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Association between vitamin D and metabolic syndrome in older adults

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Association between vitamin D and metabolic syndrome in older adults

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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ABSTRACT

Recently, low serum concentrations of 25-hydroxyvitamin D [25(OH)D] have been linked to disturbances in glucose metabolism, development of type 2 diabetes, and increased risk of metabolic syndrome (MetS). Moreover, deficiency of vitamin D is now recognized to be highly prevalent in the U.S. and worldwide, impacting between 30% and 50% of the general population. Therefore, the objective of this research study is to examine the associations between serum 25-hyroxyvitamin D and MetS. Data were collected from the Nutritional Interventions for Age-Related Muscular Function and Strength Losses Study. From this cohort, 186 independently living males and females over the age of 60, with vitamin D levels between 18 ng/mL and 30 ng/mL, without any existing liver or kidney disease or uncontrolled diabetes mellitus or type 1 diabetes mellitus requiring insulin, provided baseline data. A Pearson correlation coefficient of 0.02 with non-significant *p*-value of 0.77 was found between MetS score and 25(OH)D. Factors such as average heart rate ($p < 0.05$), weight ($p < 0.001$) , BMI ($p < 0.001$), LDL:HDL ratio ($p < 0.01$), android fat (%) ($p < 0.001$), gynoid fat mass ($p < 0.001$), and gynoid fat (%) ($p < 0.01$) were found to have significant associations with the MetS score. A backwards stepwise regression indicated that android fat (%) ($p < 0.001$), gynoid fat (%) ($p = 0.001$), total cholesterol ($p < 0.001$), VLDL cholesterol ($p < 0.001$), and LDL:HDL ratio ($p < 0.001$) were most predictive of the MetS score. In conclusion, the major finding of this study was that the combination of android fat (%), gynoid fat (%), total cholesterol, VLDL cholesterol, and the LDL:HDL ratio were predictive of the MetS score. However, no significant association between low vitamin D status and prevalence of MetS could be established.

CHAPTER 1. INTRODUCTION

The cluster of cardiovascular risk factors that define metabolic syndrome are important determinants of vascular disease and type 2 diabetes mellitus (T2DM), which are major causes of morbidity and mortality worldwide (1). Clinical diagnosis of metabolic syndrome (MetS) is based on the presence of three or more of the following markers of chronic disease: (i) greater waist circumference; (ii) elevated fasting plasma glucose (FG); (iii) hypertension referring to elevated systolic or diastolic blood pressure (SBP and DBP); (iv) elevated triglycerides (TG); and (v) low HDL cholesterol (2, 3). Recently, low serum concentrations of 25-hydroxyvitamin D or 25(OH)D have been linked to disturbances in glucose metabolism, development of type 2 diabetes, and increased risk of metabolic syndrome (4, 5, 6, 7, 8). Moreover, deficiency of vitamin D is now recognized to be highly prevalent in the U.S. and worldwide, impacting between 30% and 50% of the general population (9). Traditionally, the most characterized consequence of vitamin D deficiency has involved the musculoskeletal system, leading to rickets in children, and osteomalacia or osteoporosis in adults. However, it is now recognized that vitamin D receptors are present on a large variety of cell types. This means that vitamin D metabolites regulate a very wide range of genes involved in overall and cardiovascular health. Studies in the U.S. and Europe show that most of the general population have 25(OH)D levels below the target level of 75 nmol/L, with levels being lower in those with MetS (1). Furthermore, prospective studies have shown that low 25(OH)D levels are associated with increased all cause cardiovascular mortality. Therefore, it is vital to increase understanding and public knowledge on the importance of vitamin D status as it relates to chronic disease risk. The primary objective of this

research study was to determine whether low vitamin D status is associated with metabolic syndrome in males and females at least 60 years of age. My central hypothesis was that vitamin D status is inversely correlated with metabolic syndrome in a dose dependent manner. Data were collected from the Nutritional Interventions for Age-Related Muscular Function and Strength Losses Study. From this cohort, 186 independently living and primarily non-hispanic or latino white males and females over the age of 60, with vitamin D levels between 18 ng/mL and 30 ng/mL, without any existing liver or kidney disease or uncontrolled diabetes mellitus or type 1 diabetes mellitus requiring insulin, provided baseline data.

CHAPTER 2. BACKGROUND

Metabolic Syndrome

In the United States, where nearly two-thirds of the population is overweight or obese, more than one-fourth of the population meets diagnostic criteria for metabolic syndrome (10). Unfortunately, this problem is further reaching than the U.S. alone. Approximately one-fourth of the adult European and Latin American population is estimated to have MetS. It is also considered to be an emerging epidemic in developing East Asian countries, including China, Japan, and Korea. It is clear that MetS is a major public-health and clinical challenge worldwide, bestowing a 5-fold increased risk of T2DM and 2-fold increased risk of developing cardiovascular disease (CVD) within 5-10 years.

The idea of MetS started as a concept rather than a diagnosis when a Swedish physician demonstrated the association of hypertension, hyperglycemia, and gout in the 1920s (11). In 1947, it was identified that visceral obesity was commonly associated with the metabolic abnormalities found in CVD and T2DM. As the field moved forward, this cluster of risk factors for CVD and DM began to be described as “Syndrome X” by Reaven (1988), with the main focus being on insulin resistance. Obesity and visceral obesity were later added to the definition. Over the years the syndrome was renamed multiple times with varying definitions. More recently there has been controversy regarding the development of a single uniform definition of the disease. Currently, the most commonly used criteria for diagnosis are from the World Health Organization (WHO), the European Group for the study of Insulin Resistance (EGIR), the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III), the American

Association of Clinical Endocrinologists (AACE), and the International Diabetes Federation (IDF). While each definition possesses common features, there are certain aspects that differ. The WHO, AACE, and EGIR are largely focused on evaluating insulin resistance and requires labor-intensive testing which is primarily performed in the research laboratory. The ATP III definitions use measurements and laboratory results that are readily available to physicians. For the purpose, of this study ATP III criteria were utilized for classification of disease.

According to the ATP III report, MetS has been identified as a multiplex risk factor for cardiovascular disease that is deserving of more clinical attention (2). In the Framingham study, MetS alone predicted approximately 25% of all new-onset CVD. In addition, almost half of the population-attributable risk for diabetes could be explained by the presence of MetS. Diabetes was also shown to be a major risk factor for CVD. ATP III identifies abdominal obesity, atherogenic dyslipidemia, raised blood pressure, insulin resistance and/or glucose intolerance, and pro-inflammatory and pro-thrombic states as six components of MetS. Beyond this, each risk factor is subjected to its own regulation through both genetic and acquired factors. This regulation of risk factors can be seen with low serum concentrations of 25(OH)D, which have been linked to disturbances in glucose metabolism. In addition, vitamin D receptors are present on a large variety of cell types, meaning that vitamin D metabolites likely regulate a very wide range of genes affecting many aspects of health, especially cardiovascular health.

The current intervention for those with MetS is primarily a lifestyle modification that combines specific recommendations on diet and exercise with behavioral strategies. However, for those whose risk factors are not adequately reduced, pharmacological

interventions may be necessary (12). The clinical management of MetS is difficult because there is no recognized method to prevent or improve the whole syndrome. Thus, most physicians treat each component separately, with an emphasis placed on those that are easily treated with drugs due to the ease of prescribing a medication versus initiating a strategy to change people's lives. Therefore, while intervention in any chronic disease is of great importance, prevention of this world wide epidemic should be at the forefront of discussion. Because MetS acts as a precursor to some of the leading causes of morbidity and mortality worldwide such as Type 2 diabetes and vascular disease it is of grave concern (1). Moreover, with much of the population worldwide having inadequate serum vitamin D, achieving adequate status of nutrients such as vitamin D could serve to have a profound global impact on health (9).

Vitamin D Metabolism

Vitamin D is a fat-soluble, cholesterol derived vitamin that can be obtained from synthesis by the skin following sun exposure, food, or supplements (9). Depending on the source, vitamin D can be acquired in one of two forms: vitamin D_3 or vitamin D_2. Vitamin D_3 (cholecalciferol) is animal-derived and has high biological activity, whereas vitamin D_2 (ergocalciferol) is plant-derived and therefore has lower biological availability. Vitamin D_3 is formed from its precursor 7-dehydrocholesterol (DHC), which is found in abundant amounts in the skin of humans and animals. However, only a few foods naturally contain a significant amount of vitamin D_3, which include: liver, fish liver oils, fatty fish, and egg yolks (13,14). Salmon, mackerel, and bluefish are excellent sources of D_3, but it has been shown that farmed salmon has much less vitamin D_3 than wild-caught salmon likely due to the food supply (47). Farmed salmon commonly receive a pelleted form of food that contains much less vitamin D than what wild salmon

consume in their natural habitiat. In addition, some countries including the U.S., practice fortification of certain foods with vitamin D, such as milk products. Vitamin D_2, on the other hand, is formed following exposure to UV radiation from its precursor ergosterol, which is present in plants, yeast, and fungi (14).

The two forms of vitamin D are initially biologically inactive, and when in circulation require hepatic and renal metabolism in the liver and kidney in order to be bioavailable to cells (14, 15). After binding to a vitamin D carrier protein (DBP), vitamin D is transported to the liver, where it is enzymatically hydroxylated to 25(OH)D. 25(OH)D synthesis is catalyzed by a microsomal cytochrome P450 enzyme (CYP2R1) to make 1,25(OH)D_3. 25(OH)D then enters circulation where it has a half-life of about 15 days. Normal circulating levels of 25(OH)D in the blood are between 25 nmol and 200 nmol/L. Hydroxy vitamin D bound to DBP is then transported to the kidneys, where it is finally hydroxylated by CYP27B1 to generate the active form of 1,25(OH)D_3. 1,25(OH)D_3 has a half-life of about 10-24 hours, and is tightly regulated by parathyroid hormone (PTH), calcium, phosphate, calcitonin, fibroblast GF 23, and [1,25(OH)D3] itself. 1,25(OH)D_3 has biological effects such as increasing the resporption of calcium in the kidneys, but is also transported by DBP to other vitamin D receptor target tissues such as the bones, intestines, or parathyroid gland.

1,25D functions as a hormone that, depending how much Ca is consumed in the diet and thus into the blood, works to maintain serum calcium (Ca) and phosphorus concentrations within the normal range by enhancing the ability of the small intestine to absorb these minerals from the diet (16). When dietary calcium intake is inadequate, 1,25(OH)D_3 along with PTH, withdraws calcium stores from the bone (3, 15). This

mechanism predominates in low Ca situations. In the kidney, 1,25(OH)D increases calcium reabsorption by the distal renal tubules.

Vitamin D Status

It is well established that vitamin D has important implications on our health, but the optimal intake of vitamin D remains a matter of debate. Much debate among researchers has centered around what concentrations of 25(OH)D or 1,25(OH)D are associated with deficiency (e.g., rickets), adequacy for bone health, and optimal overall health. Multiple forms of vitamin D exist, but serum concentrations of 25(OH)D are reflective of cutaneous vitamin D_3 synthesis and dietary intake of vitamin D_2 and D_3 (13, 14, 17, 18). Thus far, cut off values have not been agreed upon by consensus. However, the Institute of Medicine concluded that persons are vitamin D deficient if serum 25(OH)D concentrations are <30 nmol/L (<12 ng/mL) (19). Vitamin D insufficiency is characterized by 25(OH)D < 50 nmol/L (12–20 ng/mL). The committee stated in their 2008 report that 50 nmol/L is the serum 25(OH)D level that covers the needs of 97.5% of the population.

Furthermore, intake reference values for vitamin D and other nutrients are provided in the Dietary Reference Intakes (DRIs) developed by the Food and Nutrition Board (FNB) at the Institute of Medicine of the National Academies (16) . DRIs describe a set of reference values used to plan and assess nutrient intakes of healthy people. These values, which vary by age and gender, include:

Recommended Dietary Allowance (RDA): average daily level of intake sufficient to meet the nutrient requirements of nearly all (97% – 98%) healthy people (see **Table 1**).

Table 1. Recommended dietary allowances (RDAs) for vitamin D (16)

Age	Male	Female	Pregnancy	Lactation
0-12 months	400 IU	400 IU	-	-
1-13 years	600 IU	600 IU	-	-
14-18 years	600 IU	600 IU	600 IU	600 IU
19-50 years	600 IU	600 IU	600 IU	600 IU
51-70 years	600 IU	600 IU	-	-
>70 years	800 IU	800 IU	-	-

Vitamin D deficiency has recently come to light as being highly prevalent in the U.S. and worldwide (18). Vitamin D deficiency as it relates to bone health has previously been well established, and is characterized by inadequate mineralization, or by demineralization of the skeleton (18, 20). Among children, vitamin D deficiency is a common cause of rickets, whereas vitamin D deficiency in adults leads to a mineralization defect in the skeleton, causing osteomalacia, and secondary hyperparathyroidism with osteoporosis.

The 600 IU/day recommendation is currently based off vitamin D levels deemed sufficient for bone health (48). However, if the body's sole input of vitamin D was only 600 IU per day, that would likely not be enough to produce a value of even 10 ng/mL. It is a generally recognized that each additional 100 IU of vitamin D per day only raises serum 25(OH)D concentrations by approximately 1 ng/mL. It is also widely recognized that 600 IU/day of vitamin D produces barely perceptible changes in individuals who are overweight or obese, which is now over 50% of the U.S. adult population. Thus, the current RDA is likely not enough to prevent deficiency in many populations who are not receiving additional sunlight exposure, Therefore, an increase in the current recommendations may be warrented.

Furthermore, vitamin D regulates a wide range of genes that play a major role in cardiovascular and metabolic health. Recently vitamin D insufficiency has been shown to be associated with increased risk of developing T2DM and CVD, as well as metabolic syndrome (10, 21). While many studies back these findings, consistent results have not been well established in the literature. This is likely due to differences in geographic locations, skin pigmentation, age, health status, study size, population diversity, and seasonal variations.

In an analysis of the Victorian Health Monitor survey in 2016, the associations between serum 25(OH)D, dietary calcium intake, and presence of the metabolic syndrome were examined (3). This analysis consisted of 3,404 participants without type 1 or type 2 diabetes mellitus from Victoria, Australia. A higher serum 25(OH)D concentration was associated with significantly reduced odds of developing MetS. In fact, for every 10 nmol/L increment that serum [25(OH)D] was reduced, the likelihood of MetS increased by 15%. Thus those in the highest levels of serum [25(OH)D] with concentrations between 65-204 nmol/L, were found to have a 60% lower chance of developing MetS. However, this was not statistically significant for every model of Ca intake tested, suggesting that vitamin D concentration can modulate the effect of Ca on MetS. It was observed that the overall effect of Ca at a moderate 25(OH)D level was stronger than at a low 25(OH)D level. This may be explained by the ability of 25(OH)D to maintain Ca homeostasis via active intestinal Ca absorption independent of its conversion to 1,25(OH)D_3. Therefore, an improvement in vitamin D status from low to adequate would further increase intestinal Ca absorption.

Premenopausal women enrolled in the Womens' Health Initiative-Calcium-Vitamin D trial exhibited serum 25(OH)D concentrations that were inversely associated with serum triglyceride concentrations and the TG:HDL ratio (22). This same study also observed that serum 25(OH)D concentrations were inversely associated with adiposity after controlling for demographic and lifestyle risk factors, suggesting that vitamin D concentrations may contribute or be independently affected by adiposity.

Role of Environment

The major contributor to serum 25(OH)D concentrations for most humans is that synthesized by the skin after exposure to sunlight or artificial sources of UVB radiation between 280-320 nm (14, 21). Under normal circumstances this contributes to more than 90% of the serum concentration of vitamin D in the human body. Furthermore, the photochemical reaction that takes place in the skin is most effective when the UVB radiation is at about 297 nm (18). This results in formation of pre-vitamin D_3 from 7-dehydrocholesterol in basal and suprabasal layers of the skin. The effectiveness of UVB on formation of pre-vitamin D_3 in the skin is influenced by several UVB absorbing molecules (i.e., chromophores in the skin such as melanin, DNA, RNA, protein, and 7-DHC). Experimental evidence indicates that about 50% of the pre-vitamin D_3 can isomerize to vitamin D_3 within 2.5 hours in the skin. Therefore, a rapid rise in vitamin D_3 levels is typically seen after exposure to UVB. Within 12-24 hours after UVB exposure the circulating concentration of vitamin D_3 is at its maximal level. In fair skinned individuals, the maximum amount of vitamin D_3 that can possibly be formed is synthesized within a few minutes of summer sun exposure (14). Longer exposures add nothing to vitamin D stores. In fact, maximal vitamin D synthesis occurs following suberthemeogenic UVB exposure, hence higher doses would cause conversion of pre-

vitamin D_3 to inactive isomers such as lumisterol, tachysterol, toxisterols, and 7-DHC. These high doses would also cause conversion of vitamin D_3 to suprasterols and 5,6-trans-vitamin D_3 to prevent toxicity from too much sun exposure.

The effectiveness of cutaneous synthesis of vitamin D is determined by: (i) the content of 7-DHC in the skin; (ii) the cutaneous concentrations of 7-DHC; (iii.) the energy of photons that depends on the wavelength of the UVB radiation; (iv) latitude, season, and time of day; (v) skin pigmentation; (vi) use of sunscreens (suppress 7-DHC); (vii) temperature (which regulates the conversion of pre-vitamin D_3 to vitamin D_3); (viii) exposure doses of UVB (maximal vitamin D synthesis occurs following subthermogenic UVB exposure, meaning that higher doses would cause conversion of pre-vitamin D_3 to inactive isomers); and (ix) age-inverse relation between concentrations of 7-DHC in the epidermis with age (the body is less efficient at producing vitamin D as it gets older) (6, 7, 8, 14). Factors such as air pollution often seen in urban environments can reduce exposure to sunlight as well (23).

Role of Ethnicity

In the United States, metabolic syndrome has a high prevalence in African Americans, particularly African American women (50). This has been attributed to the higher prevalence of obesity, hypertension, and diabetes in this population. However, the highest age adjusted prevalence of metabolic syndrome in the U.S. was found in Mexican Americans in a 1988-1994 NHANES survey. A major problem with the WHO and NCEP ATP III definitions of MetS has been their applicability to the different ethnic groups, especially when trying to define obesity cutoffs (49). This is particularly evident for the risk of T2DM, which is apparent at much lower levels of obesity in Asians compared to Europeans. As a solution to the variances in risk factors for different races and ethnicities,

the IDF proposed a set of criteria with racial/ethnic specific cut-offs. This accounts for the fact that the different populations, ethnicities, and nationalities have different distributions of norms for body weight and waist circumference as well as the variability in the relationship between these values and the risk for T2DM and CVD in different populations.

Furthermore, ethnicity, skin tone, cultural practices, or occupation may influence the amount of sun exposure and vitamin D produced. According to a study by Nair and Maseeh, ethnicity has consistently been reported to be related to circulating 25(OH)D concentrations in adults, and a high prevalence of hypovitaminosis D has been observed in several Indian studies (24). In another study of Maylay adults, female participants had significantly lower mean vitamin D levels than males after adjustment for age, central obesity, HDL cholesterol, and diastolic blood pressure (21). It was noted that possible contributing reasons could be explained by their clothing style, which consisted of long sleeves, long skirts, and a veil due to cultural or religious practices. Additionally, skin pigmentation plays a key role. Deeper skin tones absorb more UVB in the melanin of the skin than lighter-skinned individuals. Therefore, darker skinned individuals require more sun exposure to produce the same amount of vitamin D. Finally, in countries that do not fortify foods with vitamin D, intake is often lower than the already low amount consumed in the western diet. Therefore, supplementation of vitamin D may be essential in these areas of the world.

Role of Aging

Aging, even in healthy elderly people, is accompanied by a reduction in muscle mass and muscle strength, resulting in an increased risk of falls/fractures and need for increased assistance (12). Vitamin D deficiency is associated with muscle weakness, and

is common in elderly individuals. Older adults are at high risk of developing vitamin D insufficiency because of aging (21). Older adults cannot synthesize vitamin D as efficiently, and are more likely to spend more time indoors, have reduced skin thickness, are more likely to have inadequate dietary intakes of the vitamin, and experience impaired intestinal absorption, or impaired hydroxylation on the liver and kidneys. In a study of 824 elderly people aged >70 years from 11 European countries, 36% of men and 47% of women had wintertime serum 25(OH)D concentrations <30 nmol/L (53). Current available evidence indicates that vitamin D supplementation preserves muscle strength and functional abilities in high risk groups (21). Vitamin D metabolites have been found to affect muscle metabolism in 3 ways: 1) by mediating gene transcription, 2) through rapid pathways not involving DNA synthesis, and 3) by the allelic variant of the VDR. Vitamin D supplementation induces rapid changes in calcium metabolism of the muscle cell that cannot be explained by a slow genetic pathway. Evidence indicates that $1,25(OH)D_3$, possibly through a vitamin D membrane receptor, acts directly on the muscle cell membrane (54, 55). Upon $1,25(OH)D_3$ binding, several interacting second-messenger pathways were found to be activated in the muscle cell, resulting in enhanced calcium uptake (within minutes), both through voltage-dependent calcium channels and calcium release–activated calcium channels (56, 57, 58). A review of the literature regarding vitamin D deficiency on muscle function and falls in the eldery found that supplementation in this population improved muscle strength, walking distance, functional ability, and body sway (21). These findings and the observed improvements in bone density after vitamin D supplementation provide a partial explanation for the association between vitamin D supplementation and fewer falls and nonvertebral

fractures in elderly people. However, vitamin D deficiency is merely one condition that affects muscle function in elderly people, which is illustrated by the fact that even in healthy, vitamin D–replete, elderly people, muscle strength declined with age, which was not prevented by vitamin D supplementation (21).

Furthermore, published data indicate that a statistically significant relationship exists between vitamin D and a variety of metabolic risk factors that can rise dramatically with age (2). During aging, the body has been observed to be less efficient at a variety of tasks, including cutaneous production of vitamin D, and regulation of blood pressure, weight status, insulin, and blood triglycerides, all of which may contribute to and increase the risk of developing MetS with age.

Proposed Mechanisms

The biological mechanism for the inverse associations between TG and the TG:HDL ratio is not completely understood, but it may be mediated by the effects of dietary calcium (22). Higher serum 25(OH)D increases the absorption of intestinal calcium which may bind to fatty/bile acids and form insoluble lipid-calcium complexes, thus inhibiting the absorption of cholesterol and increasing its fecal excretion. Another possibility is that there are reductions in hepatic TG synthesis or secretion in response to increased hepatocellular calcium concentrations (7, 22). Excess concentrations of PTH that are seen with low 25(OH)D could also play a part in these findings, as elevated PTH reduces lipolysis (7,22). The decreased peripheral removal of TG and hypertriglyceridemia has been observed in some states of hyperparathyroidism (22).

Increased adiposity has consistently been associated with reduced serum 25(OH)D concentrations and adverse cardiometabolic outcomes (6, 22, 24). Although the mechanism is not clear, it may be that overweight individuals at increased risk of

cardiometabolic disorders are more likely to have low serum 25(OH)D because of the high lipid solubility of serum 25(OH)D and sequestration by adipose tissue that results in reduced bioavailability. Alternately, it is possible that overweight individuals simply have less exposure to ultraviolet light due to lower levels of outdoor physical activity which results in lower serum 25(OH)D concentrations.

The trend indicates that low vitamin D status may pose an increased risk of MetS; however the mechanisms proposed to explain this trend are less straightforward. A key feature in the pathophysiology of MetS is insulin resistance (3). $1,25(OH)D_3$ has a role in insulin secretion by stimulating the expression of the insulin receptor and increasing the responsiveness to glucose transport. During vitamin D deficiency, beta-cell function is inhibited, leading to a decrease in insulin secretion (3).

The renin-angiotensin system is important in the regulation of blood pressure, another marker of MetS (3). Low 25(OH)D concentrations may dysregulate control of the renin-angiotensin system. In fact, lower 25(OH)D concentrations have been found to be inversely correlated with measures of arterial stiffness and increased arterial resistance, HTN, and endothelial dysfunction. In a study by Kim et al., dosages of $1,25(OH)D_3$ suppressed renin expression in mice indicating that blood pressure may influence the renin-angiotensin system (7). The suppression of renin likely inhibits the formations of angiotensin II, which increases salt retention and blood pressure by stimulating aldosterone secretion and increasing vasoconstriction. In addition, higher vitamin D status could reduce islet beta-cell damage by reducing islet renin-angiotensin system activity, thereby reducing the risk of developing hyperglycemia (3).

Moreover, there is evidence that Ca intake may influence fat balance and hence energy balance. Dietary Ca increases whole-body fat oxidation, which could potentially reduce circulating fatty acids/lipids. For an intake of 1,200 mg/d an increase of about 5g/d in fecal fat can be expected (3). This arises from the interaction between non-absorbed calcium and dietary fat in the gastrointestinal lumen which leads to Ca-fatty acid soap formation, and thus its eventual excretion. These outcomes may contribute to lower circulating TAG and other lipids seen with Ca supplementation (3).

Finally, MetS is a chronic low-grade inflammatory state, and adequate vitamin D has a significant role in reducing inflammation in chronic disease (3). Low 25(OH)D and increases in PTH levels increase the risk of inflammation, as documented by elevated levels of C-reactive protein and interleukin-10 (51). Administration of 1,25(OH)D in a state of vitamin D deficiciency has been shown to down-regulate inflammatory biomarkers such as C-reactive protein (52).

CHAPTER 3. SIGNIFICANCE AND INNOVATION

Significance

The published studies reviewed above indicate that a relationship may exist between vitamin D and a variety of metabolic risk factors. Perhaps more worrisome is that those risk factors can rise dramatically with age as the body becomes less efficient at producing cutaneous vitamin D and regulating blood pressure, weight, insulin, and triglycerides (2). This contributes in part to the increased prevalence of MetS in the aging population. Therefore, narrowing down possible contributors to such pathologies is of great importance. If an inverse relationship between [25(OH)D] and MetS can be established, physicians, dietitians, and the public may begin to take action in promoting sufficient vitamin D intake to prevent the onset of MetS and subsequent complications to reduce a major contributor to mortality and morbidity worldwide (20).

Innovation

While previous cohort studies evaluating the effect of vitamin D on metabolic syndrome have been performed, this study provides several key differences that will set it apart from previous counterparts. First, this study focuses on a population of men and women aged 60 and older. Few previous studies conducted have focused on the aging population, and several targeted only postmenopausal women. Additionally, there is a clear advantage to focusing on the older adult population, as several studies have demonstrated an age-related decline in many metabolic steps of the vitamin D pathway (16). This includes the rate of synthesis in the skin, the rate of hydroxylation, and the response of target tissues (e.g., bone). Therefore, it is likely that much of this population

is vitamin D deficient. Advancing age in general likely affects all levels of MetS pathogenesis and thus the prevalence of MetS rises with age (2).

Furthermore, body composition changes that are associated with aging lead to increased adiposity and decreased muscle mass, making the diagnosis of obesity or increased adiposity challenging (25). In clinical practice and public health settings, anthropometric measures such as body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-hip ratio (WHR), and more recently waist-height ratio (WHtR) are used as surrogates for intra-abdominal adiposity since they require little expense or time (15). Unfortunately, these measures are known to perform poorly in assessing the adiposity of certain populations such as older adults and body builders (26). More accurate methods such as DEXA, CT, or MRI are recommended for these groups.

The distribution of fat affects the risks associated with obesity and the types of disease that result (17). Fat can be thought of as visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT), or as upper body (android or central) and lower body (gynoid or peripheral). SAT has been found to be innervated with a larger number of inflammatory and immune cells, and have a lesser pre-adipocyte differentiating capacity and a greater percentage of large adipocytes. Additionally, abdominal obesity, is a key feature of the atherothrombic and inflammatory abnormalities associated with MetS (27). There is substantial evidence linking central obesity with cardiovascular disease and the other MetS components. Therefore, it is advantageous to be able to distinguish between those at increased risk as a result of android obesity from those with the less serious gynoid fat distribution, in which fat is more evenly and peripherally distributed around the body. In other words, excess abdominal fat is generally an equal risk factor for

disease as is excess body fat in general (17). However, anthropometric measures are unable to distinguish fat versus lean mass or the amount, type, and distribution of adipose tissue. Waist circumference, the measure traditionally utilized in the diagnosis of MetS, is the simplest and most common way to measure abdominal obesity. It is the circumference of the abdomen, measured at the natural waist (in between the lowest rib and the top of the hip bone), the umbilicus (belly button), or the narrowest point of the midsection. While it is easy to measure, inexpensive, and strongly correlated with body fat in adults when measured accurately, the measurement procedure has not been standardized, and it may be difficult to measure. It is also less accurate in individuals with a BMI of 35 kg/m^2 or higher. On the contrary, gold-standard methods of body composition analysis such as CT and MRI provide accurate whole-body and regional assessment of fat and muscle but are clinically impractical and costly for routine assessment (25). Therefore, it is more reasonable for accurate measurements of adiposity to be collected using DEXA in older adults. This can eliminate the challenges associated with using waist circumference as a clinical tool with its lack of diagnostic accuracy. DEXA works via X-ray beams that pass through different body tissues at varying rates. These low-level X-ray beams are then used to develop estimates of fat-free mass, fat mass, and bone mineral density (17). Compared to other imaging techniques, DEXA scanners are widely available, radiation exposure and patient burden is low, and cost is modest (28). Although DEXA cannot distinguish between intraabdominal and subcutaneous fat, in a study with a community-based elderly population, results suggested that android fat is more strongly associated with MetS than VAT (27). Adipose tissue in the android region quantified by DEXA has been found to have effects on

plasma lipid and lipoprotein concentrations, and it correlates strongly with abdominal visceral fat. Thus, DEXA is emerging as a new standard for body composition assessment due to its high precision, reliability, and repeatability.

CHAPTER 4. RESEARCH DESIGN AND METHODS

Methods

This study was based on data collected during the Nutritional Interventions for Age-Related Muscular Function and Strength Losses Study funded by the National Institutes of Health and Metabolic Technologies Inc. and carried out by researchers at Iowa State University. Further demographic data are described in more detail elsewhere; in brief, participants of this study met the following requirements: (i) were male or female at least 60 years of age; (ii) were free from liver and kidney diseases; (iii) were free of a history of blood clots and/or taking blood thinner medication; (iv) provided no evidence of uncontrolled hypertension; (v) had a body mass index (BMI) < 40 kg/m^2; (vi) were not taking >1,000 IU of vitamin D supplement daily prior to participation in the study; (vii) were free of any serious acute or chronic medical condition or illness that would affect ability to exercise or calcium and bone metabolism; (viii) had no prior history of osteoporosis diagnosis; (ix) free of having a major surgery in past 6 weeks and/or a minor surgery in past 3 weeks at baseline; and (x) had no evidence of uncontrolled diabetes mellitus, or type 1 diabetes mellitus requiring insulin for glucose control. The data utilized in this analysis were collected between August 2014 and July 2017, with participants recruited from the Des Moines and Ames, Iowa areas.

Design

This double blind randomized controlled trial design with a 1 year follow-up period is described in more detail elsewhere, but for the purpose of this study it is briefly outlined here. Participants meeting the above criteria were randomized into one of four treatments. Group 1 received the dietary placebo plus exercise program, group 2 received

the dietary placebo without exercise, group 3 received 2,000 IU of vitamin D + HMB 3.0 g/day without the exercise program, and group 4 received 2,000 IU vitamin D + HMB 3.0 g/day plus the exercise program. The exercise program consisted of a monitored strength training program 3 times per week for 1 year.

Measurements

Of the 235 males and females aged ≥ 60 years screened for the Nutritional Interventions for Age-Related Muscular Function and Strength Losses Study, 186 individuals provided baseline data consisting of: (i) height, weight, and BMI (Body Mass Index, kg/m^2); (ii) vital signs (heart rate and blood pressure); (iii) 30 mL of blood for a fasted baseline biochemical profile (glucose, calcium, blood lipid profiles, liver enzymes, and vitamin D status); (iv) a urinalysis; (v) body composition analysis including DEXA, BodPod, and BIA analyses as well as elbow breadth measurements; (vi) health, medical history, and subject information based off questionnaires; (vii) an assessment of functional mobility, balance, and agility using an Up-&-Go test and Get-Up test; (viii) and strength measurements provided by biodex testing and hand-grip strength analyses. While individuals were followed for a 1-year period with testing every 3 months, only baseline data will be utilized in this study as to not interfere with any current ongoing investigations.

Definition of Metabolic Syndrome

Slight variances exist for defining levels of criteria for clinical diagnosis MetS, but for the purposes of this study MetS was defined according to the most recent statement by the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria (10). According to this statement an individual with MetS must meet three or more of the following criteria: triglycerides ≥150 mg/dL, low HDL

cholesterol (Men <40 mg/dL and Women <50 mg/dL), elevated blood pressure ≥130/ ≥85 mmHg, abnormal fasting glucose ≥110 mg/dL, and abdominal obesity, given as waist circumference (Men >102 cm (40 in) and Women >88 cm (35 in). Waist circumference cut-offs were developed to identify abdominal obesity based on a series of studies in Caucasians. These cut-offs were not chosen based on risk factors, but rather their relation to BMI (29). Two action levels for waist circumference were developed in which a BMI >25 kg/m^2 (action level 1) translated to 94 cm for men and 80 cm for women, and a BMI >30 kg/m^2 (action level 2) translated to 102 cm for men, and 88 cm for women (30). In order to obtain a standardized cut off for abdominal obesity using DEXA measurements, the mean of NHANES 2005-2006 examination data on android fat mass was 1,719 g. Thus anything greater than this amount is considered to be a risk factor for MetS (31).

Data Analysis

The aim of the current study was to identify determinants of MetS in older adults. Therefore, the main outcome variable was the status, or degree of MetS as characterized by the MetS Score. Multiple risk factors comprise MetS; however, the typical diagnosis supplies only a yes or no answer regarding whether and individual has it. A large contribution of this study was to propose a MetS score that characterizes the degree of severity each subject suffers from the syndrome. The MetS score was developed using Microsoft Excel. Standardized z-scores and percentile rankings for each participant were calculated based off data from the CoLaus Study (32). Percentile rankings ranging from the 1st percentile to the 99th percentile for SBP, DBP, android fat mass, FG, TG, and HDL were summed together to create a score ranging from 6-594. Further statistical analyses were conducted using SigmaPlot 11.0 software. A Pearson correlation was estimated for

a number of demographic and metabolic variables to determine strength of linear association. These results are represented in **Tables 6A** and **6B**; a p-value <0.05 was considered statistically significant. A two-sample t-test was performed to compare characteristics between groups of men and women, as well as those with MetS versus those without. An additional t-test was performed to evaluate seasonal variation within the group. Participants who underwent baseline testing between the months of November and February were arbitrarily coded as 0 and those between the months of March and October were coded as 1. Finally, three backwards step-wise regression analyses were performed. Models 1, 2, and 3 included MetS score as the dependent variable, whereas Model 4 included serum 25(OH)D as the dependent variable.

CHAPTER 5. RESULTS

Demographic characteristics of the sample are show in **Table 2.** Of the 186 participants providing baseline data, gender was split almost evenly, with 54.8% of participants being males and 46.2% being females. Of those men and women, an overwhelming majority were non-Hispanic white or Latino individuals. The remainder of the sample consisted of 1% whom identified as Asian, 1% as black or African-American, 2% Hispanic, and 4% were of an unreported or unknown race and/or ethnicity.

Table 2. Demographic characteristics of sample

		Number (%)
Characteristic		
Age (n = 186)	60-69	128 (69)
	70-79	52 (28)
	80+	6 (3)
Sex (n = 186)	Female	84 (46.8)
	Male	102 (54.2)
Race	White	180 (97)
	Asian	2 (1)
	Black/African-American	2 (1)
	Unknown	2 (1)
Ethnicity	Non-Hispanic or Latino	177 (95)
	Hispanic	4 (2)
	Not reported	4 (2)
	Unknown	1 (1)

Anthropometric and metabolic characteristics of the study population are given in **Table 4** and **Table 5**. The mean age of the population was about 67 years and was shown to be relatively constant regardless of gender or MetS diagnosis. As expected, metabolic indicators tended to increase as the number of risk factors for MetS increased. It is important to note that in the case of fat distribution, those with the lowest amount of risk factors had a lower total amount of both android and gynoid fat mass, but tended to have a higher percent of gynoid fat distribution. This indicates that those with less risk factors for MetS tended to have less central adiposity, or in other words tended to have a “pear”

shaped distribution of body fat rather than an “apple” shape. This finding agrees with previous literature that central adiposity is linked to MetS (27, 28).

Furthermore, blood calcium measurements were extremely stable across all levels of MetS. This can be explained mechanistically; when the calcium in our blood drops too low, the parathyroid glands make more PTH. Increased PTH in turn stimulates the bones to release calcium into the blood resulting in stable blood calcium levels. Therefore, while calcium measurements were constant across all levels, PTH levels increased with the increasing number of risk factors indicating that those with more risk factors for MetS had lower levels of circulating blood Ca and thus relied more heavily on Ca drawn from bone stores. Serum 25(OH)D levels were also relatively constant across all levels of MetS, and could be a result of the small range of vitamin D status within our sample.

Further group differences between those with and without MetS, as determined by having 3 or more of the 5 previous mentioned risk factors, were evaluated using a two sample *t*-test. Android fat mass, fasting glucose, blood pressure, and triglycerides were all found to be lower, while HDL cholesterol levels were found to be higher in those without MetS. Statistically significant differences ($p < 0.001$) were recorded for all risk factors except for blood pressure and serum 25(OH)D levels.

Characteristic comparison between genders resulted in finding a much larger number of women without MetS ($n = 65$) than with MetS ($n = 19$), whereas men were more evenly distributed at 50 and 52 respectively. Another finding worth noting is that men tended to have higher central obesity than women regardless of MetS diagnosis. Furthermore, two-sample *t*-tests indicated that statistically significant differences existed

between males and females when comparing android fat mass and HDL cholesterol levels $p < 0.001$ and $p < 0.004$ were reported.

To investigate further the 25(OH)D levels within the population an additional two sample *t*-test was performed comparing those with vitamin D levels below and above 25 ng/mL. Of the 186 participants, 99 had vitamin D levels below 25 ng/mL and 87 had levels at or above 25 ng/mL. No significant differences betweetn groups was significant for any of the risk factors of MetS.

Pearson correlation coefficients between anthropometric measures or metabolic characteristics are shown in **Tables 6A** and **6B**. No association was found between MetS score and serum 25(OH)D levels. Factors such as average heart rate, weight, BMI, LDL:HDL ratio, android fat (%), gynoid fat mass, and gynoid fat (%) were found to have significant associations with the MetS score. When evaluating these findings it should be taken into consideration that android fat mass, total cholesterol, and HDL cholesterol comprise part of the MetS score.

To identify the strongest predictors of the MetS score, a backwards stepwise regression model was estimated. **Table 7A** shows results from Model 1 which includes only the 5 risk factors that comprise the MetS score. This regression model demonstrates that DBP, FG, and HDL cholesterol were most highly predictive of the overall MetS score. Table 7B (Model 2) includes several anthropometric measures and metabolic outcomes as well as the risk factors for MetS as independent variables. Because of the problem of endogeneity, it was not surprising to find that many of these variables as part of the final predictive equation, and resulted in a high R^2 value of 0.88, indicating that this model highly predictive of the MetS score. However, because MetS is comprised of

these five risk factors, the final model (Model 3) did not include them as independent variables. Thus android fat (%), gynoid fat (%), total cholesterol, VLDL cholesterol, and the LDL:HDL ratio were found to be most predictive of the MetS score, with an R^2 value of 0.46. Again, due tho the problem of endogenity between a variety of risk factors, another model was ran leaving out any interrelated risk factors that could contribute to the MetS score to be certain that Model 3 was in fact accurate. We found that even once any interrelated factors were left out, no other factors such as serum vitamin D, PTH, or Ca were left. Thus, we concluded that Model 3 was in fact an accurate prediction of the MetS score.

The season in which blood measurements were performed did not appear to influence serum vitamin D levels. A two-sample *t*-test evaluating those with baseline data collected in low sun months (November through February) compared to those with baseline data collected in high sun exposure months (October through March) concluded that there was no significant difference between serum vitamin D levels in either group.

Table 3. Criteria for metabolic syndrome among adults aged ≥ 60 years.

	Total	**MetS**	**No MetS**
Number (%)	186 (100)	114 (61)	72 (39)
Criterion			
Android Fat Mass (>1718.94 g)	163 (87.6)	91 (79.8)	72 (100)
HDL (M <40; F <50 mg/dl)	49 (26.3)	7 (6.1)	42 (58.3)
TG (≥150 mg/dl)	55 (29.6)	6 (5.3)	49 (68)
Fasting Glucose (>110 mg/dl)	32 (17.2)	6 (5.3)	26 (36.1)
Blood Pressure (≥130/85 mm/Hg)	140 (75.3)	76 (66.7)	63 (87.5)

Table 4. Baseline characteristics of participants classified by MetS diagnosis and stratified by the number of risk factors (RF) present

	Total (n=186)	*No MetS (n=114)*	*MetS (n=72)*	*0 RF (n=6)*	*1 RF (n=30)*	*2 RF (n=78)*	*3 RF (n=39)*	*4 RF (n=27)*	*5 RF (n=6)*
Age	67 ± 5	68 ± 5	68 ± 6	64 ± 3	69 ± 6	67 ± 5	69 ± 6	67 ± 5	71 ± 5
HR (bpm)	65 ± 12	64 ± 11	65 ± 13	64 ± 8	64 ± 7	64 ± 13	65 ± 10	65 ± 17	65 ± 9
SBP (mmHg)	140 ± 20	138 ± 22	143 ± 16	117 ± 10	129 ± 18	143 ± 23	144 ± 15	140 ± 15	146 ± 27
DBP (mmHg)	78 ± 11	77 ± 12	80 ± 10	67 ± 7	74 ± 8	79 ± 13	80 ± 9	81 ± 11	79 ± 10
Height (cm)	170.8 ± 10.1	169.5 ± 10.7	172.8 ± 8.7	162.2 ± 7.5	166.1 ± 10.4	171.5 ± 10.5	172.8 ± 9	172.9 ± 8.8	172 ± 7.2
Weight (kg)	85.1 ± 17.9	80.3 ± 19.0	92.6 ± 12.9	61.8 ± 7.8	72.7 ± 17.2	84.9 ± 18.5	91.7 ± 12.3	92.3 ± 13.0	99.5 ± 15.8
BMI (kg/m^2)	29.0 ± 6.6	27.8 ± 5.1	31.0 ± 4.0	24.1 ± 2.4	26.2 ± 5.0	28.7 ± 5.1	30.8 ± 4.3	30.9 ± 3.6	33.5 ± 4.0
Android (kg)	3.1 ± 1.1	2.8 ± 1.2	3.5 ± 0.9	1.4 ± 0.2	2.3 ± 1.0	3.1 ±1.1	3.4 ± 0.9	3.4 ± 0.9	3.8 ± 1.0
Gynoid (kg)	5.3 ± 1.5	5.2 ± 1.6	5.4 ± 1.4	4.3 ± 0.7	5.0 ± 1.6	5.4 ± 1.6	5.5 ± 1.7	5.1 ± 1.0	6.0 ± 1.3
Android (%)	44 ± 7	43 ± 7	46 ± 5	36 ± 6	41 ± 8	45 ± 6	46 ± 6	46 ± 5	47 ± 4
Gynoid (%)	41 ± 7	42 ± 8	40 ± 7	44 ± 6	44 ± 7	41 ± 8	40 ± 8	39 ± 6	43 ± 7
TC (mg/dL)	203 ± 39	204 ± 35	201 ± 44	225 ± 53	205 ± 31	202 ± 35	202 ± 42	206 ± 47	176 ± 46
LDL (mg/dL)	188 ± 34	118 ± 31	118 ± 38	137 ± 49	118 ± 25	117 ± 32	119 ± 32	123 ± 44	87 ± 26
VLDL (mg/dL)	25 ± 12	20 ± 9	34 ± 13	17 ± 8	19 ± 6	20 ± 9	29 ± 13	38 ± 10	45 ± 7
HDL (mg/dL)	59 ± 18	66 ± 18	48 ± 12	72 ± 14	69 ± 17	65 ± 19	53 ± 12	45 ± 12	32 ± 5
LDL:HDL	2.2 ± 0.8	1.9 ± 0.8	2.5 ± 0.8	2.0 ± 1.0	1.8 ± 0.7	2.0 ± 0.8	2.3 ± 0.8	2.8 ± 0.8	2.8 ± 0.7
TG (mg/dL)	127 ± 65	98 ± 43	174 ± 68	83 ± 30	92 ± 28	101 ± 43	150 ± 65	189 ± 65	259 ± 88
FG (mg/dL)	100 ± 16	96 ± 9	108 ± 22	91 ± 8	94 ± 8	97 ± 9	100 ± 13	115 ± 29	126 ± 15
25D(ng/mL)	23.8 ± 4.1	23.9 ± 4.2	23.7 ± 4.1	24.8 ± 3.3	23.1 ± 4.1	24.2 ± 4.3	24.7 ± 3.8	22.9 ± 4.1	21.1 ± 4.5
PTH (pg/mL)	45.5 ± 20.5	43.6 ± 17.5	48.8 ± 24.5	35.2 ± 5.2	42.0 ± 15.8	44.9 ± 18.6	43.6 ± 24.2	51.9 ± 22.7	56.7 ± 20.2
Ca (mg/dL)	9.4 ± 0.3	9.4 ± 0.3	9.4 ± 0.3	9.4 ± 0.3	9.3 ± 0.3	9.4 ± 0.4	9.3 ± 0.3	9.48 ± 0.3	9.4 ± 0.3

Data are expressed as means ± standard deviations. Abbreviations: HR (heart rate), SBP (average systolic blood pressure), DBP (average diastolic blood pressure), BMI (body mass index), TC (total cholesterol), LDL (low density lipoprotein), VLDL (very low density lipoprotein), HDL (high density lipoprotein), TG (triglycerides), FG (fasting glucose), 25D (25-hydroxy vitamin D), PTH (parathyroid hormone), Ca (calcium).

Table 5. Baseline characteristics of participants classified by gender

	Female (n=84)	*Male (n=102)*	*Females No MetS (n=65)*	*Females MetS (n=19)*	*Males No MetS (n=50)*	*Males MetS (n=52)*
Age	68 ± 6	68 ± 5	68 ± 6	69 ± 6	67 ± 5	68 ± 5
HR (bpm)	66 ± 11	63 ± 12	65 ± 12	70 ± 7	63 ± 9	63 ± 14
SBP (mmHg)	139 ± 25	140 ± 16	137 ± 27	147 ± 13	139 ± 14	141 ± 17
DBP (mmHg)	78 ± 13	79 ± 10	76 ± 14	83 ± 9	79 ± 9	79 ± 10
Height (cm)	162.7 ± 6.2	177.4 ± 7.5	162.6 ± 6.4	163.1 ± 5.6	178.4 ± 8.3	176.5 ± 6.5
Weight (kg)	74.1 ± 15.0	94.1 ± 14.7	70.4 ± 13.4	86.4 ± 13.9	93.0 ± 17.5	95.2 ± 11.5
BMI (kg/m^2)	28.1 ± 5.6	39.9 ± 4.3	26.7 ± 5	32.6 ± 5.3	29.2 ± 5	30.6 ± 3.4
Android Fat (kg)	2.7 ± 1.1	3.4 ± 1.1	2.3 ± 1.0	3.4 ± 1.0	3.3 ± 1.2	3.5 ± 0.9
Gynoid Fat (kg)	5.7 ± 1.7	4.9 ± 1.3	5.4 ± 1.6	6.6 ± 1.6	4.9 ± 1.3	4.9 ± 1.1
Android Fat (%)	45 ± 7	43 ± 6	44 ± 7	50 ± 4	42 ± 7	44 ± 5
Gynoid Fat (%)	47 ± 5	36 ± 5	47 ± 6	50 ± 4	36 ± 6	36 ± 4
TC (mg/dL)	202 ± 34	203 ± 42	201 ± 35	206 ± 31	207 ± 34	200 ± 48
LDL (mg/dL)	115 ± 29	121 ± 37	115 ± 31	116 ± 23	122 ± 32	119 ± 42
VLDL (mg/dL)	24 ± 11	26 ± 13	20 ± 8	35 ± 14	19 ± 10	33 ± 12
HDL (mg/dL)	64 ± 19	56 ± 17	66 ± 20	54 ± 14	66 ± 16	46 ± 11
LDL:HDL	2.0 ± 0.7	2.3 ± 0.9	1.9 ± 0.7	2.3 ± 0.7	2.0 ± 0.8	2.6 ± 0.8
TG (mg/dL)	117.7 ± 56.0	134.6 ± 71.4	100.6 ± 37.3	176.3 ± 69.6	96.4 ± 52.1	171.4 ± 52.6
FG (mg/dL)	100.2 ± 17.9	100.5 ± 14.8	95.9 ± 10.0	115.1 ± 28.7	96.4 ± 8.9	104.4 ± 18.1
25(OH)D (ng/mL)	23.8 ± 4.1	23.9 ± 4.2	23.6 ± 4.1	24.3 ± 4.2	24.2 ± 4.3	23.6 ± 4.1
PTH (pg/mL)	41.4 ± 14.4	48.6 ± 23.6	41.5 ± 14.5	41 ± 14.6	46 ± 20.4	51.1 ± 26.5
Ca (mg/dL)	9.4 ± 0.4	9.4 ± 0.3	9.4 ± 0.4	9.3 ± 0.4	9.4 ± 0.3	9.4 ± 0.3

Data are expressed as means ± standard deviations. Abbreviations: HR (heart rate), SBP (average systolic blood pressure), DBP (average diastolic blood pressure), BMI (body mass index), TC (total cholesterol), LDL (low density lipoprotein), VLDL (very low density lipoprotein), HDL (high density lipoprotein), TG (triglycerides), FG (fasting glucose), PTH (parathyroid hormone), Ca (calcium).

Table 6A. Correlation coefficients between MetS Score and a variety of anthropomentric and metabolic risk factors

	MetS	Age	Height	Weight	BMI	HR	SBP	DBP	FG	TG
MetS Score	--									
Age	0.11	--								
Height (cm)	-0.02	-0.10	--							
Weight (kg)	0.29***	-0.001	0.59***	--						
BMI (kg/m^2)	0.38***	0.04	-0.006	0.55***	--					
HR (bpm)	0.16*	0.07	-0.01	0.06	0.02	--				
SBP (mmHg)	0.12	0.25***	0.01	0.05	0.01	0.12	--			
DBP (mmHg)	0.13	-0.09	0.09	0.18	0.07	0.25***	0.57***	--		
FG (mg/dL)	0.36***	0.11	-0.008	0.06	0.03	0.11	0.04	0.05	--	
TG (mg/dL)	0.57***	-0.001	0.08	0.24**	0.20*	-0.06	-0.09	-0.09	0.31***	--
TC (mg/dL)	0.13	-0.07	-0.03	-0.07	0.06**	-0.10	-0.04	-0.04	-0.09	0.14*
LDL (mg/dL)	-0.03	-0.11	0.02	-0.08	0.04	-0.13	-0.07	-0.06	-0.08	0.07
VLDL (mg/dL)	0.07	-0.009	0.06	0.20**	0.18	0.06	-0.12	-0.09	0.32***	0.99***
HDL (mg/dL)	-0.48***	0.05	-0.15*	-0.17*	-0.09	0.06	0.12	0.09	-0.26***	-0.5***
LDL:HDL	-0.22**	-0.12	0.12	0.06	0.09	-0.14	-0.14	-0.1	0.11	0.45***
25(OH)D (ng/mL)	-0.02	-0.04	-0.004	0.08	0.12	0.05	0.01	-0.05	-0.19*	-0.03
PTH (pg/mL)	0.03	0.10	0.12	0.10	-0.02**	-0.06	0.10	-0.03	0.04	0.12
Ca (mg/dL)	0.09	0.14	-0.04	-0.004	0.01	0.03	0.63	-0.02	0.18*	0.12
Android (g)	0.28***	0.01	0.34***	0.80***	0.51	0.03	0.09	0.18*	0.07	0.24**
Gynoid (g)	0.34***	-0.002	-0.03	0.51***	0.46***	0.15*	0.01	0.11	-0.03	0.02
Android (%)	0.47***	-0.03	-0.11	0.39***	0.42***	-0.03	0.04	0.13	0.09	0.17
Gynoid (%)	0.20**	0.03	-0.51***	-0.11	0.20***	0.15	-0.06	-0.01	-0.04	-0.11

*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$. Abbreviations: BMI (body mass index), HR (heart rate), SBP (average systolic blood pressure), DBP (average diastolic blood pressure), FG (fasting glucose), TG (triglycerides), TC (total cholesterol), LDL (low density lipoprotein), VLDL (very low density lipoprotein), HDL (high density lipoprotein), PTH (parathyroid hormone), Ca (calcium).

Table 6B. Correlation coefficient between MetS Score and a variety of anthropometric and metabolic risk factors

	TC	LDL	VLDL	HDL	LDL:HDL	25(OH)D	PTH	Ca	Andriod Mass	Gynoid Mass	Android %
TC (mg/dL)	--										
LDL (mg/dL)	0.92***	--									
VLDL (mg/dL)	0.11	0.05	--								
HDL (mg/dL)	0.33***	0.04	-0.53***	--							
LDL:HDL	0.43***	0.67***	0.43***	-0.63***	--						
25(OH)D (ng/mL)	-0.06	-0.10	0.04	0.14	-0.13	--					
PTH (pg/mL)	-0.11	-0.11	0.13	-0.11	0.04	-0.36***	--				
Ca (mg/dL)	0.13	0.08	0.17*	0.39	0.01	0.04	-0.14	--			
Android (g)	-0.04	-0.07	0.19**	-0.13	0.03	-0.01	0.09	-0.04	--		
Gynoid (g)	-0.06	-0.13	-0.003	0.08	-0.16*	0.06	-0.02	-0.06	0.96***	--	
Android (%)	0.01	-0.04	0.14	-0.03	-.001	-0.07	0.47	0.01	0.96***	0.98***	--
Gynoid (%)	-0.05	-0.13	-0.11	0.22**	-0.24**	-0.02	-0.09	-0.05	0.92***	0.98***	0.99***

*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$. Abbreviations: TC (total cholesterol), LDL (low density lipoprotein), VLDL (very low density lipoprotein), HDL (high density lipoprotein), PTH (parathyroid hormone), Ca (calcium).

Table 7A. Backwards stepwaise regression analysis predicting MetS score (Model 1)

Dependent Variable	Independent Variable	Coefficient	Std. Coefficient	Std. Error	*p-value*
MetS Score	Average SBP	0.99	0.30	0.14	<0.001
	Average DBP	1.98	0.33	0.26	<0.001
	Android Fat Mass	0.03	0.44	0.002	<0.001
	Fasting Glucose	1.08	0.26	0.15	<0.001
	HDL Cholesterol	1.27	0.34	0.15	<0.001
	Triglycerides	0.29	0.27	0.04	<0.001

Final Equation: MetS score = -305.00 + (0.99 SBP) + (1.98 DBP) + (0.03 Android fat) + (1.08 FG) + (1.27 HDL) + (0.29 TG)

Table 7B. Backwards stepwaise regression analysis predicting MetS score (Model 2)

Dependent Variable	Independent Variable	Coefficient	Std. Coefficient	Std. Error	*p-value*
MetS Score	Average SBP	1.40	0.38	0.13	<0.001
	Average DBP	2.57	0.38	0.24	<0.001
	Android Fat Mass	0.03	0.39	0.01	<0.001
	Fasting Glucose	0.85	0.22	0.15	<0.001
	Average Height	-0.86	-0.13	0.40	0.033
	Average Weight	0.97	0.25	0.49	0.051
	Gynoid Fat Mass	-0.01	-0.27	0.01	0.036
	Gynoid % Fat	2.19	0.24	1.06	0.042
	Total Cholesterol	0.44	0.25	0.05	<0.001
	VLDL Cholesterol	1.38	0.23	0.26	<0.001
	LDL:HDL Ratio	-26.67	-0.33	3.52	<0.001

Final Equation: MetS score = -297.97 + (1.40 SBP) + (2.57 DBP) + (0.03 Android fat) + (0.85 FG) + (-0.86 Ht.) + (0.97 Wt.) + (-0.01 Gynoid %) + (0.44 TC) + (1.38 VLDL) + (-26.67 LDL:HDL) R^2:0.88

The following variables did not significantly add to the ability of the equation to predict MetS Sscore adnwere not indluced in the final equation: age, TG, HDL cholesterol, average HR, BMI, android % fat, LDL cholesterol, serum PTH, calcium, serum 25(OH)D.

Table 7C. Backwards stepwise regression analysis predicting MetS score (Model 3)

Dependent Variable	Independent Variable	Coefficient	Std. Coefficient	Std. Error	*p-value*
MetS Score	Android Fat (%)	6.87	0.61	0.93	<0.001
	Gynoid Fat (%)	-2.55	-0.28	0.77	0.001
	Total Cholesterol	0.49	0.28	0.13	<0.001
	VLDL Cholesterol	2.02	0.33	0.48	<0.001
	LDL:HDL	-43.4	-0.54	7.10	<0.001
Final Equation: MetS score = 33.44 + (6.87 Android Fat %) + (-2.55 Gynoid Fat %) + (0.49 TC) + (2.02 VLDL) + (-43.41 LDL:HDL)					R^2:0.46

The following variables did not significantly add to the ability of the equation to predict MetS score and were not included in the final equation: age, average HR, average height, average weight, BMI, gynoid fat mass, LDL cholesterol, serum PTH, calcium, and serum 25(OH)D.

Table 8. Backwards stepwise regression analysis predicting serum 25(OH)D levels

Dependent Variable	Independent Variable	Coefficient	Std. Coefficient	Std. Error	p-value
Serum 25(OH)D	Android Fat Mass	-0.002	-0.55	0.001	0.002
	Average Height	0.08	0.21	0.04	0.031
	BMI	0.52	0.60	0.15	<0.001
	PTH	-0.07	-0.36	0.02	<0.001
Final Equation: Serum 25(OH)D = 4.20 (-0.002 Android Mass) + (0.08 Ht.) + (0.52 BMI) + (-0.07 PTH)					R^2: 0.21

The following variables did not significantly add to the ability of the equation to predict serum 25(OH)D and were not included in the final equation: MetS score, age, average DBP, FG, TG, HDL cholesterol, average HR, average weight, gynoid fat mass, android % fat, gynoid % fat, TC , VLDL cholesterol, LDL cholesterol, LDL:HDL, and calcium.

CHAPTER 6. DISCUSSION

The major finding in this study was that the combination of android fat (%), gynoid fat (%), total cholesterol, VLDL cholesterol, and the LDL:HDL ratio were predictive of the MetS score with a multiple regression coefficient of 33.44 and a standard error of the estimate of 50.34 (Model 3). While there is a certain extent of interrlatedness between the risk factors that comprise the MetS score and the independent variables that were found to be predictive of MetS, it is worth noting that body fat appears to play an important role in predicting MetS.

Vitamin D was excluded from the model and therefore the hypothesis that vitamin D status would explain a significant portion of the variance in MetS was rejected. However, the android fat mass, average height, BMI, and PTH were all predictive of serum 25(OH)D in a stepwise backward regression model. Previous literature has indicated that large amounts of fat mass could lead to sequestration of the fat soluble vitamin D, and thus lower serum 25(OH)D concentrations (6, 22, 24). The finding that android fat mass, but not gynoid fat mass was predictive of 25(OH)D takes this a step further by indicating that distribution of fat may be more predictive of health outcomes than just fat mass alone.

Therefore, while no significant correlation between vitamin D and MetS score was detected, previous literature has demonstrated obesity and low HDL levels to be associated with vitamin D deficiency, in fact the risk of vitamin D deficiency was almost double among obese adults compared to normal weight adults (40). Similar findings have been reported worldwide. Research on 3,100 women in northeast Scotland found that those with an average BMI of 34 kg/m^2 produced 10% less vitamin D than those of

average weight (41). In Spain it was reported that that over half of those with a BMI ≥ 40 kg/m^2 were diagnosed with vitamin D deficiency (42). BMI was also significantly correlated with 25(OH)D concentrations after adjusting for insulin-sensitivity, HDL cholesterol, LDL cholesterol, total cholesterol, and triglycerides in a study performed in Italy (43).

The relationship between vitamin D deficiency and obesity is still unknown, and it is not clear whether vitamin D deficiency causes obesity or the other way around. However, a possible mechanism could be that excess fat tissue could absorb and retain vitamin D, and thus reduce circulatory vitamin D available to the body (40). The absence of vitamin D could also create interference with the functioning of a hormone called leptin, which signals the brain when the stomach is full, therefore, stop eating. Additionally there is the possibility that overweight people may tend to spend more time indoors and receive less ultraviolet rays from sun.

The association between vitamin D deficiency and weight could also be a result of the link between vitamin D and MetS, as overweightness was shown to be a major component of MetS. Previous studies have shown an inverse relationship between vitamin D concentrations and the prevalence of the metabolic syndrome, including insulin resistance, high total cholesterol and triglyceride levels, low HDL cholesterol level, and high blood pressure (44, 45, 46). The onset of T2DM, a common consequence of metabolic syndrome, has also been associated with vitamin D deficiency in several studies (41, 46). This is thought to be because vitamin D has effects on insulin action that may impact several pathways important in the development of T2DM (40).

The small range of serum 25(OH)D levels within the sample population could have contributed to the lack of significant associations detected between vitamin D status and the MetS score in this group. Due to the recruitment process of the Nutritional Interventions for Age-Related Muscular Function and Strength Losses Study, participants who provided baseline data were required to have serum vitamin D levels between 18 ng/mL and 30 ng/mL at the time of screening. While research has shown that older adults tend to have lower serum vitamin D levels, our data may not be entirely representative of the older adult population as a whole (21,33).

Results of this study differ from other recent reports evaluating vitamin D status on metabolic syndrome in the elderly. In a study embedded within the Rotterdam Study, serum 25(OH)D concentrations were found to be inversely associated with the prevalence of MetS (33). This study was a population-based cohort of 3,240 middle-aged and elderly adults (median age 71.2 years), free of T2DM at baseline. The association they found between vitamin D and MetS appeared to be primarily driven by elevated waist circumference, but was also inversely associated with the prevalence of elevated serum TG, reduced HDL cholesterol levels, and elevated fasting glucose levels.

It is interesting to note that no significant association was found between serum 25(OH)D and blood pressure in the Rotterdam Study; however, vitamin D has been implicated in controlling the production of renin, one of the most important hormones for regulating blood pressure (3,7, 34). The results of this study indicated no significant association between either SBP or DBP and vitamin D or MetS; however, **Table 3**. indicates that hypertension and android fat mass were the two largest contributing risk factors to MetS in this study population. A large majority (75.3%) of the population suffered from blood

pressure>135/80 mmHg. Therefore, while high blood pressure was extremely prevalent in this population with low vitamin D status, no statistically significant associations between the two factors could not be concluded.

Addional studies have previously found an inverse association between vitamin D status and MetS, but were typically observed in younger populations (35, 36, 37, 38). Within a population of older adults, the LASA study found a significant association between higher serum 25(OH)D (>50nmol/L) and lower prevalence of MetS. Similar to the Rotterdam Study, the LASA study found HDL cholesterol and waist circumference to be significant predictors of vitamin D status.

Furthermore, it is important to point out the variability in data sources utilized for calculation of the MetS score. The initial goal was to obtain NHANES data of adults aged 60 years or older for all five risk factors of MetS in order to develop a comparative standard for population. However, data separated out by age or gender was found to be unavailable through this dataset. The NHANES database included data from males and females aged 8-69 years old. Therefore, BP, FG, HDL cholesterol, and TG standardized values were all based off data from the CoLaus study (32). Data from this study was collected by a random sample of 6,188 extensively phenotyped Caucasian subjects (3,251 women and 2,937 men) aged 35 to 75 years living in Lausanne, Switzerland. Because the population of the current study is also extensively Caucasian, similar characteristics between populations could be expected. Unfortunately, due to the limited amount of large population based DEXA results, the comparative standard for android fat mass utilized in this study was based off the mean of the 2005-2006 NHANES dataset (31). While this limitation was recognized, it is likely that this only resulted in a small variation in the

mean value for android fat mass. Thus, when compiled with the other four risk factors making up the total overall score, it is probable that this had little impact on the overall results. At any rate, it is more representative than utilizing the mean of our own refined population of older adults as a comparative standard. It should be mentioned that this same dataset was used to determine a cutoff point for the diagnosis of central adiposity when determining MetS status within our population as well.

In regards to the demographic characteristics of our study population, 97% of this sample was white with 95% of the population identifying as non-hispanic or latino as shown in **Table 2**. According to NCEP/ATP III guidelines and based on NHANES 2005-2006 data, a little more than one-third of the adults in the United States meet the criteria for MetS diagnosis (39). In an effort to determine the correlates of vitamin D deficiency, researchers found that vitamin D deficiency was common in U.S. adults, especially among minority groups (40). Using serum 25(OH)D concentrations $\leq$ 20 ng/mL as a cut off, they found that over 80% of black adults, both men and women, would be categorized as vitamin D deficient. Compared with white adults, other minorities were also at a higher risk for vitamin D deficiency, especially Hispanic men. Because the skin pigment melanin absorbs sunlight, people of color are at particularly high risk for vitamin D deficiency (22, 40). The association between race and vitamin D deficiency may be related to several factors. Sun exposure is the primary determinant of vitamin D status and non-whites require more sunlight exposure to obtain adequate vitamin D levels because of skin pigmentation. Another possible explanation could be because of the different dietary patterns, particularly the intake of dairy products in different population groups (13, 14, 40).

Furthermore, the prevalence of metabolic syndrome also varied by race and ethnicity (39). Non-Hispanic black males were less likely than non-Hispanic white males to have metabolic syndrome, but non-Hispanic black and Mexican American females were more likely than non-Hispanic white females to have it. Among the five diagnostic criteria for MetS abdominal obesity, hypertension, and hyperglycemia were the most prevalent. Thus the limited racial and ethnic variation within the sample of this study, prevented further examination into many of these variables.

Moreover, this study was based on a one-time measurement of vitamin D, thus it could not show the variation of vitamin D concentration during different seasons. The two sample *t-test* conducted evaluating differences between vitamin D levels based on month of blood draw was simply a snapshot in time. It in turn tells us more about the difference between two people rather than a difference in overall seasonal variation within the population. If participants were evaluated overtime seasonal variations would likely have been detected; however, due to the nature of the ongoing study it could not be analyzed at this time.

Major Strengths and Limitations

The strengths of this study were the large sample size, the use of DEXA in the evaluation of android and gynoid fat distribution, and the propostion of a collective MetS score to determine the severity of metabolic syndrome. An existing issue in clinical diagnoiss of MetS is that MetS is simply a compilation of risk factors with no clear agreed upon diagnosis. While the NCEP ATP III criteria used in this study is most broadly used in the medical field, this diagnosis fails to indicate any degree of severity (11). A standardized score that communicates degree of severity to patients could have

profound impacts on public comprehension of current disease state, and their motivation to comply with health recommendations.

On the other hand, the cross-sectional design of the study permits only an examination of associations between vitamin D and MetS, no causal relationship can be established. Furthermore, there is no way to establish whether MetS or low vitamin D status preceeded the other. Therefore the question remains, does low vitamin D status cause MetS, or does MetS cause low vitamin D status.

Conclusion

This study did not find a statistically significant association between low vitamin D status and prevalence of MetS as represented by the MetS score likely due to the limited range of vitamin D levels within the sample. However, the combination of android fat (%), gynoid fat (%), total cholesterol, VLDL cholesterol, and LDL:HDL ratio were predictive of the MetS score. While limitations of this study prevented significant association between vitamin D and MetS to be detected, previous literature indicates that older adults may benefit from higher serum 25(OH)D, especially women who are at greater risk for osteoporosis and bone deterioration. However, the causality between vitamin D status and MetS still needs to be investigated. Therefore, randomized controlled supplementation trials are needed.

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