magnesium, and zinc which was represented by Melatonin = Folate x Protein x Magnesium x Zinc. These equations were used to create a map to graphically represent relationships between variables in each group. We then

used bootstrap analysis to develop regression weight estimates for each path that predicted the degree of change in the response variables with every one unit change in the predictor variables.

Finally, the percent contribution of melatonin obtained from each food group were calculated to examine how different foods contribute to total melatonin intake. We included the food groups of fruit, vegetables, grains, dairy, and nuts and seeds, and calculated percentages within the chronic and normal inflammatory groups.

Results

The results from the factor analysis are displayed graphically in Figure 3. Factors 1, 2 and 3 were included in the analysis, as they provided the best separation and explanation for variation between the chronic inflammatory and low inflammatory groups. We compared the significant variables that had the highest loadings for each factor with the subsequent means and nonparametric test results. We found that participants of the low inflammatory group were distinguished from individuals with chronic inflammation by consuming higher levels of magnesium, B vitamins, protein, calories, zinc, vitamin C, and melatonin, and by having higher femur BMD and HDL cholesterol, and lower alkaline phosphatase. The chronic inflammatory group was best distinguished by having greater mean CRP, more hours of sleep, and lower dietary zinc, caloric intake, protein, and vitamin C (Figure 3).

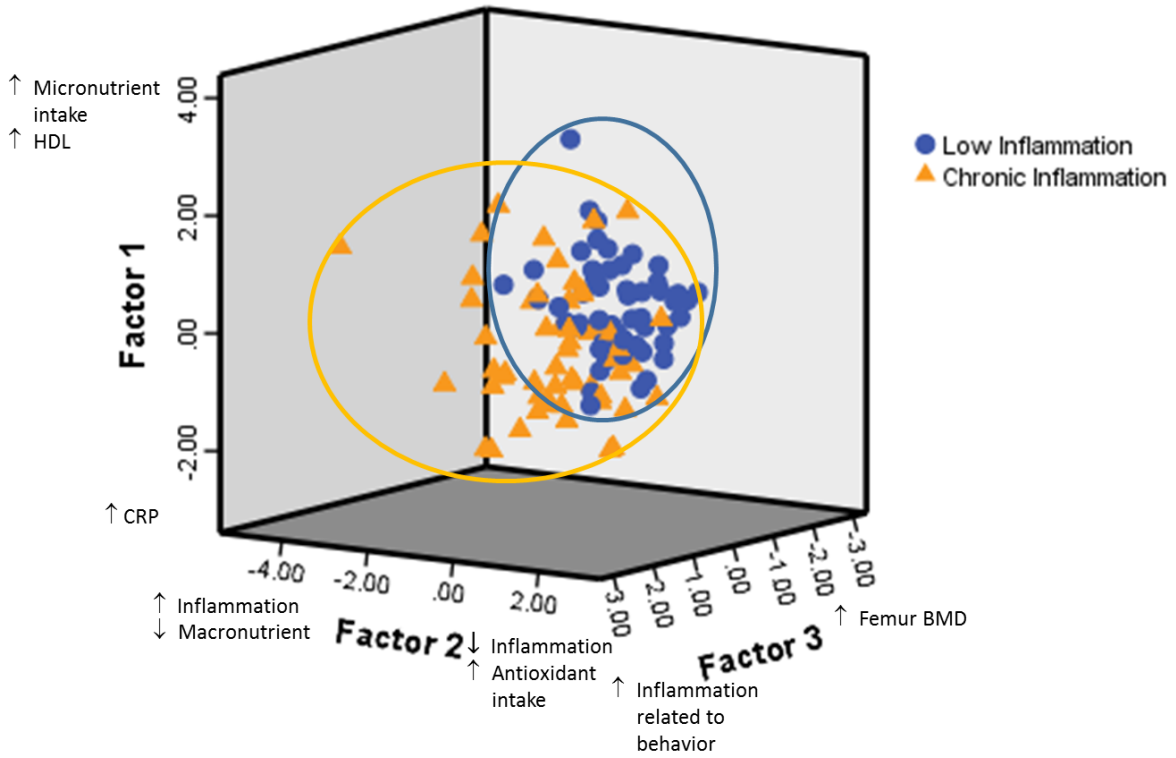


Figure 3. Graphical representation of factor 1 vs factor 2 vs factor 3 from factor analysis with associated variables on each axis. Variable factor loadings of > 0.3 were considered significant. Factor 1 is associated with micronutrient intake (magnesium, vitamin B6, protein, folate, energy, zinc, vitamin C, melatonin, vitamin B12) and HDL cholesterol on positive end and increased inflammation on the negative end. Factor 2 is associated with high CRP and low macronutrient intake (energy, protein, zinc) on the negative end and low inflammation (high HDL, low alkaline phosphatase) and high antioxidant intake (vitamin C, Melatonin) on the positive end. Factor 3 is associated with increased CRP and sleep, and low vitamin C intake on the positive end, and high femur BMD on the negative end.

Discriminant function analysis revealed that the ANOVA variables contributing a significant amount of prediction to separate the chronic and low inflammatory groups included folate, magnesium, HDL cholesterol, and melatonin (Appendix C). The analysis revealed that folate is the most important variable for discrimination between the groups ($p = 0.001$). One discriminant function was included, as the associated chi-square test indicated that this function best separated the chronic and low inflammatory groups ($p = 0.001$). Standardized canonical discriminant function revealed the variables with the highest coefficients that had the greatest ability to discriminate between the two groups, which included folate, vitamin B6, magnesium, calories, protein, melatonin, and vitamin C (Appendix C).

We used means tests, including t-tests and nonparametric ANOVAs, to characterize differences in variable means between the chronic inflammatory and low inflammatory groups (Table 2). Variables with significant differences between group means included CRP, HDL cholesterol, femur BMD, dietary melatonin, folate, vitamin B6, and magnesium. Individuals exhibiting chronic inflammation had significantly higher CRP and significantly lower HDL cholesterol, femur BMD, dietary melatonin, folate, vitamin B6, and magnesium than subjects with low inflammation. While the difference in mean alkaline phosphatase between the two groups was not significant, the borderline significant p-value indicates that alkaline phosphatase may still be of biological significance in these two populations.

Table 2. Statistical output of t-tests and nonparametric ANOVAs comparing mean values between the low and chronic inflammatory groups. A p-values of <0.05 was considered statistically significant. * denotes statistically significant variables, † denotes untransformed data was used to derive the mean.

		Means Test Statistics				
Variable	**N**	**Chronic Inflammation (M)**	**Low Inflammation (M)**	**t-value**	**F-value**	**p**
CRP (mg/dL)	110	3.43	0.42	.	146.95	< 0.0001*
Alkaline Phosphatase (U/L)	106	81.33†	75.49†	.	2.89	0.092
HDL (mg/dL)	107	54.33†	59.66†	.	5.52	0.021*
Femur BMD (g/cm^2)	110	0.84	0.9	-2	.	0.049*
Melatonin (ng)	109	958.33†	972.04†	-2.15	.	0.034*
Total Energy (kcal)	108	1625	1690	.	0.39	0.53
Protein (g)	109	63.34	66.83	.	0.69	0.41
Folate (mcg)	109	293.38	390.3	-4.09	.	< 0.0001*
Vitamin B6 (mg)	105	1.4	1.77	.	12.87	0.0005*
Vitamin B12 (mcg)	103	4.03	4.5	.	1.52	0.22
Vitamin C (mg)	110	79.92†	84.83†	-1.37	.	0.17
Magnesium (mg)	109	229.5†	283.41†	.	12.84	0.0005*
Zinc (mg)	109	8.99†	9.98†	.	2.53	0.12
Sleep (hours)	107	7.04	6.94	.	0.12	0.73
Sedentary Time (min/day)	77	311.71	330	.	0.28	0.59

Simple linear regression was conducted on variables identified in factor analysis that provided a significant contribution to the graphical model and significant variables identified by discriminant function analysis. This included HDL cholesterol, alkaline phosphatase, femur BMD, melatonin, energy, protein, folate, B6, B12, vitamin C, magnesium, zinc, sleep. HDL cholesterol exhibited a non-significant negative relationship with CRP in the low inflammatory group, as well as in the chronic inflammatory (Figure 4). Alkaline phosphatase showed no relationship to CRP in both inflammatory groups (Figure 5).

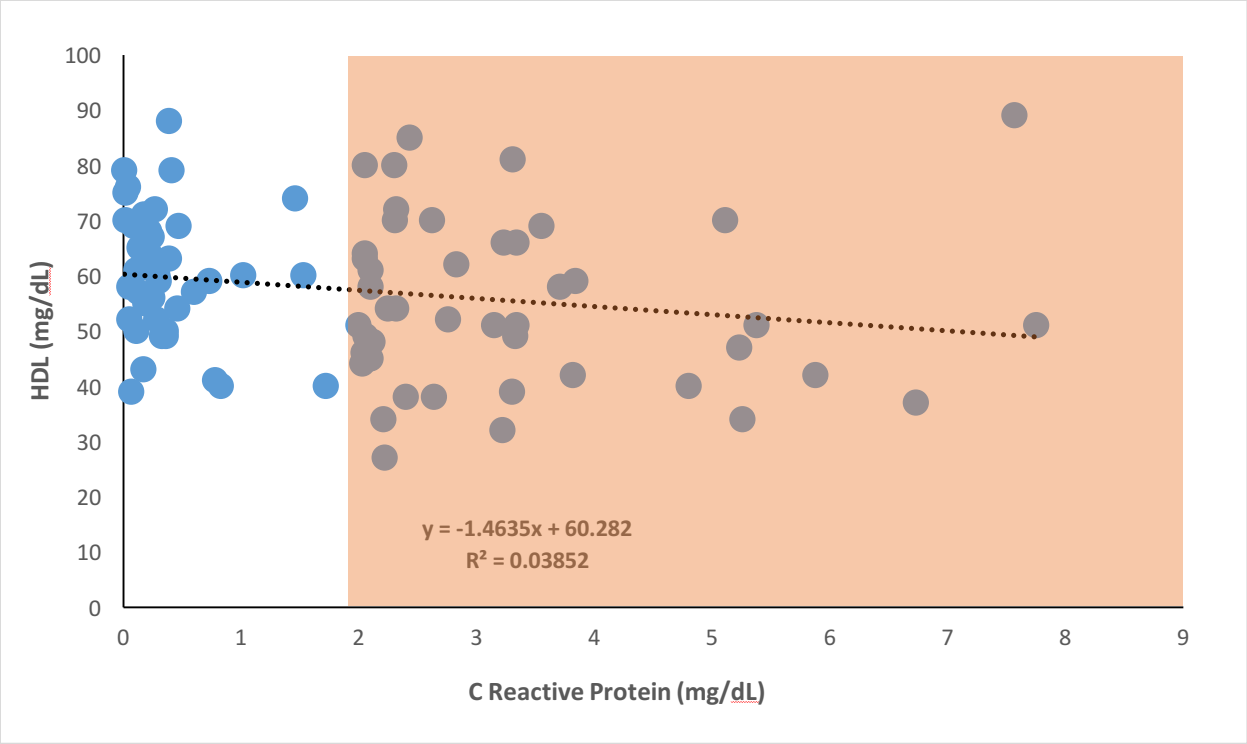


Figure 4. Simple linear regression comparing untransformed HDL cholesterol to CRP in both low and chronic inflammatory individuals (n = 107). The orange shaded region represents individuals with chronic inflammation. The regression equation for both inflammatory groups(normal and chronic) combined was borderline significant [b=-1.46, R^2=0.039, F (1,106) = 3.36, p=0.07,] but explained less than 4% of the observed variation in HDL. Individual regression equations for each group were also not significant (Normal group, ln transformed data, b = -0.11, R^2 = 0.05, F(1, 51) = 2.48, p = 0.12 and chronic group, ln transformed data b = -0.01, R^2 = 0.01, F(1, 52) = 0.30, p = 0.59). Data for each inflammatory group were log-normalized as the smaller sample size produced non-normal distributions.

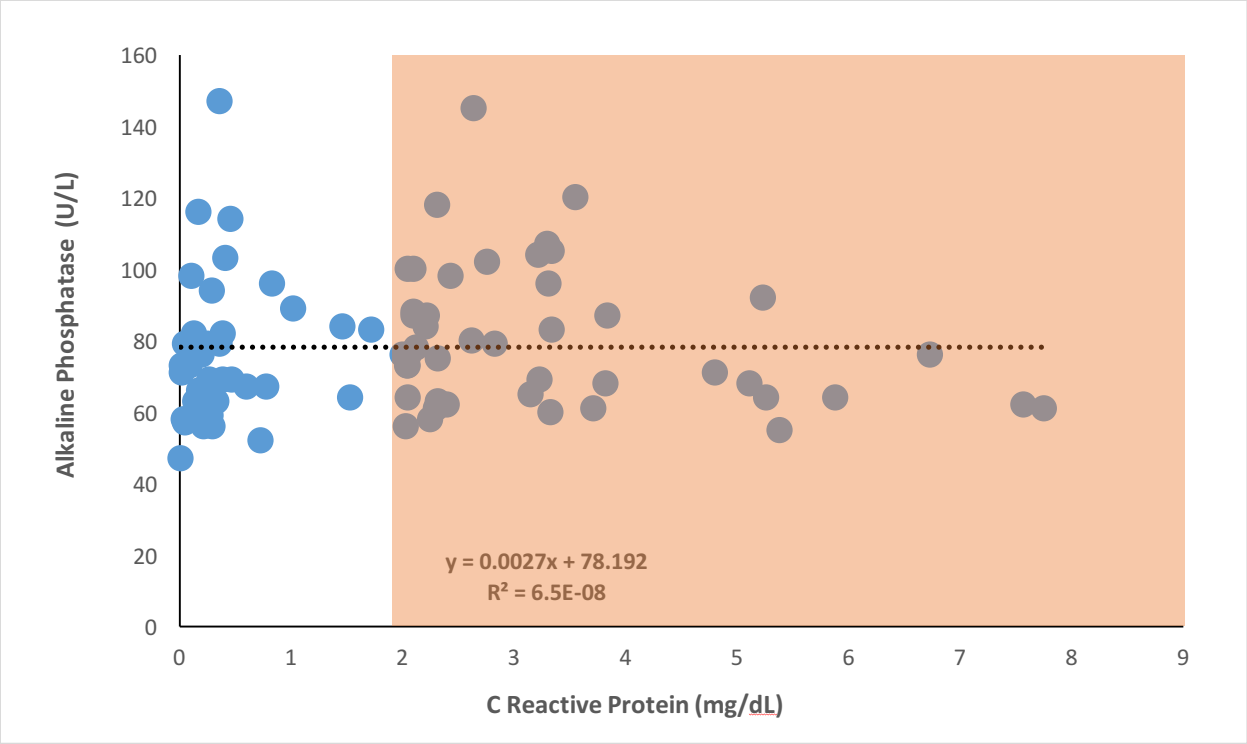


Figure 5. Simple linear regression comparing untransformed alkaline phosphatase to CRP in low and chronic inflammatory individuals (n = 106). The orange shaded region represents individuals with chronic inflammation. The regression equation for both inflammatory groups (normal and chronic) combined was not significant [b=0.003, R^2=6.49-08F (1,105)= 5.45 E-06, p=0.998]. Individual regression equations for each group were also not significant. (Normal group, ln transformed data, b = -0.0007, R^2 = 0.009, F(1, 49) = 0.43, p = 0.5 and chronic group ln transformed data, b = 0.00008, R^2 = 0.002, F(1, 53) = 0.12, p = 0.73. Data for each inflammatory group were log-normalized as the smaller sample size produced non-normal distributions.

Femur BMD also exhibited different linear relationships between the normal and chronic inflammatory groups. Subjects with chronic inflammation showed reduced BMD with increasing CRP that was statistically significant, while femur BMD in subjects with low inflammation had an unexpected positive trend with CRP that approached statistical significance (Figure 6). Additional plots of simple linear regression between CRP and dietary melatonin, calories, protein, folate, B12, B6, vitamin C, magnesium, zinc, and hours of sleep are in Appendix E.

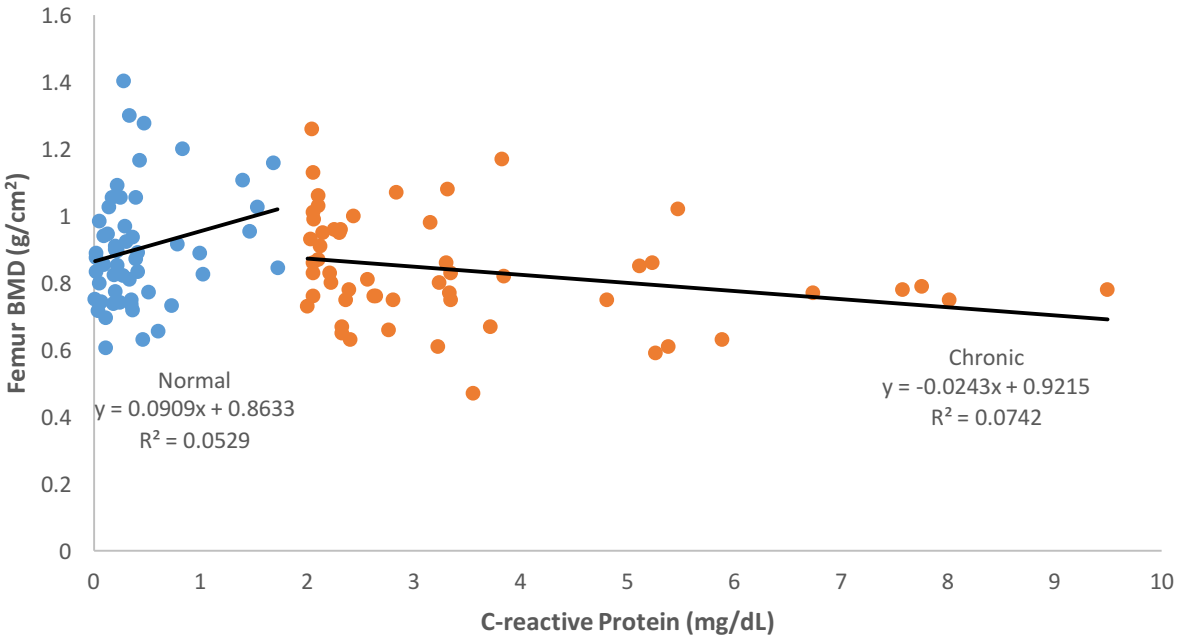


Figure 6. Simple linear regression of femur BMD and CRP in low and chronic inflammatory individuals (n = 110). Normal group b = 0.09, R^2 = 0.05, F(1, 53) = 2.96, p = 0.09 and chronic group b = -0.02, R^2 = 0.07, F(1, 53) = 4.25, p = 0.04.

We also conducted multiple linear regression to analyze the relationships between all variables of interest within each group (Table 3 and Table 4). Within the chronic inflammatory group, CRP and femur BMD showed a significant negative correlation, which was expected, but no dietary components were related to BMD (Table 3). HDL cholesterol and magnesium shared a positive relationship and melatonin was significantly correlated with a number of nutrients, including protein, folate, vitamin B12, magnesium, and zinc. Total caloric intake and protein shared a positive correlation with one other and with the majority of the nutrients included in the analysis. The majority of the B vitamins and minerals showed significant positive correlations with one another and vitamin C was related to intake of magnesium and vitamin B6. Hours of sleep exhibited a positive relationship with CRP, which was also observed in our simple linear regression.

Among individuals with low inflammation, more variables showed a significant relationship with HDL cholesterol than in the chronic inflammatory group. Alkaline phosphatase, caloric intake, and magnesium were all positively correlated with HDL cholesterol while sedentary time had a negative correlation (Table 4). BMD shared a significant positive correlation with protein intake, but melatonin consumption had an unexpected negative relationship to vitamin B12 intake. As with the chronic inflammatory group, calories and protein shared a positive relationship, and the majority of the nutrients included in our dataset were significantly correlated with one another. Again, vitamin C shared a positive correlation with magnesium and vitamin B6. Hours of sleep shared a positive relationship with zinc consumption.

Table 3. Correlation coefficient matrix of all biomarkers, BMD, dietary, and lifestyle variables for the chronic inflammatory group. (Significant correlations in bold).

	C_Reactive_Protein	Alkaline_Phosphatase__1_x_normal	HDL_ln_normalized	Femur_BMD	Melatonin__ln_normal_	Energy	Protein	Folate	B12	B6	Vitamin_C_ln	Magnesium_ln	Zinc_ln	Sedentary_Time
C_Reactive_Protein														
Alkaline_Phosphatase__1_x_normal	0.0484													
	0.7256													
	55													
HDL_ln_normalized	-0.07598	0.139												
	0.585	0.3161												
	54	54												
Femur_BMD	***-0.27237***	-0.11374	0.11296											
	0.0442	0.4083	0.4161											
	55	55	54											
Melatonin__ln_normal_	0.0579	0.12765	0.17968	0.00489										
	0.6745	0.353	0.1936	0.9717										
	55	55	54	55										
Energy	0.04466	-0.01291	0.11569	-0.02051	0.24156									
	0.7485	0.9262	0.4048	0.883	0.0785									
	54	54	54	54	54									
Protein	0.10089	0.16767	0.0185	0.11879	***0.36845***	***0.67503***								
	0.4636	0.2211	0.8944	0.3877	***0.0056***	***<.0001***								
	55	55	54	55	***55***	***54***								
Folate	0.05545	0.09674	0.13139	-0.01763	***0.45998***	***0.58513***	***0.58957***							
	0.6905	0.4865	0.3483	0.8993	***0.0005***	***<.0001***	***<.0001***							
	54	54	53	54	***54***	***53***	***54***							
B12	0.17797	-0.00914	-0.02727	-0.03622	***0.30769***	***0.4926***	***0.65134***	***0.59154***						
	0.2115	0.9493	0.8493	0.8008	***0.0281***	***0.0002***	***<.0001***	***<.0001***						
	51	51	51	51	***51***	***51***	***51***	***50***						
B6	-0.05872	0.20769	0.0619	0.08674	0.22505	***0.42603***	***0.60559***	***0.61384***	***0.58582***					
	0.6823	0.1436	0.6693	0.545	0.1123	***0.002***	***<.0001***	***<.0001***	***<.0001***					
	51	51	50	51	51	***50***	***51***	***50***	***48***					
Vitamin_C_ln	0.26037	0.09834	0.22949	0.09547	0.08713	0.11573	0.13894	0.25055	0.24382	***0.33132***				
	0.0549	0.475	0.0951	0.4881	0.5271	0.4046	0.3117	0.0676	0.0847	***0.0176***				
	55	55	54	55	55	54	55	54	51	***51***				
Magnesium_ln	0.18302	0.26722	***0.27846***	0.02641	***0.52804***	***0.59664***	***0.63194***	***0.67885***	***0.56189***	***0.70229***	***0.47248***			
	0.1853	0.0508	***0.0435***	0.8496	***<.0001***	***<.0001***	***<.0001***	***<.0001***	***<.0001***	***<.0001***	***0.0003***			
	54	54	***53***	54	***54***	***53***	***54***	***53***	***50***	***50***	***54***			
Zinc_ln	0.01683	0.15956	0.08441	-0.0069	***0.47376***	***0.59904***	***0.83769***	***0.72572***	***0.6916***	***0.58183***	0.16732	***0.68969***		
	0.9038	0.2491	0.5479	0.9605	***0.0003***	***<.0001***	***<.0001***	***<.0001***	***<.0001***	***<.0001***	0.2265	***<.0001***		
	54	54	53	54	***54***	***53***	***54***	***53***	***51***	***50***	54	***53***		
Sedentary_Time	0.04292	0.1429	-0.2129	0.04567	-0.24209	-0.1758	-0.19338	-0.24928	-0.14053	-0.1908	-0.24684	-0.29647	-0.29099	
	0.7899	0.3728	0.1814	0.7768	0.1273	0.2716	0.2257	0.1209	0.3935	0.2512	0.1197	0.0632	0.0649	
	41	41	41	41	41	41	41	40	39	38	41	40	41	
Sleep__hours_	***0.27569***	-0.03755	-0.0655	-0.1458	0.03155	-0.1114	-0.00411	-0.01312	0.12295	0.10043	0.08146	0.00353	0.05723	0.19483
	0.0457	0.7895	0.6446	0.2975	0.8225	0.4317	0.9767	0.9264	0.395	0.4877	0.562	0.9802	0.687	0.2346
	53	53	52	53	53	52	53	52	50	50	53	52	52	39

Table 4. Correlation coefficient matrix for all biomarkers, BMD, dietary, and lifestyle variables for the normal inflammatory group. (Significant correlations in bold).

	C_Reactive_Protein	Alkaline_Phosphatase__1_x_normal	HDL_ln_normalized	Femur_BMD	Melatonin__ln_normal_	Energy	Protein	Folate	B12	B6	Vitamin_C_ln	Magnesium_ln	Zinc_ln	Sedentary_Time
Alkaline_Phosphatase__1_x_normal	-0.09364													
	0.5134													
	51													
HDL_ln_normalized	-0.21518	***0.31909***												
	0.1218	***0.0254***												
	53	***49***												
Femur_BMD	0.23003	-0.04689	-0.08556											
	0.0911	0.7439	0.5424											
	55	51	53											
Melatonin__ln_normal_	0.12909	0.15652	-0.10948	-0.10334										
	0.3522	0.2777	0.4398	0.4571										
	54	50	52	54										
Energy	0.01332	-0.15817	***0.34492***	0.21217	-0.26644									
	0.9238	0.2726	***0.0123***	0.1235	0.0538									
	54	50	***52***	54	53									
Protein	0.03411	-0.1085	0.19866	***0.30197***	-0.05829	***0.61902***								
	0.8066	0.4532	0.158	***0.0265***	0.6785	***<.0001***								
	54	50	52	***54***	53	***53***								
Folate	0.06189	0.18769	0.06063	0.10504	0.00322	***0.34051***	0.23636							
	0.6535	0.1872	0.6663	0.4454	0.9816	***0.0118***	0.0853							
	55	51	53	55	54	***54***	54							
B12	0.04054	-0.02368	0.11404	-0.0039	***-0.30598***	***0.32026***	***0.39583***	***0.51549***						
	0.7754	0.8731	0.4304	0.9781	***0.029***	***0.022***	***0.004***	***<.0001***						
	52	48	50	52	***51***	***51***	***51***	***52***						
B6	0.09081	0.11964	0.01704	0.0516	0.04173	***0.32206***	***0.42873***	***0.55438***	***0.55633***					
	0.5137	0.4079	0.9046	0.711	0.7667	***0.0187***	***0.0014***	***<.0001***	***<.0001***					
	54	50	52	54	53	***53***	***53***	***54***	***51***					
Vitamin_C_ln	0.02511	0.08055	0.11143	-0.02668	0.26109	0.09988	0.05801	***0.35847***	0.04735	***0.38013***				
	0.8556	0.5742	0.427	0.8467	0.0565	0.4724	0.677	***0.0072***	0.7389	***0.0046***				
	55	51	53	55	54	54	54	***55***	52	***54***				
Magnesium_ln	0.05324	0.02184	***0.28744***	0.18597	0.05128	***0.6311***	***0.6753***	***0.4699***	***0.30258***	***0.50111***	***0.31981***			
	0.6995	0.8791	***0.0369***	0.174	0.7127	***<.0001***	***<.0001***	***0.0003***	***0.0292***	***0.0001***	***0.0173***			
	55	51	***53***	55	54	***54***	***54***	***55***	***52***	***54***	***55***			
Zinc_ln	0.12917	0.02539	0.22784	0.21281	0.00614	***0.68503***	***0.7397***	***0.37983***	***0.3964***	***0.44631***	0.0967	***0.63893***		
	0.3473	0.8596	0.1008	0.1188	0.9649	***<.0001***	***<.0001***	***0.0042***	***0.0036***	***0.0007***	0.4825	***<.0001***		
	55	51	53	55	54	***54***	***54***	***55***	***52***	***54***	55	***55***		
Sedentary_Time	0.27207	-0.04074	***-0.35837***	0.20509	0.22199	-0.08809	0.16676	0.05604	0.00596	0.12162	0.08511	0.2584	0.14729	
	0.1084	0.8219	***0.0345***	0.2302	0.2	0.6148	0.3383	0.7455	0.9729	0.4798	0.6217	0.1281	0.3913	
	36	33	***35***	36	35	35	35	36	35	36	36	36	36	
Sleep__hours_	0.19498	-0.05441	-0.00201	-0.0586	0.04008	0.14648	0.19315	0.21685	0.2048	0.09264	0.15137	0.17281	***0.27324***	-0.07199
	0.1577	0.7074	0.9887	0.6738	0.7757	0.2953	0.1658	0.1153	0.1494	0.5094	0.2746	0.2114	***0.0456***	0.6765
	54	50	52	54	53	53	53	54	51	53	54	54	***54***	36

The path analysis revealed a number of differences between the chronic and normal inflammatory groups in variable correlations and predictive relationships (Figure 7 and Figure 8). Within the chronic inflammatory group, CRP, femur BMD, melatonin, and dietary protein were included as predictive variables (Figure 7). With regards to CRP, hours of sleep was a significant positive predictor and folate was a significant negative predictor. CRP was a significant negative predictor of femur BMD. Magnesium and zinc were significant positive predictors of dietary melatonin and protein intake was negatively correlated with melatonin. Protein intake was negatively predicted by folate, and positively predicted by caloric intake, vitamins B12 and B6, melatonin, and zinc.

In the normal inflammatory group, the variables CRP, hours of sleep, HDL cholesterol, femur BMD, melatonin, and protein were identified as predictive variables in the model (Figure 8). However, there were fewer statistically significant relationships identified in the normal inflammatory group with caloric intake, vitamin B6, and zinc being positive predictors of protein intake.

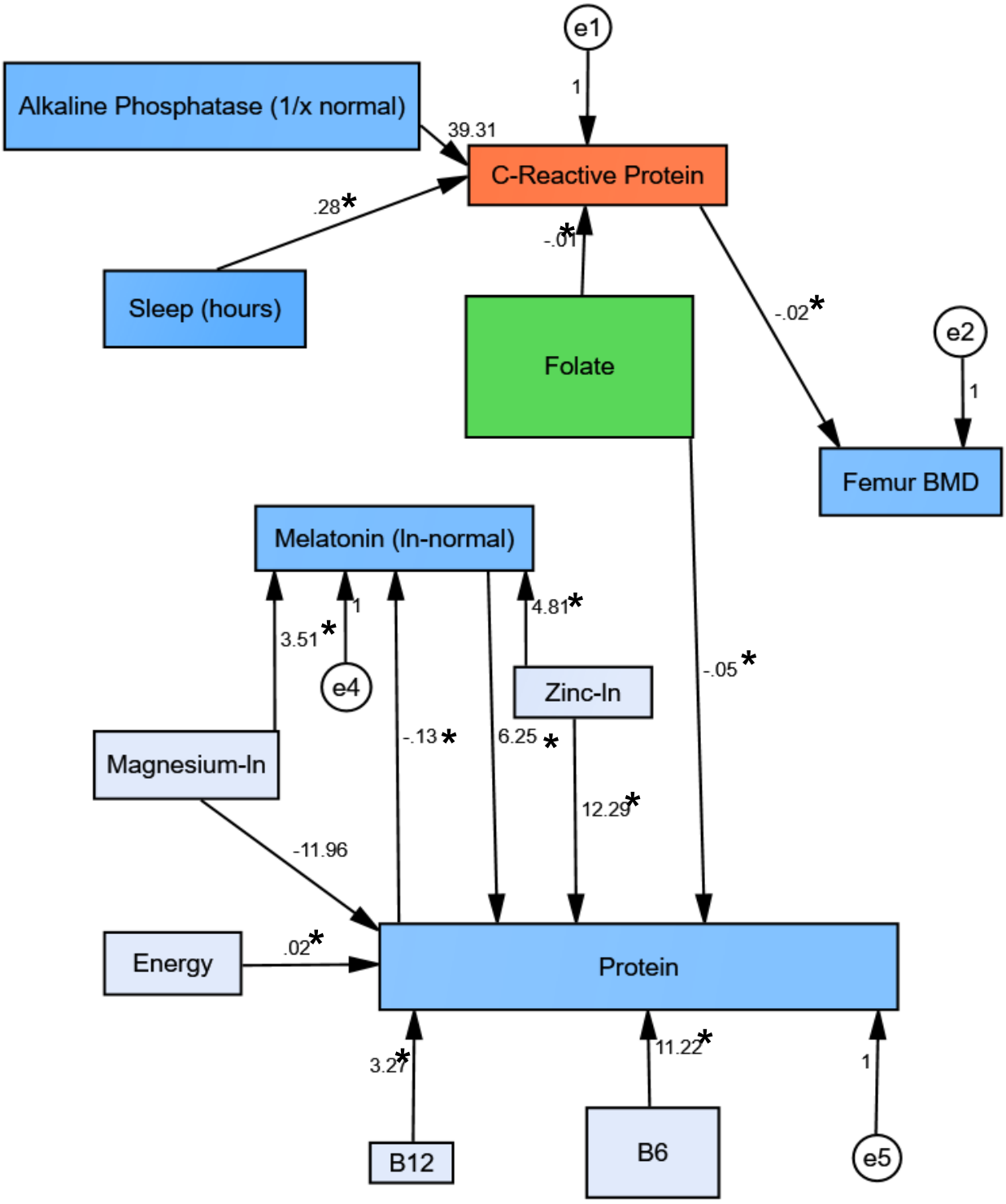


Figure 7. Path analysis of chronic inflammatory group multiple linear regression variables. Arrow numbers indicate regression weights that define the degree to which the response variable changes with every one unit increase in the predictor variable. * indicates significant relationships.

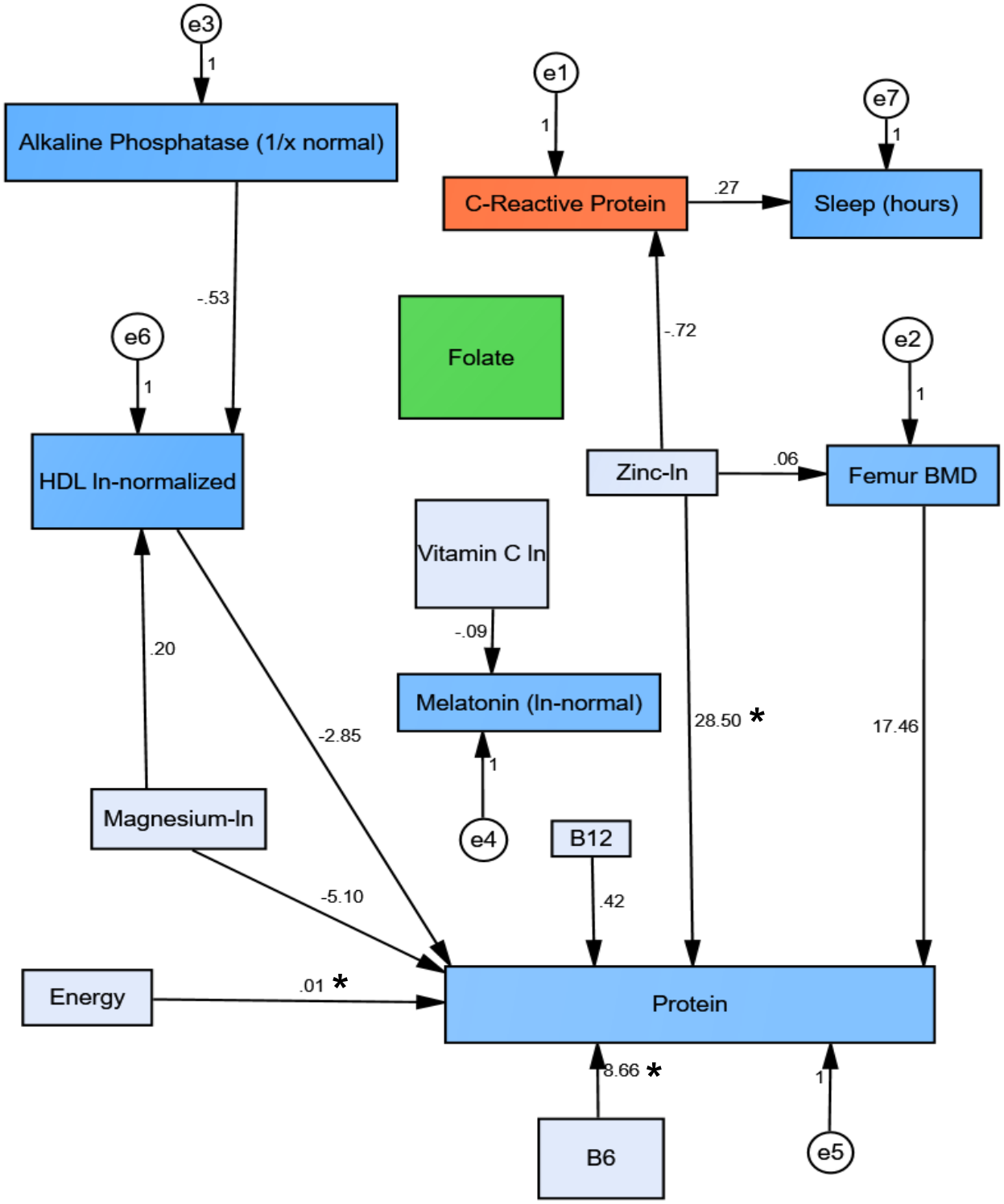


Figure 8. Path analysis of normal inflammatory group multiple linear regression variables. Arrow numbers indicate regression weights that define the degree to which the response variable changes with every one unit increase in the predictor variable. * indicates significant relationships.

Finally, we calculated the percent contribution of each food group to the total melatonin consumed in each group (Figures 9 and Figure 10). Grain products constituted the majority of dietary melatonin in both the chronic and low inflammatory groups. Fruits and vegetables provided the next greatest percent contribution, with nuts and dairy providing the least. While individuals in the normal inflammatory group consumed a significantly greater amount of melatonin than individuals in the chronic inflammatory group, less of their dietary melatonin was consumed from fruits and vegetables with a greater percentage from grains compared to the chronic inflammatory group.

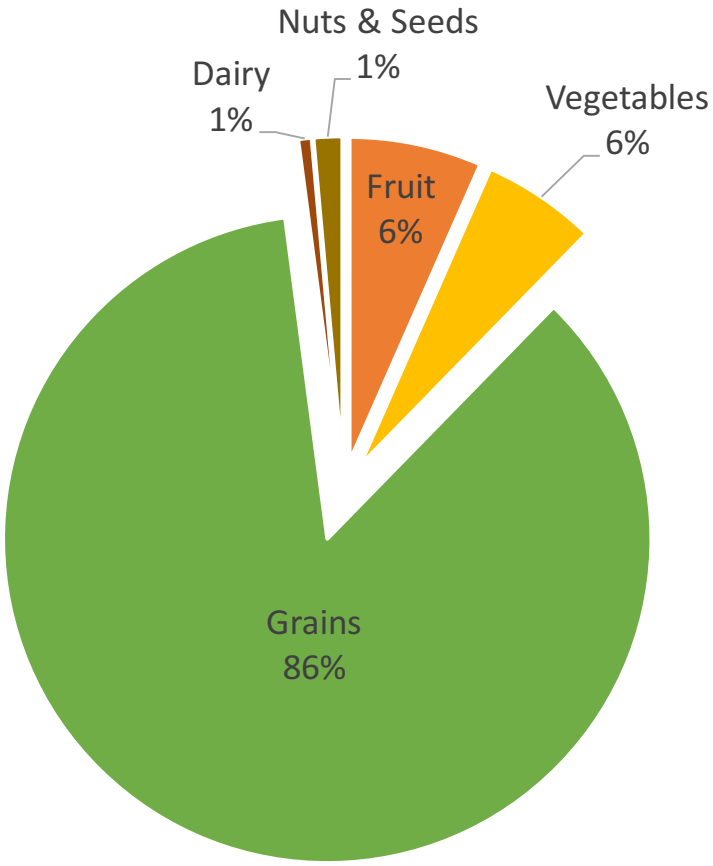


Figure 9. Percent contribution of each food group to the total melatonin consumed by individuals in the chronic inflammatory group.

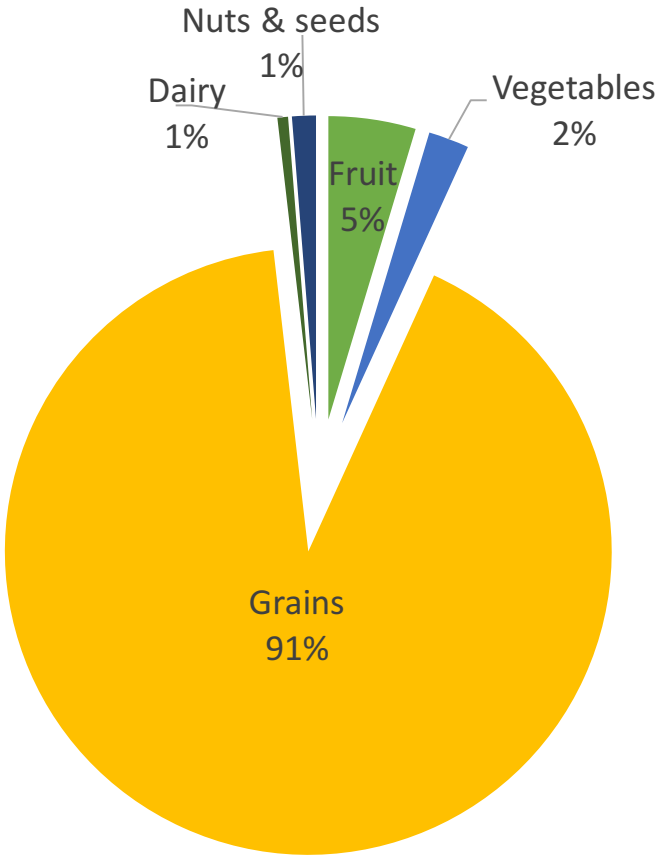


Figure 10. Percent contribution of each food group to the total melatonin consumed by individuals in the normal inflammatory group.

Discussion

Nutrients and Endogenous Melatonin Synthesis

The results from the factor analysis revealed key dietary, inflammatory, and lifestyle factors that distinguished the normal and chronic inflammatory groups from one another. A notable result was that individuals with low CRP exhibited the highest intake of B vitamins and minerals that are important for endogenous production of melatonin. The low inflammatory group consumed significantly greater amounts of folate, vitamin B6, and magnesium than those with chronic inflammation. The low inflammatory group also had a higher, although not statistically significant, intake of vitamin B12, vitamin C, and zinc. Folate is a coenzyme for TPH and provides methyl groups for the final step of the pathway where HIOMT converts N-acetylserotonin to melatonin (Figure 1). Vitamin B6 is a coenzyme for AADC in the endogenous pathway and B12 is needed in the folate cycle to allow methionine synthase to obtain methyl groups from folate, which would then be passed to HIOMT (Figure 1). Further, zinc and magnesium are required cofactors for AANAT, which converts serotonin to N-acetylserotonin (Figure 1). Since the chronic inflammatory group consumed less of these nutrients, it is possible that a lack of cofactors and coenzymes might prevent or decrease the activity of the endogenous melatonin pathway. Reduced synthesis of endogenous melatonin would reduce the beneficial effects of melatonin on inflammation, oxidative stress, and circadian rhythm. A lack of endogenous melatonin could also limit synthesis of new bone, since melatonin has the ability to stimulate osteoblasts, and unchecked inflammation and oxidative stress could negatively impact BMD by stimulating bone resorption.

Folate and Melatonin Synthesis

The discriminant function analysis revealed that folate was of the nutrient of greatest importance in differentiating the low and chronic inflammation groups. Folate consumption showed a significant positive correlation with melatonin consumption in the chronic inflammatory group as evidenced by multiple linear regression analysis. Means tests, however, showed that the low inflammatory individuals consumed significantly more folate overall than those with chronic inflammation. The significance of this result is highlighted by the physiological role of folate as a methyl donor that allows *S*-adenosyl-methionine to deliver a methyl group to N-acetylserotonin to create melatonin (Figure 1). In an animal model of folate deficiency, rats that did not consume folate over a 4-week period had significantly reduced melatonin concentration in the pineal gland, as well as reduced excretion of 6-SMT in the urine, compared to animals that consumed adequate melatonin.[52] The authors suggested that reduced melatonin synthesis due to folate deficiency could contribute to a number of health problems, since melatonin is important for regulating inflammation and ROS.[52] Another study evaluated the effects of melatonin and folic acid supplementation in an animal model of oxidative stress and inflammation.[53] When rats were provided with either melatonin, folic acid, or a combination of both, there was a greater improvement in parameters associated with cellular damage and antioxidant activity with the combined melatonin and folic acid treatment.[53] Therefore, folate and melatonin may work synergistically to control inflammation and oxidative stress, where methyl groups from folate enhance endogenous melatonin production and increase melatonin synthesis, combined with exogenous melatonin supplementation, would provide an improved ability to mitigate inflammation and ROS. Furthermore, since melatonin is primarily found in plant foods, such as fruits, vegetables, and whole grains, that are good sources of folate, individuals who

consume more of these foods would also obtain more dietary melatonin. This is one plausible theory that could explain why folate appeared as the variable of primary importance in the discriminant function analysis.

Vitamin B12 and Melatonin Synthesis

While mean vitamin B12 consumption did not differ significantly between each inflammatory group, B12 consumption showed a significant positive correlation with melatonin consumption in the chronic inflammatory group and significant negative correlation in the normal inflammatory group in the multiple linear regression. Vitamin B12 is a necessary component of the folate cycle to allow methyl groups from folate to be passed to N-acetylserotonin (Figure 1). However, B12 and folate are typically obtained from different food groups. Since B12 is primarily found in animal foods and melatonin in plant foods, individuals of the normal inflammatory group who consumed fewer animal products rich in vitamin B12 and more plant foods rich in melatonin could have contributed to the apparent negative correlation. This result may also be a function of individuals in the chronic inflammatory group consuming greater amounts of meat than the normal group while attempting to increase intake of fruits and vegetables, which is medical advice that would be common for most diseases of inflammation (e.g., obesity, heart disease, cardiovascular problems, diabetes, cancer, etc). It is also possible that individuals with chronic inflammation consuming more dietary melatonin are more likely to obtain vitamin B12 through supplements, fortified foods, and meal replacements that contain large amounts of B12.

This result is of interest as there is evidence that B12 is an important component for adequate endogenous melatonin release. In a rat model of circadian rhythm, an injection of methyl-B12 prior to taking samples from the pineal gland resulted in a significant increase in melatonin secretion.[54] The authors of this study ascertained that vitamin B12 both increased endogenous synthesis and secretion of melatonin while elevating the sensitivity of the rat circadian clock to the effects of melatonin.[54] An additional study examined vitamin B12 supplementation in humans and found that urinary excretion of the melatonin metabolite, 6-SMT, peaked during the night following vitamin B12 supplementation.[55] Further, individuals taking a methylcobalamin supplement tended to have improved sleep and more alertness upon waking.[55] While more information is needed to understand the exact role of vitamin B12 in melatonin synthesis and sleep cycles, it is plausible that inadequate B12 consumption could compromise melatonin production, therefore limiting the ability of melatonin to reduce inflammation, oxidative stress, and to promote bone health.

Vitamin B6 and Melatonin Synthesis

Vitamin B6 consumption was significantly higher in the normal group than in the chronic inflammatory group with no significant correlations observed through multiple linear regression. Although B6 is a coenzyme for the pathway of endogenous melatonin synthesis, limited research has examined its effects on melatonin production. One study found no significant increase in serum melatonin levels after supplementation with vitamin B6 compared to a placebo in adult males.[56] However, other research has found that pyridoxine deficiency causes a significant decrease in melatonin production in rats.[57] Pyridoxine deficiency negatively altered the activity of 5-hydroxytryptophan decarboxylase, also known as AADC, which resulted in a buildup of 5-

hydroxytryptophan and a reduction in 5-hydroxytrytamine or serotonin.[57] Although this study was performed in animals, vitamin B6 deficiency may have the potential to alter melatonin synthesis and prevent melatonin from exerting its beneficial effects on inflammation, oxidative stress, and BMD.

Magnesium, Zinc and Melatonin Synthesis

Magnesium and zinc exhibited significant positive correlations with dietary melatonin in the chronic inflammatory group. Similar to previously described dietary patterns, the normal inflammatory group consumed significantly more magnesium than the chronic group. Magnesium and zinc were also significant predictors of melatonin intake in the path analysis of individuals with chronic inflammation. Foods that are good sources of magnesium and zinc, such as whole grains, nuts, seeds, and dairy also contain significant levels of melatonin, contributing to the observed positive correlations. A few studies have explored how magnesium and zinc are related to melatonin production and circadian rhythm. An animal model of magnesium deficiency found that after four weeks of consumption of a magnesium-deficient diet, rats had a significant reduction in plasma melatonin concentration compared to the animals receiving an appropriate amount of dietary melatonin.[58] The authors suggested that since magnesium is a required cofactor for the enzyme AANAT, which converts serotonin to N-acetylserotonin, inadequate dietary magnesium could negatively affect the ability of this pathway to function.[58] A double-blind, randomized controlled trial evaluated the effects of daily supplemental magnesium, zinc, and melatonin on insomnia and quality of sleep in long-term care facility residents.[59] It was found that study participants supplemented with 225 mg magnesium, 11.25 mg zinc, and 5 mg melatonin had significant improvements in overall sleep quality and sleep time compared to

study participants receiving a placebo.[59] However, it is difficult to ascertain whether the supplemental melatonin, magnesium and zinc, or both increased serum melatonin and resulted in improved circadian rhythm. Based on these studies, magnesium and zinc have the potential to enhance endogenous synthesis of melatonin and to improve outcomes of melatonin on sleep, inflammation and BMD. However, more studies are needed to evaluate the separate and combined effects of supplemental magnesium and zinc on endogenous melatonin production, and whether inadequate intake has a negative impact on serum melatonin in humans.

Calories, Protein, and Melatonin Synthesis

Although not statistically significant, the low inflammatory group consumed more calories and protein than those with chronic inflammation. Protein, however, was significantly correlated with melatonin in individuals with chronic inflammation. Increased protein levels would be beneficial for endogenous melatonin production through metabolic pathways related to tryptophan. Calorie restriction may significantly reduce blood concentration of melatonin; in this circumstance, adequate tryptophan would be necessary to serve as the starting substrate for melatonin production.[5] A lack of both calories and protein could therefore interfere with endogenous melatonin synthesis in the chronic inflammatory group. Furthermore, consuming an inadequate amount of calories would make it difficult to obtain optimal amounts of the vitamins and minerals necessary for endogenous production of melatonin. Since melatonin is involved in reducing inflammation by inhibiting nuclear factor-κβ, TNF-α, and IL-1, consuming adequate amounts of nutrients needed for optimal endogenous melatonin production is important.[6,7,8,9]

Tryptophan, Niacin, and Melatonin

It is important to note that some tryptophan, which is the starting substrate for melatonin synthesis, is converted to niacin (Figure 1). Therefore, not all dietary tryptophan is directed to the pathway of endogenous melatonin synthesis. If an individual is niacin deficient, it is possible that more tryptophan would be diverted away from the pathway of endogenous melatonin synthesis to produce additional niacin. This would limit the amount of endogenous melatonin produced and could lead to excess inflammation, oxidative stress, reduced BMD, and disrupted circadian rhythm that would be mitigated under conditions of normal melatonin synthesis. This situation would make dietary melatonin of even greater importance to compensate for a lack of endogenous melatonin. Although this study did not evaluate niacin consumption of study participants, it would be important to assess the relationship between niacin, tryptophan, and dietary and endogenous melatonin to better understand how the pathways of tryptophan metabolism are prioritized within the body.

Dietary Melatonin Consumption

Mean dietary melatonin consumption was significantly higher in the normal group, was an important variable in the initial factor analysis, and melatonin consumption was predicted by a number of nutrients in the path analysis. However, we did not find significant direct associations between dietary melatonin and femur BMD, inflammatory markers, or hours of sleep. It is possible that melatonin did not explain the majority of variation in our outcome variables of interest because melatonin may work with other nutrients to mitigate inflammation and oxidative stress, rather than functioning on its own. Melatonin was significantly correlated with other

nutrients, such as protein, folate, B12, magnesium, and zinc, which means that melatonin may be obtained from foods that also contain these nutrients. While melatonin has its own unique functions of promoting BMD, regulating circadian rhythm, and reducing inflammation and ROS, its actions may be enhanced by the presence of other important nutrients where melatonin is one key player in the overall pathway of reducing inflammation and oxidative stress, and improving bone health.

Dietary melatonin and the foods that contain nutrients important for melatonin synthesis should be considered from an integrative perspective in that no single food source is the best at providing melatonin. Nutrients are not single components that act alone; rather, they interact with one another to improve health via synergistic mechanisms.[60,61] It is therefore likely that any positive health effects of dietary melatonin occur through its interactions with other nutrients. Further, having a deficiency in a single nutrient could reduce the effectiveness of dietary melatonin if the absent nutrient is needed to enable melatonin to perform its metabolic functions.[60,61] This therefore supports consumption of a diet containing a variety of foods rich in melatonin to ensure that the array of nutrients needed for melatonin metabolism are present. The idea of food synergy also suggests that isolated sources of melatonin from supplements, without the presence of synergistic nutrients, may not be as effectively utilized within the body as melatonin from whole foods.

Nutrients and BMD: Protein

The differences in nutrient consumption between each inflammatory group complement our findings on femur BMD. Individuals with low inflammation had significantly higher femur

BMD than those with chronic inflammation, which may be associated with having greater mean consumption of a number of the nutrients discussed above. Protein consumption showed a significant positive correlation with femur BMD in the normal inflammatory group, with femur BMD being a positive predictor of protein intake in the path analysis of this group. Having adequate dietary protein intake would improve BMD in a number of ways. Protein is essential for promoting proper collagen matrix formation, as well as increasing levels of IGF-1, which is involved in activating bone growth and promoting activation of calcitriol in the kidneys to lead to increased dietary calcium absorption at enterocytes.[62] In a prospective cohort study evaluating protein intake and BMD in individuals ages 50 and older, postmenopausal women consuming less than 12% of their total energy intake from protein had a significantly greater risk of developing fractures than those consuming at least 15% of total calories from protein.[63] Further, postmenopausal women consuming protein in the lowest quartile had a significantly greater risk of fragility fractures than women in the second highest quartile.[63] It has also been suggested that postmenopausal women who consume greater than 0.8 g/kg body weight of protein per day not only have higher BMD, but slower bone loss over time.[62]

B Vitamins and BMD

Folate, vitamin B12, and vitamin B6 may also contribute to the significant differences in femur BMD observed between the low and chronic inflammatory groups, since the normal inflammatory group showed a significantly higher consumption of folate and vitamin B6 than the chronic group. A number of studies have examined whether supplementation with these vitamins has the ability to increase BMD with mixed results. Women older than 54 years were provided with supplementation with 500 µg folic acid, 50 mg vitamin B6, and µg 500 vitamin B12, as

well as vitamin D and calcium over one year, and were found to have significantly reduced alkaline phosphatase.[64] This indicates that the B vitamins may reduce bone turnover and prevent bone loss in older females.[64] Other studies providing B vitamin supplementation have found no significant impact on markers of bone turnover, which may have been due to reduced length of time taking the supplements and differences in quantity of each vitamin.[64] A number of cohort studies examining folate consumption and hip fractures indicate that adequate folate status may be of more importance for promoting optimal femur BMD in females rather than males.[64] Limited data on vitamin B12 status and associations with BMD show a significant decrease in BMD with inadequate serum vitamin B12 in older women, but other studies indicate that there is no significant association between these two variables.[64] In regards to vitamin B6, it was found that increased pyridoxine consumption in older adults was associated with higher femur BMD with another study indicating that pyridoxal-5'-phosphate may be of particular importance for preventing osteoporotic fractures.[65]

Research points to homocysteine and lack of vitamin B12 and folate as a possible link to less than optimal BMD. It is known that folate, B12, and B6 are necessary to run the folate cycle to prevent the buildup of homocysteine, and that elevated homocysteine leads to inflammation, oxidative stress, and endothelial damage.[65] In vitro and animal models examining the effects of homocysteine have found that even slightly higher than normal concentrations of homocysteine lead to activation of osteoclasts, reduction in bone formation, and inhibition of protein cross-linking that is necessary to produce a healthy bone matrix.[65] Elevated homocysteine and the resulting damage to blood vessels has also been suggested to interfere with bone vascularization and delivery of nutrients necessary for building healthy bones.[65] Studies in mice have found that those with vitamin B12 deficiencies had reduced numbers of osteoblasts and increased rates of

osteoporosis.[65] A randomized, double-blind, placebo-controlled trial investigated whether supplementation with 400 µg folic acid, 500 µg vitamin B12, and 600 IU vitamin D compared to a control group with only 600 IU vitamin D would increase bone health in adults older than 65 years with mild hyperhomocysteinemia.[66] The authors found that after 2 years, there was a significant increase in BMD of the lumbar spine but a significant decrease in femoral neck BMD.[66] However, subjects ages 80 and older were more responsive to supplementation since they exhibited greater calcaneal broadband ultrasound attenuation, which is an indicator of higher bone quality.[66]

While studies are inconclusive regarding the role of supplemental folate and vitamins B12 and B6 on bone health, it is important to consider that habitual and long-term consumption of these B vitamins from whole foods could have a beneficial impact on BMD. These nutrients could affect BMD via multiple pathways, such as directly influencing osteoblast and osteoclast activity, reducing the production of homocysteine, or increasing endogenous melatonin production, which is known to increase bone formation and reduce bone damage caused by oxidative stress and inflammation.

Magnesium, Zinc, and BMD

Magnesium and zinc consumption also appeared as important variables linked to BMD in factor analysis. The chronic inflammatory group had a significantly lower intake of magnesium as compared to individuals with low inflammation. Zinc was also a positive predictor of femur BMD in the normal inflammatory group path analysis, which was not statistically significant. As with the B vitamins, magnesium and zinc have been shown to be involved in BMD and are of

importance for bone health in postmenopausal women. Both are important for promoting formation of a healthy bone matrix, where zinc may be a necessary component of bone mineralization and magnesium may be involved in hydroxyapatite crystal formation and regulation of parathyroid hormone release.[67] A cross-sectional study compared dietary intake of magnesium and zinc, serum magnesium and zinc, and level of femur BMD classified by either osteopenia or osteoporosis.[67] This study found that the mean consumption of magnesium and zinc were below the RDA for postmenopausal women, with subjects having low femur BMD consuming around 48% of the RDA for zinc and 35% of the RDA for magnesium.[67] Participants with low BMD had reduced serum zinc and 40% of the participants had low serum magnesium.[67] Therefore, it is possible that poor magnesium and zinc intake may be related to loss of BMD in postmenopausal women and may have contributed to the significantly reduced BMD seen in the chronic inflammatory group.

Zinc may play a larger role by influencing BMD directly, as well as through regulation of the immune system and chronic inflammation that are known to negatively affect bone health. Zinc is involved in the regulation of osteoblasts and osteoclasts, enzymatic reactions that promote formation of the collagen matrix and bone mineralization, and production of growth factors needed for bone formation.[68] Zinc deficiency results in alterations of the immune system that favor a low-grade pro-inflammatory state, including increased activation of T-cells and monocytes, and elevated production of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β, which have been shown to normalize with addition of zinc in an in vitro model of zinc deficiency.[68] Therefore, increased production of pro-inflammatory cytokines, due to zinc deficiency, and inflammatory-mediated activation of osteoclasts and inhibition of osteoblasts would reduce BMD in individuals consuming inadequate zinc.[68] In an animal model of

inflammation, BMD was significantly reduced in animals experiencing low-grade inflammation but zinc deficiency with low-level inflammation did not result in significant bone loss.[68] While the authors concluded that a moderate zinc deficiency with inflammation would negatively affect bone biomechanics, it is possible that, given the important role of zinc in humans, inadequate zinc intake could have detrimental effects on BMD in postmenopausal women. While there was no significant difference in zinc intake between the low and chronic inflammatory groups in our study, individuals with lower mean zinc consumption had significantly higher levels of CRP, significantly lower HDL cholesterol and femur BMD, and borderline significantly higher levels of alkaline phosphatase compared to the low inflammatory group. This suggests that zinc may play an underlying role in regulating low-grade inflammation and promoting BMD.

Outliers and Supplementation

It is important to note that we found some unusual patterns while normalizing our data and removing outliers with values greater than or less than two standard deviations from the mean. Several of the outliers identified in the chronic inflammatory group had nutrient intakes that were well above the mean. These nutrient intakes were at a level that would be difficult to obtain from whole foods alone, leading us to suggest that individuals with chronic inflammation may have been more likely to obtain nutrients from supplements rather than food. While we did not evaluate supplementation among subjects included in our analysis, outliers who may have obtained the majority of their nutrients from supplements might not be consuming other beneficial components of whole foods, such as dietary melatonin, that would help mitigate inflammation and oxidative stress.

Inflammatory Markers and BMD

In regards to the markers of inflammation, CRP concentration was significantly higher in the chronic inflammatory group; this was expected, as this was the variable we used to initially place individuals in each inflammatory group. However, CRP was also independently identified as a variable of importance to distinguish each inflammatory group in the factor analysis, which aligned with the means tests.

CRP was positively predicted by alkaline phosphatase and hours of sleep, and negatively predicted by dietary folate in the chronic inflammatory group path analysis. Elevated alkaline phosphatase is related to increased bone turnover and bone loss in postmenopausal women, and may be used as a marker of osteomalacia and osteoporosis.[49] A cross-sectional study that assessed the ability of an osteoporosis predictive tool to identify women with osteopenia found that women with lower BMD had significantly higher alkaline phosphatase than women with a healthy BMD.[50] Since chronic inflammation and elevated CRP are known to negatively affect BMD, it makes sense that increased alkaline phosphatase would be associated with an increase in CRP in the chronic inflammatory group path analysis.[1,2,3] This observation was further validated by our finding that individuals with chronic inflammation had significantly reduced mean femur BMD and borderline significantly higher alkaline phosphatase compared to individuals of the normal inflammatory group. Multiple linear regression also revealed that CRP had a significant negative association with femur BMD in the chronic inflammatory group, which was identified as a significant relationship in path analysis and which is in agreement with literature describing the detrimental impact of inflammation on BMD.[1,2,3] Folate consumption was a significant negative predictor of CRP in the chronic inflammatory group, a result that aligns with folate's role of providing methyl groups to run the folate cycle. Since elevated homocysteine leads to

inflammation, oxidative stress, and elevated CRP, adequate folate consumption would provide the methyl groups needed to convert homocysteine to methionine, therefore mitigating the detrimental effects of elevated homocysteine and, therefore, inflammation.[65]

While the normal inflammatory group exhibited significantly greater femur BMD, significantly lower CRP, significantly higher HDL cholesterol, and borderline significantly lower alkaline phosphatase, none of the inflammatory markers in this group were significantly correlated with femur BMD. In contrast, alkaline phosphatase showed a nonsignificant, negative correlation with HDL cholesterol in the path analysis. Since elevated alkaline phosphatase is associated with bone loss and possibly inflammation in postmenopausal women, and HDL cholesterol is an indirect marker of chronic inflammation, our observations support the negative association observed between these two variables in the normal inflammatory group.[47,49,50]

However, it is important to note that other studies have reported a differing role of alkaline phosphatase in BMD. A case-control study was performed to evaluate biochemical markers that can be used to predict low BMD in postmenopausal women, and found that study participants with osteoporosis and the highest fracture rates exhibited the lowest serum concentrations of alkaline phosphatase compared to control subjects with a normal BMD.[51] The authors suggested that since alkaline phosphatase is also an indicator for bone formation, postmenopausal women with low levels of bone growth would be at a higher risk for osteopenia, osteoporosis, and fractures.[51] Therefore, it is important to consider factors that could increase alkaline phosphatase in individuals with a normal BMD and low levels of inflammation, such as weight-bearing exercise that would stimulate the production of new bone.

Sleep and Inflammation

In regards to sleep, an increase in the number of hours of sleep per day was positively correlated with CRP in the chronic inflammatory group (multiple linear regression) and was an important variable for distinguishing individuals with chronic inflammation from those with low levels of inflammation in the factor analysis. In the path analysis, sleep was also a significant positive predictor of CRP in the chronic inflammatory group and CRP was a positive predictor of sleep in the normal inflammatory group. These results suggest that longer sleep time is associated with elevated markers of inflammation. A randomized-controlled trial evaluated the effects of longer sleep duration on pro-inflammatory cytokines and depressive symptoms in healthy individuals, the majority of which were women. The treatment group exposed to three extra hours of time in bed experienced doubled IL-6 concentration and increased parameters of depression, compared to control subjects that remained in bed for their usual mean sleep time.[69] An additional study evaluated associations between sleep quality and plasma markers of inflammation in perimenopausal and postmenopausal women.[70] The authors found that increases in sleep disturbance exhibited a positive correlation with CRP, IL-6, a macrophage inflammatory protein-1β, and interferon gamma-inducible protein-10.[70]

On the other hand, a cross-sectional study reported that for every one-hour decrease in amount of sleep at night in middle-aged men and women, CRP and IL-6 were elevated by 8.1% and 4.5% respectively.[71] It is also plausible that excess time spent sleeping and remaining sedentary in bed would reduce the amount of time an individual engages in physical activity, which could result in elevated levels of inflammation. While our study was not able to effectively evaluate sleep disturbances and although there was no significant difference in mean hours of sleep between the chronic and normal inflammatory groups, the available literature

suggests that sleep either in excess of or below recommendations may contribute to a chronic inflammatory state. When looking at the distribution of hours of sleep in the chronic inflammatory group, it is clear that a number of individuals in this group have an average number of hours of sleep in excess of 10 hours per night, which could contribute to differences in inflammation and melatonin consumption between each inflammatory group (Appendix D). The distribution of individuals with less than six hours of sleep was similar in both the normal and chronic inflammatory groups (Appendix D).

Sedentary Time and Inflammation

While average minutes of sedentary time per day was included in our factor analysis and multiple linear regression, there were no significant differences in mean sedentary time between each inflammatory group. HDL cholesterol exhibited a significant negative correlation with sedentary time in the normal inflammatory group, which is plausible since physical activity is a mechanism that increases serum HDL cholesterol. We did not find any other significant relationships between sedentary time and our variables of interest. This may have occurred since only two-thirds of the subjects included in our analysis had a value for sedentary time, as the NHANES physical activity questionnaires were not the same between each of the three datasets we used for this study. The questions asking participants about type and level of physical activity were also inconsistent between years of data collection. Therefore, we were unable to accurately quantify amount and level of physical activity or lack of physical activity among our study participants; this prevented us from assessing the relationship between activity level, BMD, inflammation, sleep, and dietary melatonin.

Dietary-Derived Melatonin

In regards to the percent contribution of each food group towards the total melatonin consumption, both groups obtained the majority of dietary melatonin from grains. Fruits, vegetables, nuts and seeds, and dairy made up the smallest percentages of overall melatonin intake in all cases. Much of this result was driven by how we assessed melatonin intake and whether there were available data to describe the effects of cooking on the melatonin content of these foods, since melatonin degrades with heat exposure. Grain products made up the majority of dietary melatonin since there was available literature describing the effects of hydrothermal processing on the melatonin content of these foods.[30] After the highest temperature exposure of 400° F, the grains included in the study still contained a significant concentration of melatonin per gram of food.[30] We were only able to include raw fruits and vegetables in the average melatonin consumed by each individual, due to an absence of data on the melatonin content of cooked fruits and vegetables. Had there been studies available quantifying the degree of melatonin degradation in fruits and vegetables with varying amount and time of cooking, it is possible that we could have counted more of these foods in our analysis. This may have allowed produce to constitute a larger percentage of total dietary melatonin consumed by each inflammatory group.

It is also important to consider that the overall diet quality of each individual could have contributed to the majority of dietary melatonin being obtained from grains. Many individuals consumed a diet with large amounts of refined, and possibly fortified, grains that contained melatonin due to yeast leavening. However, an overabundance of these foods would reduce the nutritional quality and variety of an individual's diet and prevent individuals from obtaining other nutrients that are important for maintaining BMD, endogenous melatonin production,

reducing chronic inflammation, and regulating sleep. Evaluating the relationship between diet quality and dietary melatonin intake would be a method of further evaluating the contribution of each food group towards total melatonin consumption. Within this study, diet quality could be assessed by comparing each group's mean intake of vitamins and minerals to the dietary reference intake (DRI) for postmenopausal women. Neither the chronic or normal inflammatory group met the recommended dietary allowance (RDA) for folate of 400 mcg, although the normal group consumed an average of 10 mcg less than recommendations. The chronic group was below the RDA for vitamin B6, 1.5 mg, and both groups did not meet the RDA for magnesium of 320 mg. However, both inflammatory groups met the recommended intake for vitamin B12, C, and zinc of 2.5 mcg, 75 mg, and 8 mg, respectively.

Although animal foods, such as meats, fish, and eggs were included in our table of foods containing melatonin, these foods were not counted towards the dietary melatonin consumption of each subject. While a single study identified animal foods as containing melatonin that could contribute to dietary intake, the samples of meats were raw and the eggs were dried solids.[24] Therefore, we did not have enough data on the degree of melatonin degradation in animal products after cooking to enable us to include these foods in our analysis. The dairy products included did not make a large contribution to overall dietary melatonin consumption, as milk is a poor source of melatonin with only 0.014 ng/g and few subjects in our dataset consumed yogurt every day. Nuts and seeds may have made up a small percentage of total melatonin intake since a limited number of subjects in the dataset consumed these foods.

Interestingly, the chronic inflammatory group obtained a greater percentage of their total melatonin intake from fruits and vegetables and a smaller percentage from grains than did the normal inflammatory group. Although we had expected that individuals with less inflammation

would consume more dietary melatonin from fruits and vegetables, there are some plausible explanations for this unintuitive outcome. First, there were only two days of 24-hour dietary recall data available from the NHANES datasets; the available data simply provide a snapshot of each subject's dietary intake for a very brief window of time. This dietary information may not be representative of each inflammatory group's usual dietary intake, which could have accounted for the differences in melatonin consumption from produce. Response bias may have also influenced these results. Since the two 24-hour dietary recalls were spaced only 3-10 days apart, it is possible that subjects in the chronic inflammatory group could have altered their dietary intake after completing the first dietary recall to make their dietary choices appear healthier. Finally, the individuals with chronic inflammation may also have had a greater prevalence of conditions under the active care of a physician. These individuals may therefore have been encouraged by healthcare professionals or influenced by other sources to consume a healthier diet to reduce the risks associated with disease.

Study Limitations and Strengths

There are a number of limitations to this study that should be considered. First, the cross-sectional nature of this study did not allow us to determine cause and effect relationships between our variables of interest. We were only able to establish whether there are associations between dietary melatonin, inflammation, BMD, and sleep, since the NHANES data we used was collected at a single point in time. Only two, 24-hour dietary recalls were used in our datasets, which may not be representative of each subject's usual dietary intake. If a different method had been used to collect dietary data, such as a food frequency questionnaire, we would have been able to assess each subject's typical intake of certain types of food and obtained a

better estimate of habitual dietary melatonin consumption, and possibly tryptophan intake, from each food group. The dietary data we used did not allow us to evaluate whether seasonality plays a role melatonin consumption, as the month in which each subject's 24-hour dietary recalls were collected was not included in the data.

The melatonin content that we established for each type of food is a best estimate, and could vary considerably with plant exposure to different environmental conditions, genotype, degree of processing and cooking, animal quality of life, and animal diet. Since we cannot determine the exact source and genotype of foods through NHANES data, the melatonin concentration used for each food may not be completely accurate. The melatonin content of foods that were identified by the studies included in our literature review could also differ depending on the laboratory methods that were used to quantify melatonin concentration. Since not all of the studies used the same melatonin quantification techniques, there may have been differences in measured melatonin content. The amount of dietary tryptophan consumed was not available in the NHANES datasets we used. Since tryptophan is the starting substrate for endogenous melatonin synthesis, it would have been useful to assess the relationships between tryptophan consumption, inflammation, BMD, and sleep in our study sample. If subjects with greater tryptophan consumption had less inflammation, higher BMD, and adequate sleep compared to those with a low tryptophan intake, it is possible that some of this dietary tryptophan could have been used for endogenous melatonin production, therefore increasing the amount of melatonin available to mitigate inflammation, oxidative stress, and increase BMD.

We used CRP as the primary marker of inflammation to separate the postmenopausal women into the chronic and normal inflammatory groups. However, CRP may be elevated due to minor infections or a low-grade, acute activation of the immune system, rather than from long-term,

chronic inflammation. Therefore, using CRP could have classified individuals as being chronically inflamed when their CRP level was elevated for a different reason. Since the three NHANES datasets used for this study did not include physical activity questionnaires with identical questions, it was difficult for us to evaluate the relationship between type and degree of physical activity on inflammation, BMD, sleep, and consumption of dietary melatonin. The physical activity questionnaires did not distinguish between weight-bearing activities and non-weight bearing activities, each of which would have differing effects on BMD. Further, the frequency and amount of physical activity were not listed as continuous variables, which would have made it difficult for us to include physical activity in our statistical analyses that require continuous variables. We did not include all possible covariates in our analysis, such as age, smoking, alcohol consumption, socioeconomic status, or race and ethnicity, as we already had a large number of variables in our dataset that made our analysis complex. Multivariate analysis requires that the number of observations exceed the number of variables included in a given model. Although our sample sizes were reasonably larger, we could not include additional variables without violating the basic premise of the multivariate analysis. However, excluding these covariates prevented us from assessing whether they had any influence on our outcome variables of interest. It also prevented us from evaluating whether there are trends in inflammation, melatonin consumption, BMD, or sleep according to socioeconomic status or race and ethnicity. To include these covariates in the analysis, we would have required many additional years of data to significantly increase our sample sizes.

In spite of its limitations, there are a number of strengths to this study. NHANES data is accurate, reliable, and samples a group of individuals who are representative of the general United States population. This means that our sample is likely representative of the average

postmenopausal woman in the United States. We also had a large sample size of postmenopausal women, with 55 individuals in each inflammatory group, which provided us with enough power to detect statistically significant differences between each group. Furthermore, it was beneficial that we separated individuals with chronic inflammation from those with a normal level of inflammation. This not only allowed us to look for differences in mean values between individuals of different inflammatory states, but also enabled us to assess the unique ways in which our variables of interest were related to one another within each inflammatory group. Separating the study participants in this way also allowed us to evaluate the most important variables that accounted for the differences between each group. While 24-hour dietary recalls may not represent usual dietary intake, this method of dietary data collection has advantages. This method is objective and does not require a large amount of time to complete, therefore reducing participant burden. The 24-hour recall provides detailed information of an individual's dietary intake, such as food brands, serving sizes, and preparation methods, and multiple pass methods can help identify missing or commonly underreported foods.

This study was novel in that we assessed the relationships between key variables of interest, including inflammation, BMD, and sleep in a relatively unexplored area of dietary melatonin. We collected literature on research that has already been done in each of these areas, and created a study that integrated each of these variables, allowing us to begin to identify possible relationships of biological significance. We also conducted a thorough literature review to identify as many foods as possible that contain melatonin. By averaging multiple reported melatonin concentrations for each food, and only including cooked foods with evidence of melatonin remaining after heat exposure, our melatonin concentrations used were a conservative estimate. This ensured that we did not overestimate the potential contribution of each food to the

total dietary melatonin intake, and that we did not assume that cooked produce and meats contain melatonin when there is insufficient evidence to support this. This study is also strong in that we considered the biological impact of dietary-derived melatonin, rather than supplemental melatonin. Since melatonin is an antioxidant and anti-inflammatory compound found in whole foods, it is important to assess the impact of consuming low-grade doses of melatonin from whole foods over a long period of time, rather than pharmacological doses that can only be obtained from supplements.

Conclusions and Future Directions

To the best of our knowledge, this is the first study to examine the relationships between melatonin derived from whole foods and inflammation, BMD, and sleep in postmenopausal women. While recent studies have begun to assess whether dietary melatonin consumption increases plasma levels of melatonin metabolites, we successfully applied available data on food melatonin concentrations to the 24-hour dietary recalls of a sample of postmenopausal women representative of the general US population. Our results support our proposal that dietary-derived melatonin may have an impact on inflammation, BMD, and sleep, as individuals with normal levels of inflammation had significantly greater BMD and melatonin consumption compared to the chronic inflammatory group. While we did not see a direct association between melatonin consumption and inflammation, BMD, and sleep, our results suggest that melatonin from whole foods may indirectly affect these outcomes and may work with other nutrients to reduce inflammatory and oxidative processes that could negatively affect BMD and circadian rhythm.

Therefore, our findings support the need for further investigation to understand the exact mechanisms by which dietary melatonin is absorbed, assimilated, and the biological pathways in which it is involved in promoting BMD and reducing inflammation. Our study supports the need to identify additional foods that contain melatonin, and to obtain a better understanding of how heat, cooking, and processing affect the melatonin concentration of foods. This study also illustrates that dietary-derived melatonin may be part of a healthy dietary pattern for improving BMD and reducing the risk for osteopenia and osteoporosis in postmenopausal women. While previous studies have found improvements in BMD after high-dose melatonin supplementation, our study supports the need for more investigation into the effects of long-term, low-dose consumption of melatonin from whole foods.

Future research should incorporate covariates into the analysis, including age, socioeconomic status, education level, smoking, and alcohol consumption to understand whether they reveal patterns in melatonin consumption, BMD, inflammation, and sleep. Examining melatonin consumption and relationships with our variables of interest among different ethnic groups would make it possible to identify whether individuals with certain genotypes have an increased or reduced capacity to make endogenous melatonin or to process exogenous sources of melatonin. Including tryptophan in the dataset would be beneficial for evaluating endogenous synthesis of melatonin, since this is the starting substrate for melatonin production. It would also be important to evaluate the effects of estrogen replacement therapy on the actions of melatonin in postmenopausal women, as studies have illustrated that melatonin and estrogen may work synergistically to improve BMD. Further, using a dietary data collection method that provides information on usual dietary intake over time would be a more accurate way of estimating usual intake of dietary melatonin. Finally, dietary data collection should consider the role of

seasonality and geography in melatonin consumption, as fruits and vegetables that are rich in melatonin may only be available during certain times of the year, melatonin-rich foods may be grown in specific locations, and environmental exposures in different climate regions could change the food melatonin content.

Appendix A

Variables Included in Initial Factor Analysis

Dietary: Melatonin, Calories, Protein, Folate, Vitamin B6, Vitamin B12, Vitamin C, Magnesium, Zinc

Bone: Femur BMD

Biochemical: C-reactive Protein, HDL Cholesterol ($\log_{10}$-transformed), Alkaline Phosphatase (1/x-transformed)

Lifestyle: Hours of Sleep, Sedentary Time

Appendix B

Factor Analysis

a) Descriptive Statistics

Descriptive Statistics

	Mean	Std. Deviation[a]	Analysis N[a]	Missing N
C-Reactive Protein	1.92	1.99	110.00	0.00
Alkaline Phosphatase (1/x normal)	0.01	0.01	110.00	4.00
HDL ln-normalized	4.01	0.25	110.00	3.00
Femur BMD	0.87	0.17	110.00	0.00
Melatonin (ln-normal)	5.59	1.91	110.00	1.00
B12	4.92	4.17	110.00	0.00
Folate	345.19	135.27	110.00	0.00
B6	1.65	0.68	110.00	0.00
Energy	1673.20	555.78	110.00	1.00
Protein	65.07	21.73	110.00	1.00
Magnesium	255.02	92.47	110.00	0.00
Zinc	9.98	6.59	110.00	0.00
Vitamin C ln	4.09	0.83	110.00	0.00
Sleep (hours)	7.04	1.61	110.00	0.00
Sedentary Time	320.26	126.07	110.00	33.00

a. For each variable, missing values are replaced with the variable mean.

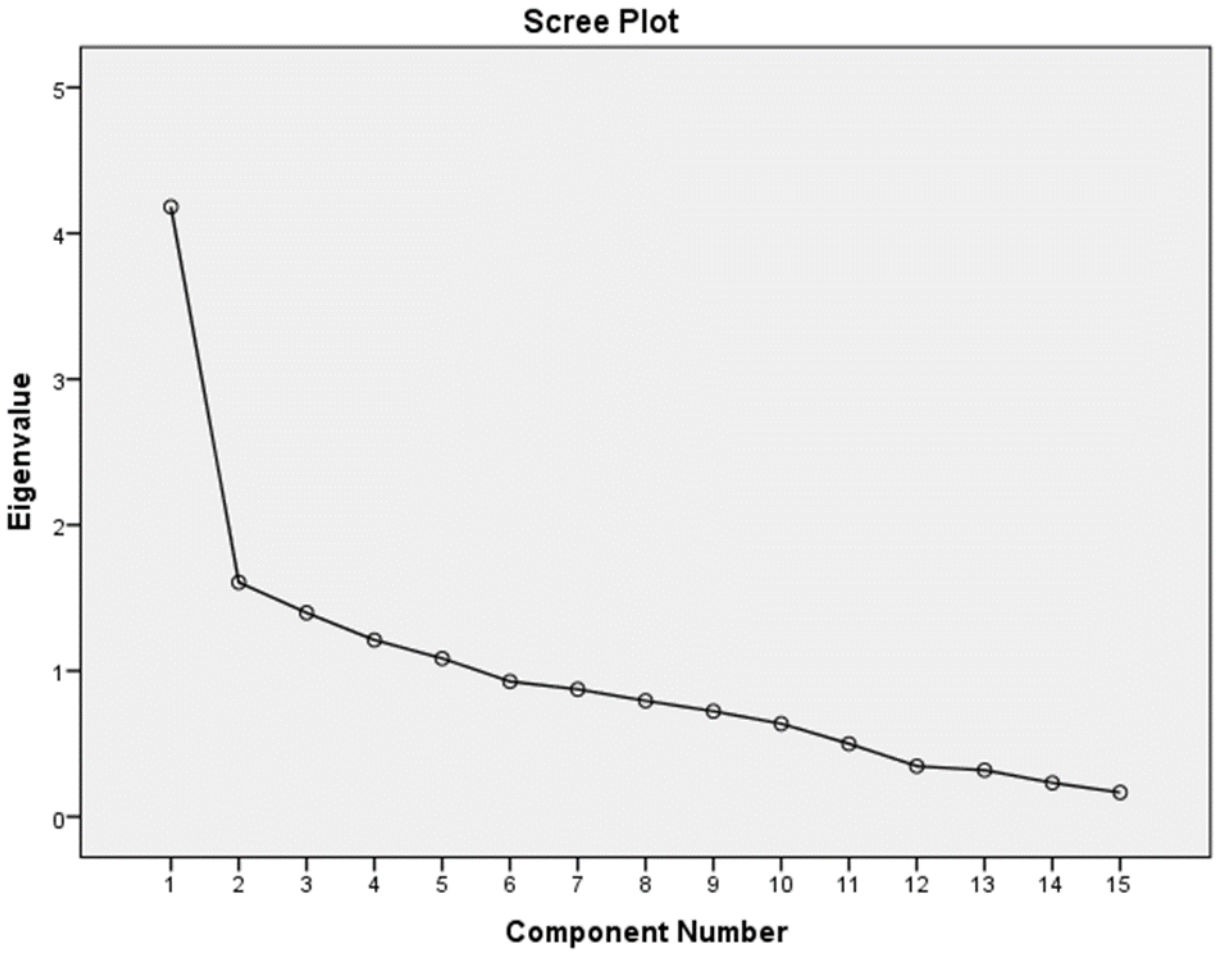
b) Total Variance Explained

Total Variance Explained

	Initial Eigenvalues			Extraction Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.182	27.878	27.878	4.182	27.878	27.878
2	1.607	10.711	38.589	1.607	10.711	38.589
3	1.398	9.319	47.908	1.398	9.319	47.908
4	1.211	8.075	55.984	1.211	8.075	55.984
5	1.085	7.232	63.216			
6	.927	6.181	69.397			
7	.874	5.824	75.221			
8	.795	5.297	80.518			
9	.723	4.818	85.336			
10	.637	4.249	89.585			
11	.500	3.332	92.917			
12	.346	2.309	95.226			
13	.318	2.123	97.350			
14	.232	1.547	98.896			
15	.166	1.104	100.000			

Extraction Method: Principal Component Analysis.

c) Scree Plot


Scree Plot
Eigenvalue
0
1
2
3
4
5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Component Number

d) Component Matrix

Component Matrix[a]

	Component			
	1	2	3	4
C-Reactive Protein	-.183	-.337	.656	-.120
Alkaline Phosphatase (1/x normal)	.223	.468	-.090	-.153
HDL ln-normalized	.326	.479	-.269	-.393
Femur BMD	.208	-.028	-.509	.509
Melatonin (ln-normal)	.369	.393	.175	.151
B12	.349	-.252	-.304	-.418
Folate	.777	.201	.037	.062
B6	.831	.031	.227	.094
Energy	.696	-.380	-.057	.011
Protein	.788	-.364	-.003	.029
Magnesium	.871	.133	.088	.135
Zinc	.557	-.529	-.198	-.185
Vitamin C ln	.431	.429	.329	.055
Sleep (hours)	.171	-.113	.543	-.057
Sedentary Time	-.086	-.111	.008	.700

Extraction Method: Principal Component Analysis.

a. 4 components extracted.

Appendix C

Discriminant Function Analysis

a) Tests of Equality of Group Means

Tests of Equality of Group Means

	Wilks' Lambda	F	df1	df2	Sig.
HDL ln-normalized	.946	5.683	1	99	.019
Alkaline Phosphatase (1/x normal)	.971	2.984	1	99	.087
Melatonin (ln-normal)	.958	4.303	1	99	.041
Vitamin C ln	.988	1.224	1	99	.271
Femur BMD	.971	2.986	1	99	.087
Folate	.886	12.692	1	99	.001
Magnesium	.908	10.078	1	99	.002
B6	.968	3.257	1	99	.074
Energy	.992	.846	1	99	.360
Zinc	.999	.086	1	99	.770
B12	.972	2.865	1	99	.094
Protein	.994	.596	1	99	.442
Sleep (hours)	1.000	.001	1	99	.980

b) Test for Multivariate Normality

Test Results

Box's M		1.059
F	Approx.	1.048
	df1	1
	df2	29012.954
	Sig.	.306

Tests null hypothesis of equal population covariance matrices.

c) Variables Entered/Removed

Variables Entered/Removed[a,b,c,d]

		Wilks' Lambda							
						Exact F			
Step	Entered	Statistic	df1	df2	df3	Statistic	df1	df2	Sig.
1	Folate	.886	1	1	99.000	12.692	1	99.000	.001

At each step, the variable that minimizes the overall Wilks' Lambda is entered.

a. Maximum number of steps is 26.

b. Minimum partial F to enter is 3.84.

c. Maximum partial F to remove is 2.71.

d. F level, tolerance, or VIN insufficient for further computation.

d) Wilks' Lambda Test of Function(s)

Wilks' Lambda

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1	.886	11.881	1	.001

e) Standardized Canonical Discriminant Function Coefficients

Standardized Canonical Discriminant Function Coefficients

	Function
	1
Folate	1.000

f) Structure Matrix

Structure Matrix

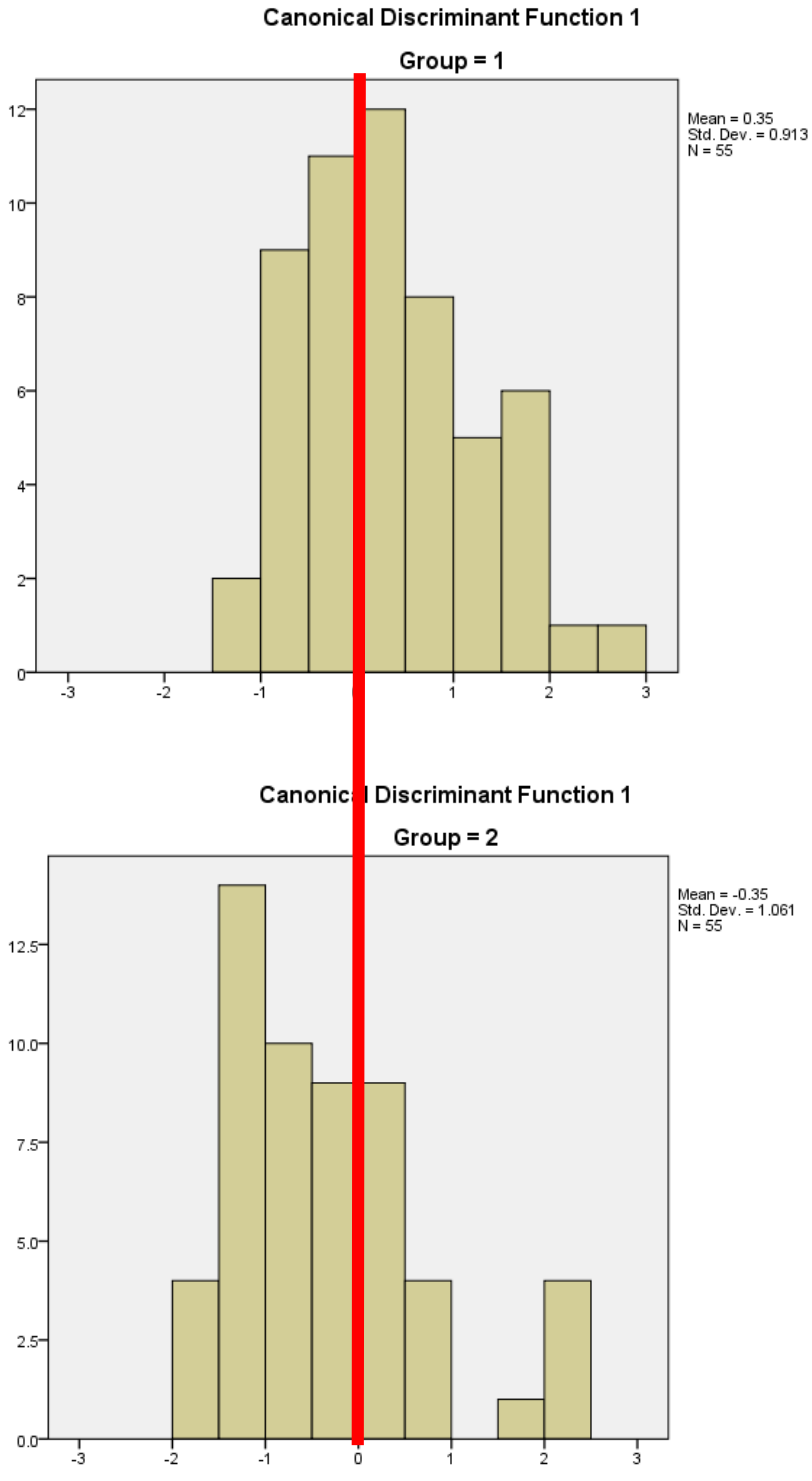
	Function 1
Folate	1.000
B6[a]	.640
Magnesium[a]	.606
Energy[a]	.493
Protein[a]	.486
Melatonin (ln-normal)[a]	.321
Vitamin C ln[a]	.316
Zinc[a]	.280
Alkaline Phosphatase (1/x normal)[a]	.163
B12[a]	.150
HDL ln-normalized[a]	.123
Sleep (hours)[a]	.105
Femur BMD[a]	.052

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions

Variables ordered by absolute size of correlation within function.

a. This variable not used in the analysis.

g) Canonical Discriminant Function Histograms



Group 1 = Low inflammatory

Group 2 = Chronic inflammatory

Appendix D

Means Tests

a) CRP

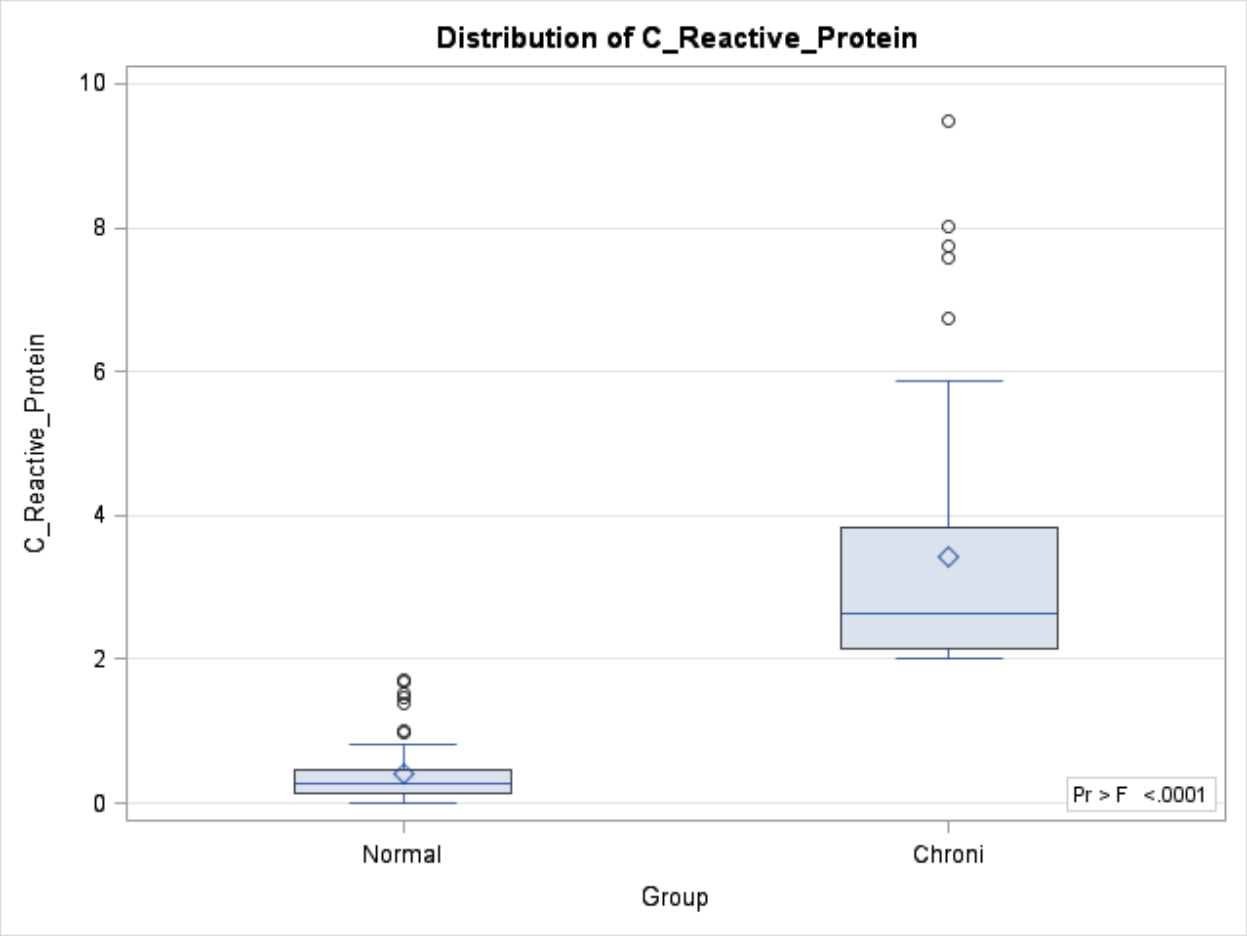
The NPAR1WAY Procedure

Analysis of Variance for Variable C_Reactive_Protein Classified by Variable Group

Group	N	Mean
Normal	55	0.415818
Chronic	55	3.426909

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Among	1	249.333383	249.333383	146.9541	<.0001
Within	108	183.240913	1.696675		

Average scores were used for ties.



Wilcoxon Scores (Rank Sums) for Variable C_Reactive_Protein Classified by Variable Group

Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Normal	55	1540.0	3052.50	167.258624	28.0
Chronic	55	4565.0	3052.50	167.258624	83.0

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	1540.0000

Normal Approximation

Z	-9.0399
One-Sided Pr < Z	<.0001
Two-Sided Pr > |Z|	<.0001

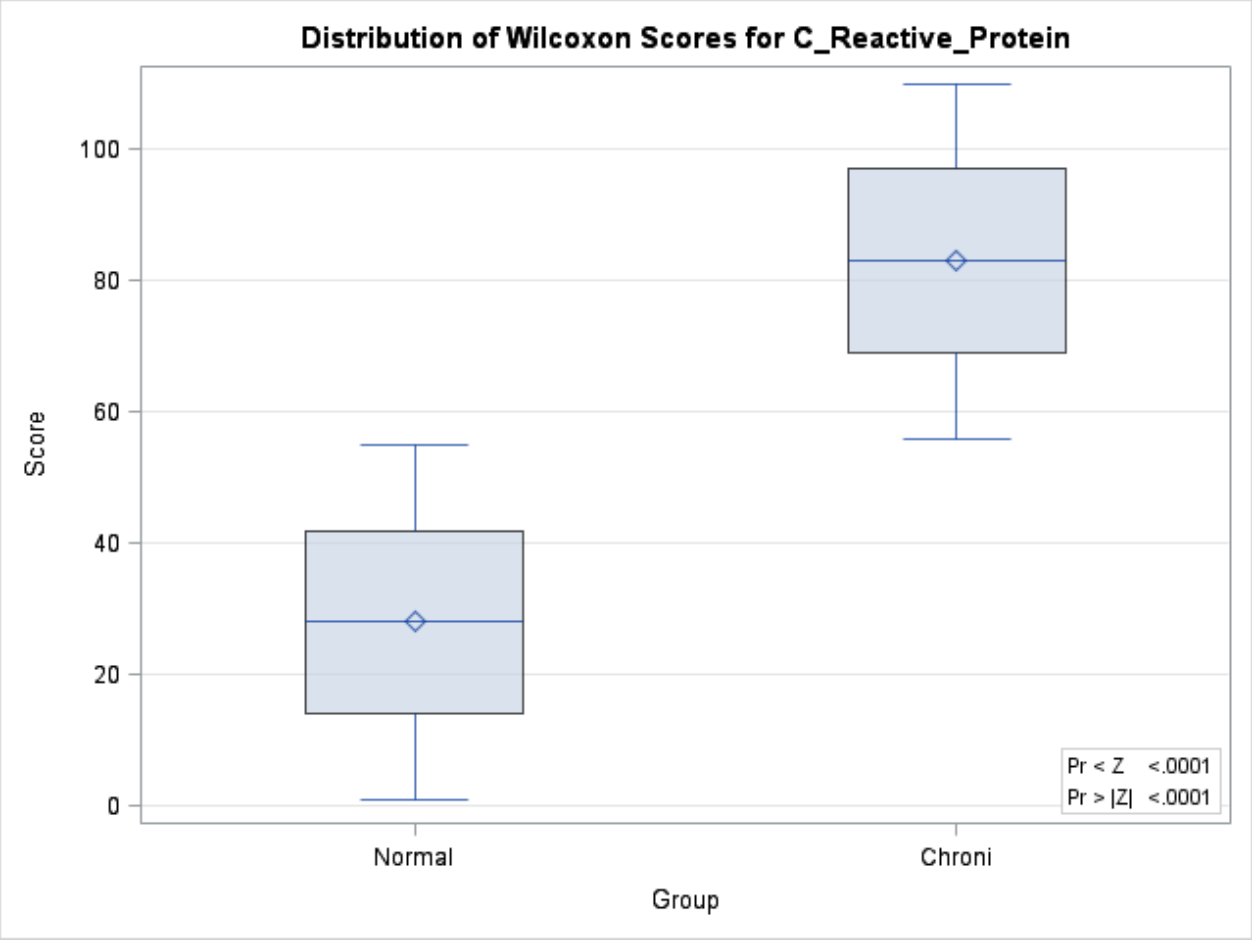
t Approximation

One-Sided Pr < Z	<.0001
Two-Sided Pr > |Z|	<.0001

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	81.7737
DF	1
Pr > Chi-Square	<.0001


Distribution of Wilcoxon Scores for C_Reactive_Protein
Score
100
80
60
40
20
0
Normal
Chroni
Group
Pr < Z <.0001
Pr > |Z| <.0001

b) Alkaline Phosphatase

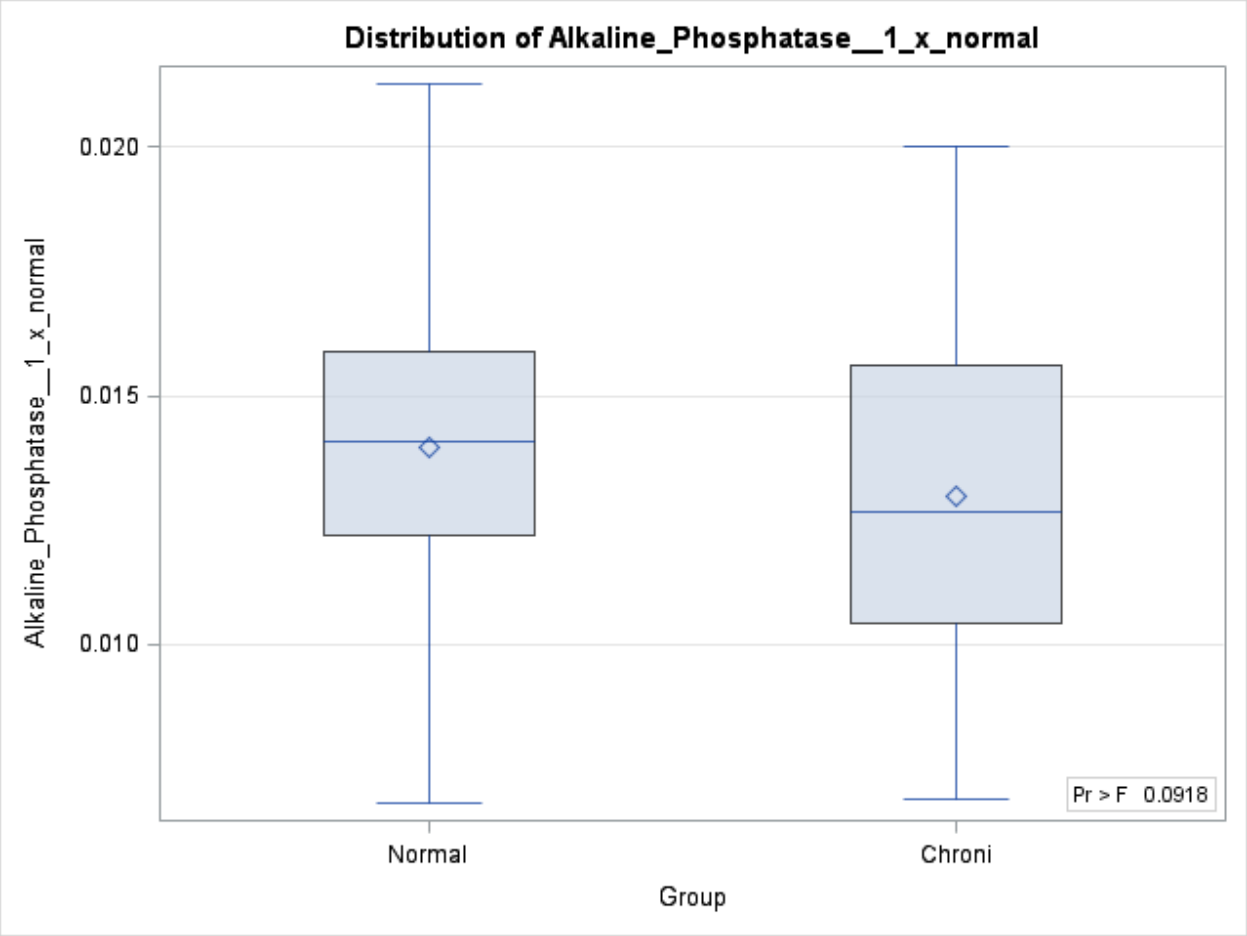
The NPAR1WAY Procedure

Analysis of Variance for Variable Alkaline_Phosphatase__1_x_normal Classified by Variable Group

Group	N	Mean
Normal	51	0.013952
Chronic	55	0.012972

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Among	1	0.000025	0.000025416	2.8956	0.0918
Within	104	0.000913	8.777366E-6		

Average scores were used for ties.



Wilcoxon Scores (Rank Sums) for Variable Alkaline_Phosphatase__1_x_normal Classified by Variable Group

Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Normal	51	3012.0	2728.50	158.072546	59.058824
Chronic	55	2659.0	2942.50	158.072546	48.345455

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	3012.0000

Normal Approximation

Z	1.7903
One-Sided Pr > Z	0.0367
Two-Sided Pr > |Z|	0.0734

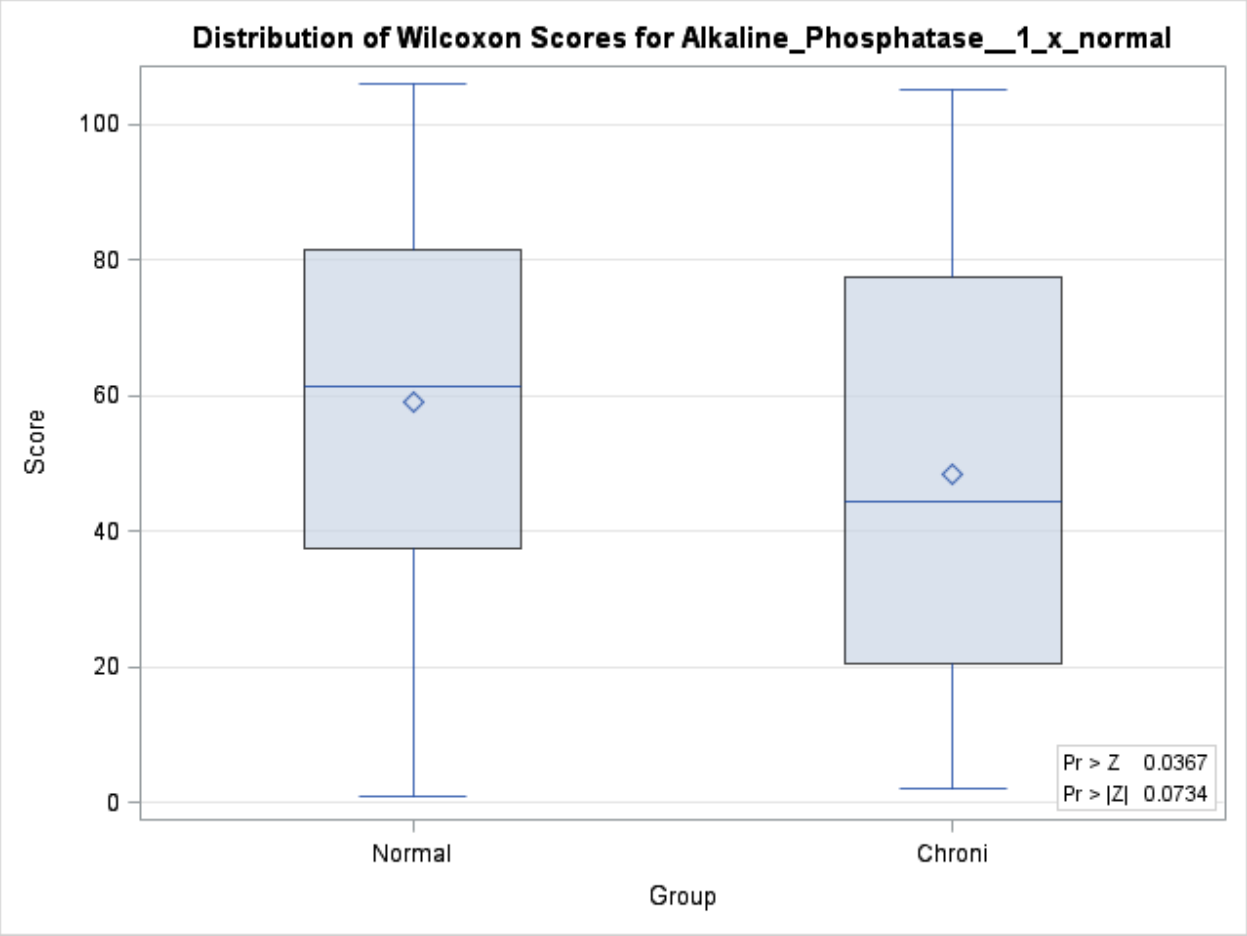
t Approximation

One-Sided Pr > Z	0.0381
Two-Sided Pr > |Z|	0.0763

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	3.2166
DF	1
Pr > Chi-Square	0.0729


Distribution of Wilcoxon Scores for Alkaline_Phosphatase__1_x_normal
Score
100
80
60
40
20
0
Normal
Chroni
Group
Pr > Z 0.0367
Pr > |Z| 0.0734

c) HDL Cholesterol

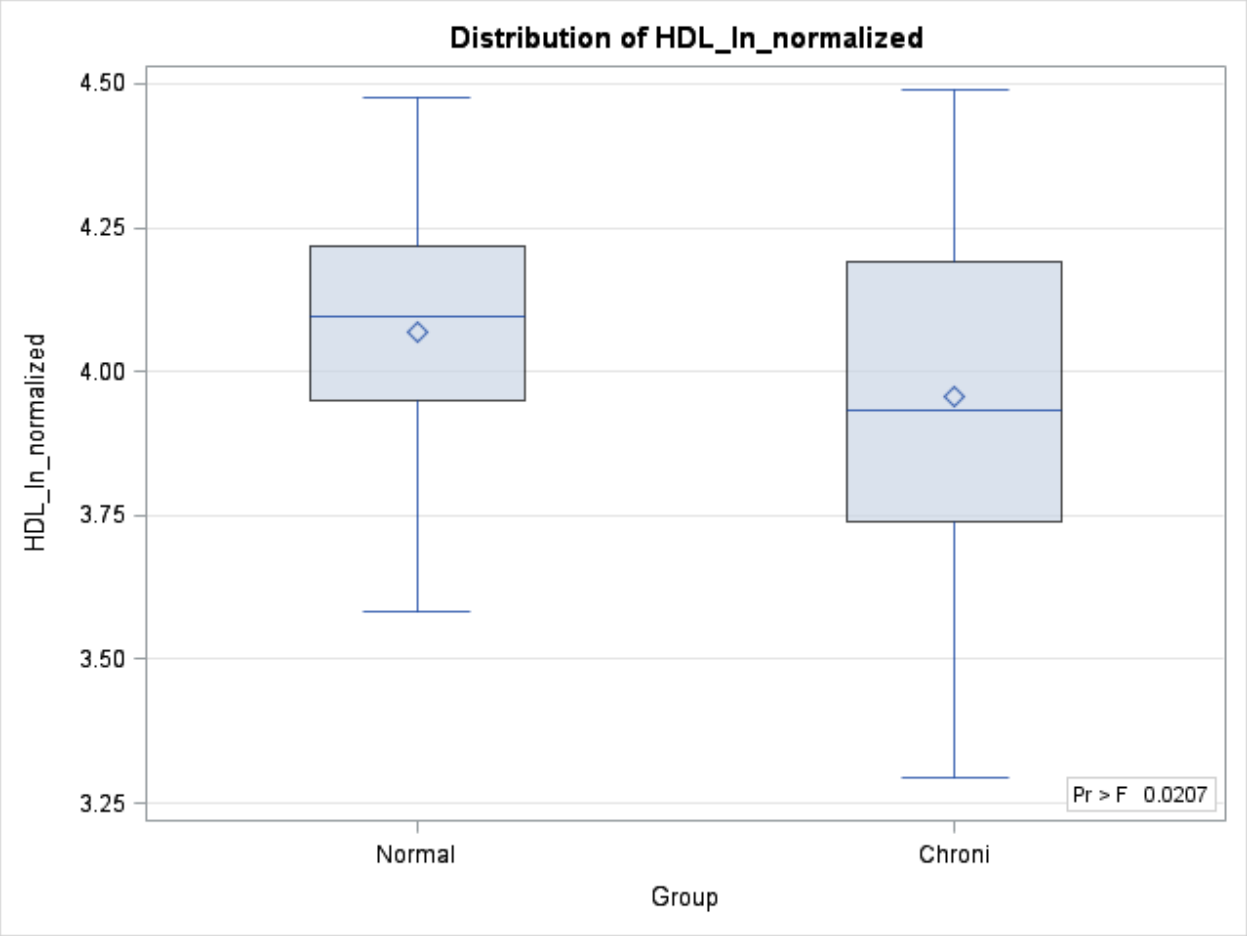
The NPAR1WAY Procedure

Analysis of Variance for Variable HDL_ln_normalized Classified by Variable Group

Group	N	Mean
Normal	53	4.068723
Chronic	54	3.955954

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Among	1	0.340147	0.340147	5.5172	0.0207
Within	105	6.473458	0.061652		

Average scores were used for ties.



Wilcoxon Scores (Rank Sums) for Variable HDL_ln_normalized Classified by Variable Group

Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Normal	53	3198.0	2862.0	160.434413	60.339623
Chronic	54	2580.0	2916.0	160.434413	47.777778

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	3198.0000

Normal Approximation

Z	2.0912
One-Sided Pr > Z	0.0183
Two-Sided Pr > |Z|	0.0365

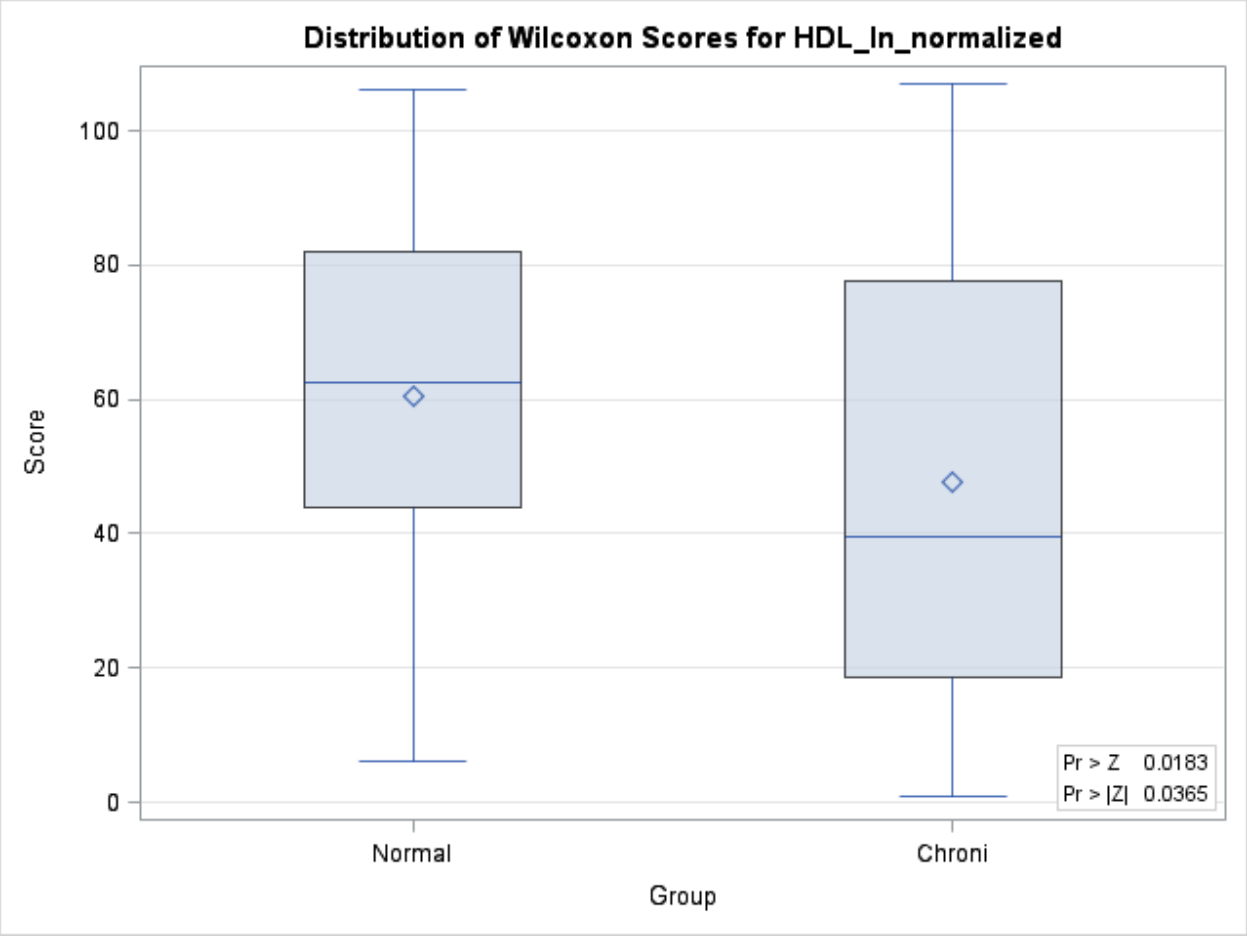
t Approximation

One-Sided Pr > Z	0.0195
Two-Sided Pr > |Z|	0.0389

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	4.3862
DF	1
Pr > Chi-Square	0.0362


Distribution of Wilcoxon Scores for HDL_ln_normalized
Score
100
80
60
40
20
0
Normal
Chroni
Group
Pr > Z 0.0183
Pr > |Z| 0.0365

d) Femur BMD

The TTEST Procedure

Variable: Femur_BMD

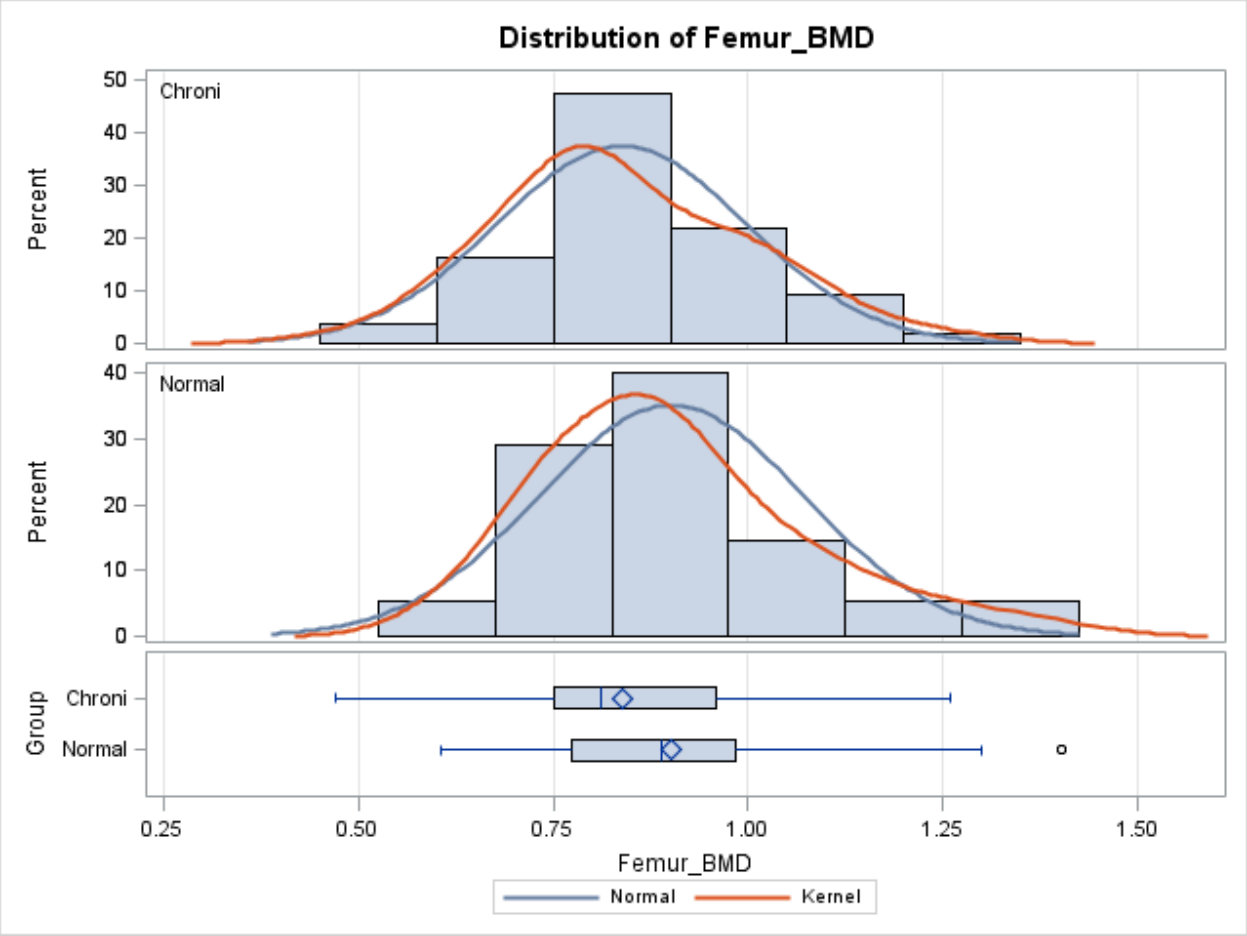
Group	N	Mean	Std Dev	Std Err	Minimum	Maximum
Chronic	55	0.8382	0.1599	0.0216	0.4700	1.2600
Normal	55	0.9011	0.1707	0.0230	0.6060	1.4030
Diff (1-2)		-0.0629	0.1654	0.0315		

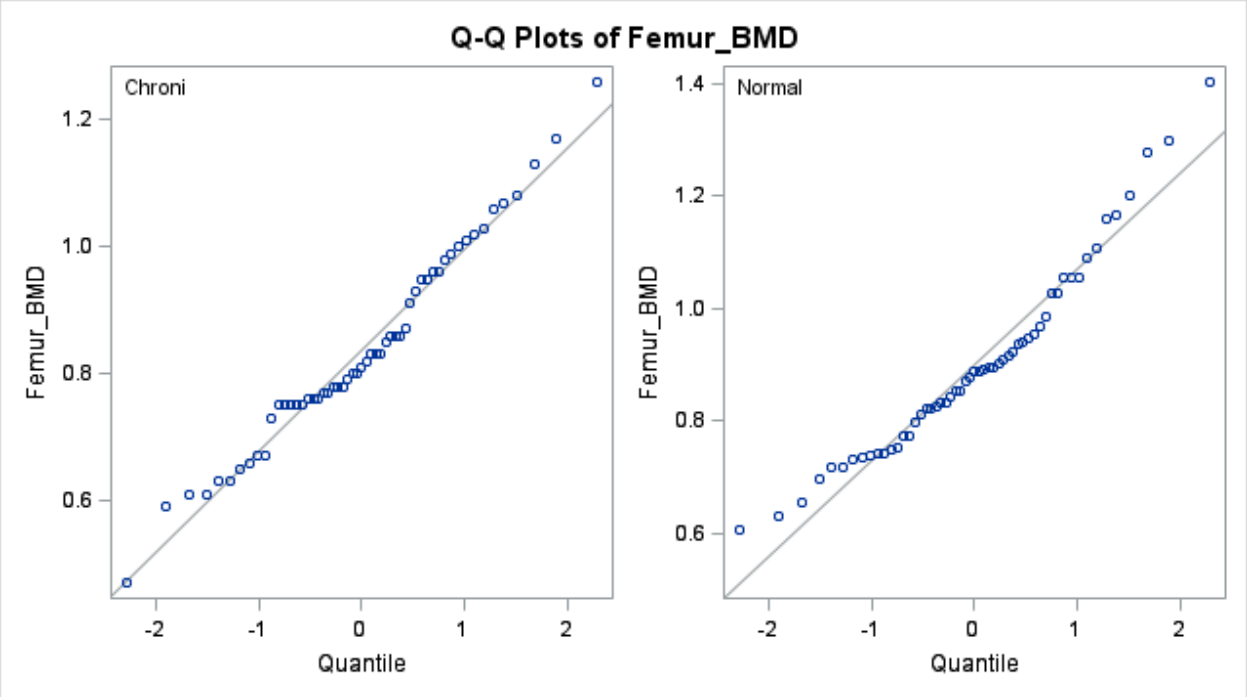
Group	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Chronic		0.8382	0.7950	0.8814	0.1599	0.1346	0.1970
Normal		0.9011	0.8550	0.9473	0.1707	0.1437	0.2103
Diff (1-2)	**Pooled**	-0.0629	-0.1255	-0.00043	0.1654	0.1460	0.1908
Diff (1-2)	**Satterthwaite**	-0.0629	-0.1255	-0.00043			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	108	-2.00	0.0485
Satterthwaite	Unequal	107.54	-2.00	0.0485

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	54	54	1.14	0.6331


Distribution of Femur_BMD
Chroni
Normal
Percent
Group
Chroni
Normal
0.25
0.50
0.75
1.00
1.25
1.50
Femur_BMD
Normal
Kernel


Q-Q Plots of Femur_BMD
Chroni
Normal
Femur_BMD
Quantile

e) Melatonin

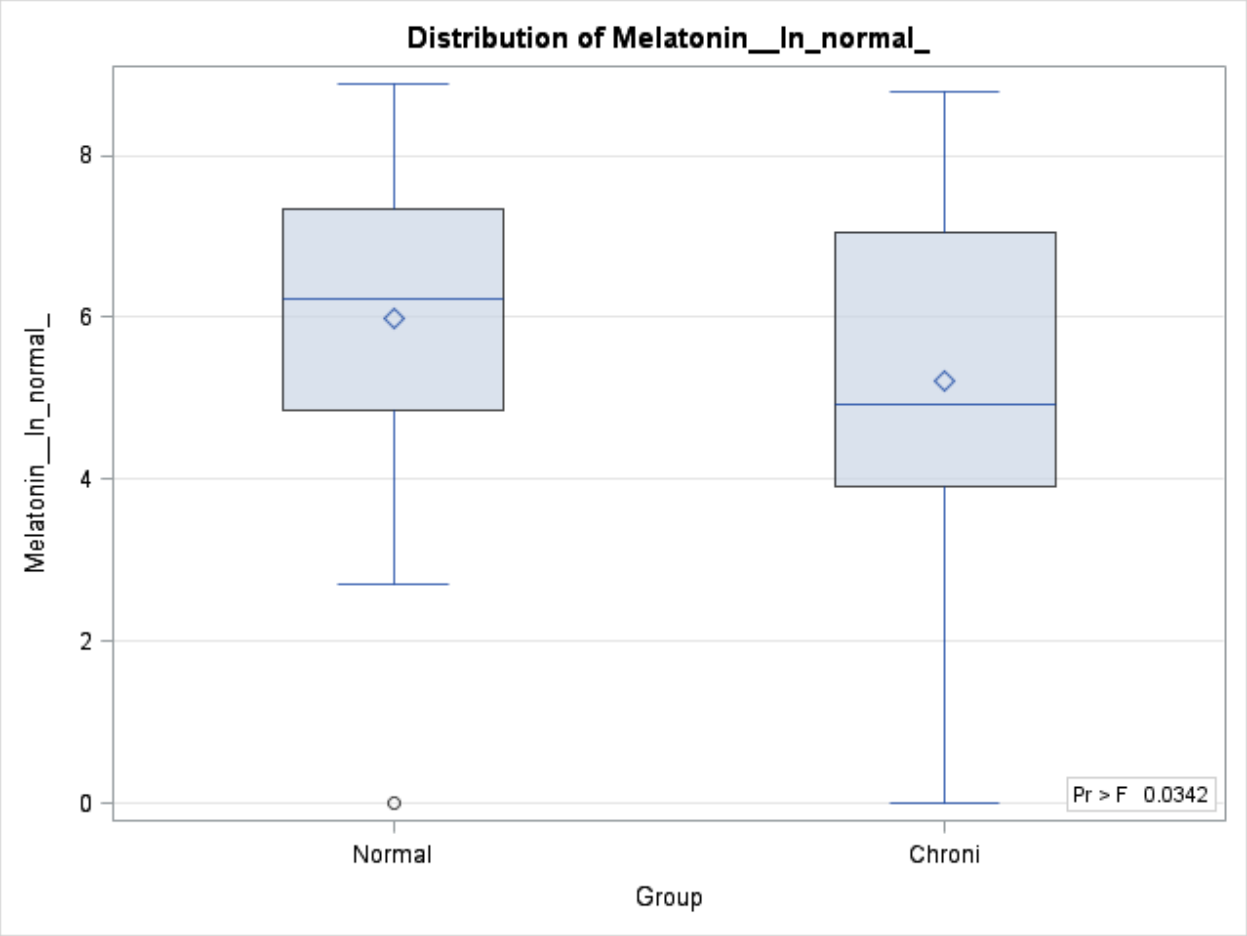
The NPAR1WAY Procedure

Analysis of Variance for Variable Melatonin__ln_normal_ Classified by Variable Group

Group	N	Mean
Normal	54	5.983812
Chronic	55	5.209435

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Among	1	16.339374	16.339374	4.6008	0.0342
Within	107	380.002233	3.551423		

Average scores were used for ties.



Wilcoxon Scores (Rank Sums) for Variable Melatonin__ln_normal_ Classified by Variable Group

Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Normal	54	3312.0	2970.0	164.998471	61.333333
Chronic	55	2683.0	3025.0	164.998471	48.781818

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	3312.0000

Normal Approximation

Z	2.0697
One-Sided Pr > Z	0.0192
Two-Sided Pr > |Z|	0.0385

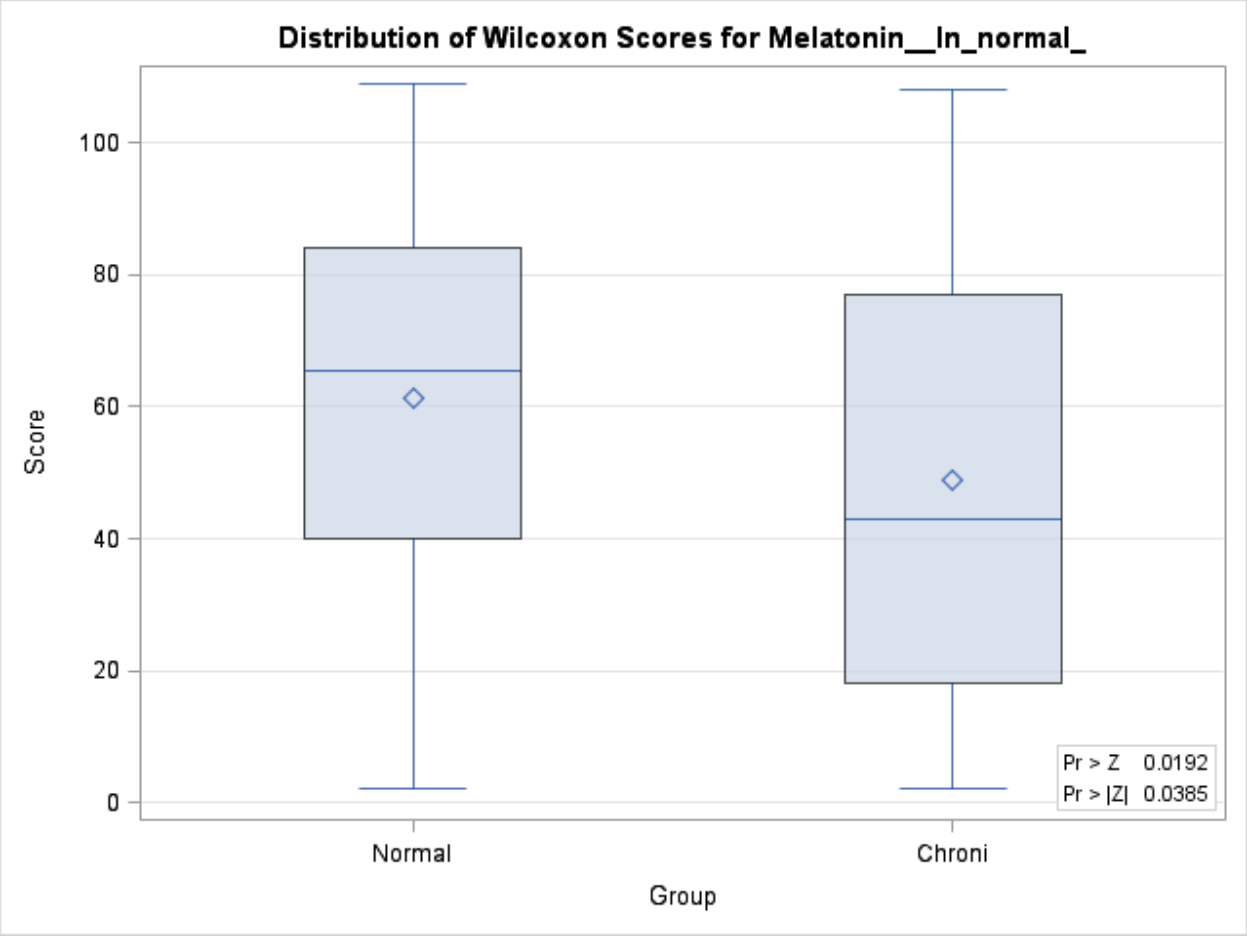
t Approximation

One-Sided Pr > Z	0.0204
Two-Sided Pr > |Z|	0.0409

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	4.2963
DF	1
Pr > Chi-Square	0.0382


Distribution of Wilcoxon Scores for Melatonin__In_normal_
Score
100
80
60
40
20
0
Normal
Chroni
Group
Pr > Z 0.0192
Pr > |Z| 0.0385

f) Calories

The TTEST Procedure

Variable: Energy

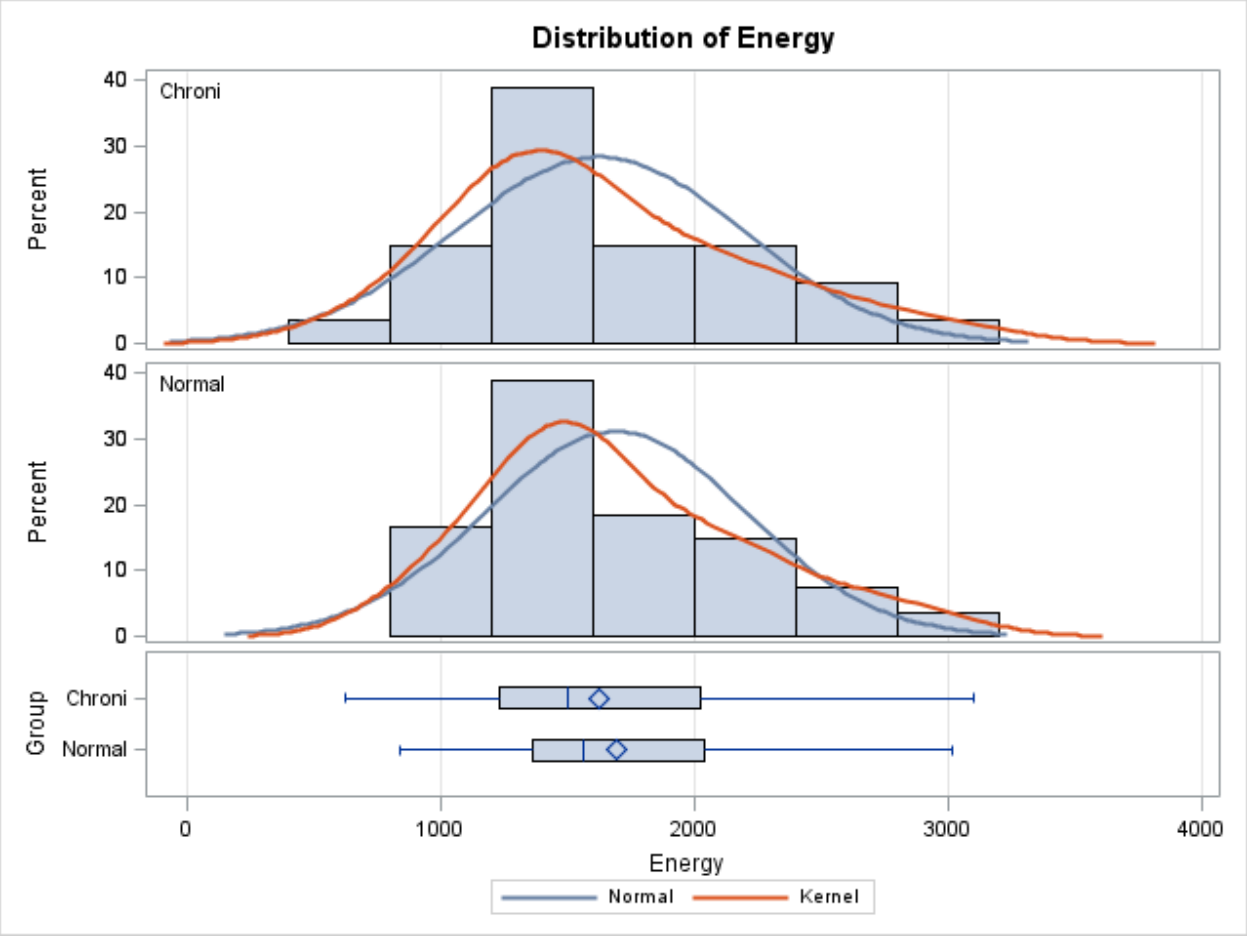
Group	N	Mean	Std Dev	Std Err	Minimum	Maximum
Chronic	54	1625.6	563.5	76.6867	623.5	3098.5
Normal	54	1690.2	513.6	69.8895	837.5	3015.5
Diff (1-2)		-64.6204	539.1	103.8		

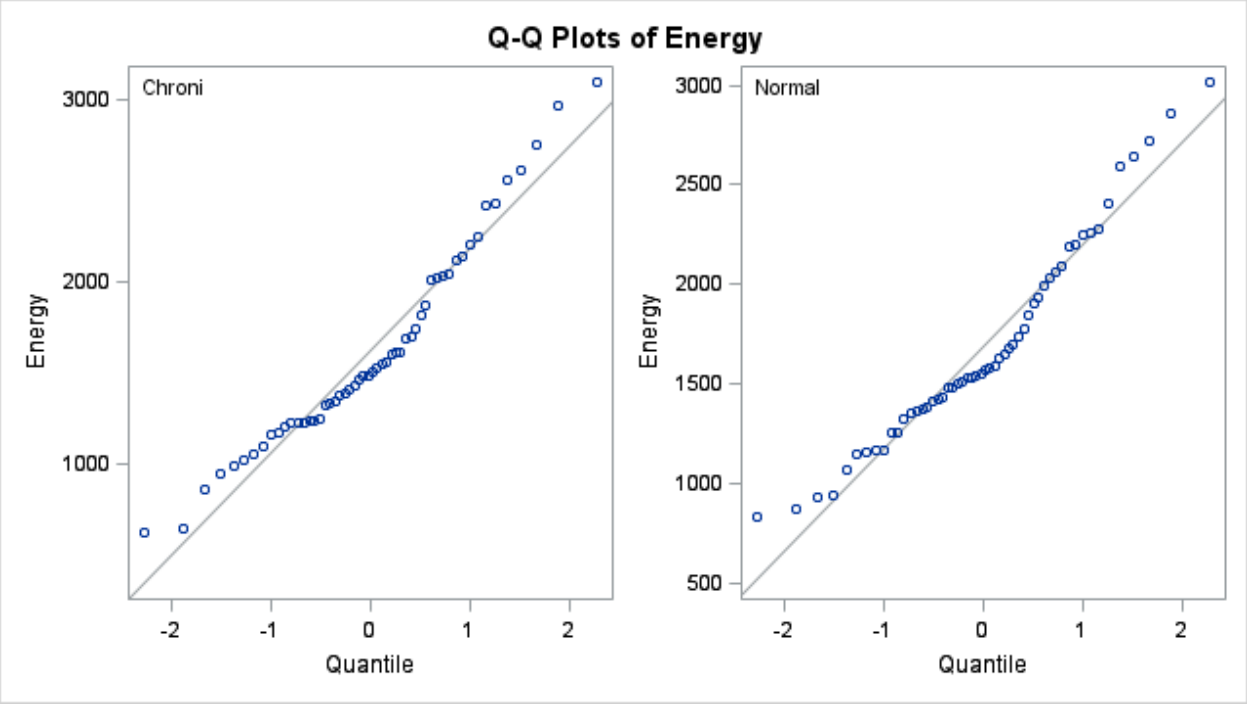
Group	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Chronic		1625.6	1471.8	1779.4	563.5	473.7	695.7
Normal		1690.2	1550.1	1830.4	513.6	431.7	634.0
Diff (1-2)	**Pooled**	-64.6204	-270.3	141.1	539.1	475.3	622.9
Diff (1-2)	**Satterthwaite**	-64.6204	-270.3	141.1			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	106	-0.62	0.5347
Satterthwaite	Unequal	105.1	-0.62	0.5348

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	53	53	1.20	0.5016


Distribution of Energy
Chroni
Normal
Percent
Group
Chroni
Normal
0
1000
2000
3000
4000
Energy
Normal
Kernel


Q-Q Plots of Energy
Chroni
Normal
Energy
Quantile
3000
2500
2000
1500
1000
500
-2
-1
0
1
2

g) Protein

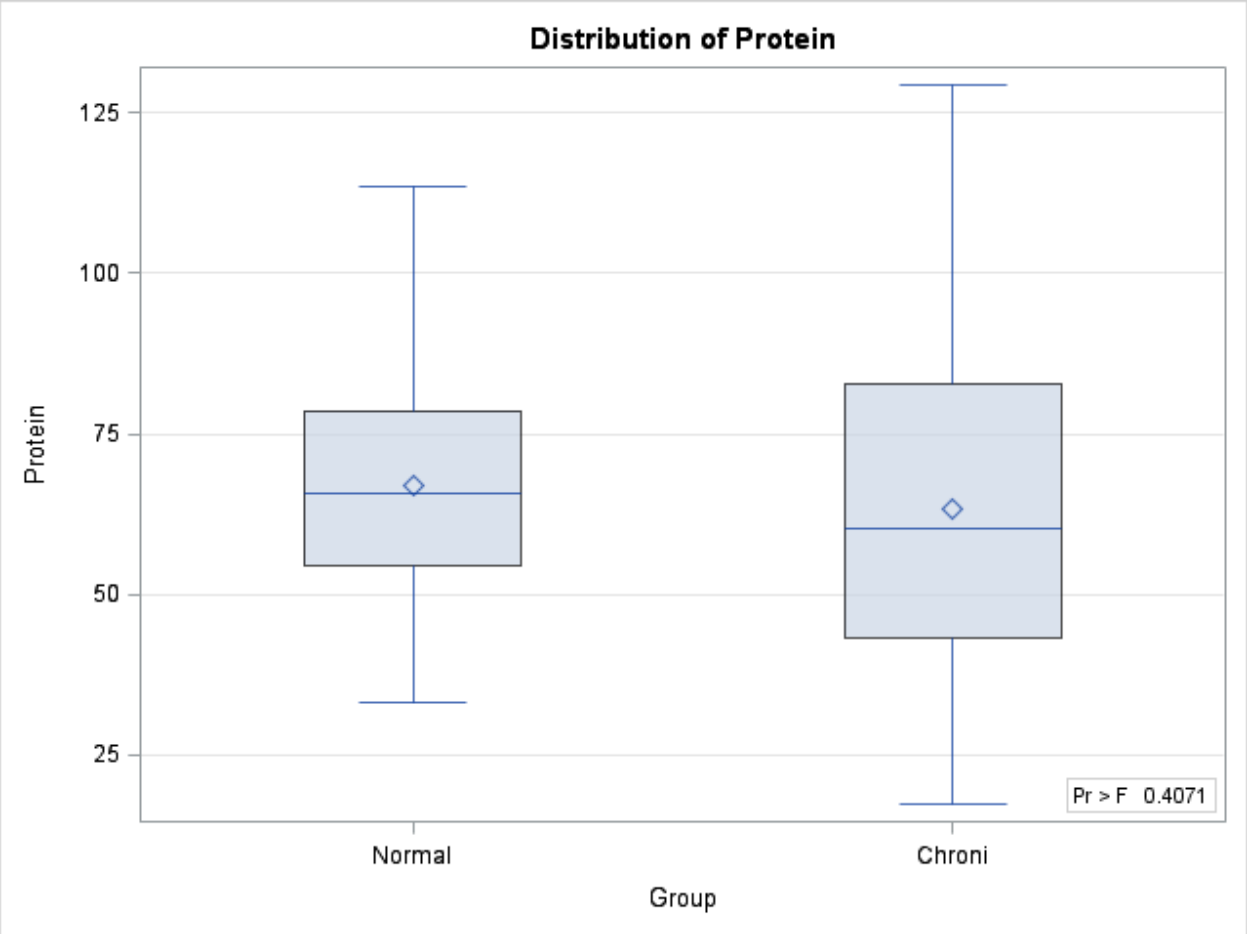
The NPAR1WAY Procedure

Analysis of Variance for Variable Protein Classified by Variable Group

Group	N	Mean
Normal	54	66.828056
Chronic	55	63.342000

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Among	1	331.130023	331.130023	0.6927	0.4071
Within	107	51148.020751	478.018886		

Average scores were used for ties.



Wilcoxon Scores (Rank Sums) for Variable Protein Classified by Variable Group

Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Normal	54	3136.50	2970.0	164.999618	58.083333
Chronic	55	2858.50	3025.0	164.999618	51.972727

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	3136.5000

Normal Approximation

Z	1.0061
One-Sided Pr > Z	0.1572
Two-Sided Pr > |Z|	0.3144

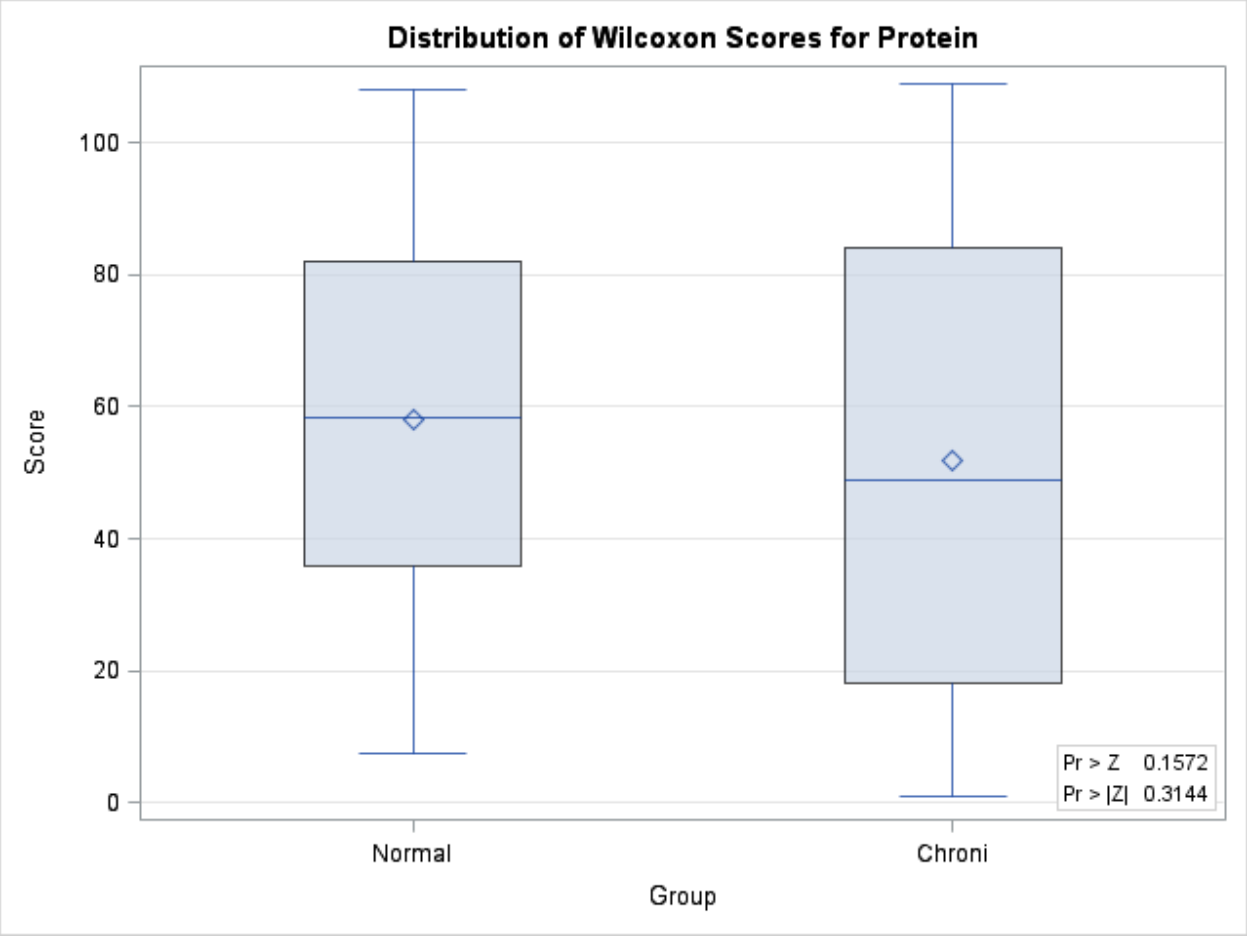
t Approximation

One-Sided Pr > Z	0.1583
Two-Sided Pr > |Z|	0.3166

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	1.0183
DF	1
Pr > Chi-Square	0.3129


Distribution of Wilcoxon Scores for Protein
Score
100
80
60
40
20
0
Normal
Chroni
Group
Pr > Z 0.1572
Pr > |Z| 0.3144

h) Folate

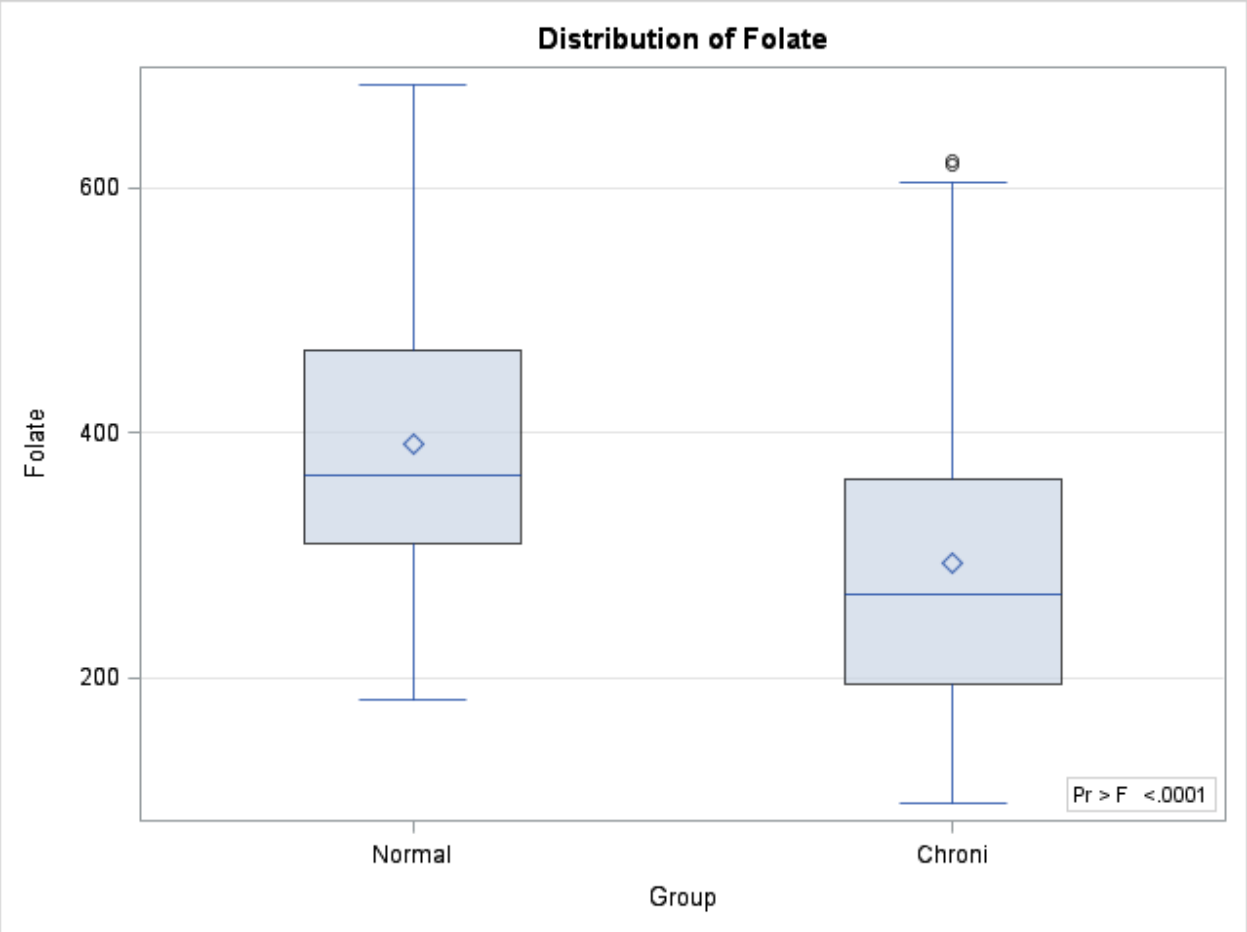
The NPAR1WAY Procedure

Analysis of Variance for Variable Folate Classified by Variable Group

Group	N	Mean
Normal	55	390.300000
Chronic	54	293.379630

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Among	1	255952.915894	255952.9159	16.7283	<.0001
Within	107	1637164.267593	15300.6006		

Average scores were used for ties.



Wilcoxon Scores (Rank Sums) for Variable Folate Classified by Variable Group

Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Normal	55	3690.50	3025.0	164.998853	67.100000
Chronic	54	2304.50	2970.0	164.998853	42.675926

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	2304.5000

Normal Approximation

Z	-4.0303
One-Sided Pr < Z	<.0001
Two-Sided Pr > |Z|	<.0001

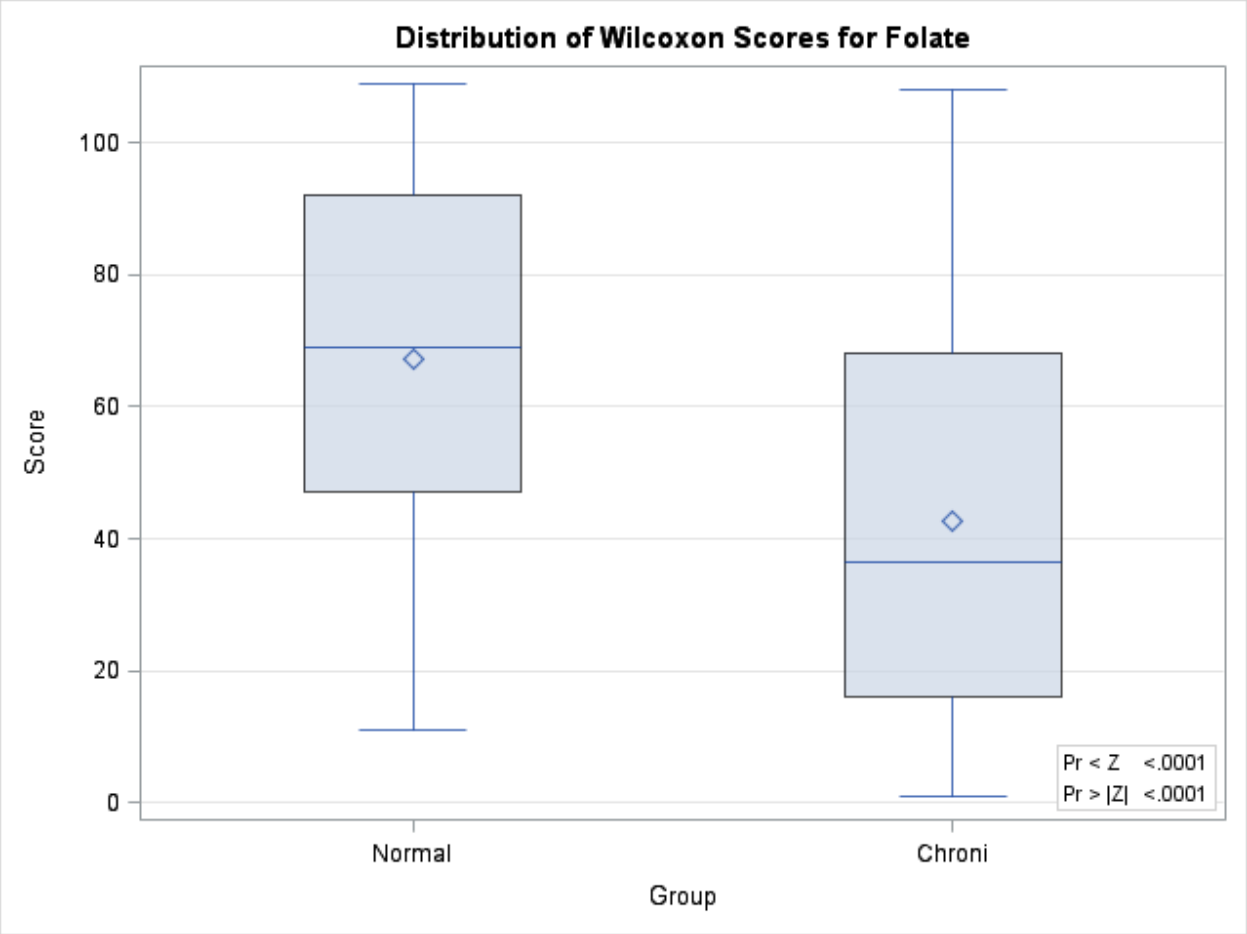
t Approximation

One-Sided Pr < Z	<.0001
Two-Sided Pr > |Z|	0.0001

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	16.2680
DF	1
Pr > Chi-Square	<.0001


Distribution of Wilcoxon Scores for Folate
Score
100
80
60
40
20
0
Normal
Chroni
Group
Pr < Z <.0001
Pr > |Z| <.0001

i) Vitamin B12

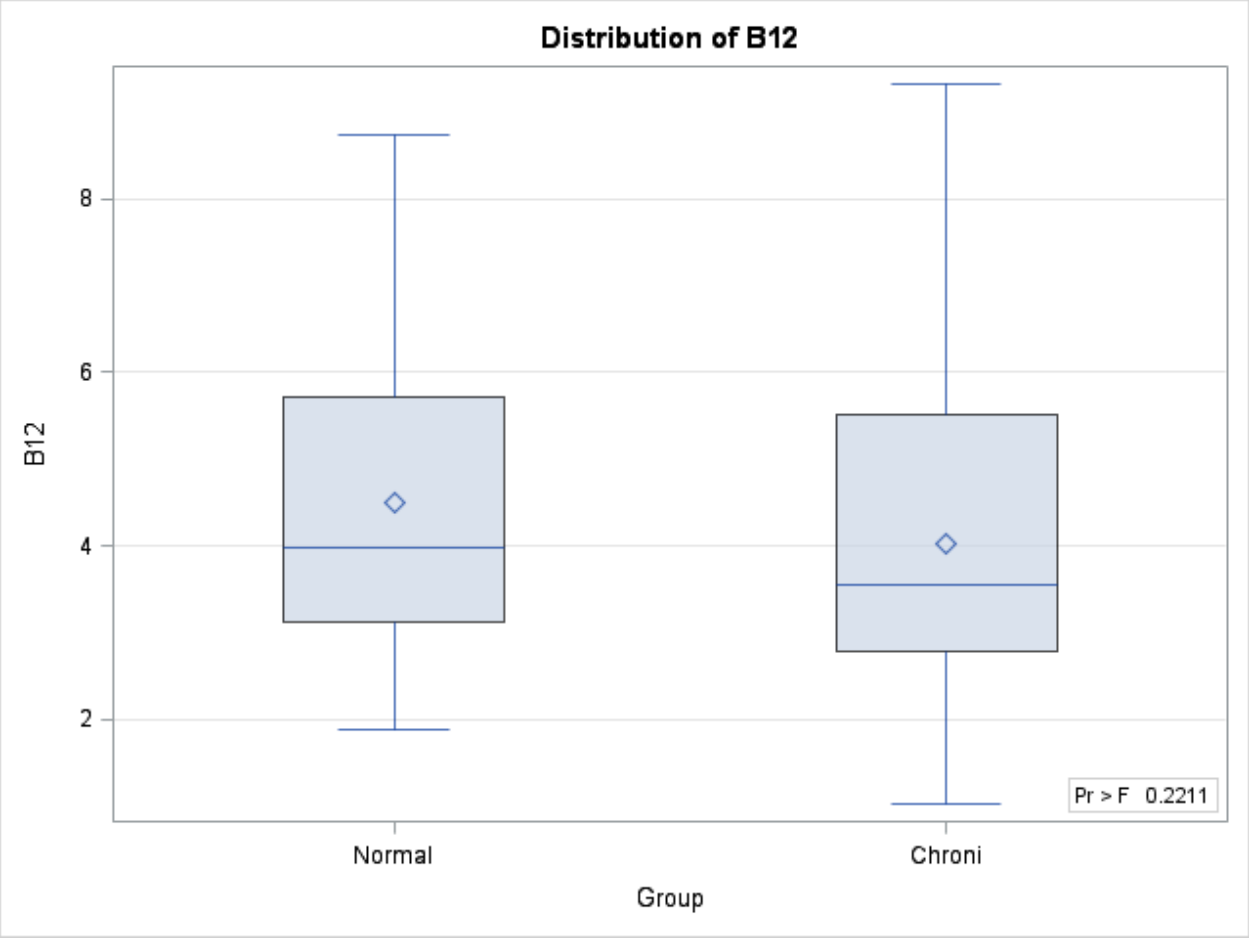
The NPAR1WAY Procedure

Analysis of Variance for Variable B12 Classified by Variable Group

Group	N	Mean
Normal	52	4.501827
Chronic	51	4.033137

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Among	1	5.655969	5.655969	1.5159	0.2211
Within	101	376.838049	3.731070		

Average scores were used for ties.



Wilcoxon Scores (Rank Sums) for Variable B12
Classified by Variable Group

Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Normal	52	2912.50	2704.0	151.602668	56.009615
Chronic	51	2443.50	2652.0	151.602668	47.911765

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	2443.5000

Normal Approximation

Z	-1.3720
One-Sided Pr < Z	0.0850
Two-Sided Pr > |Z|	0.1701

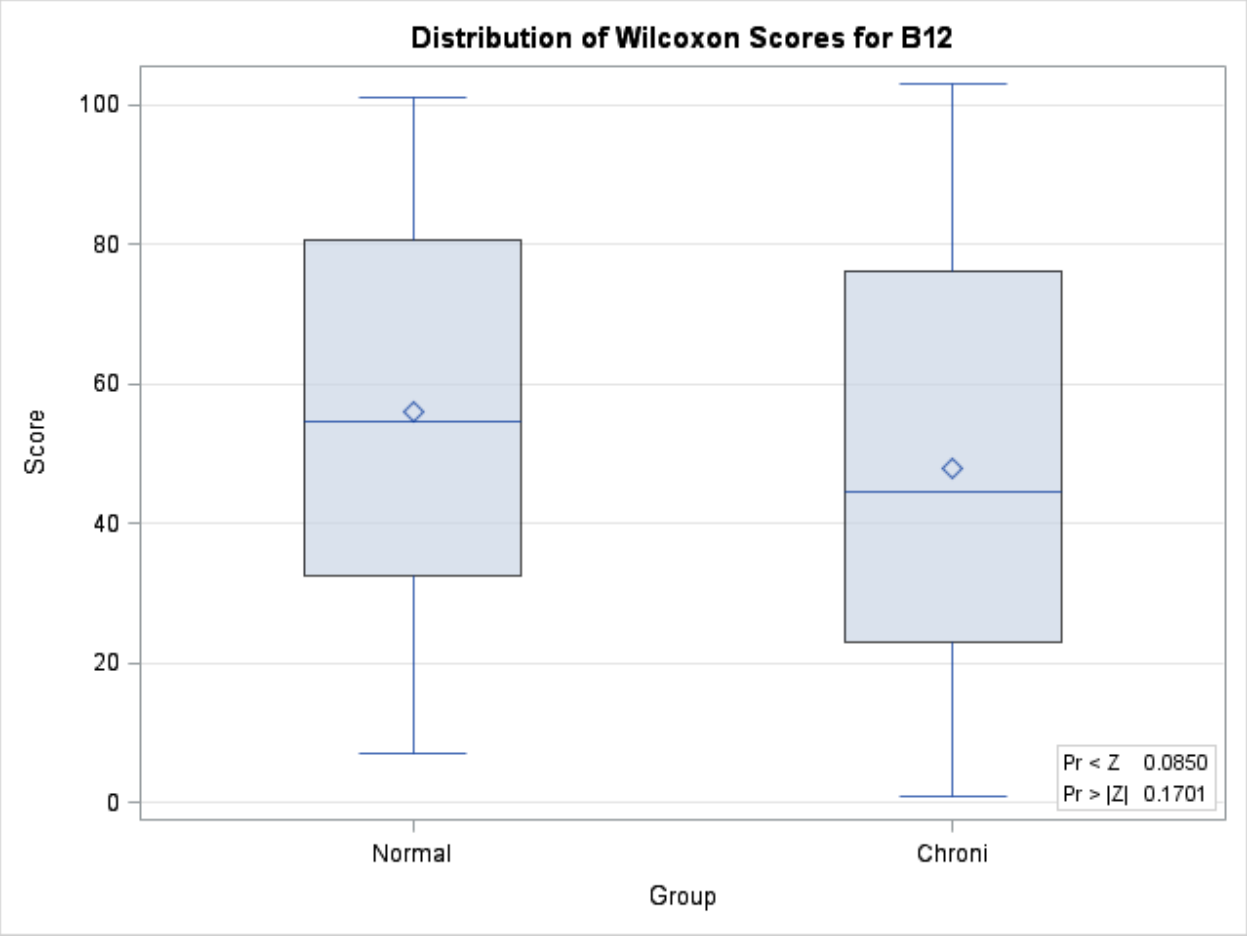
t Approximation

One-Sided Pr < Z	0.0865
Two-Sided Pr > |Z|	0.1731

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	1.8915
DF	1
Pr > Chi-Square	0.1690


Distribution of Wilcoxon Scores for B12
Score
100
80
60
40
20
0
Normal
Chroni
Group
Pr < Z 0.0850
Pr > |Z| 0.1701

j) Vitamin B6

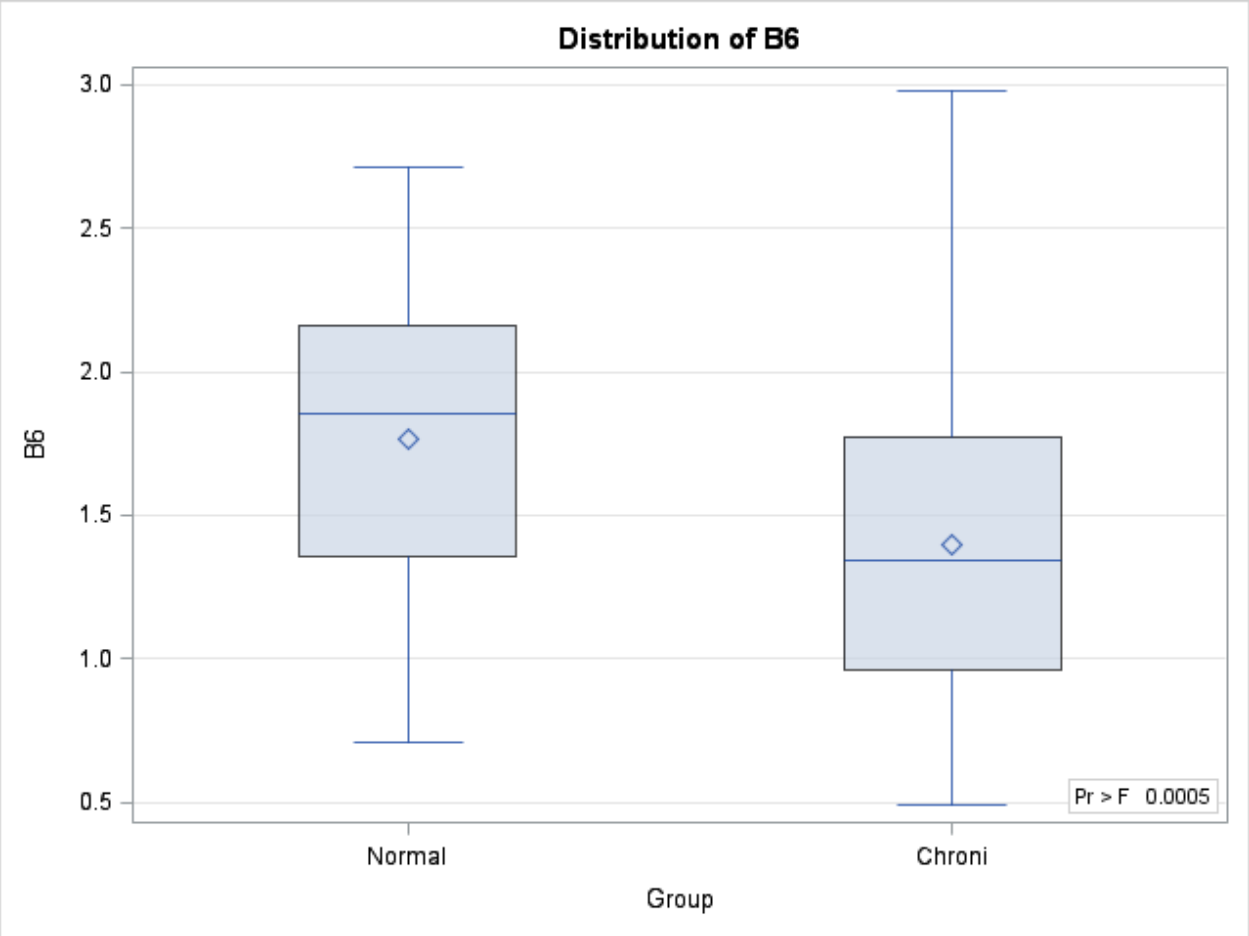
The NPAR1WAY Procedure

Analysis of Variance for Variable B6 Classified by Variable Group

Group	N	Mean
Normal	54	1.767620
Chronic	51	1.397059

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Among	1	3.601599	3.601599	12.8673	0.0005
Within	103	28.830029	0.279903		

Average scores were used for ties.



Wilcoxon Scores (Rank Sums) for Variable B6
Classified by Variable Group

Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Normal	54	3425.0	2862.0	155.967917	63.425926
Chronic	51	2140.0	2703.0	155.967917	41.960784

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	2140.0000

Normal Approximation

Z	-3.6065
One-Sided Pr < Z	0.0002
Two-Sided Pr > |Z|	0.0003

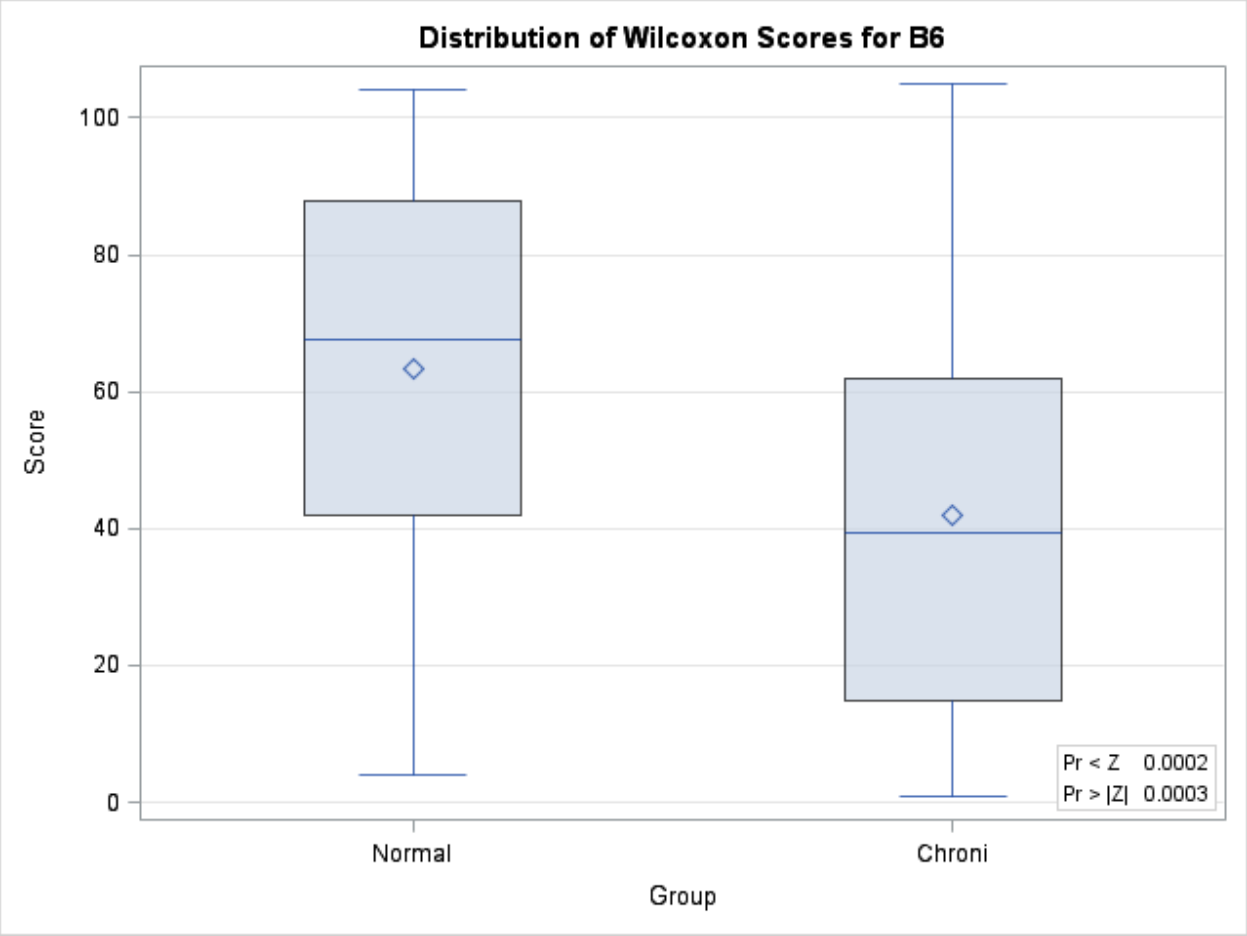
t Approximation

One-Sided Pr < Z	0.0002
Two-Sided Pr > |Z|	0.0005

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	13.0301
DF	1
Pr > Chi-Square	0.0003


Distribution of Wilcoxon Scores for B6
Score
100
80
60
40
20
0
Normal
Chroni
Group
Pr < Z 0.0002
Pr > |Z| 0.0003

k) Vitamin C

The TTEST Procedure

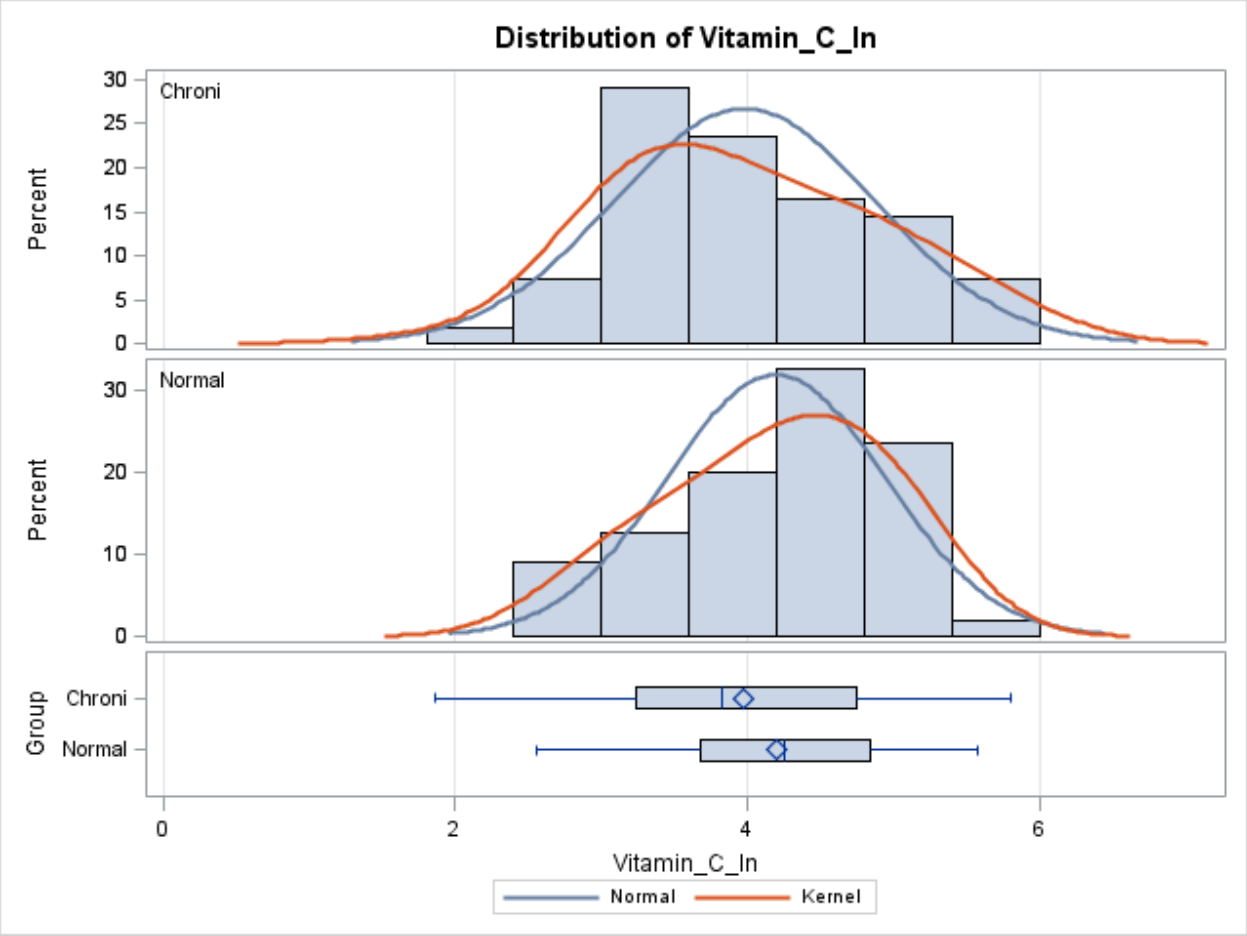
Variable: Vitamin_C_ln

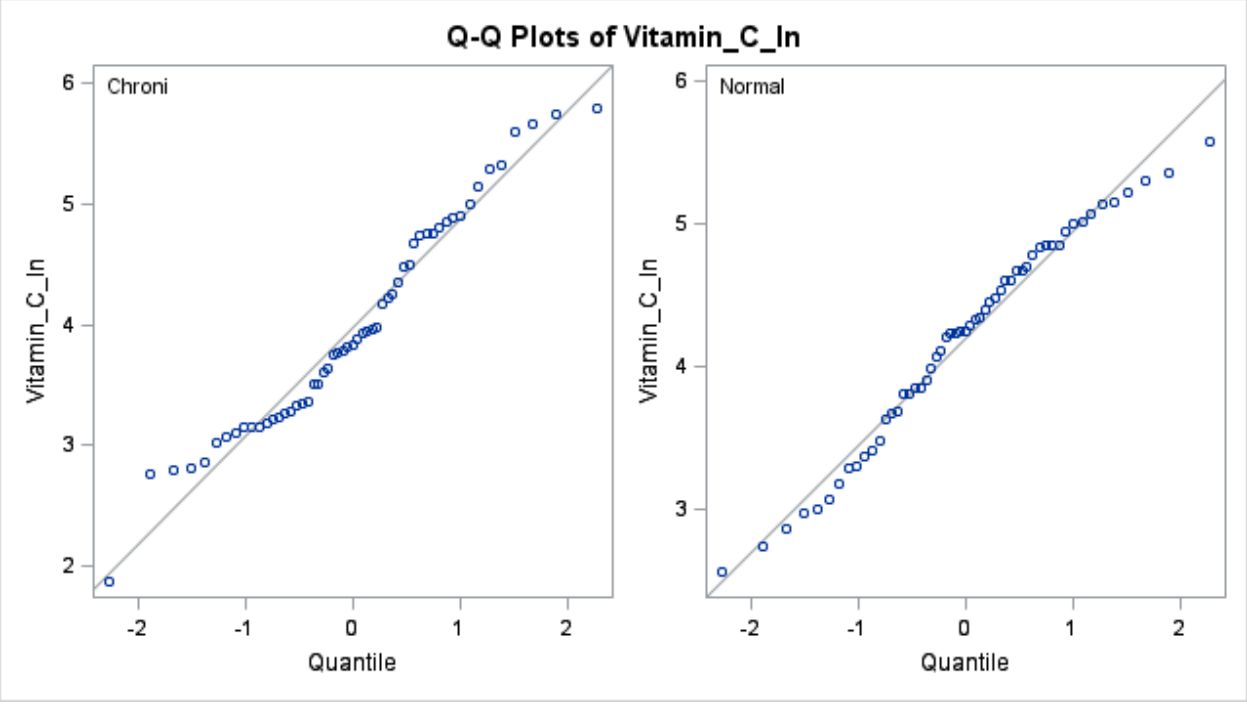
Group	N	Mean	Std Dev	Std Err	Minimum	Maximum
Chronic	55	3.9778	0.8972	0.1210	1.8641	5.8003
Normal	55	4.1942	0.7480	0.1009	2.5533	5.5744
Diff (1-2)		-0.2164	0.8260	0.1575		

Group	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Chronic		3.9778	3.7352	4.2203	0.8972	0.7553	1.1052
Normal		4.1942	3.9919	4.3964	0.7480	0.6297	0.9214
Diff (1-2)	**Pooled**	-0.2164	-0.5286	0.0958	0.8260	0.7290	0.9530
Diff (1-2)	**Satterthwaite**	-0.2164	-0.5287	0.0960			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	108	-1.37	0.1724
Satterthwaite	Unequal	104.61	-1.37	0.1725

Equality of Variances				
Method	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Folded F	54	54	1.44	0.1845


Distribution of Vitamin_C_ln
Chroni
Normal
Percent
Group
Chroni
Normal
Vitamin_C_ln
Normal
Kernel


Q-Q Plots of Vitamin_C_ln
Chroni
Normal
Vitamin_C_ln
Quantile

l) Magnesium

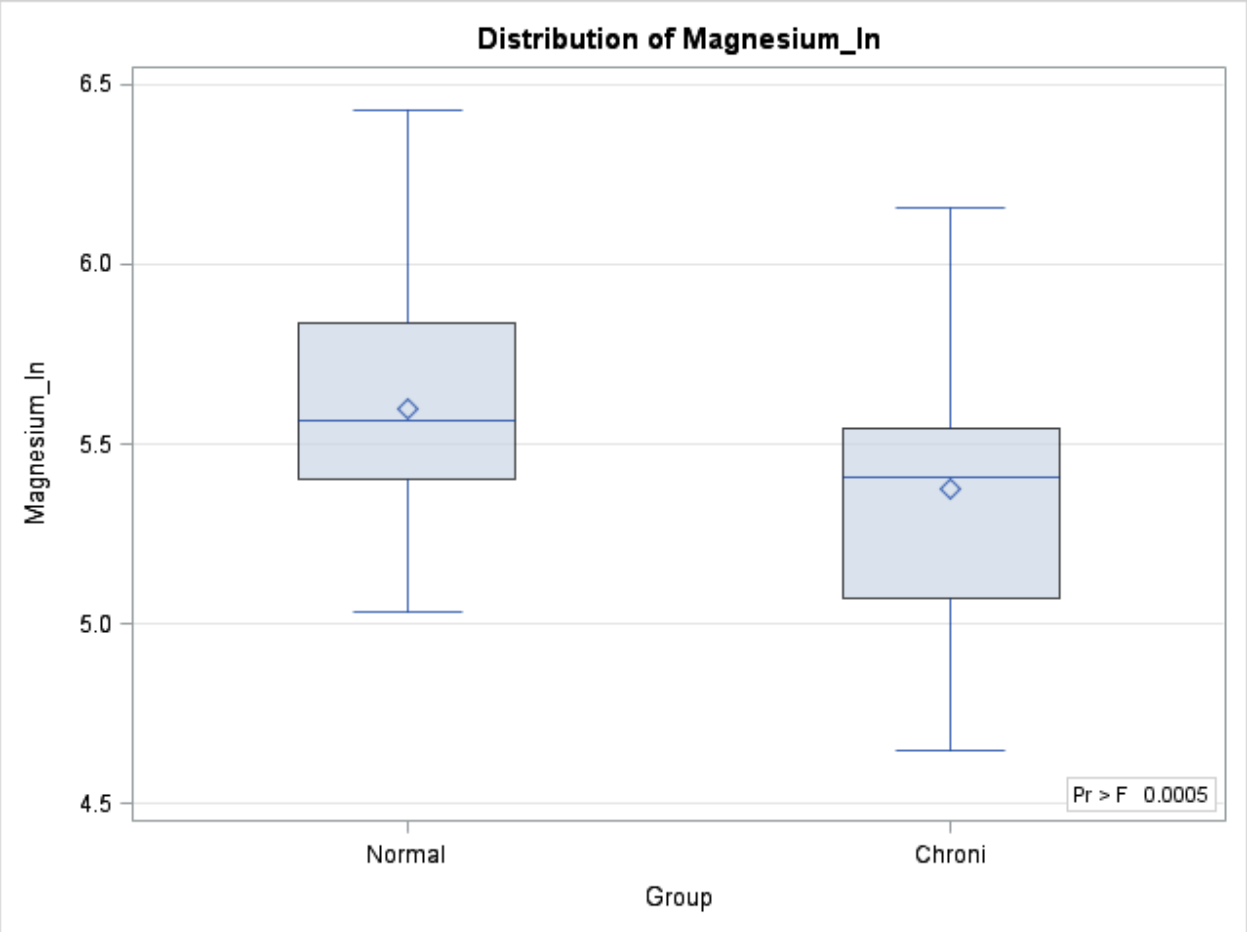
The NPAR1WAY Procedure

Analysis of Variance for Variable Magnesium_ln Classified by Variable Group

Group	N	Mean
Normal	55	5.599662
Chronic	54	5.377465

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Among	1	1.345255	1.345255	12.8381	0.0005
Within	107	11.212155	0.104786		

Average scores were used for ties.



Wilcoxon Scores (Rank Sums) for Variable Magnesium_ln Classified by Variable Group

Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Normal	55	3591.50	3025.0	164.996177	65.300000
Chronic	54	2403.50	2970.0	164.996177	44.509259

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	2403.5000

Normal Approximation

Z	-3.4304
One-Sided Pr < Z	0.0003
Two-Sided Pr > |Z|	0.0006

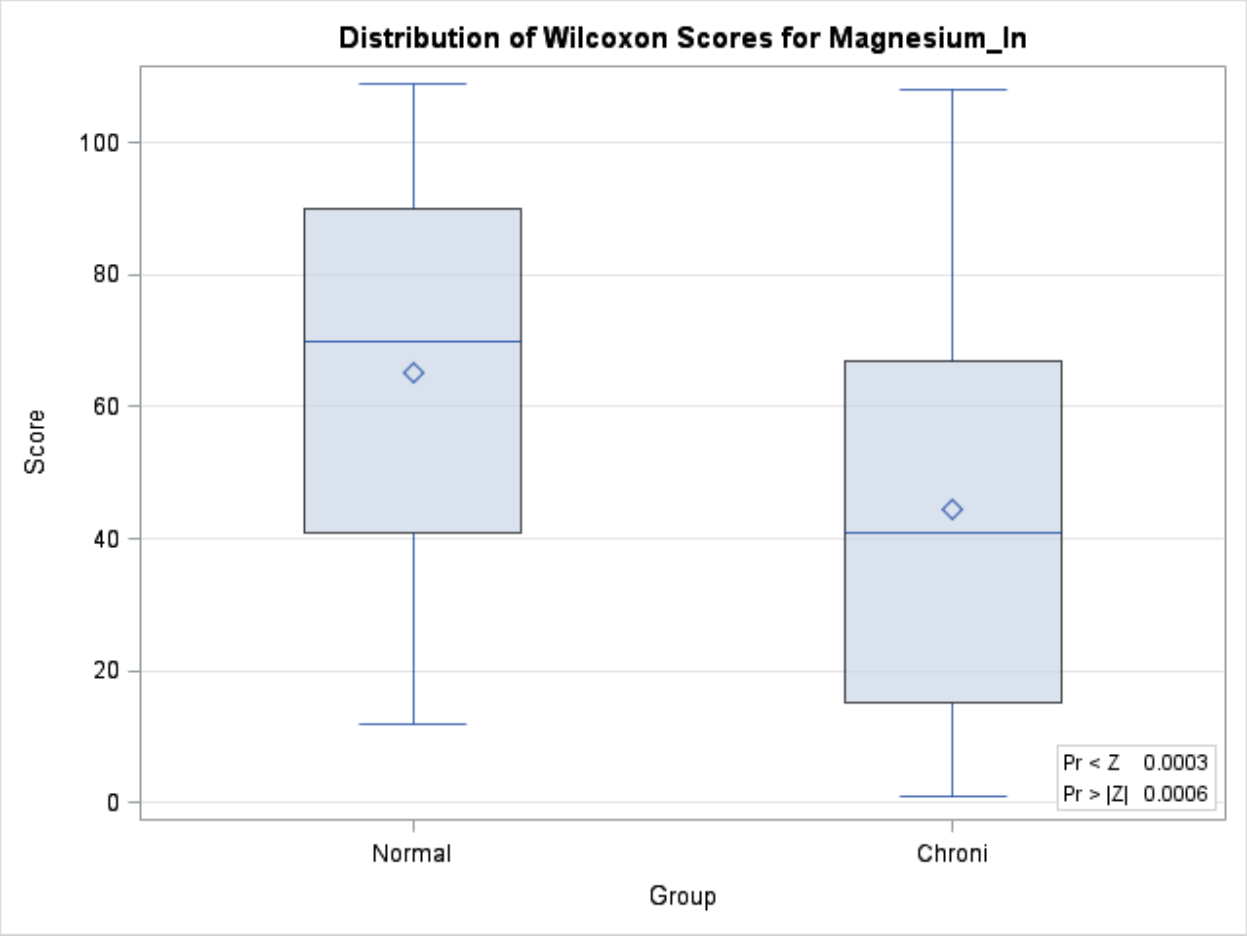
t Approximation

One-Sided Pr < Z	0.0004
Two-Sided Pr > |Z|	0.0009

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	11.7883
DF	1
Pr > Chi-Square	0.0006


Distribution of Wilcoxon Scores for Magnesium_ln
Score
100
80
60
40
20
0
Normal
Chroni
Group
Pr < Z 0.0003
Pr > |Z| 0.0006

m) Zinc

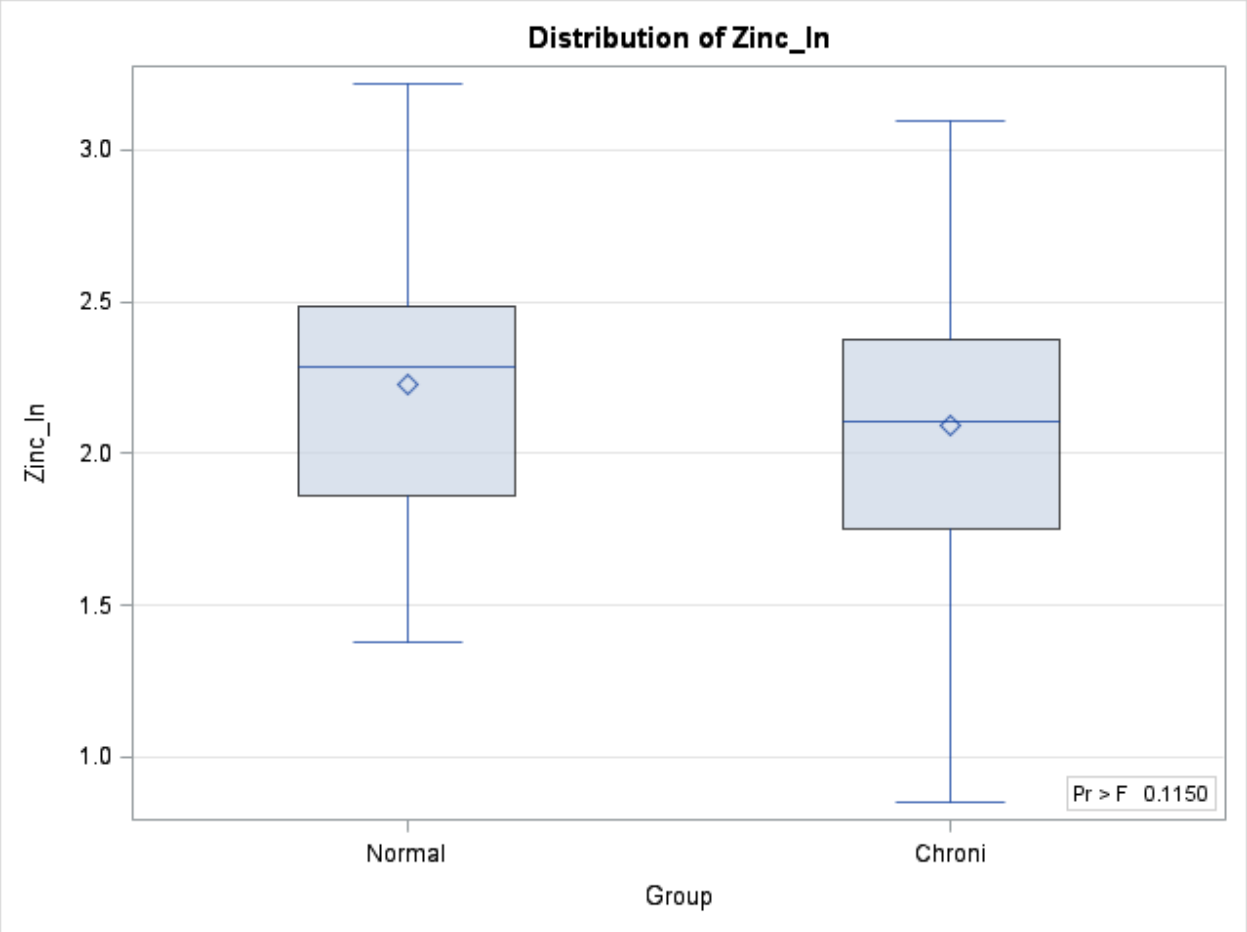
The NPAR1WAY Procedure

Analysis of Variance for Variable Zinc_ln Classified by Variable Group

Group	N	Mean
Normal	55	2.224702
Chronic	54	2.094350

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Among	1	0.462983	0.462983	2.5254	0.1150
Within	107	19.616405	0.183331		

Average scores were used for ties.



Wilcoxon Scores (Rank Sums) for Variable Zinc_ln Classified by Variable Group

Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Normal	55	3274.50	3025.0	164.998089	59.536364
Chronic	54	2720.50	2970.0	164.998089	50.379630

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	2720.5000

Normal Approximation

Z	-1.5091
One-Sided Pr < Z	0.0656
Two-Sided Pr > |Z|	0.1313

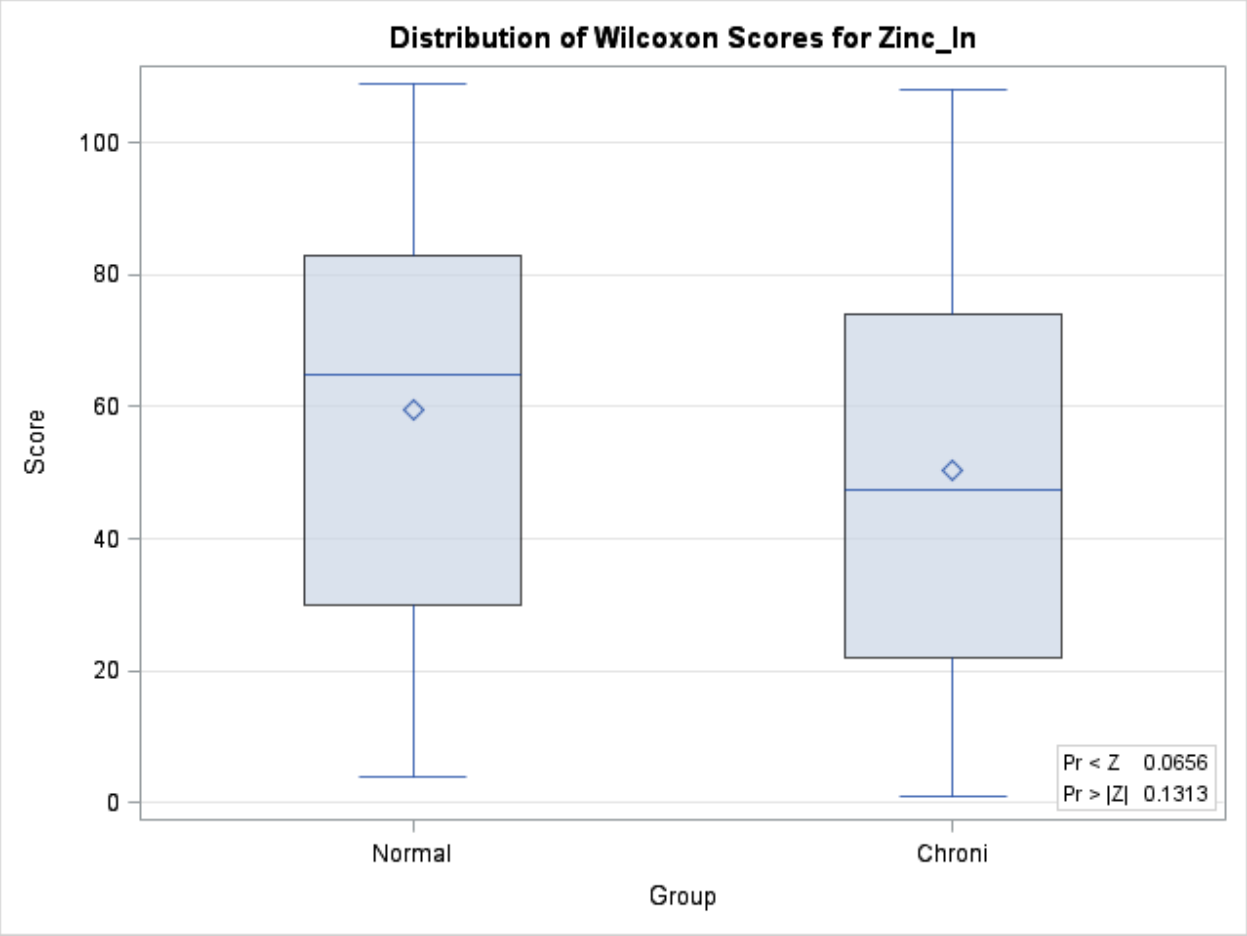
t Approximation

One-Sided Pr < Z	0.0671
Two-Sided Pr > |Z|	0.1342

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	2.2866
DF	1
Pr > Chi-Square	0.1305


Distribution of Wilcoxon Scores for Zinc_ln
Score
100
80
60
40
20
0
Normal
Chroni
Group
Pr < Z 0.0656
Pr > |Z| 0.1313

n) Sedentary Time

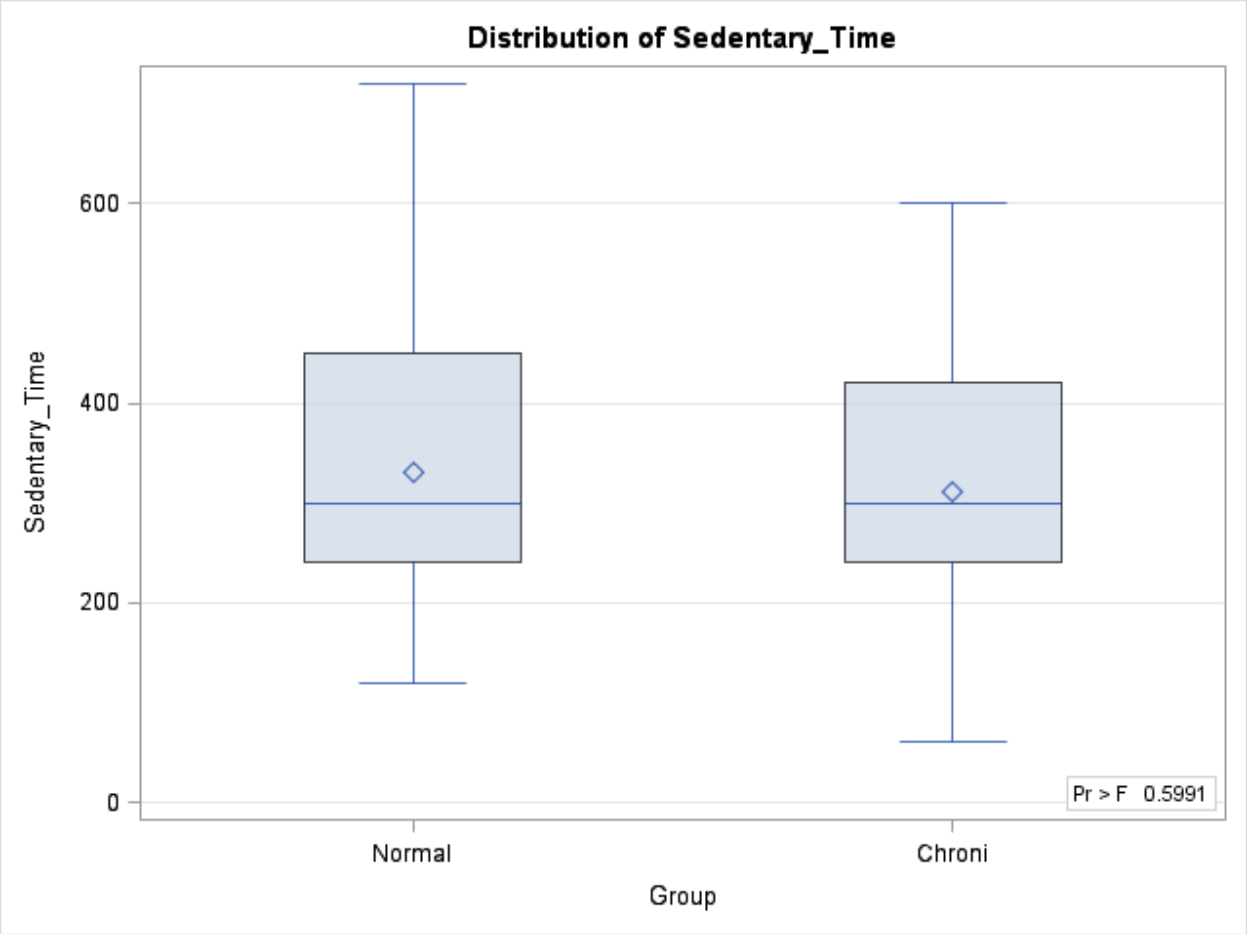
The NPAR1WAY Procedure

Analysis of Variance for Variable Sedentary_Time Classified by Variable Group

Group	N	Mean
Normal	36	330.000000
Chronic	41	311.707317

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Among	1	6414.317390	6414.31739	0.2787	0.5991
Within	75	1725980.487805	23013.07317		

Average scores were used for ties.



Wilcoxon Scores (Rank Sums) for Variable Sedentary_Time Classified by Variable Group

Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Normal	36	1429.0	1404.0	97.040272	39.694444
Chronic	41	1574.0	1599.0	97.040272	38.390244

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	1429.0000

Normal Approximation

Z	0.2525
One-Sided Pr > Z	0.4003
Two-Sided Pr > |Z|	0.8007

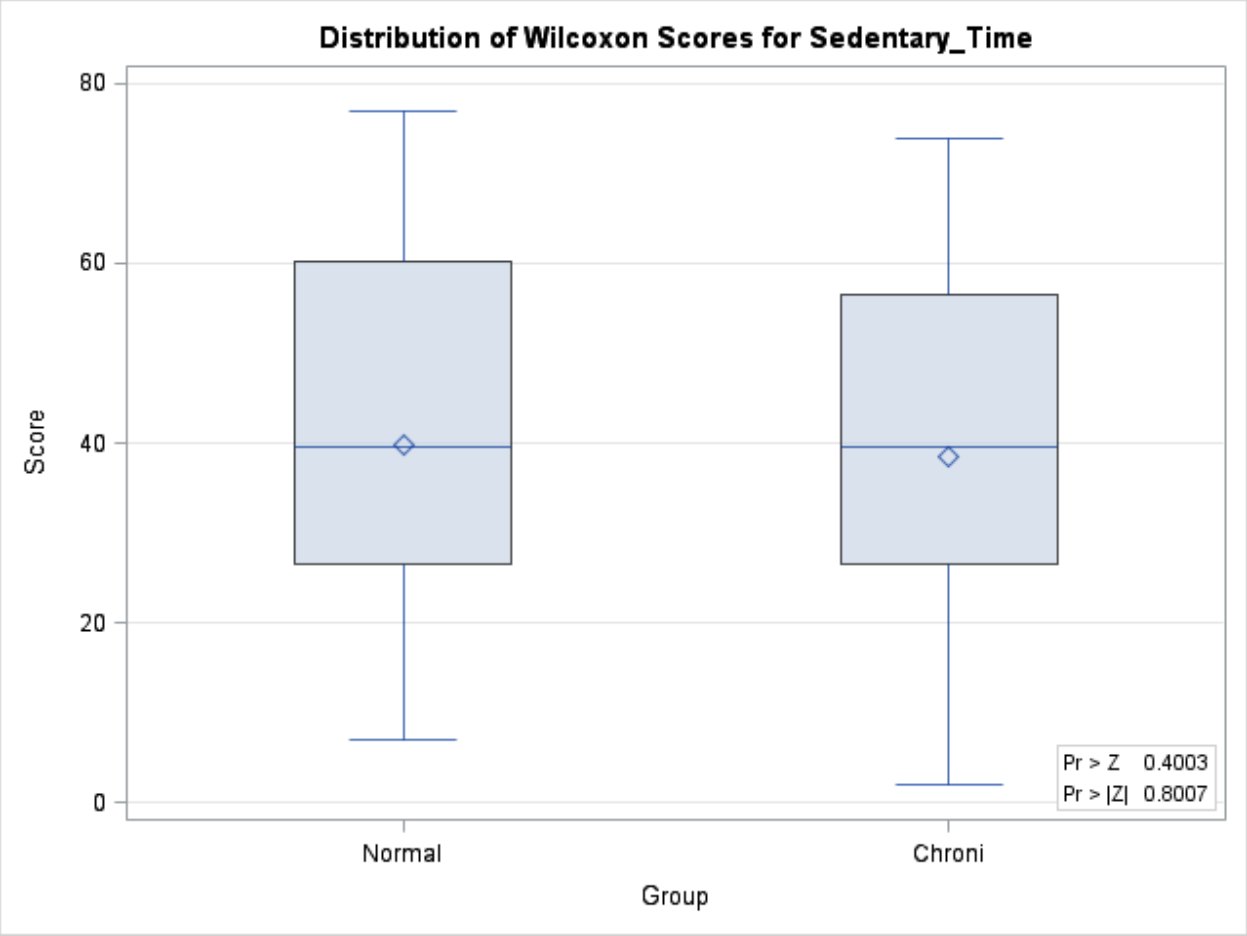
t Approximation

One-Sided Pr > Z	0.4007
Two-Sided Pr > |Z|	0.8014

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	0.0664
DF	1
Pr > Chi-Square	0.7967


Distribution of Wilcoxon Scores for Sedentary_Time
80
60
40
20
0
Score
Normal
Chroni
Group
Pr > Z 0.4003
Pr > |Z| 0.8007

o) Hours of Sleep

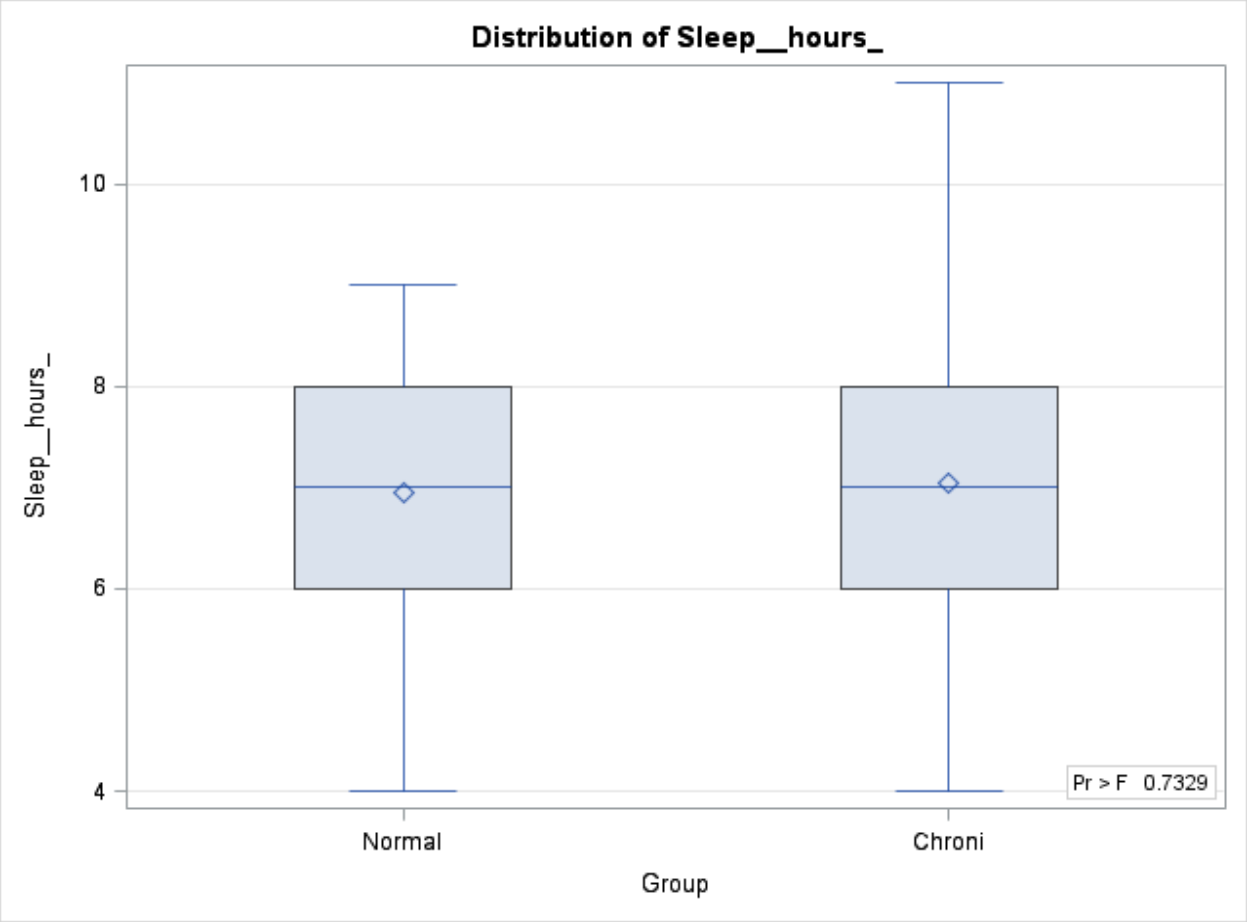
The NPAR1WAY Procedure

Analysis of Variance for Variable Sleep__hours_ Classified by Variable Group

Group	N	Mean
Normal	54	6.944444
Chronic	53	7.037736

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Among	1	0.232793	0.232793	0.1171	0.7329
Within	105	208.757862	1.988170		

Average scores were used for ties.



Wilcoxon Scores (Rank Sums) for Variable Sleep__hours_ Classified by Variable Group

Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Normal	54	2925.0	2916.0	156.474297	54.166667
Chronic	53	2853.0	2862.0	156.474297	53.830189

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	2853.0000

Normal Approximation

Z	-0.0543
One-Sided Pr < Z	0.4783
Two-Sided Pr > |Z|	0.9567

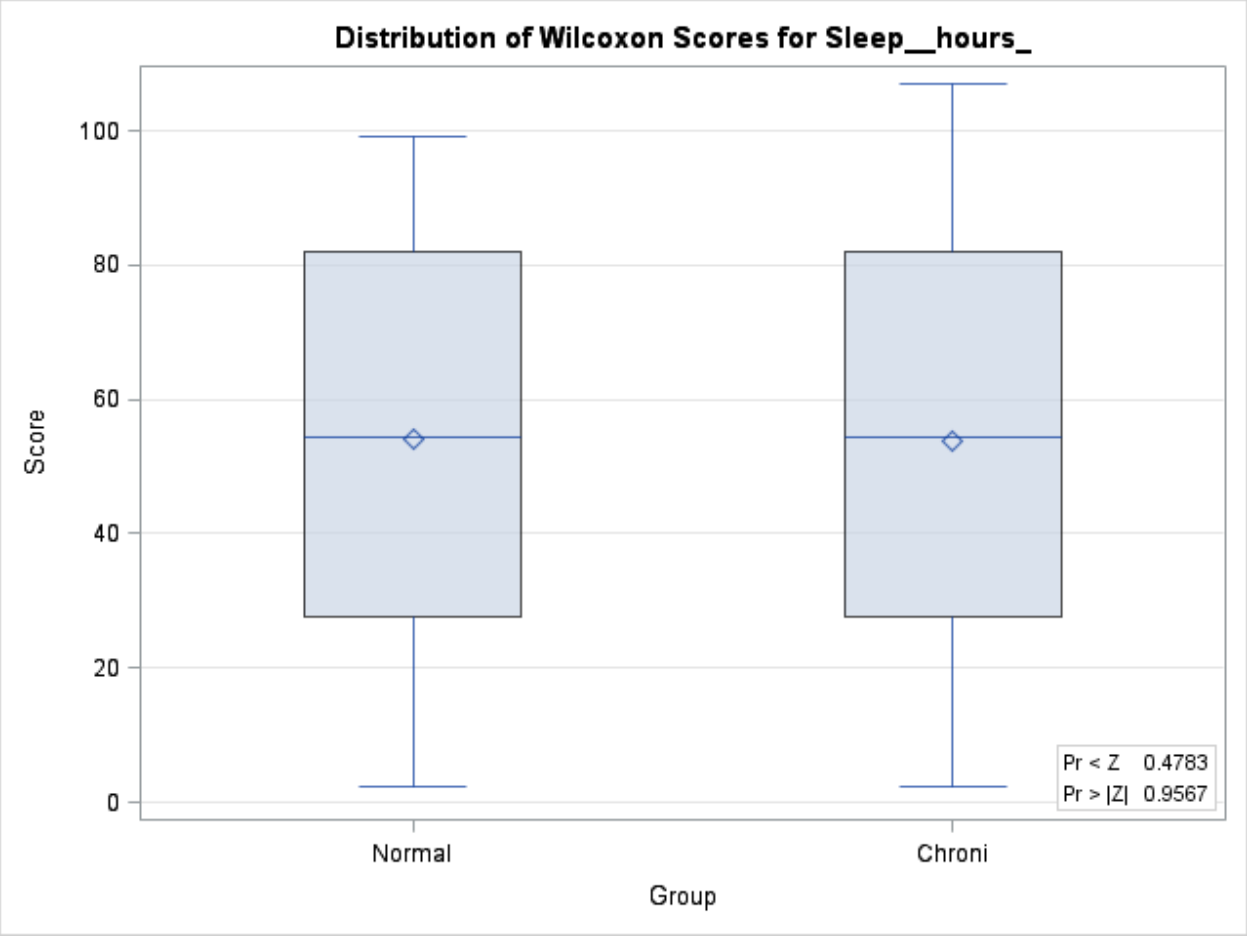
t Approximation

One-Sided Pr < Z	0.4784
Two-Sided Pr > |Z|	0.9568

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

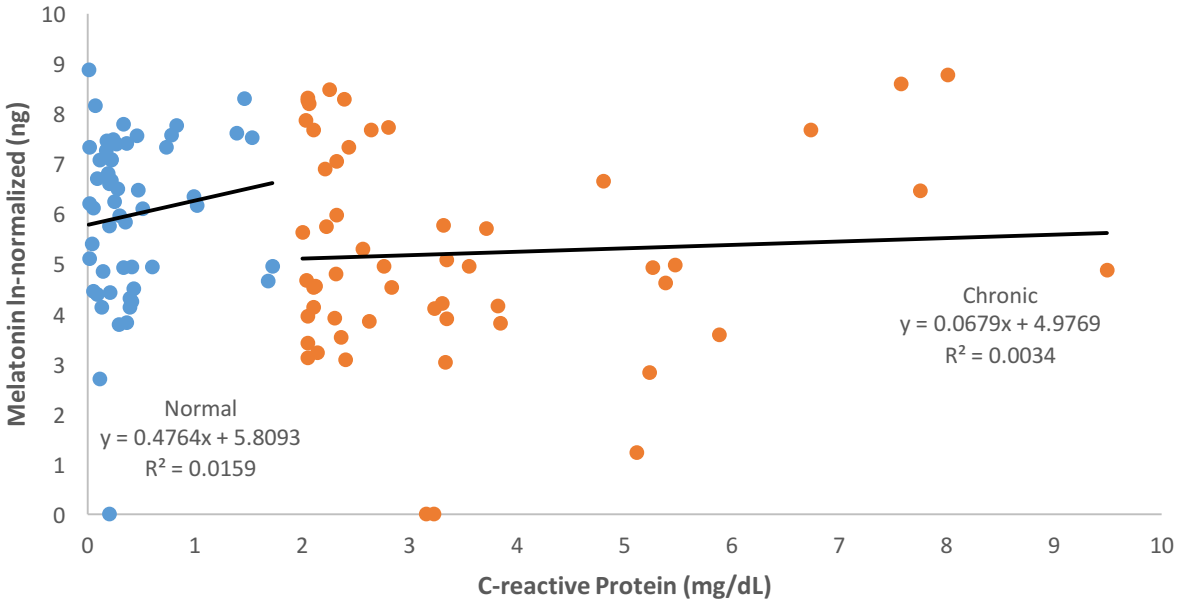
Chi-Square	0.0033
DF	1
Pr > Chi-Square	0.9541


Distribution of Wilcoxon Scores for Sleep__hours_
100
80
60
40
20
0
Score
Normal
Chroni
Group
Pr < Z 0.4783
Pr > |Z| 0.9567

Appendix E

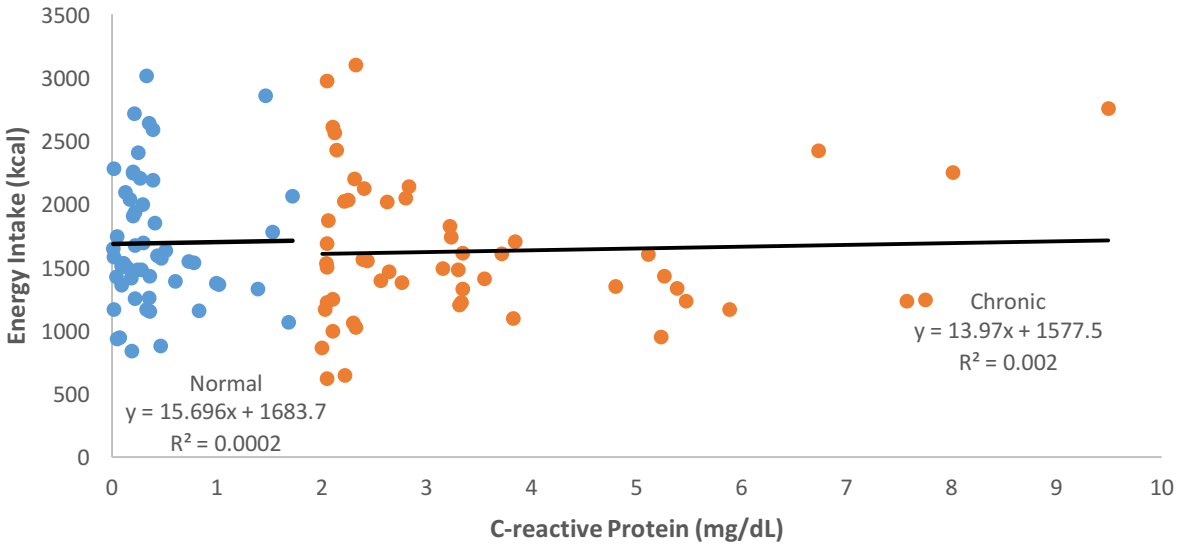
Simple Linear Regression

a) CRP and Dietary Melatonin



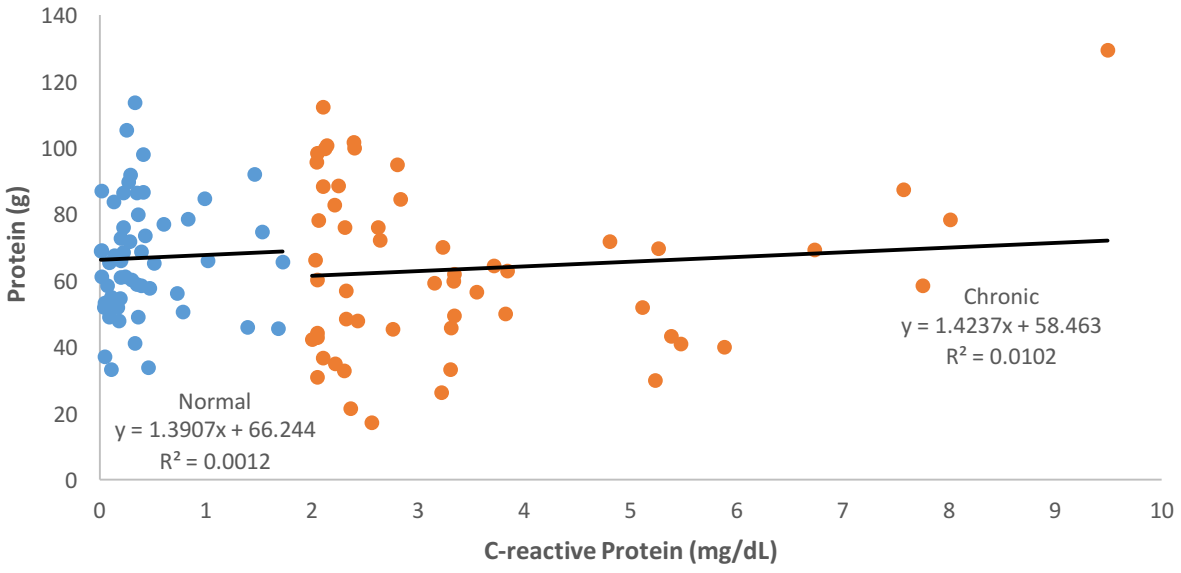
Simple linear regression between dietary melatonin and CRP in low and chronic inflammatory individuals (n = 109). Normal group b = 0.48, R^2 = 0.02, F(1, 52) = 0.88, p = 0.35 and chronic group b = 0.07, R^2 = 0.003, F(1, 53) = 0.18, p = 0.67.

b) CRP and Caloric Intake



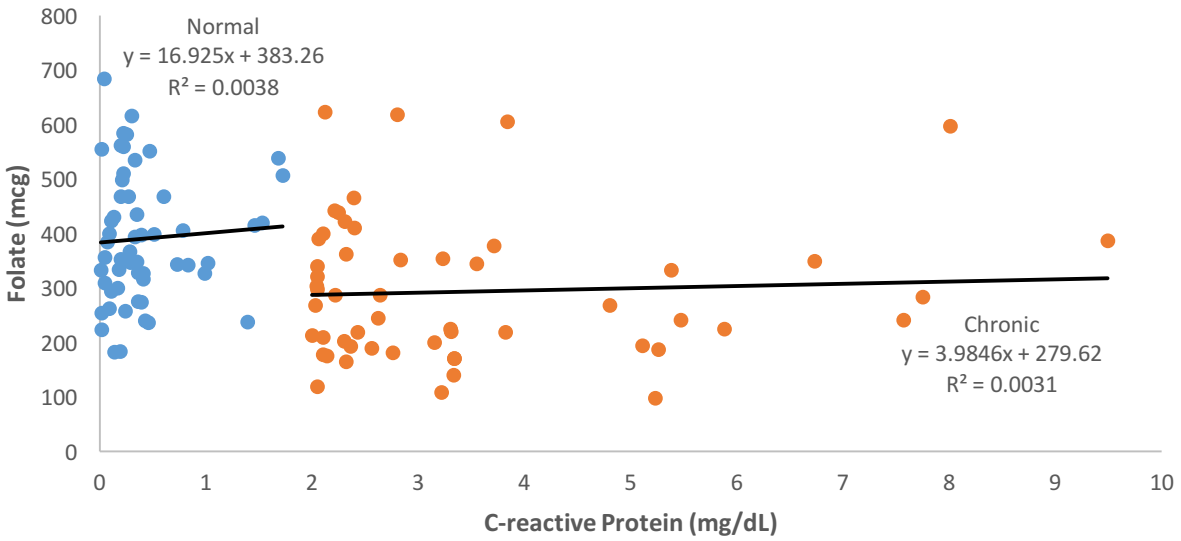
Simple linear regression between caloric intake and CRP in low and chronic inflammatory individuals (n = 108). Normal group b = 15.69, R^2 = 0.0002, F(1, 52) = 0.009, p = 0.92 and chronic group b = 13.97, R^2 = 0.002, F(1, 52) = 0.10, p = 0.75.

c) CRP and Protein



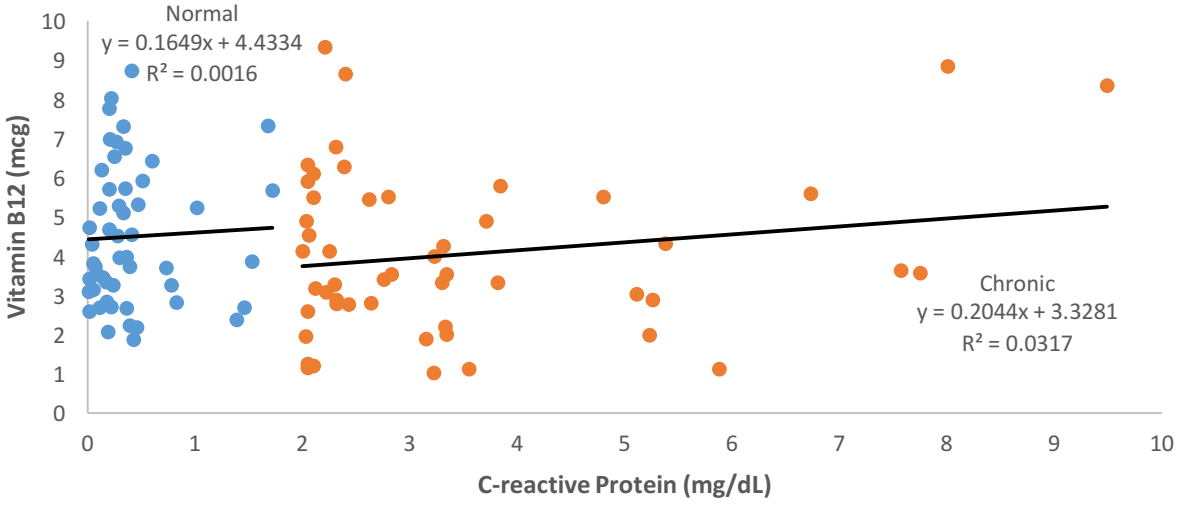
Simple linear regression between dietary protein and CRP in low and chronic inflammatory individuals (n = 109). Normal group b = 1.39, R^2 = 0.001, F(1, 52) = 0.06, p = 0.81 and chronic group b = 1.42, R^2 = 0.01, F(1, 53) = 0.55, p = 0.46.

d) CRP and Folate



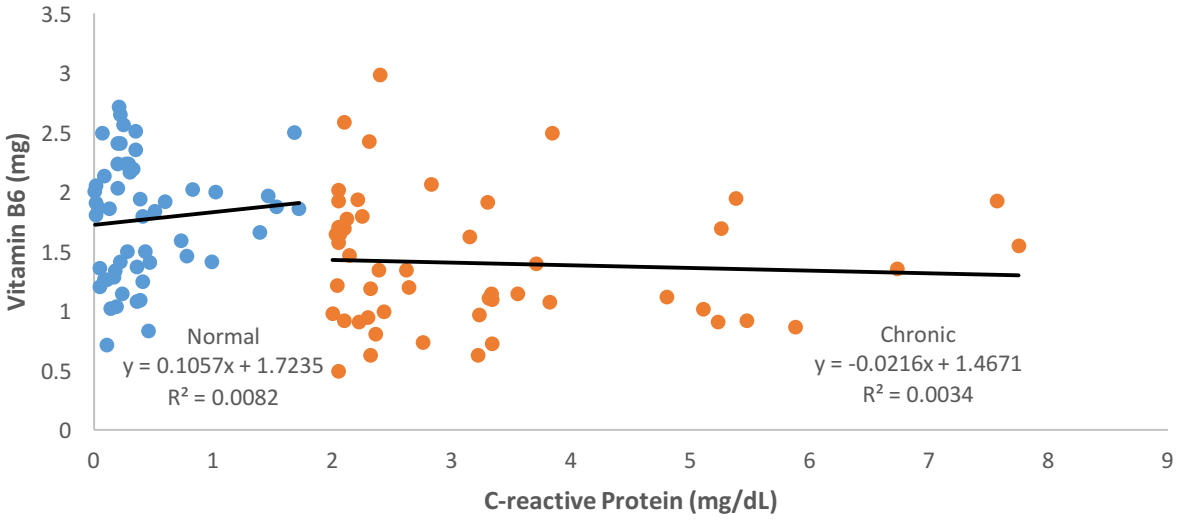
Simple linear regression between folate and CRP in low and chronic inflammatory individuals (n = 109). Normal group b = 16.93, R^2 = 0.004, F(1, 53) = 0.20, p = 0.65 and chronic group b = 3.98, R^2 = 0.003, F(1, 52) = 0.16, p = 0.69.

e) CRP and Vitamin B12



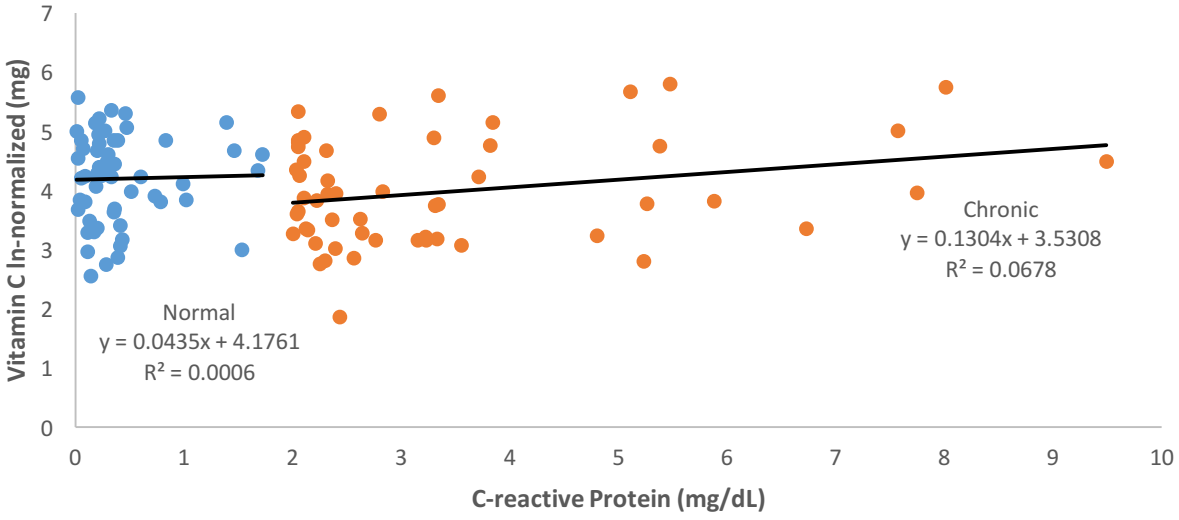
Simple linear regression between vitamin B12 and CRP in low and chronic inflammatory individuals (n = 103). Normal group b = 0.16, R^2 = 0.002, F(1, 50) = 0.08, p = 0.78 and chronic group b = 0.20, R^2 = 0.03, F(1, 49) = 1.6, p = 0.21.

f) CRP and Vitamin B6



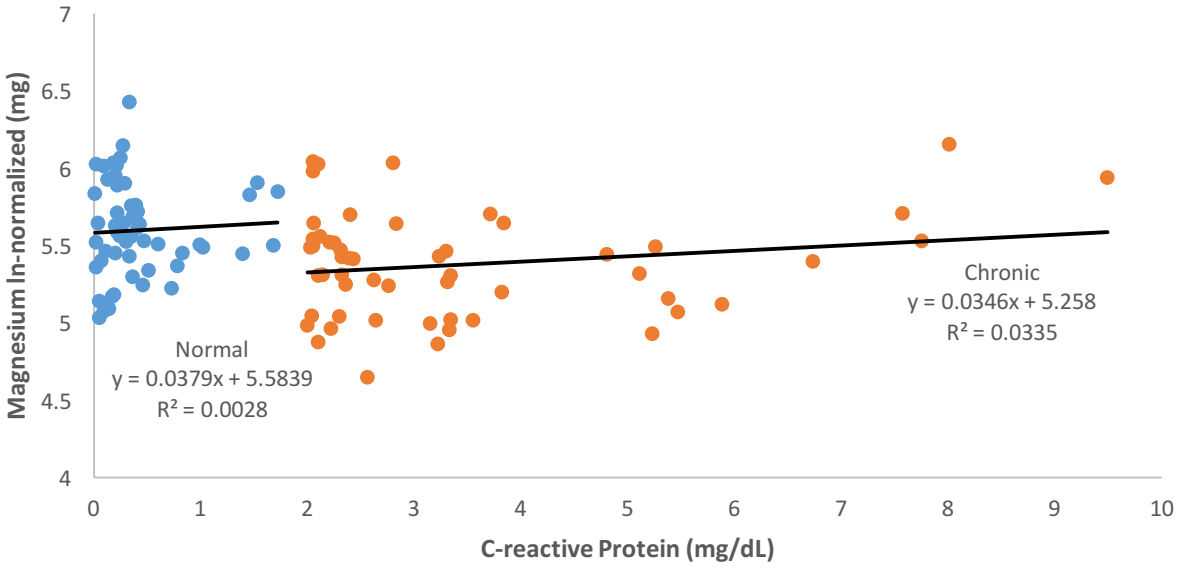
Simple linear regression between vitamin B6 and CRP in low and chronic inflammatory individuals (n = 105). Normal group b = 0.12, R^2 = 0.008, F(1, 52) = 0.43, p = 0.51 and chronic group b = -0.02, R^2 = 0.003, F(1, 49) = 0.17, p = 0.68.

g) CRP and Vitamin C



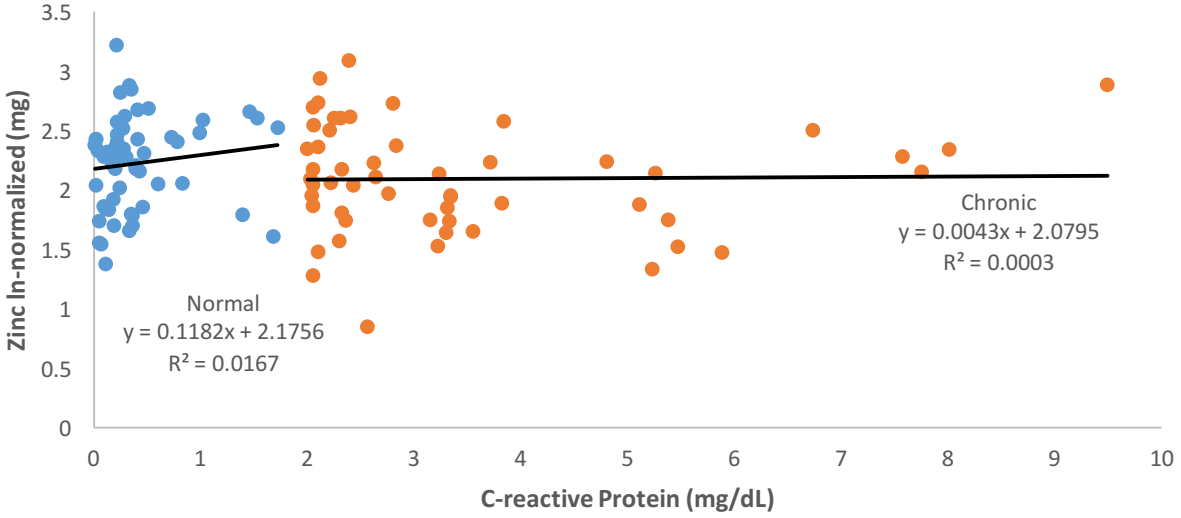
Simple linear regression between vitamin C and CRP in low and chronic inflammatory individuals (n = 110). Normal group b = 0.04, R^2 = 0.0006, F(1, 53) = 0.03, p = 0.86 and chronic group b = 0.13, R^2 = 0.07, F(1, 53) = 3.85, p = 0.055.

h) CRP and Magnesium



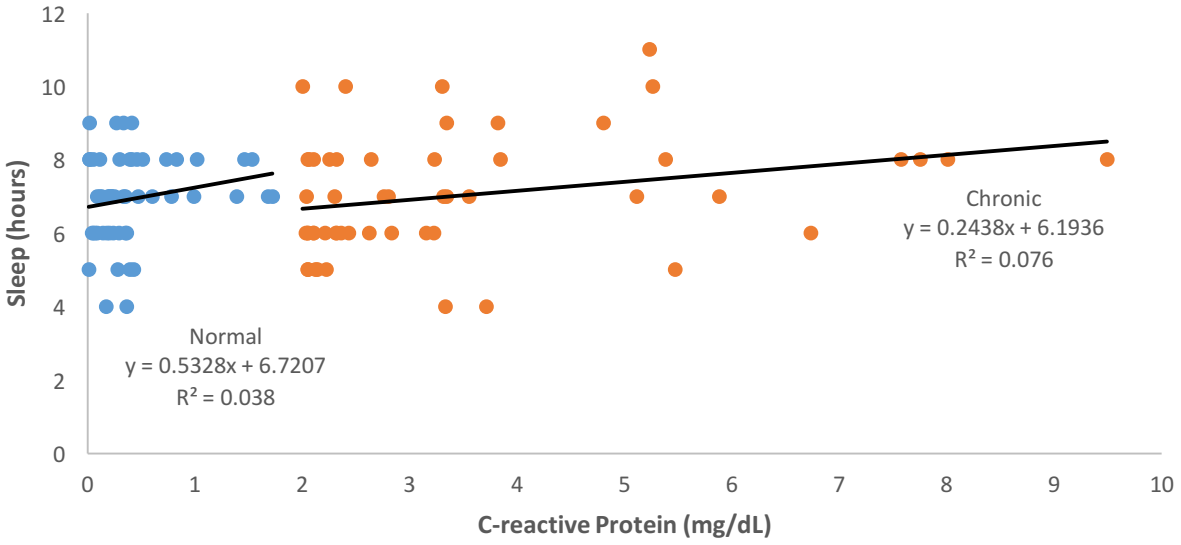
Simple linear regression between magnesium and CRP in low and chronic inflammatory individuals (n = 109). Normal group b = 0.04, R^2 = 0.003, F(1, 53) = 0.15, p = 0.70 and chronic group b = 0.03, R^2 = 0.03, F(1, 52) = 1.80, p = 0.19.

i) CRP and Zinc



Simple linear regression between zinc and CRP in low and chronic inflammatory individuals (n = 109). Normal group b = 0.12, R^2 = 0.02, F(1, 53) = 0.90, p = 0.35 and chronic group b = 0.004, R^2 = 0.0003, F(1, 52) = 0.01, p = 0.90.

j) CRP and Sleep



Simple linear regression between hours of sleep and CRP in low and chronic inflammatory individuals (n = 107). Normal group b = 0.53, R^2 = 0.04, F(1, 52) = 2.06, p = 0.16 and chronic group b = 0.24, R^2 = 0.08, F(1, 51) = 4.20, p = 0.045.

Appendix F

Multivariate Linear Regression

a) Descriptive Statistics – Chronic Inflammatory Group

Simple Statistics						
Variable	**N**	**Mean**	**Std Dev**	**Sum**	**Minimum**	**Maximum**
C_Reactive_Protein	55	3.43	1.79	188.48	2.00	9.49
Alkaline_Phosphatase__1_x_normal	55	0.01	0.00	0.71	0.01	0.02
HDL_ln_normalized	54	3.96	0.28	213.62	3.29	4.49
Femur_BMD	55	0.84	0.16	46.10	0.47	1.26
Melatonin__ln_normal_	55	5.21	2.09	286.52	0.00	8.78
Energy	54	1626.00	563.53	87784.00	623.50	3099.00
Protein	55	63.34	25.27	3484.00	17.22	129.43
Folate	54	293.38	129.17	15843.00	97.50	622.50
B12	51	4.03	2.09	205.69	1.02	9.33
B6	51	1.39	0.55	71.25	0.49	2.98
Vitamin_C_ln	55	3.98	0.89	218.78	1.86	5.80
Magnesium_ln	54	5.38	0.34	290.38	4.65	6.15
Zinc_ln	54	2.09	0.46	113.09	0.85	3.09
Sedentary_Time	41	311.71	147.71	12780.00	60.00	600.00
Sleep__hours_	53	7.04	1.60	373.00	4.00	11.00

b) Descriptive Statistics – Normal Inflammatory Group

Simple Statistics						
Variable	**N**	**Mean**	**Std Dev**	**Sum**	**Minimum**	**Maximum**
C_Reactive_Protein	55	0.42	0.43	22.87	0.01	1.72
Alkaline_Phosphatase__1_x_normal	51	0.01	0.00	0.71	0.01	0.02
HDL_ln_normalized	53	4.07	0.20	215.64	3.58	4.48
Femur_BMD	55	0.90	0.17	49.56	0.61	1.40
Melatonin__ln_normal_	54	5.98	1.64	323.13	0.00	8.88
Energy	54	1690.00	513.58	91273.00	837.50	3016.00
Protein	54	66.83	17.73	3609.00	33.06	113.56
Folate	55	390.30	118.07	21467.00	182.00	684.00
B12	52	4.50	1.76	234.09	1.88	8.74
B6	54	1.77	0.51	95.45	0.71	2.71
Vitamin_C_ln	55	4.19	0.75	230.68	2.55	5.57
Magnesium_ln	55	5.59	0.31	307.98	5.03	6.43
Zinc_ln	55	2.22	0.39	122.36	1.38	3.22
Sedentary_Time	36	330.00	156.13	11880.00	120.00	720.00
Sleep__hours_	54	6.94	1.19	375.00	4.00	9.00

Appendix G

Path Analysis

a) Regression Weights – Chronic Inflammatory Group

Regression Weights: (Group number 1 - Default model)

			Estimate	S.E.	C.R.	P
MelatoninlnnormaI	<---	Magnesiumln	3.51	.83	4.24	***
Protein	<---	Energy	.02	.00	4.83	***
Protein	<---	Magnesiumln	-11.96	6.87	-1.74	.08
Protein	<---	Zincln	12.29	5.22	2.36	.02
Protein	<---	B12	3.26	.98	3.31	***
Protein	<---	B6	11.22	3.24	3.46	***
MelatoninlnnormaI	<---	Zincln	4.81	1.04	4.62	***
Protein	<---	Folate	-.05	.014	-3.74	***
CReactiveProtein	<---	Sleephours	.28	.13	2.24	.03
CReactiveProtein	<---	AlkalinePhosphatase1xnormal	39.31	60.63	.65	.52
CReactiveProtein	<---	Folate	-.01	.00	-4.03	***
FemurBMD	<---	CReactiveProtein	-.02	.01	-2.27	.02
MelatoninlnnormaI	<---	Protein	-.13	.03	-4.02	***
Protein	<---	MelatoninlnnormaI	6.25	1.72	3.63	***

b) Model: Melatonin = Folate x Protein x Magnesium x Zinc

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Zincln, Magnesiumln, Folate, Protein[b]	.	Enter

a. Dependent Variable: MelatoninInnormal

b. All requested variables entered.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.530[a]	.281	.209	1.836125690000000

a. Predictors: (Constant), Zincln, Magnesiumln, Folate, Protein

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	52.659	4	13.165	3.905	.009[b]
	Residual	134.854	40	3.371		
	Total	187.514	44			

a. Dependent Variable: MelatoninInnormal

b. Predictors: (Constant), Zincln, Magnesiumln, Folate, Protein

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	-13.090	6.106		-2.144	.038
	Folate	-.002	.004	-.114	-.566	.575
	Protein	-.006	.021	-.060	-.282	.779
	Magnesiumln	2.892	1.283	.403	2.254	.030
	Zincln	1.753	1.399	.316	1.253	.217

a. Dependent Variable: MelatoninInnormal

c) Model: CRP = Femur BMD x Alkaline Phosphatase

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	FemurBMD, AlkalinePhosphatase1xnormal[b]	.	Enter

a. Dependent Variable: CReactiveProtein

b. All requested variables entered.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.411[a]	.169	.130	1.41945045200 0000

a. Predictors: (Constant), FemurBMD, AlkalinePhosphatase1xnormal

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	17.237	2	8.618	4.277	.020[b]
	Residual	84.623	42	2.015		
	Total	101.860	44			

a. Dependent Variable: CReactiveProtein

b. Predictors: (Constant), FemurBMD, AlkalinePhosphatase1xnormal

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	3.771	1.534		2.458	.018
	AlkalinePhosphatase1xnormal	135.931	75.586	.254	1.798	.079
	FemurBMD	-2.671	1.264	-.299	-2.114	.041

a. Dependent Variable: CReactiveProtein

d) Model: Femur BMD = CRP

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	CReactiveProtein[b]	.	Enter

a. Dependent Variable: FemurBMD

b. All requested variables entered.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.324[a]	.105	.084	.162864211000000

a. Predictors: (Constant), CReactiveProtein

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.134	1	.134	5.058	.030[b]
	Residual	1.141	43	.027		
	Total	1.275	44			

a. Dependent Variable: FemurBMD

b. Predictors: (Constant), CReactiveProtein

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	.957	.059		16.299	.000
	CReactiveProtein	-.036	.016	-.324	-2.249	.030

a. Dependent Variable: FemurBMD

e) Model: Protein = Zinc x Melatonin x Energy x B6 x B12 x Folate x Magnesium

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Zincln, MelatoninInnormal, Energy, B6, B12, Folate, Magnesiumln[b]	.	Enter

a. Dependent Variable: Protein

b. All requested variables entered.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.825[a]	.681	.621	12.880538610000000

a. Predictors: (Constant), Zincln, MelatoninInnormal, Energy, B6, B12, Folate, Magnesiumln

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	13098.961	7	1871.280	11.279	.000[b]
	Residual	6138.606	37	165.908		
	Total	19237.567	44			

a. Dependent Variable: Protein

b. Predictors: (Constant), Zincln, MelatoninInnormal, Energy, B6, B12, Folate, Magnesiumln

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	23.783	55.325		.430	.670
	MelatoninInnormal	1.210	1.223	.119	.989	.329
	B12	.334	1.444	.030	.231	.818
	Folate	-.033	.028	-.184	-1.181	.245
	B6	13.571	6.091	.359	2.228	.032
	Energy	.013	.005	.313	2.433	.020
	Magnesiumln	-12.109	12.241	-.166	-.989	.329
	Zincln	31.100	9.449	.553	3.291	.002

a. Dependent Variable: Protein

f) Model: Sleep = CRP

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	CReactiveProtein[b]	.	Enter

a. Dependent Variable: Sleephours

b. All requested variables entered.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.253[a]	.064	.042	1.570

a. Predictors: (Constant), CReactiveProtein

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	7.225	1	7.225	2.932	.094[b]
	Residual	105.975	43	2.465		
	Total	113.200	44			

a. Dependent Variable: Sleephours

b. Predictors: (Constant), CReactiveProtein

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	6.317	.566		11.157	.000
	CReactiveProtein	.266	.156	.253	1.712	.094

a. Dependent Variable: Sleephours

g) Regression Weights – Normal Inflammatory Groups

Regression Weights: (Group number 1 - Default model)

			Estimate	S.E.	C.R.	P
HDLlnnormalized	<---	Magnesiumln	.21	.15	1.41	.16
CReactiveProtein	<---	Zincln	-.72	.61	-1.19	.24
FemurBMD	<---	Zincln	.06	.07	.81	.42
HDLlnnormalized	<---	AlkalinePhosphatase1xnormal	-.53	14.62	-.04	.97
Protein	<---	Energy	.01	.01	2.77	.01
Protein	<---	Magnesiumln	-5.10	6.33	-.81	.42
Melatoninlnnormal	<---	VitaminCln	-.09	.37	-.25	.80
Protein	<---	B12	.42	.95	.44	.66
Protein	<---	FemurBMD	17.46	10.53	1.66	.09
Protein	<---	B6	8.66	3.22	2.69	.01
Protein	<---	Zincln	28.49	4.82	5.91	***
Protein	<---	HDLlnnormalized	-2.85	6.45	-.44	.66
Sleephours	<---	CReactiveProtein	.27	.15	1.73	.08

h) Model: Melatonin = Vitamin C x B12

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	VitaminCln, B12[b]	.	Enter

a. Dependent Variable: MelatoninInnormal

b. All requested variables entered.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.411[a]	.169	.125	1.664789741000000

a. Predictors: (Constant), VitaminCln, B12

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	21.419	2	10.709	3.864	.030[b]
	Residual	105.318	38	2.772		
	Total	126.737	40			

a. Dependent Variable: MelatoninInnormal

b. Predictors: (Constant), VitaminCln, B12

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	4.892	1.623		3.015	.005
	B12	-.337	.164	-.304	-2.053	.047
	VitaminCln	.629	.343	.271	1.835	.074

a. Dependent Variable: MelatoninInnormal

i) Model: CRP = Zinc

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Zincln[b]	.	Enter

a. Dependent Variable: CReactiveProtein

b. All requested variables entered.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.405[a]	.164	.143	.379755872000000

a. Predictors: (Constant), Zincln

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.107	1	1.107	7.674	.009[b]
	Residual	5.624	39	.144		
	Total	6.731	40			

a. Dependent Variable: CReactiveProtein

b. Predictors: (Constant), Zincln

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	-.619	.367		-1.690	.099
	Zincln	.459	.166	.405	2.770	.009

a. Dependent Variable: CReactiveProtein

j) Model: Alkaline phosphatase = HDL Cholesterol

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	HDLlnnormalized[b]	.	Enter

a. Dependent Variable: AlkalinePhosphatase1xnormal

b. All requested variables entered.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.331[a]	.110	.087	.002930879190

a. Predictors: (Constant), HDLlnnormalized

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.000	1	.000	4.809	.034[b]
	Residual	.000	39	.000		
	Total	.000	40			

a. Dependent Variable: AlkalinePhosphatase1xnormal

b. Predictors: (Constant), HDLlnnormalized

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	-.007	.009		-.710	.482
	HDLlnnormalized	.005	.002	.331	2.193	.034

a. Dependent Variable: AlkalinePhosphatase1xnormal

k) Model: HDL Cholesterol = Alkaline phosphatase x Energy x Protein x Magnesium x Zinc

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Zincln, AlkalinePhosphatase1xnormal, Magnesiumln, Energy, Protein[b]	.	Enter

a. Dependent Variable: HDLInnormalized

b. All requested variables entered.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.560[a]	.313	.215	.178070599000000

a. Predictors: (Constant), Zincln, AlkalinePhosphatase1xnormal, Magnesiumln, Energy, Protein

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.507	5	.101	3.195	.018[b]
	Residual	1.110	35	.032		
	Total	1.616	40			

a. Dependent Variable: HDLInnormalized

b. Predictors: (Constant), Zincln, AlkalinePhosphatase1xnormal, Magnesiumln, Energy, Protein

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	3.012	.607		4.960	.000
	AlkalinePhosphatase1xnormal	23.412	9.587	.357	2.442	.020
	Energy	.000	.000	.343	1.755	.088
	Protein	.001	.003	.087	.404	.689
	MagnesiumIn	.090	.133	.138	.681	.501
	ZincIn	-.035	.127	-.064	-.280	.781

a. Dependent Variable: HDLInnormalized

l) Model: Femur BMD = Zinc x Protein

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Zincln, Protein[b]	.	Enter

a. Dependent Variable: FemurBMD

b. All requested variables entered.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.365[a]	.133	.087	.158266918000000

a. Predictors: (Constant), Zincln, Protein

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.146	2	.073	2.911	.067[b]
	Residual	.952	38	.025		
	Total	1.098	40			

a. Dependent Variable: FemurBMD

b. Predictors: (Constant), Zincln, Protein

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	.549	.153		3.584	.001
	Protein	.002	.002	.159	.752	.456
	Zincln	.108	.096	.235	1.116	.271

a. Dependent Variable: FemurBMD

m) Model: Protein = HDL Cholesterol x Femur BMD x B12 x B6 x Energy x Magnesium x Zinc

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Zincln, HDLlnnormalized, FemurBMD, B12, B6, Energy, Magnesiumln[b]	.	Enter

a. Dependent Variable: Protein

b. All requested variables entered.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.793[a]	.629	.550	11.403644200000000

a. Predictors: (Constant), Zincln, HDLlnnormalized, FemurBMD, B12, B6, Energy, Magnesiumln

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	7270.350	7	1038.621	7.987	.000[b]
	Residual	4291.422	33	130.043		
	Total	11561.773	40			

a. Dependent Variable: Protein

b. Predictors: (Constant), Zincln, HDLlnnormalized, FemurBMD, B12, B6, Energy, Magnesiumln

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	-101.880	53.104		-1.919	.064
	HDLInnormalized	6.691	10.571	.079	.633	.531
	FemurBMD	18.284	12.394	.178	1.475	.150
	B12	2.206	1.317	.208	1.675	.103
	B6	4.542	4.734	.133	.959	.344
	Energy	.004	.005	.107	.715	.480
	Magnesiumln	13.912	8.768	.251	1.587	.122
	Zincln	10.410	8.232	.222	1.264	.215

a. Dependent Variable: Protein

n) Model: Sleep = CRP

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	CReactiveProtein[b]	.	Enter

a. Dependent Variable: Sleephours

b. All requested variables entered.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.267[a]	.071	.047	1.179

a. Predictors: (Constant), CReactiveProtein

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	4.147	1	4.147	2.982	.092[b]
	Residual	54.243	39	1.391		
	Total	58.390	40			

a. Dependent Variable: Sleephours

b. Predictors: (Constant), CReactiveProtein

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	6.578	.253		25.963	.000
	CReactiveProtein	.785	.455	.267	1.727	.092

a. Dependent Variable: Sleephours

References

1. de Pablo P, Cooper M, Buckley C. Association between bone mineral density and C-reactive protein in a large population-based sample. *Arthritis & Rheumatism*. 2012;64(8):2624-2631.
2. Nabipour I, Larijani B, Vahdat K et al. Relationships among serum receptor of nuclear factor-κB ligand, osteoprotegerin, high-sensitivity C-reactive protein, and bone mineral density in postmenopausal women. *Menopause*. 2009;16(5):950-955.
3. Lian C, Wu Z, Gao B et al. Melatonin reversed tumor necrosis factor-alpha-inhibited osteogenesis of human mesenchymal stem cells by stabilizing SMAD1 protein. *Journal of Pineal Research*. 2016;61(3):317-327.
4. Amstrup A, Sikjaer T, Mosekilde L, Rejnmark L. Melatonin and the skeleton. *Osteoporosis International*. 2013;24(12):2919-2927.
5. Peuhkuri K, Sihvola N, Korpela R. Dietary factors and fluctuating levels of melatonin. *Food & Nutrition Research*. 2012;56.
6. Herman A, Bochenek J, Król K et al. Central interleukin-1β suppresses the nocturnal secretion of melatonin. *Mediators of Inflammation*. 2016;2016:1-15.
7. Parekh P, Oldfield IV E, Challapallisri V, Ware J, Johnson D. Sleep disorders and inflammatory disease activity: chicken or the egg?. *The American Journal of Gastroenterology*. 2014;110(4):484-488.
8. Pandi-Perumal S, Srinivasan V, Maestroni G, Cardinali D, Poeggeler B, Hardeland R. Melatonin. *FEBS Journal*. 2006;273(13):2813-2838.
9. Tresguerres I, Tamimi F, Eimar H et al. Melatonin dietary supplement as an anti-aging therapy for age-related bone loss. *Rejuvenation Research*. 2014;17(4):341-346.
10. Papas AM. Antioxidant Status, Diet, Nutrition, and Health. 1st ed. New York, NY: CRC Press LLC;1999.
11. Korkmaz A, Reiter RJ, Topal T, Manchester LC, Oter S, Tan D. Melatonin: an established antioxidant worthy of use in clinical trials. *Mol Med.* 2009;15(1-2):43-50.
12. Reiter R, Tan D, Burkhardt S, Manchester L. Melatonin in plants. *Nutrition Reviews*. 2001;59(9):286-290.
13. Tan D, Hardeland R, Manchester L et al. Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science. *Journal of Experimental Botany*. 2011;63(2):577-597.
14. Nawaz M, Huang Y, Bie Z et al. Melatonin: Current status and future perspectives in plant science. *Frontiers in Plant Science*. 2016;6(1236).
15. Riga P, Medina S, García-Flores L, Gil-Izquierdo Á. Melatonin content of pepper and tomato fruits: Effects of cultivar and solar radiation. *Food Chemistry*. 2014;156:347-352.
16. Howatson G, Bell P, Tallent J, Middleton B, McHugh M, Ellis J. Effect of tart cherry juice (Prunus cerasus) on melatonin levels and enhanced sleep quality. *European Journal of Nutrition*. 2011;51(8):909-916.
17. Iriti M, Varoni E. Melatonin in Mediterranean diet, a new perspective. *Journal of the Science of Food and Agriculture*. 2015;95(12):2355-2359.
18. McCune L, Kubota C, Stendell-Hollis N, Thomson C. Cherries and health: a review. *Critical Reviews in Food Science and Nutrition*. 2010;51(1):1-12.
19. Pasko P, Sulkowska-Ziaja K, Muszynska B, Zagrodzki P. Serotonin, melatonin, and certain indole derivatives profiles in rutabaga and kohlrabi s18eeds, sprouts, bulbs, and roots. *LWT - Food Science and Technology*. 2014;59(2):740-745.

20. Posmyk M, Janas K. Melatonin in plants. *Acta Physiologiae Plantarum*. 2008;31(1):1-11.
21. Badria F. Melatonin, serotonin, and tryptamine in some Egyptian food and medicinal plants. *Journal of Medicinal Food*. 2002;5(3):153-157.
22. Reiter RJ, Tan DX. Melatonin: an antioxidant in edible plants. *Ann. N.Y. Acad. Sci.* 2002;957:341-344.
23. Yılmaz C, Kocadağlı T, Gökmen V. Formation of melatonin and its isomer during bread dough fermentation and effect of baking. *J Agric Food Chem*. 2014;62(13):2900-2905.
24. Tan D, Zanghi B, Manchester L, Reiter R. Melatonin identified in meats and other food stuffs: potentially nutritional impact. *Journal of Pineal Research*. 2014;57(2):213-218.
25. Jouan P, Pouliot Y, Gauthier S, Laforest J. Hormones in bovine milk and milk products: A survey. *International Dairy Journal*. 2006;16(11):1408-1414.
26. Hattori A, Migitaka H, Iigo M et al. Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptor in vertebrates. *Biochemistry and Molecular Biology International.* 1995;35(3):627-634.
27. Feng X, Wang M, Zhao Y, Han P, Dai Y. Melatonin from different fruit sources, functional roles, and analytical methods. *Trends in Food Science & Technology*. 2014;37(1):21-31.
28. Reiter R, Manchester L, Tan D. Melatonin in walnuts: Influence on levels of melatonin and total antioxidant capacity of blood. *Nutrition*. 2005;21(9):920-924.
29. Johns N, Johns J, Porasuphatana S, Plaimee P, Sae-Teaw M. Dietary intake of melatonin from tropical fruit altered urinary excretion of 6-sulfatoxymelatonin in healthy volunteers. *J Agric Food Chem*. 2013;61(4):913-919.
30. Zielinski H, Kozlowska H, Lewczuk B. Bioactive compounds in the cereal grains before and after hydrothermal processing. *Innovative Food Science & Emerging Technologies*. 2001;2(3):159-169.
31. Terzieva DD, Matevna ND, Vladimirova-Kitova LG. Melatonin reference limits at 3:00 AM and 8:00 AM in healthy adults. *Clin Lab.* 2009;55(9-10):359-61.
32. PanLaboratories In-Vitro Diagnostic Testing. Melatonin, serum/plasma. http://panlaboratories.com/melatonin-serumplasma/. Accessed November 18, 2016.
33. Genova Diagnostics. Comprehensive melatonin profile: Sample report salivary melatonin. 2015. Accessed November 18, 2016.
34. Nagata C, Nagao Y, Shibuya C, Kashiki Y, Shimizu H. Association of vegetable intake with urinary 6-sulfatoxymelatonin level. *Cancer Epidemiology Biomarkers & Prevention*. 2005;14(5):1333-1335.
35. Oba S, Nakamura K, Sahashi Y, Hattori A, Nagata C. Consumption of vegetables alters morning urinary 6-sulfatoxymelatonin concentration. *Journal of Pineal Research*. 2008;45(1):17-23.
36. Sae-Teaw M, Johns J, Johns N, Subongkot S. Serum melatonin levels and antioxidant capacities after consumption of pineapple, orange, or banana by healthy male volunteers. *Journal of Pineal Research*. 2012;55(1):58-64.
37. Bravo R, Matito S, Cubero J et al. Tryptophan-enriched cereal intake improves nocturnal sleep, melatonin, serotonin, and total antioxidant capacity levels and mood in elderly humans. *AGE*. 2012;35(4):1277-1285.
38. Brown JE. Nutrition through the life cycle. 5th ed. Stamford, CT: Cengage Learning; 2014.

39. Position of the American Dietetic Association and Dietitians of Canada: Nutrition and Women's Health. *Journal of the American Dietetic Association*. 2004;104(6):984-1001.
40. Gunn C, Weber J, Kruger M. Diet, weight, cytokines and bone health in postmenopausal women. *J Nutr Health Aging*. 2014;18(5):479-486.
41. Taleb-Belkadi O, Chaib H, Zemour L, Fatah A, Chafi B, Mekki K. Lipid profile, inflammation, and oxidative status in peri- and postmenopausal women. *Gynecological Endocrinology*. 2016:1-4.
42. Gubbels Bupp M. Sex, the aging immune system, and chronic disease. *Cellular Immunology*. 2015;294(2):102-110.
43. Kolesnikova L, Madaeva I, Semenova N et al. Pathogenic role of melatonin in sleep disorders in menopausal women. *Bulletin of Experimental Biology and Medicine*. 2013;156(1):104-106.
44. Amstrup A, Sikjaer T, Heickendorff L, Mosekilde L, Rejnmark L. Melatonin improves bone mineral density at the femoral neck in postmenopausal women with osteopenia: a randomized controlled trial. *Journal of Pineal Research*. 2015;59(2):221-229.
45. NHANES - National Health and Nutrition Examination Survey Homepage. *Cdcgov*. 2016. Available at: https://www.cdc.gov/Nchs/Nhanes/index.htm. Accessed November 19, 2016.
46. Shivappa N, Steck S, Hurley T et al. A population-based dietary inflammatory index predicts levels of C-reactive protein in the Seasonal Variation of Blood Cholesterol Study (SEASONS). *Public Health Nutr*. 2013;17(08):1825-1833.
47. Mahan LK, Raymond JL. Krause's Food and the Nutrition Care Process. 14th ed. St. Louis, MO: Elsevier;2017.
48. Mesri Alamdari N, Mahdavi R, Roshanravan N, Lotfi Yaghin N, Ostadrahimi A, Faramarzi E. A Double-Blind, Placebo-controlled trial related to the effects of melatonin on oxidative stress and inflammatory parameters of obese women. *Hormone and Metabolic Research*. 2014;47(07):504-508.
49. Mukaiyama K, Kamimura M, Uchiyama S, Ikegami S, Nakamura Y, Kato H. Elevation of serum alkaline phosphatase (ALP) level in postmenopausal women is caused by high bone turnover. *Aging Clinical and Experimental Research*. 2015;27(4):413-418.
50. Chen J, Chen Y, Tsai M et al. Predicting the risk of osteopenia for women aged 40–55 years. *Journal of the Formosan Medical Association*. 2017:1-9
51. Formosa M, Xuereb-Anastasi A. Biochemical Predictors of Low Bone Mineral Density and Fracture Susceptibility in Maltese Postmenopausal Women. *Calcified Tissue International*. 2016;98(1):28-41.
52. Fournier I, Ploye F, Cottet-Emard J, Brun J, Claustrat B. Folate deficiency alters melatonin secretion in rats. *The Journal of Nutrition.* 2002;132:2781-2784.
53. Ebaid H, Bashandy SAE, Alhazza IM, Rady A, El-Shehry S. Folic acid and melatonin ameliorate carbon tetrachloride-induced hepatic injury, oxidative stress and inflammation in rats. *Nutrition & Metabolism*. 2013;10(1):20.
54. Ikeda M, Asai M, Moriya T, Sagara M, Inoué S, Shibata S. Methylcobalamin amplifies melatonin-induced circadian phase shifts by facilitation of melatonin synthesis in the rat pineal gland. *Brain Research*. 1998;795(1-2):98-104.
55. Mayer G, Kröger M, Meier-Ewert K. Effects of vitamin B12 on performance and circadian rhythm in normal subjects. *Neuropsychopharmacology.* 1996;15(5):456-464.

56. Luboshitzky R, Ophir U, Nave R, Epstein R, Shen-Orr Z, Herer P. The effect of pyridoxine administration on melatonin secretion in normal men. *Neuro Endocrinol Lett.* 2002;23(3):213-7.
57. Dakshinamurti K, Paulose CS, Viswanathan M, Siow YL, Sharma SK. Neurobiology of pyridoxine. *Annals New York Academy of Sciences.* 1990;585:128-44.
58. Billyard AJ, Eggett DI, Franz KB. Dietary magnesium deficiency decreases plasma melatonin in rats. *Magnesium Research.* 2006;19(3):157-61.
59. Rondanelli M, Opizzi A, Monteferrario F, Antoniello N, Manni R, Klersy C. The effect of melatonin, magnesium, and zinc on primary insomnia in long-term care facility residents in Italy: a double-blind, placebo-controlled clinical trial. *Journal of the American Geriatrics Society*. 2011;59(1):82-90.
60. McCann JC, Shigenaga MK, Mietus-Snyder ML et al. A multicomponent nutrient bar promotes weight loss and improves dyslipidemia and insulin resistance in the overweight/obese: chronic inflammation blunts these improvements. *The FASEB Journal.* 2015;29(8):3287-3301.
61. Mietus-Snyder ML, Shigenaga MK, Suh JH et al. A nutrient-dense, high-fiber, fruit-based supplement bar increases HDL cholesterol, particularly large HDL, lowers homocysteine, and raises glutathione in a 2-wk trial. *The FASEB Journal.* 2012;26(8):3515-3527.
62. Rizzoli R, Bischoff-Ferrari H, Dawson-Hughes B, Weaver C. Nutrition and bone health in women after the menopause. *Women's Health*. 2014;10(6):599-608.
63. Langsetmo L, Barr S, Berger C et al. Associations of protein intake and protein source with bone mineral density and fracture risk: A population-based cohort study. *The Journal of Nutrition, Health & Aging*. 2015;19(8):861-868.
64. Bailey R, van Wijngaarden J. The role of B-vitamins in bone health and disease in older adults. *Current Osteoporosis Reports*. 2015;13(4):256-261.
65. Fratoni V, Brandi M. B vitamins, homocysteine and bone health. *Nutrients*. 2015;7(4):2176-2192.
66. Enneman AW, Swart KMA, van Wijngaarden JP et al. Effect of vitamin B12 and folic acid supplementation on bone mineral density and quantitative ultrasound parameters in older people with an elevated plasma homocysteine level: B-PROOF, a randomized controlled trial. *Calcified Tissue International*. 2015;96(5):401-409.
67. Mahdavi-Roshan M, Ebrahimi M, Ebrahimi A. Copper, magnesium, zinc and calcium status in osteopenic and osteoporotic post-menopausal women. *Clinical Cases in Mineral and Bone Metabolism*. 2015;12(1):18-21.
68. Smith BJ, Chongwatpol P, Rendina-Ruedy E, Stoecker BJ, Clarke SL, Lucas EA. Implications of compromised zinc status on bone loss associated with chronic inflammation in C57BL/6 mice. *Journal of Inflammation Research*. 2015;8:117-128.
69. Reynold AM, Bowles ER, Saxena A, Fayad R, Youngstedt SD. Negative effects of time in bed extension: a pilot study. *J Sleep Med Disord.* 2014;1(1):1-13.
70. Huang W, Huang C, Chang C, Kor C, Chen T, Wu H. Associations of self-reported sleep quality with circulating interferon gamma-inducible protein 10, interleukin 6, and high-sensitivity c-reactive protein in healthy menopausal women. *PLOS ONE*. 2017;12(1):e0169216.

71. Ferrie JE, Kivimaki M, Akbaraly TN et al. Associations between change in sleep duration and inflammation: findings on c-reactive protein and interleukin 6 in the Whitehall II Study. *American Journal of Epidemiology*. 2013;178(6):956-961.

VITA
Martha R. Wasserbauer

EDUCATION

M.S. Nutrition Science *June 2017*
Syracuse University, Department of Nutrition Science & Dietetics

- Cumulative GPA of 4.0
- Awarded graduate assistantship for 2015-2016 and 2016-2017
- Nutrition Science Graduate Program Marshall and Graduate Student Speaker for Falk College Convocation, May 2017
- Falk College Master's Prize, May 2017
- Nutrition Science and Dietetics Graduate Research Award, May 2017

B.S. Biology *May 2014*
St. Lawrence University, Department of Biology

- Cumulative GPA of 3.72
- Graduated Magna Cum Laude

TEACHING EXPERIENCE

Graduate Teaching Assistant
Syracuse University, Nutrition Science Department *August 2015 – May 2017*

- Assisted in teaching undergraduate nutritional biochemistry and advanced nutrition under Dr. Margaret Voss
- Held weekly office hours to assist students in understanding course material and preparing for exams, and was responsible for grading exams and homework assignments and proctoring exams.

RESEARCH EXPERIENCE

Master's Thesis *August 2016 – June 2017*
Syracuse University, Nutrition Science Department

- Guided by Dr. Margaret Voss
- Researched the associations between dietary-derived melatonin, bone mineral density, inflammation, and circadian rhythm in postmenopausal women. The study was a secondary data analysis of data obtained from the National Health and Nutrition Examination Survey and focused on quantifying melatonin intake from whole foods.
- Results were presented at the New York State Dietetic Association Annual Meeting & Expo 2017 poster session.

Get FRUVED! Study *January 2016 – May 2016*
Syracuse University, Nutrition Science Department

- Collected data for Get FRUVED, which is a USDA funded study to improve college student dietary intake, physical activity, and wellness.
- Completed environmental audits of convenience stores near Syracuse University to assess the availability, quality, and cost of healthy foods.
- Completed physical assessments of student subjects, including measuring student weight, height, waist, hip and neck circumference, and blood pressure.

Senior Year Research Experience *January 2014 – May 2014*
St. Lawrence University, Biology Department

- Guided by Dr. Karin Heckman
- Developed a qualitative study exploring the effects of gut microbiota on metabolic mechanisms of obesity and researched the role of *Lactococcus lactis* and *Bifidobacterium breve* on regulation of immune cell activity.
- Developed a survey to assess college study knowledge and consumption of probiotics.
- Results presented at the New York State Dietetic Association Annual Meeting & Expo 2014 poster session.

Fellowship Research Program *January 2013 – September 2013*
St. Lawrence University, Biology Department

- Guided by Dr. Ana Estevez
- Completed a randomized controlled trial to understand the mechanisms by which cerium oxide nanoparticles scavenge free-radicals and mitigate oxidative stress in caenorhabdidtis elegans nematodes.
- Was responsible for running the lab, preparing treatment plates, maintaining nematode populations, and using florescence microscopy to quantify accumulation of lipofuscin.
- Results were published in the Journal of Free Radical Biology and Medicine.

PROFESSIONAL EXPERIENCE

Central New York Dietetic Association *September 2016 – May 2017*
Student Representative

- Managed communication between CNYDA board of directors and Syracuse University nutrition students to increase student membership and plan professional activities.
- Planned a networking event for professionals and students featuring registered dietitians of the Central New York area.

Childtime Learning Center - Syracuse, NY *September 2014 – July 2015*
Food Specialist

- Oversaw meal preparation for daycare children, created menus in compliance with the USDA Child and Adult Care Food Program, and maintained food inventory on a budget.
- Prepared daily breakfast, lunch, and afternoon snack for up to 60 children and ensured that serving size requirements were met.
- Compiled food records for CACFP reimbursement, completed food orders, and followed proper food safety and sanitation.

PKU Cooking Workshop – Onondaga Community College *April 2015*
Chef's Assistant

- Assisted with a cooking demo and workshop for individuals with phenylketonuria by organizing cooking stations, preparing recipe ingredients, and helping workshop participants with recipe preparation. Collected prepared food to organize a buffet-style lunch for participants.

Language Resource Center, St. Lawrence University *August 2011 – May 2014*
Intern

- Managed classroom technology, computer software, and media library of the Department of Modern Languages and Literatures.
- Assisted faculty and students with classroom technology, recorded language teaching assistant hours, and kept track of films and cameras that were distributed from the media library.

Laurentian Singers, St. Lawrence University *August 2013 – May 2014*
President

- Served as student leader to choir members of the Laurentian Singers by integrating new members, coordinating annual auditions, providing members with music assistance, and providing communication between students and the director.
- Assisted in planning the annual tour to Trinidad and helped coordinate activities during the tour.

Camp Stella Maris – Livonia, NY *June 2011 – August 2012*
Kitchen Staff

- Prepared breakfast, lunch, and dinner for 450 children in a fast-paced, demanding, and team-based environment while promoting facilities cleanliness, which provided campers with a quality and enjoyable dining experience.
- Was responsible for meal preparation, dishwashing, cleaning and sanitizing.

VOLUNTEER EXPERIENCE

VA Medical Center – Syracuse, NY *September 2016 – December 2016*
Nutrition Office Volunteer

- Shadowed inpatient dietitians conduct the ADIME process, prioritize patients, and assess patients. Observed outpatient counseling sessions, assisted with the Move! Program education sessions, and assisted with food preparation in the hospital kitchen.
- Reviewed hospital menus to provide suggestions for increasing recipe nutritional value, variety, and sustainability.

Samaritan Center – Syracuse, NY *June 2016 – September 2016*
Volunteer

- Served meals to homeless and food insecure individuals who attended mealtimes.
- Received food donations, prepared meal components, and cleaned and sanitized the facilities.

Crouse Hospital – Syracuse, NY *September 2015 – December 2015*
Nutrition Aide

- Visited patients to provide them with education about the hospital room service menu and weekly specials. Answered patient questions about the menu, helped patients choose items adhering to their dietary needs, and assisted patients with ordering meals.
- Completed menu item nutrient analysis, helped compile a database of hospital foods containing high fructose corn syrup, and shadowed dietitians.

POSTER PRESENTATIONS

Does Dietary Melatonin Play a Role in Bone Mineral Density? 2017. MR Wasserbauer, MA Voss. New York State Academy of Nutrition and Dietetics Annual Meeting & Expo, Lake Placid, NY. May 2017.

Knowledge and Consumption of Probiotics in College Students. MR Wasserbauer, K Heckman. New York State Academy of Nutrition and Dietetics Annual Meeting & Expo, Westchester, NY. April 2014.

PUBLICATIONS

Estevez AY, Wasserbauer MR, Airoldi BL, Alvin JW, Reed K, Erlichman JS. Comparison of commercial and custom-synthesized cerium oxide nanoparticles in mitigating oxidative stress markers in the nematode caenorhabditis elegans. *Free Radical Biology and Medicine.* 2013;65:S123-S124.

PROFESSIONAL MEMBERSHIPS

Academy of Nutrition and Dietetics *May 2016 – Present*
Central New York Dietetic Association *September 2016 - Present*

HONORS AND AWARDS

Adirondack 46er Membership *October 2015*

- Awarded to individuals who have summited all 46 high peaks of the Adirondack Mountains.

J. Kenneth Munson Award *May 2014*

- Annual award given by St. Lawrence University Department of Music in recognition of a student who has demonstrated excellence in choral musicianship.

Beta Beta Beta Biology Honor Society *April 2014*

- A professional honor society for outstanding students studying biological sciences, and is dedicated to increasing an appreciation for and understanding of biological sciences.

Omicron Delta Kappa *May 2013*

- A national leadership honor society that recognizes students who exhibit superior scholarship, leadership, and character. An emphasis is placed on developing members' contribution to the college community and to improving society.

Most Outstanding Media Project *May 2011*

- An annual award presented by the St. Lawrence University Department of Modern Languages and Literatures to a student who has created an outstanding languages project. This award recognized my completion of an Advanced German media presentation on solar energy.

CERTIFICATIONS

ServSafe Certification – National Restaurant Association *Valid until November 2019*
Collaborative Institutional Training Program *Valid until September 2018*
CRP and First Aid – American Red Cross *February 2015*

STUDY ABROAD EXPERIENCE

Southeast Asia Study Abroad Program to India – Syracuse University *June 2016*
AMIDEAST Study Abroad - Amman, Jordan *August 2012 – December 2012*
Rotary Youth Exchange Program – Vienna, Austria *August 2009 – July 2010*