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25-Hydroxyvitamin D, IGF-1, and Waist Circumference A Cross-Sectional Study

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25-HYDROXYVITAMIN D, IGF-1, AND WAIST CIRCUMFERENCE A CROSS-
SECTIONAL STUDY

By

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Bachelor of Science in Nutrition Sciences
University of Nevada Las Vegas
2011

A thesis submitted in partial fulfillment
of the requirements for the

Masters of Science - Exercise Physiology

Department of Kinesiology and Nutrition Sciences
School of Allied Health Sciences
Division of Health Sciences
The Graduate College

University of Nevada, Las Vegas
August 2014

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THE GRADUATE COLLEGE

We recommend the thesis prepared under our supervision by

Damon McCune

entitled

25-Hydroxyvitamin D, IGF-1, and Waist Circumference A Cross-Sectional Study

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ABSTRACT

25-Hydroxyvitamin D, IGF-1, and Waist Circumference A Cross-Sectional Study

by

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Purpose: Waist circumference has been shown to have a link to various forms of chronic disease and low levels of Vitamin D (25(OH)D) and IGF-1. The purpose of this study is to assess if there is a relationship with Vitamin D (25(OH)D) and IGF-1 levels and waist circumference.

Methods: As part of a cross-sectional correlation study, participants were males and females (n=26), ages 25-65, who were students at UNLV and/or individuals who attend local health and wellness facilities as well as attendees of classes held by the UNLV Nutrition Center. Participants reported to UNLV to review and sign informed consent and submit a finger stick blood draw under sterile conditions as well as resting blood pressure and waist circumference. The finger stick blood draw sample was used to obtain serum 25(OH)D and IGF-1 levels using ELISA kits. Participants completed food frequency questionnaires asking about eating habits prior to study and surveys were administered to determine level and type of physical activity. Participants then reported to a local Quest Diagnostics at their convenience to obtain a fasting comprehensive metabolic panel. Data were collected and evaluated using Microsoft Excel and SPSS software.

Results: Subjects were separated into two groups according to waist circumference (Female >35", Male >40") Data reflected no significant difference between large waist and normal waist circumference groups in regards to both IGF-1 and 25(OH)D. Data did reflect, however, that a significant relationship between IGF-1 and waist circumference did exist ($P=.006$). Although, a significant relationship between 25(OH)D and waist circumference did not exist.

Conclusion: Waist circumference has an inverse relationship to IGF-1. Abdominal obesity may cause a reduction in the amount of free IGF-1 produced by the body which could increase risk for chronic disease. Obtaining waist circumference as well as activity level is relatively convenient in medical treatment settings and could provide clinicians with more valuable information regarding patient's level of risk for disease.

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CHAPTER 1

INTRODUCTION

Risk of Abdominal Obesity

Waist circumference has been shown to be reflected to many aspects of adverse health conditions including cardiovascular disease (CVD), hypertension, hyperlipidemia, and diabetes mellitus. Since CVD is the leading cause of morbidity and mortality in many countries worldwide, the high correlation to waist circumference and risk of CVD needs to be further investigated.¹

Conditions Associated with Abdominal Obesity-Metabolic Syndrome

Waist circumference has also been linked to indicators of Metabolic Syndrome. Metabolic syndrome is the co-occurrence of the health conditions previously mentioned related to waist circumference. While this body of work will not be focusing on all indicators of Metabolic Syndrome, understanding the conditions associated with Metabolic Syndrome and how those relate to waist circumference is crucial. To assess Metabolic Syndrome, it is important to understand that it is not a condition defined by one disease condition but rather a cluster of several co-occurring cardiovascular risk factors that significantly increase an individual's risk for a cardiovascular event. These factors include; insulin resistance, hypertension, atherogenic dyslipidemia, and obesity (mainly abdominal obesity).¹ Although these factors all have individual parameters for diagnosis and individual health threats, when combined they pose increased risk for a cardiovascular event to occur even if they only carry a borderline diagnosis. All may be controlled through dietary and lifestyle changes.

Insulin resistance is a condition in which the body produces insulin but does not utilize it properly. The biochemistry of this action can be described partially by the binding of insulin mainly to insulin receptors on cells and also through insulin like growth factor-1 (IGF-1) receptors. IGF-1, a polypeptide hormone, plays a role in proliferation and differentiation of cells and has apoptosis properties. These traits may be the reason it has a relationship to chronic diseases. Insulin sensitivity can be adversely altered due to obesity, in particular abdominal obesity.² Cellular actions of insulin depend on a wide variety of effects on post receptor signaling pathways within target cells. The beta subunit of the insulin receptor is a tyrosine kinase, which is activated when insulin binds to the alpha subunit. The kinase autophosphorylates and mediates multiple activities of insulin. Insulin receptor concentration or affinity may be altered due to obesity, physical inactivity, ambient insulin levels, various physiological and disease states, and drugs. The secretion of insulin and insulin sensitivity are reciprocally related which means that insulin resistance results in an increase in insulin secretion to maintain normal glucose and lipid homeostasis. There is also an inverse association between serum 25(OH)D and insulin resistance. Some researchers hypothesize that the insulin resistance is caused by low levels of circulating 25(OH)D, while other researchers believe that obesity, in particular central obesity, is the cause of both low levels of circulating 25(OH)D and insulin resistance.²⁻³

The final component of metabolic syndrome is obesity. Some believe this to be the cause and others find it as a byproduct to the other conditions associated. Regardless of the view of this condition, it plays a major role in the risk and systemic

pathophysiology of cardiovascular disease. It should be noted that having too much body fat is considered different from being overweight, or simply weighing too much. The most common measurement of obesity is the Body Mass Index (BMI), and is a measurement of height (in meters)/weight (in kilograms²). A normal range is between 18-25 m/kg², overweight is considered between 25-29.9 m/kg², obese is considered over 30 m/kg², and underweight is below 18 m/kg². Recent literature has introduced a modified approach to also include abdominal obesity to the measurement by including a waist circumference measurement. This method is referred to as the Body Shape Index (ABSI), and was introduced by Nir Krakauer, et al. The ABSI formula is $WC/(BMI^{2/3}Height^{1/2})$.¹

Population at Risk

An accumulation of body fat, usually twenty percent or more over an individual's ideal body weight is also considered to make an individual obese and increases risk for chronic disease. The accumulation of excess body fat in the abdominal area in particular also increases risk for cardiovascular events and chronic disease. The cause of this condition is simply consuming more energy than is expended by the body. While the cause of this condition is simple and easy to counteract, gaining compliance from patients seems to be one of the most difficult tasks facing medical professionals. ⁴

Clinical Indicators of Metabolic Syndrome

The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria was used to define large waistline and high fasting glucose. The

NCEP-ATP III defines criteria of metabolic syndrome as any three of the following traits in the same individual: ³

- Large Waistline – Men: 40 inches (102cm) or more, and women: 35 inches (88cm) or more.
- High Triglycerides – 150 mg/dL or more
- Low HDL Cholesterol – Men: 40 mg/dL or less, and women: 50 mg/dL or less
- High Blood Pressure – 130/85 mmHg or more
- High Fasting Glucose – 110 mg/dL or more

It has been shown that individuals borderline on these indicators also show an increased risk for a cardiovascular event as well as those with extremely high values. Also, according to the American Heart Association estimated \$475.3 billion cost for cardiovascular disease and stroke in the United States for 2009.⁵

Impact on Organs

Organs usually affected by metabolic syndrome include, but are not limited to; heart, brain, pancreas, kidneys, lungs, liver, and arteries. The main organ impacted by the condition would be the heart. Due to the increase in resistance of blood flow in arteries, cardiac output is decreased thus forcing the heart to work harder. This is compounded by the fluid retention that is common in those who are obese or suffer from kidney complications which further increases strain to the heart. To further add to this strain, liver complications can further increase dyslipidemia in patients which also compounds these previously mentioned complications to the heart. Finally, possibly at the forefront of the complications, pancreatic dysfunction or insulin resistance does not allow for the utilization of

glucose and lipids properly. Lack of glucose to supply the brain with usable fuel can lead to dysfunction systemically in the body including respiratory dysfunction and all of these systemic complications, when co-occurring, compound each other and further impose strain on the heart until it can no longer handle the load.

Individuals that meet criteria of metabolic syndrome are usually found to be over-nourished and have a large waist circumference. These individuals are generally not deficient in nutrients, although acute deficiency may occur depending on lifestyle or secondary conditions. Among these deficiencies, vitamin D deficiency seems to be one of the most prevalent conditions. These acute deficiencies are easily manageable when compared to combatting the over-intake of macronutrients and energy.⁴

The purpose of this study is to explore the relationship between 25-hydroxyvitamin D (25(OH)D), IGF-1, and waist circumference.

Question 1: Is there a relationship that exists between 25(OH)D and waist circumference?

Ho: Serum 25(OH)D levels have no association with waist circumference

H1: Serum 25(OH)D levels will be inversely associated with waist circumference.

Question 2: Is there a relationship that exists between IGF-1 and waist circumference?

Ho: Serum free IGF-1 levels have no association with waist circumference.

H1: Serum free IGF-1 levels will be inversely associated with waist circumference.

Question 3: Do individuals with large waist circumference have lower levels of serum IGF-1 than those with normal waist circumferences?

Ho: Individuals with large waist circumference will not have a lower level of serum IGF-1 than those with normal waist circumference.

H1: Individuals with large waist circumference will have a lower level of serum IGF-1 than those with normal waist circumference.

Question 4: Do individuals with large waist circumference have lower levels of serum 25(OH)D than those with normal waist circumference?

Ho: Individuals with large waist circumference will not have a lower level of serum 25(OH)D than those with normal waist circumference.

H1: Individuals with large waist circumference will have a lower level of serum 25(OH)D than those with normal waist circumference.

Recently, several studies have supported dietary and lifestyle approaches for reducing risk factors related to cardiovascular disease (CVD) that provide information as to why maintaining a healthy waist circumference is vital.

CHAPTER 2

REVIEW OF LITERATURE

Body Shape Index Validity

Recently, researchers have sought to produce a formula to include some form of body composition to the Body Mass Index (BMI) even in complex settings when conventional tools of measurement may not be accessible. This measurement could provide clinicians with a more accurate depiction of risk in patients since the BMI does not always reflect accurately on individuals whose weight is primarily skeletal muscle with little body fat. Nir Krakauer, et al, introduced the Body Shape Index (ABSI) to provide a useful and practical tool for those situations where patients are non-ambulatory conventional composition tools are not available.¹

The World Health Organization (WHO) rank weight and obesity fifth among causes of death worldwide. Validity of BMI-based obesity guidelines has come into question since BMI does not distinguish between muscle and fat mass and accumulation. Abdominal fat accumulation has been shown to increase risk of disease more so than accumulation of fat in other areas of the body. This reflection of increased risk has projected waist circumference (WC) as the leading compliment to BMI.¹

Research was focused on individuals using outcome data from the National Health and Nutrition Examination Survey (NHANES) 1999-2004. Fourteen thousand, one hundred and five individuals were sampled of all ages, races, and gender. Z scores were calculated to control for age and gender. A fraction of

population mortality hazard from high values was calculated and uncertainty in this expression was approximated only to uncertainty in the numerator.¹

Krakauer, et al, followed up this initial research with a follow up to account for limitations identified in the NHANES sample. Waist to hip ratio (WHR) and waist to height ratio (WHtR) was not directly evaluated in initial sample. Also, anthropometrics measurements were only taken on one occasion so there was no way to assess ABSI over time. Follow up with participants was also relatively short at five years which further raises cause for concern of accuracy of ABSI over time. This body of work expanded samples to include information obtained from the Health and Lifestyle Survey (HALS). Researchers introduced HALS data to analysis methods, compared calculations to widely used anthropometric measures including WHR, and evaluated mortality as a function of change spaced 7 years apart. This research concluded that ABSI outperformed other measures of abdominal obesity as an indicator of mortality. Also, ABSI was a consistent predictor of mortality over at least 20 years of follow up.²

Other researchers took notice to this new and seemingly effective formula for obtaining risk of disease and applied it to populations in China to see if the calculation could predict new onset diabetes mellitus (DM) in the general population. Sen He, et al, applied the ABSI's predictive power to the follow up data collected over 15 years from a general Chinese population. 711 participants from an urban community submitted data in 1992 and then again in 2007. Twenty four participants were diagnosed with DM in 1992 so their data was excluded. Complete data were obtained for 687 participants. Results supported the conclusion that ABSI

was an effective predictor of new onset diabetes mellitus. More research is needed involving other populations to confirm if ABSI is an effective predictor of new onset diabetes mellitus.³

Causes of insulin resistance include genetic or primary target cell defects, autoantibodies to insulin, and accelerated degradation of insulin. Mitochondrial dysfunction may play an important role in development of insulin resistance and complications. Obesity is the most common cause of insulin resistance due to the association with decreased number of receptors and post receptor failure to activate tyrosine kinase.⁴⁻⁶ Physical inactivity also lowers the body's sensitivity to insulin by similar mechanisms to obesity. This condition seems to be the cornerstone for the cause of other conditions involved with metabolic syndrome. Although, evidence suggests that there is an inverse relationship between IGF-1 and Metabolic Syndrome serum IGF-1 levels may be controlled to an extent with appropriate nutrition and activity.²

The next condition contributing to metabolic syndrome is hypertension. Blood pressure is the measurement of the force against the walls of arteries as the heart pumps blood through the body. Hypertension is defined as a consistent measurement of systolic over 130 or diastolic over 85 (NCEP-ATP III).⁴ Two types of hypertension have been identified and are referred to as primary or essential hypertension and secondary hypertension.

For most adults, primary hypertension has no identifiable cause of high blood pressure. This type tends to develop gradually over many years and is the result of several lifestyle factors including; diet (excess sodium intake, decreased

potassium intake), physical inactivity, smoking, stress, and obesity. Again, abdominal obesity being most correlated. There are no symptoms of hypertension so it may go years without any diagnosis.⁷

Secondary hypertension is high blood pressure caused by an underlying condition. This condition tends to appear suddenly and cause a higher blood pressure than primary hypertension. Various conditions and medications can lead to secondary hypertension including; renal insufficiency or disease, adrenal gland tumors, certain congenital blood vessel defects, or medications such as birth control pills, cold remedies and decongestants, over the counter pain relievers, and prescription medications. Neither primary nor secondary hypertension has visible symptoms which is why hypertension is known as the “Silent Killer”.⁸

Another contributing factor in metabolic syndrome is atherogenic dyslipidemia which is defined as a triad of increased blood concentrations of small, dense low-density lipoprotein (LDL) particles, decreased high-density lipoprotein (HDL) particles, and increased triglycerides. HDL levels in men are considered low if under 40 mg/dL and under 50 mg/dL for women. Triglycerides are considered high in either gender if over 150 mg/dL. When combined with hypertension, the elevated LDL levels in the blood will react with cytokines released due to inflammation within blood vessels. Damage is usually found at a branch of the vessel caused by hypertension. LDL-cholesterol collects at the site of inflammation to form foam cells which, after time will cause hardening and thickening of the arterial walls compounding the problems associated with hypertension and increased cardiac output. Causes contributing to dyslipidemia may be primary (genetic) or secondary

and include; sedentary lifestyle, excess intake of saturated fatty acids, cholesterol, and trans-fatty acids, excess simple carbohydrate intake, alcohol abuse, chronic kidney disease, hypothyroidism, and medications such as corticosteroids and glucocorticosteroids.⁹ Much like hypertension, atherogenic dyslipidemia has little to no visible symptoms until severe elevations are present. The relationship between abdominal obesity and atherogenic dyslipidemia has been well established. Therefore, this research will focus on relationships of abdominal obesity with less information in current literature.

Those at risk for these conditions are individuals with poor eating habits and sedentary lifestyles. Usually individuals who are consuming an excess of a certain macronutrient such as saturated fat are also consuming an excess of energy as well. This is usually accompanied by a sedentary lifestyle and the two behavioral habits compound each other's complications. Also, highly stressful lifestyles contribute to increasing risk of conditions. Furthermore, risks for conditions of metabolic syndrome as well as abdominal obesity increase with age. According to the Center for Disease Control, thirty four percent of individuals over the age of twenty meet the criteria for metabolic syndrome, men and women age forty to fifty nine are more than three times likely to meet criteria than those age twenty to thirty nine, men over age sixty are more than four times more likely to meet criteria than those age twenty to thirty nine, and women over age sixty are more than six times likely to meet criteria than those age twenty to thirty nine.¹⁰

25(OH)D and IGF-1

Further research supports the notion that vitamin D and IGF-1 are reduced with obesity. Since IGF-1 shares is able to bind with insulin receptors on cells, obesity leads to less insulin receptors to be present on cells for binding. A cross-sectional cohort study conducted on primarily Caucasian individuals, age 45 years exhibited that lower serum concentrations of vitamin D and IGF-1 have been associated with adverse lipid profiles, hypertension, cardiovascular disease and disturbed glucose metabolism. 6,810 participants were contacted to answer questionnaires and submit blood sample to assess non fasting glucose and lipid profiles as well as serum 25(OH)D and IGF-1 levels. 78% of participants completed questionnaires and 81% submitted blood samples. Men resulted as having disturbed glucose metabolism, high blood pressure, and high triglycerides, although, low HDL-C and abdominal obesity was less prevalent in men. Abdominal obesity was most the most common indicator of Metabolic Syndrome present in 94.5% of men and 95.8% of women. All of these conditions will raise risk of metabolic syndrome.¹¹⁻¹⁴

IGF-1 levels are strongly associated with changes with production and secretion of human growth hormone (HGH). Low secretion of HGH results in low levels of IGF-1 and acromegaly results in increased IGF-1. Age and sex also impact serum IGF-1 concentrations and at age 65 years, daily spontaneous secretion of HGH is reduced by 50-70% thus causing a decline in IGF-1 progressively. Normally, the male gender will exhibit a higher level of IGF-1 when compared to females of the same age.¹⁵

Subtle changes in IGF-1 have also been linked to changes in blood pressure (BP) and insulin sensitivity. IGF-1 levels in the upper normal range have produced results of reduced BP, vascular tone, and increased insulin sensitivity.

Epidemiological studies have suggested IGF-1 levels in the lower normal range have been associated with increased risk of heart disease and stroke. A study of 174 healthy individuals investigated IGF -1, IGFBP3, and intima-media thickness (IMT) of carotid artery and found that IGF-1 levels were the best predictors of total cholesterol and HDL-C.¹⁵

Studies investigating relationships between IGF-1 and atherosclerosis have resulted in conflicting conclusions; some authors found a positive association between IGF-1 and IMT in both men and women, while others found an inverse association between IGF-1 and carotid IMT in healthy women.¹⁶

Even though normal IGF-1 ranges have been established based on age and gender, they are quite contradictory. The normal reference range used in this study was established and used by the Mayo Clinic, however, other organizations as well as research studies have established various ranges as normal. The Mayo Clinic's range was used because it reflected the lowest reference range based on age and gender. A study conducted on 1798 men and women age 25-85 years determined IGF-1 concentrations using chemiluminescent immunometric assay. Quantile regressions were then performed to calculate the 2.5 and 97.5 percentile. These reference ranges were substantially higher than those established by the Mayo Clinic.¹⁷

Impact of Treatment on Vitamin D and IGF-1 Status

Historically, clinicians' first instinct to combating large waistlines and high levels of lipids and cholesterol in patients was to eliminate nearly all dietary fat in the diet. An example of one of these diets that is still in use by many physicians today is the Ornish Diet, developed by Dr. Dean Ornish. Dr. Ornish is a cardiologist with many accolades who also happens to be a vegetarian. His diet plan compliments his personal plant-based approach to reducing risk for coronary artery disease (CAD). However, according to the research energy and protein restriction may actually cause a reduction in serum IGF-1 and IGFBP3.¹²

Controversy exists in the effectiveness of following a plant-based diet to lower serum lipids. Comparing the Ornish diet to other diets high in animal fat and cholesterol resulted in unexpected outcomes. Subjects on a diet that did not limit cholesterol intake showed a greater decrease in systolic and diastolic blood pressures, a greater increase in insulin sensitivity, a greater decrease in triglycerides, and an increase in HDL-C as well as a greater reduction in body mass and body fat when compared to subjects following the plant-based diet. The only category of secondary outcomes that the plant-based diet subjects showed a greater reduction was LDL-C.¹¹ This information was supplied by a study comparing dietary intake of whole eggs versus egg substitute among participants. In a randomized, single-blind, parallel design study, 37 participants were recruited to either consume 3 whole eggs daily or the equivalent amount of yolk-free egg substitute over a 12 week period. 20 participants were assigned to the whole egg group and 17 to the yolk-free egg substitute. Plasma lipids, apolipoproteins, oxidized LDL, cholesteryl

ester transfer protein (CETP) and lecithin-cholesterol acyltransferase (LCAT) activities were assessed at baseline and week 12. Plasma triglycerides, apoC-III, apoE, oxLDL, VLDL particle diameter, large VDL, total IDL, small LDL, and medium LDL particles were reduced in all participants. HDL-C, large LDL, and large HDL particles also increased in all participants. However, there were greater increases in HDL-C and large HDL and reductions in VLDL and medium VLDL particles for those consuming whole egg compared to substitute. LDL-C is an important risk factor pertaining to CAD, however, the combination of all of the other categories have a far greater impact on risk for cardiovascular event.

Another study focusing on nutritional approaches to reduce risk of CVD focused on dietary intake of low saturated fat but a higher intake of red meat presented results suggesting the intake of non-conventional foods for reducing lipids and cholesterol could actually have a great impact on reducing risk of CAD. Research based on beef in an optimal lean diet (BOLD diet) and the effects on lipids, lipoproteins, and apolipoproteins contained data that reflected that participants with the highest intake of red meat per day (~5.4 oz/day) had the most positive impact on triglycerides and LDL-C levels. Participants all presented with hypercholesterolemia (LDL-C >2.8 mmol/L) and were housed in a metabolic kitchen. There is no mention of a structured physical activity program that may have been followed. However, researchers were able to closely monitor daily behaviors and provide detailed, nutrient dense meals including lean cuts of red meat that still contains cholesterol. These lifestyle behaviors could have a positive impact on serum 25(OH)D and IGF-1.¹⁸

This is supported by research conducted exploring the inverse relationship between HDL-C or apolipoprotein A-I and cardiovascular events in women. Results showed a consistent inverse association with HDL-C and coronary events among participants with a range of LDL-C levels. An interesting portion of data within this study also reflected that those participants with the highest HDL-C levels also had the highest percentage of participants who exercised at least once or more per week. This infers that those who were most active also had the highest level of HDL-C. This group also resulted with the lowest percentage of individuals developing diabetes, lowest percentage of smokers, lowest percentage of individuals developing hypertension, the lowest level of apolipoprotein B100, the lowest triglyceride level, and very nearly the lowest level of LDL-C.¹⁹

Impact of Physical Activity on Cardiovascular Disease

The concept of physical activity is a major catalyst when it comes to reducing risk for CVD as well as reducing risk for obesity. Countless studies have reflected the beneficial impact of physical activity on the human body. One such study conducted suggested that time spent in vigorous activity is a significant indicator of total cholesterol and LDL-C with waist girth being a sole predictor of triglycerides and HDL-C. The study sought to explore the correlation of habitual exercisers, lean sedentary men, and abdominally obese sedentary men to risk of CVD. A cross-sectional study recruited 113 non-smoking men age 30-45 years. CVD risk factors were assessed in these individuals and compared to exercisers, sedentary men, and in subgroups of lean men, lean sedentary men, and obese sedentary men. Blood pressure, physical activity (assessed with 7 day recall), physical fitness (assessed by

VO2 max), fasted lipoproteins, apolipoprotein B, triglycerides, glucose, and fibrinogen were assessed. Results showed exercisers were leaner and fitter than sedentary men and that leanness was associated with favorable HDL-C levels and triglycerides with or without exercise. However, exercise contributed to also having lower levels of total cholesterol, LDL-C, and apolipoprotein B. The subject group for this study was for individuals aged 30-45 years which reflects an age group that may still be active because they are still very ambulatory.²⁰

Further research on physical activity indicates that muscle damage caused by exercise can up-regulate isoforms of IGF-1, primarily IGF-1Ea, IGF-1Eb, and IGF-1Ec. These isoforms are known as mechano growth factors (MGF) and are produced locally within the muscle. 10 healthy men were recruited, age 23-27 years, that were physically active and had not participated in any type of resistance training or regular exercise regime for at least 6 months prior to the study. Participants were instructed to not consume any medication, nutritional supplements, and maintain their habitual diet plans refraining from consuming alcohol through the duration of the study. Maximal eccentric exercise protocol of the knee extensor muscles in each leg was assessed in each participant. Biopsies and blood draws were taken in intervals prior, during, and post exercise. Serum levels of IGF-1 did not reflect any appreciable change, although MGF levels increased significantly. Increase in local growth factors could result in a major complimentary reaction within the body and increase insulin sensitivity.²¹

Physical Activity in the Elderly

The elderly may not be able to participate in vigorous activity for various reasons including disease or injury. Another study conducted included individuals from a more elderly population. 2,772 individuals were randomly enrolled and stratified by age and gender. Age ranged between 18-88 with the average age of men 45 +/- 12 yrs and average age of women 45 +/- 13 yrs. Physically active women showed a significantly lower level of serum cholesterol, LDL-C, and triglycerides. Men did not show a significant difference between physically active men compared to sedentary men, although the levels are more favorable in physically active men reinforcing the concept that the more physically active an individual is, the lower their risk of CVD.²²

Further research focused on physical activity primarily in the elderly population. Long-term follow up data was collected on a total of 89 coronary artery bypass graft (CABG) patients ranging from 55-88 years old. Subjects were divided into two groups based on daily physical activity being greater or less than thirty minutes. Surprisingly, only 26% of participants achieved the recommended thirty minutes per day of moderate intensity activity. These individuals who were most active again showed a more favorable profile of indicators of cardiovascular risk as well as lower body mass. Subjects were objectively monitored with double-labeled water, indirect calorimetry, pedometers, heart rate monitors, and accelerometry to assess true daily activity level. Higher physical activity was associated with higher apolipoprotein A-I levels and smaller apoB/apoAI ratios.²⁰

Vitamin D and Skeletal Muscle

Emerging research suggests that there may be a relationship with vitamin D and skeletal muscle mass, strength, and performance. No definitive mechanism has been identified to how vitamin D exerts these benefits, however, there are many rodent model as well as human model studies to support these suggestions. Vitamin D has traditionally been regarded as a regulator of bone metabolism and serum calcium and phosphate levels. Although, recent evidence suggests that the biologically active form of vitamin D, 1, 25-dihydroxyvitamin D (1,25(OH)₂D) has a role in nuclear transcription as well as several components of cellular metabolism via the vitamin D receptor (VDR). Mechanism of action and characterization has not been well established for nonnuclear VDR, however, the binding of 1,25(OH)₂D to nonnuclear VDR is proposed to result in rapid cellular effects via formation of a second messenger of intracellular proteins. Localized VDR have been identified in human skeletal muscle through the use of biopsies.²³⁻³⁴

Human aging may decrease expression of VDR and a positive association has been found between 25(OH)D and VDR concentration in skeletal muscle. Recent findings reflected an increase in VDR mRNA in human myoblasts treated with 1,25(OH)₂D. Another study on 14 older, vitamin D deficient women discovered that intramyonuclear VDR concentration increased 30% with vitamin D₃ supplementation compared with placebo over a 4 month period.²³

1, 25(OH)₂D is theorized to alter the activity of calcium pumps via a calcium binding protein located in the sarcoplasmic reticulum and sarcolemma which will impact muscle contraction by modulating cell calcium flux. Administration of 1,

25(OH)2D regulates the expression of genes involved in these cellular processes of calcium handling. Also, 1,25(OH)2D may increase calmodulin synthesis resulting in influence on muscle contraction.^{23,26-34}

Research focusing on individuals with insulin resistance revealed that decreased levels of serum 25(OH)D, 1,25(OH)2D, and elevated levels of serum parathyroid hormone can lead to an increase in intracellular calcium in adipocytes, which can stimulate lipogenesis further predisposing individuals to weight gain and increase their risk of developing type 2 diabetes. Furthermore, administering vitamin D to individuals with type 2 diabetes has been shown to decrease insulin resistance. However, similar administration of vitamin D in individuals with prediabetes has not been well established. 157 participants with prediabetes were evaluated in the study and results showed that serum 25(OH)D had a relatively strong inverse correlation with measures of insulin resistance and positive correlation with insulin sensitivity.³¹⁻³⁴

Vitamin D has also been shown to play a role in muscle growth by its effect on anabolic signaling pathways. In a rat model study, rats consuming an alkali diet receiving vitamin D3 supplementation enhanced phosphorylated Akt protein kinase B (Akt) concentration which is an established component of the skeletal muscle anabolic cascade. Upregulation of Akt will stimulate myogenesis in cells potentially through a VDR mechanism.²³

Regulation of non-muscle tissue inflammation has also been related to vitamin D status through suppression of several serum proinflammatory cytokines.

This suggests that maintaining optimal levels of vitamin D may reduce risk of atherosclerosis.²³

The notion of inflammation increasing risk for CVD is further supported by research focusing on the sub-acute inflammation that accompanies obesity. Obesity has been shown to increase inflammation in immune as well as parenchymal cells within adipose tissue, liver, and vasculature. Within these tissues there is an abnormal production of cytokines and chemokines that may further attract and activate immune cells. Obese subjects also illicit increases in both amount and activation of peripheral mononuclear cells and exhibit an increase in serum levels of cytokines. Large scale studies contain evidence that markers of inflammation in aggregate predict incident type 2 diabetes. A particularly interesting inclusion in the study investigated reduction on inflammatory markers in patients who had undergone Roux-en-Y bypass procedures and while the procedure resulted in a marked reduction in adipose tissue macrophages and crown like structures, the degree of improvement in insulin sensitivity 3 months post-surgery did not correlate to adipose tissue macrophages.³⁴

Abdominal obesity has been shown to be a strong independent indicator of 25(OH)D status in humans with obese individuals presenting with insufficient or deficient status. Although appropriate 25(OH)D status is still under debate in the medical community, the Institute of Medicine currently defines vitamin D deficiency as $<50 \text{ nmol/L}^{-1}$ or $<20 \text{ ng/dL}^{-1}$.³⁵ Two perspectives on why this occurs have come to the forefront of research, however, evidence is limited for both. The first perspective is that in vitro studies have reflected that the VDR and 1- α -hydroxylase

enzyme are present in adipose tissue. Researchers observed that 3T3-L1 pre-adipocytes express the VDR and subsequently that by 10 days after differentiation, these adipocytes express receptors for vitamin D and parathyroid hormone (PTH). Rat models recently found the presence of the 1- α -hydroxylase enzyme in these 3T3-L1 pre-adipocytes as well which is why adipose tissue is regarded as metabolically active and may be regulated and regulate vitamin D.³⁶⁻⁴²

The second perspective focuses on obese individuals having altered vitamin D and PTH physiology. Several studies on obese patients reflect low levels of circulating 25(OH)D and elevated levels of PTH. Much of the most recent information on this phenomenon, is derived from research on bariatric surgery patients. These patients report preoperatively with low circulating 25(OH)D (<80 nmol/L on average). Other studies on these patients also indicated that BMI and body fat were inversely related to 25(OH)D. Despite the growing amount of evidence to support the low levels of circulating 25(OH)D in obese individuals, the relationship between obesity and the active form of vitamin D remains unclear due to the natural endocrine response to elevate PTH when low circulating 25(OH)D is present. This is where controversy lies. Some researchers have observed that the best indicator of vitamin D status in overweight and obese individuals is the substrate 25(OH)D while others have observed that the best indicator is the active form 1, 25(OH)₂D itself.³⁶⁻⁴² This raises the question, does obesity cause poor vitamin D status or does poor vitamin D status cause obesity? Our research hopes to contribute valuable information to further answer these questions.

CHAPTER 3

METHODOLOGY

Subjects and Design

Subjects: As part of a cross-sectional correlation study, participants were males (7) and females(19) (n=26), ages 25-65, who are students at UNLV and/or individuals who attend local health and wellness facilities as well as attendees of classes held by the UNLV Nutrition Center. Individuals were excluded if there were pregnant as this may cause unusual hormone status.

Research Procedures: Participants reported to UNLV to review and sign informed consent, complete a food frequency and physical activity questionnaire, and submit a finger stick blood draw under sterile conditions as well as resting blood pressure and waist circumference. Blood Pressure was obtained using a sphygmomanometer and stethoscope taken on the right arm of each participant while in a seated position. Waist circumference was obtained at the umbilicus using a flexible tape measure.⁴³ Questionnaires were administered prior to collecting blood pressure to allow blood pressure to reach a resting rate. This study was reviewed and approved by the UNLV Institutional Review for Biomedical Research.

Framingham Food Frequency Questionnaires (FFQ) were used to establish if there were any unusual eating patterns as well as any supplementation taken by participants. Framingham scores are considered the most reliable when predicting risk of developing cardiovascular disease.⁴⁴ The International Physical Activity Questionnaires (IPAQ) was used to assess if there were unusual levels of physical activity or unusual time spent indoors/outdoors. Both questionnaires have been

validated to produce repeatable results.⁴⁴⁻⁴⁵ If participants were to report unusual eating or physical activity behavior it could lead to obscure results.

Participants were then escorted to BHS 119 laboratory where sterile instruments were used to obtain finger stick blood samples. The ring finger of the participant's non-dominant hand was prepped using 70% isopropyl alcohol swabs. Terumo 1.5 mm width by 1.5 mm diameter were then used to penetrate the skin of the finger and initiate blood draw. 600 μ L were collected in Multivette 600 capillary tubes. Once the target amount of blood was acquired, participants were given sterile gauze dressing to be applied to wound for no less than two minutes as well as a band aid to wear following the gauze protocol.

Blood was then spun using a centrifuge for no less than five minutes to separate plasma. Plasma was then collected using a micro pipette with a sterile tip for each sample and stored in Eppendorf tubes in -70 degree freezer for later assessment. Remaining fluid was discarded following appropriate protocol in biohazard waste containers.

Plasma samples collected were used to obtain serum 25(OH)D and IGF-1 levels using Immunodiagnostic System ELISA kits. 25-Hydroxyvitamin D enzymeimmunoassay (EIA), product code AC-57F1, and IGF-1 immunoenzymometric assay (IEMA), product code AC-27F1 were used to determine serum levels of each respective substrate.

25(OH)D EIA Procedures: 25 µL of each calibrator and sample were combined with 1mL of 25-D Biotin Solution and mixed with a vortex machine thoroughly for 10 seconds. 200 µL of each diluted calibrator and sample were then placed in appropriate wells in duplicate on the Antibody Coated Plate. Plate was then covered with adhesive plate sealer (provided with kit) and left to incubate for 2 hours at room temperature. Following incubation period, all wells were then washed 3 times with 300 µL of Wash Solution. Following third wash, wells were then decanted by inverting sharply. 250 µL of wash was added to all wells and decanting process was repeated twice. 200 µL of Enzyme Conjugate was added to all wells using multichannel pipette. Wash and decant process was then repeated. 200 µL of TMB Substrate was added to all wells. Plate was covered again with adhesive plate sealer and set aside to incubate for 30 minutes. Following incubation, 100 µL of Stop Solution was added to all wells. Absorbance was then measured using microplate reader and results were calculated.

Figure 1: 25-Hydroxyvitamin D ELISA Results

IGF-1 IEMA Procedures: 25 µL of each calibrator and sample were combined with 100 µL of Releasing Reagent, vortexed for thoroughly, and set aside to incubate at room temperature for 10 minutes. 1.0 mL of Sample Diluent was added to each and vortexed thoroughly. 50 µL of each diluted calibrator and sample was then added in duplicate to appropriate wells of the Antibody Coated Plate. 200 µL of Enzyme Conjugate was added to all wells using a multichannel pipette. Plate was then set aside to incubate for 2 hours at room temperature. Following incubation, wells were washed 3 times with 300 µL of Wash Solution. Following third wash, wells were then decanted by inverting sharply. 250 µL of wash was added to all wells and decanting process was repeated twice. 200 µL of TMB Substrate was added to all wells using a multichannel pipette and set aside to incubate for 30 minutes. Following incubation, 100 µL of Stop Solution was added to all wells. Absorbance was then measured using microplate reader and results were calculated.

Figure 2: IGF-1 ELISA Results

Metabolic Panel: Participants then reported to a local Quest Diagnostics at their convenience to obtain a fasting comprehensive metabolic panel. Data were collected and analyzed using a paired t-test.

CHAPTER 4

FINDINGS

Table 1: Participant Data

Participant	Gender	Waist Cir	25(OH)D nmol/L	IGF-1 µg/L
1	F	29	163.9	109.5
2	M	34.5	54.4	63.2
3	M	34	104.6	47.1
4	F	25.75	170.1	90.2
6	F	26	133.9	53.7
7	F	31.25	102.9	57.2
10	F	33.5	297.5	41.5
11	F	30.5	216.5	78.1
12	F	28.5	239.5	69.1
A	F	29.5	97.8	53.7
C	F	29	115.7	50.9
E	M	31	150.8	66.8
H	M	36	147.1	48.3
J	F	26.5	144.1	75.6
K	F	28.5	140.2	79.4
L	M	30.5	301.3	59.3
MEAN		30.25	161.26875	65.225
STDEV		3.050956	69.93581551	17.94096

Participant		Waist Cir	25(OH)D nmol/L	IGF-1 µg/L
5	M	48	268.9	40.6
8	F	39	67.3	78
9	F	38	158.9	68.6
13	M	48	154.9	63.8
B	F	40.5	65.6	36.6
D	F	35.5	83.2	46.6
F	F	45	157.1	41.2
G	F	36.5	144.8	54.7
I	F	37	45	75.5
M	F	39	258	46.2
MEAN		40.65	140.37	55.18
STDEV		4.672912	77.8205921	15.25988

Table 1 reflects data collected from each participant regarding gender, waist

circumference, 25(OH)D, and IGF-1 levels. Participants are separated according to waist circumference group with normal waist circumference in the upper portion and large waist circumference in the lower portion.

Table 2: t-Test Assuming Equal Variances: Waist Circumference

t-Test: Two-Sample Assuming Equal Variances	Waist Circumference	
	<i>Variable 1</i>	<i>Variable 2</i>
Mean	30.25	40.65
Variance	9.308333	21.83611
Observations	16	10
Pooled Variance	14.00625	
Hypothesized Mean Difference	0	
df	24	
t Stat	-6.89359	
P(T<=t) one-tail	1.98E-07	
t Critical one-tail	1.710882	
P(T<=t) two-tail	3.96E-07	
t Critical two-tail	2.063899	

Table 2 reflects data collected regarding the waist circumference of participants of both genders resulted in a significant difference between the normal waist circumference group and the large waist circumference group ($P = 0.000002$).

Relationship between IGF-1, and Waist Circumference

Figure 3: Scatter Plot Correlation of IGF-1 to Waist Circumference

Figure 3 reflects data collected regarding the correlation between waist circumference and IGF-1. Results found a significant correlation between waist circumference and IGF-1 ($P=.006$)

Table 3: Correlation of IGF-1 to Waist Circumference

Correlations		Waist_Circumference	IGF1
Waist_Circumference	Pearson Correlation	1	-.525**
	Sig. (2-tailed)		.006
	N	26	26
IGF1	Pearson Correlation	-.525**	1
	Sig. (2-tailed)	.006	
	N	26	26

Table 3 reflects data collected regarding the correlation between waist circumference and IGF-1. Results found a significant correlation between waist circumference and IGF-1 (P=.006)

Relationship between 25-Hydroxyvitamin D and Waist Circumference

Figure 4: Scatter Plot of 25(OH)D to Waist Circumference

Figure 4 reflects data collected regarding the correlation between waist circumference and 25(OH)D. No significant difference was found ($P = 0.649$).

Table 4: Correlation of 25(OH)D to Waist Circumference

Correlations		Waist_Circumference	VITD
Waist_Circumference	Pearson Correlation	1	-.094
	Sig. (2-tailed)		.649
	N	26	26
VITD	Pearson Correlation	-.094	1
	Sig. (2-tailed)	.649	
	N	26	26

Table 4 reflects data collected regarding the correlation between waist circumference and 25(OH)D. No significant difference was found ($P = 0.649$).

Table 5: t-Test Assuming Equal Variances IGF-1

t-Test: Two-Sample Assuming Equal Variances		IGF-1	
	<i>Variable 1</i>	<i>Variable 2</i>	
Mean	65.225	55.18	
Variance	321.878	232.864	
Observations	16	10	
Pooled Variance	288.4978		
Hypothesized Mean Difference	0		
df	24		
t Stat	1.467074		
P(T<=t) one-tail	0.077669		
t Critical one-tail	1.710882		
P(T<=t) two-tail	0.155339		
t Critical two-tail	2.063899		

Table 5 reflects data in regards to large waist and normal waist circumference groups and their level of IGF-1. No significant difference was found between the groups ($P=0.08$).

Table 6: t-Test Assuming Equal Variances 25(OH)D

t-Test: Two-Sample Assuming Equal Variances 25(OH)D

	<i>Variable</i>	
	<i>1</i>	<i>Variable 2</i>
Mean	161.2688	140.37
Variance	4891.018	6056.044556
Observations	16	10
Pooled Variance	5327.903	
Hypothesized Mean Difference	0	
df	24	
t Stat	0.710257	
P(T<=t) one-tail	0.242195	
t Critical one-tail	1.710882	
P(T<=t) two-tail	0.48439	
t Critical two-tail	2.063899	

Table 6 reflects data in regards to large waist and normal waist circumference groups and their level of 25(OH)D. No significant difference was found between the groups (P=0.24).

Groups were also separated according to gender and the same statistical analysis were evaluated. Within our data set, gender did not seem to influence results, however, our sample group contained primarily female participants. Data collected reflects a definitive separation of participant groups according to waist circumference to be used to assess other relationships within the data.

CHAPTER 5

DICUSSION AND IMPLICATIONS

Discussion

The purpose of this study was to assess if there was a relationship between 25(OH)D, IGF-1, and waist circumference. A significant relationship was found between IGF-1 and waist circumference, however, 25(OH)D was not found to have a significant relationship to waist circumference. No significant difference was found in both IGF-1 and 25(OH)D between large waist and normal waist circumference groups. It is worth mentioning that only 5 of the 26 participants presented with sufficient IGF-1 (according to normal reference range¹⁷). Also, only 1 participant presented with 25(OH)D levels borderline insufficient.

Table 7: Normal Reference Range IGF-1

21-25 years	83-344	
26-30 years	75-275	
31-35 years	71-241	
36-40 years	69-226	
41-45 years	64-210	
46-50 years	59-201	
51-55 years	56-201	
56-60 years	51-194	
61-65 years	47-191	

Males

21-25 years	84-323	
26-30 years	77-271	
31-35 years	73-244	
36-40 years	68-225	
41-45 years	62-205	
46-50 years	56-194	
51-55 years	53-191	
56-60 years	45-173	
61-65 years	41-168	

Females

Table 7 indicates normal reference ranges of free IGF-1 according to information derived from Mayo Clinic Laboratories.¹⁷

This research project was unique from other similar research projects in the ability of investigators to interact personally and obtain detailed information regarding nutritional intake and physical activity. According to data collected in food frequency and physical activity questionnaires, all participants ate a relatively well-balanced diet that included decent sources of vitamin D. All participants also maintained a relatively active lifestyle. Active lifestyles could enhance the participant's insulin sensitivity as well as up-regulate IGF-1 production allowing for their IGF-1 to operate more efficiently. However, our results indicated that the majority of participants still exhibited low levels of IGF-1.

Another aspect to consider is that data was collected in the later part of spring and early summer in a desert climate. Participants could easily obtain additional vitamin D from being outside. Within our data set, gender did not seem to influence results, however, our sample group contained primarily female participants. Future research including a larger representation of male population would be beneficial to identify possible relationships between 25(OH)D, and waist circumference.

Conclusion

Risks associated with large waist circumference, low IGF-1, and low 25(OH)D can lead to metabolic syndrome and if left untreated can be fatal. These risks also include a variety of complications such as; higher risk of cardiovascular event, sleep apnea, aortic stiffness, hypertension, joint pain, respiratory distress, and all of the complications involved will compound each other furthering progression of the syndrome. All of the conditions of metabolic syndrome can be controlled through

diet, exercise, and lifestyle changes, however, few patients are able to make or adhere to the necessary changes to reduce the risk involved.

Both ELISA kits displayed acceptable accuracy. Despite all participants displaying sufficient amounts of serum 25(OH)D, only 5 participants displayed sufficient serum IGF-1. Insufficient serum IGF-1 may suggest possible human growth hormone (HGH) deficiency, however, more information regarding participants' IGF-1 binding proteins would be required to fully assess this condition. Theoretically, evidence reviewed in this project suggests that insufficient serum IGF-1 levels can be treated pharmaceutically as well as through lifestyle modification.

Future Research

Future research including accurate information regarding all binding proteins of IGF-1 as well as a more detailed account of daily behavior by a larger sample of participants would very useful in assessing relationships between 25(OH)D, IGF-1, and waist circumference. Large waist circumference could be related to many risk factors for disease, however, maintaining a relatively active lifestyle may slightly reduce the risk for individuals who have large waist circumferences. Obtaining waist circumference as well as activity level is relatively convenient in medical treatment settings and could provide clinicians with more valuable information regarding patient's level of risk for disease. This information could provide researchers and clinicians with practical information that could be used to formulate patient care plans that could greatly reduce their risk for cardiovascular event.

APPENDIX I: INFORMED CONCENT

INFORMED CONSENT

Department of Kinesiology and Nutrition Sciences

TITLE OF STUDY: 25-Hydroxyvitamin D, IGF-1, and Metabolic Syndrome A cross-sectional study

INVESTIGATOR(S): Damon McCune, Dr. Laura Kruskall, Dr. Richard Tandy, Dr. James Navalta, and Dr. Sue Schuerman

For questions or concerns about the study, you may contact **Dr. Laura Kruskall at (702) 895-4985** or **Damon McCune at (702) 300-4250**.

For questions regarding the rights of research subjects, any complaints or comments regarding the manner in which the study is being conducted, contact **the UNLV Office of Research Integrity – Human Subjects at 702-895-2794, toll free at 877-895-2794 or via email at IRB@unlv.edu**.

Purpose of the Study

You are invited to participate in a research study. The purpose of this study is to evaluate the relationship of insulin-like growth factor (IGF-1) and vitamin D with risk for metabolic syndrome in individuals of various ages and lifestyles.

Participants

You are being asked to participate in the study because you fit this criteria: Male or non-pregnant female age 25-65.

You will not be allowed to participate in this study if you are pregnant. The reason for this is that pregnancy could alter hormone levels which could present misleading data. For any female, there is a possibility that you are pregnant but do not know that you are. If it is found that you are pregnant after the study, you should know that the potential for damage of the exposed fetus is extremely low.

Procedures

If you volunteer to participate in this study, you will be asked to do the following: Allow 60 minutes for testing at each location. All testing will be conducted in the Bigelow Health Science Building as well as at a local Quest Diagnostic location of your choosing.

1. Report to the UNLV Nutrition Sciences Office (BHS 313). You will be escorted to a private room to review the study protocol, give consent, and provide a finger prick sample of blood (~0.12 tsp; ~600 µL) to assess vitamin D and insulin-like growth hormone (IGF-1) status. Blood pressure, body weight, and circumference measurements will be taken of the waist, arm, and thigh.
2. Complete short questionnaires on food intake, vitamin D intake/synthesis, and activity level/type.

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Approved by the UNLV IRB. Protocol #1312-4666

Received: 01-26-14 Approved: 01-30-14 Expiration: 01-29-15

3. Report to a convenient local Quest Diagnostic office and submit a blood sample for a metabolic panel to be evaluated.

Benefits of Participation

The direct benefits to you for participating in this study include knowledge of your blood lipid levels, your circulating IGF-1 level and your serum vitamin D level. The main benefit of the study will be in determining if there is a relationship between IGF-1 and vitamin D on risk of metabolic syndrome.

Risks of Participation

There is a risk for infection with the finger-stick, however, all materials will be sterilized and sealed; although it may be temporarily painful, the puncture wound is insignificant.

Cost /Compensation

There will not be any financial cost to you to participate in this study. The study will take 120 minutes of your time; however there is no compensation for your time.

Confidentiality

All information gathered in this study will be kept completely confidential. Only those persons who are directly related to this study (i.e. researchers, data analysts) will have access to your data. No reference will be made in written or oral materials, which could link you to this study. All records will be stored in the laboratory for a period of 3 years. After 3 years, any documentation with identifiable information (e.g., name) will be destroyed. Unidentifiable data will be stored in locked storage indefinitely.

Voluntary Participation

Your participation in this study is voluntary. You may refuse to participate in this study or in any part of this study. You may withdraw at any time without prejudice to your relations with UNLV. You are encouraged to ask questions about this study at the beginning or any time during the research study.

Participant Consent:

I have read the above information and agree to participate in this study. I have been able to ask questions about the research study. I am at least 18 years of age. A copy of this form has been given to me.

Signature of Participant

Date

Participant Name (Please Print)

APPENDIX II: FOOD FREQUENCY QUESTIONNAIRE

Instructions for Completing the Food Frequency Questionnaire

Thank you for participating in this research study. An important part of this study is the Food Frequency Questionnaire, designed to measure your dietary pattern over the past year. Remember, the information we get from the study is only as good as the information you give us. Accuracy is essential!

Please complete this form and bring it with you at the time of your appointment, or complete prior to the time of your home visit.

- 1) Please use a No. 2 pencil, and make sure the circles are completely darkened.**
- 2) Please do not leave any questions blank. If the section does not apply to you, please fill in the "never" section.**
- 3) Please do not separate, staple or rip the booklet.**
- 4) Please do not leave any stray marks. Make sure all erasures are complete.**

DIET ASSESSMENT

ID: _____

1. Do you currently take multiple vitamins? (Please report individual vitamins under question 2.)

☐ No ☐ Yes → If yes, a) How many do you take per week? ☐ 2 or less ☐ 6-9 ☐ 3-5 ☐ 10 or more

b) What specific brand do you usually use? _____ Specify exact brand and type

2. Not counting multiple vitamins, do you take any of the following preparations:

a) Vitamin A? ☐ No ☐ Yes, seasonal only ☐ Yes, most months } If Yes, { How many years? → ☐ 0-1 yr. ☐ 2-4 yrs. ☐ 5-9 yrs. ☐ 10+ yrs. ☐ Don't know
What dose per day? → ☐ Less than 8,000 IU ☐ 8,000 to 12,000 IU ☐ 13,000 to 22,000 IU ☐ 23,000 IU or more ☐ Don't know

b) Vitamin C? ☐ No ☐ Yes, seasonal only ☐ Yes, most months } If Yes, { How many years? → ☐ 0-1 yr. ☐ 2-4 yrs. ☐ 5-9 yrs. ☐ 10+ yrs. ☐ Don't know
What dose per day? → ☐ Less than 400 mg. ☐ 400 to 700 mg. ☐ 750 to 1250 mg. ☐ 1300 mg. or more ☐ Don't know

c) Vitamin B₆? ☐ No ☐ Yes → If yes, { How many years? → ☐ 0-1 yr. ☐ 2-4 yrs. ☐ 5-9 yrs. ☐ 10+ yrs. ☐ Don't know
What dose per day? → ☐ Less than 10 mg. ☐ 10 to 39 mg. ☐ 40 to 79 mg. ☐ 80 mg. or more ☐ Don't know

d) Vitamin E? ☐ No ☐ Yes → If yes, { How many years? → ☐ 0-1 yr. ☐ 2-4 yrs. ☐ 5-9 yrs. ☐ 10+ yrs. ☐ Don't know
What dose per day? → ☐ Less than 100 IU ☐ 100 to 250 IU ☐ 300 to 500 IU ☐ 600 IU or more ☐ Don't know

e) Selenium? ☐ No ☐ Yes → If yes, { How many years? → ☐ 0-1 yr. ☐ 2-4 yrs. ☐ 5-9 yrs. ☐ 10+ yrs. ☐ Don't know
What dose per day? → ☐ Less than 80 mcg. ☐ 80 to 130 mcg. ☐ 140 to 250 mcg. ☐ 260 mcg. or more ☐ Don't know

f) Iron? ☐ No ☐ Yes → If yes, { How many years? → ☐ 0-1 yr. ☐ 2-4 yrs. ☐ 5-9 yrs. ☐ 10+ yrs. ☐ Don't know
What dose per day? → ☐ Less than 51 mg. ☐ 51 to 200 mg. ☐ 201 to 400 mg. ☐ 401 mg. or more ☐ Don't know

g) Zinc? ☐ No ☐ Yes → If yes, { How many years? → ☐ 0-1 yr. ☐ 2-4 yrs. ☐ 5-9 yrs. ☐ 10+ yrs. ☐ Don't know
What dose per day? → ☐ Less than 25 mg. ☐ 25 to 74 mg. ☐ 75 to 100 mg. ☐ 101 mg. or more ☐ Don't know

h) Calcium? (Include Calcium in Dolomites) ☐ No ☐ Yes → If yes, { How many years? → ☐ 0-1 yr. ☐ 2-4 yrs. ☐ 5-9 yrs. ☐ 10+ yrs. ☐ Don't know
What dose per day? → ☐ Less than 400 mg. ☐ 400 to 900 mg. ☐ 901 to 1300 mg. ☐ 1301 mg. or more ☐ Don't know

i) Are there other supplements that you take on a regular basis? Please mark if yes:

☐ Folic acid ☐ Cod liver Oil ☐ Iodine ☐ Beta-Carotene ☐ Other (please specify): _____
☐ Vitamin D ☐ Copper ☐ Brewer's Yeast ☐ Magnesium
☐ B-Complex Vitamins ☐ Omega-3 Fatty-acids

3. For each food listed, fill in the circle indicating how often on average you have used the amount specified during the past year.

in the circle indicating you have used the amount last year.		AVERAGE USE LAST YEAR									P
		Never, or less than once per month	1-3 per mo.	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day	2
DAIRY FOODS											
Skim or low fat milk (8 oz. glass)		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Whole milk (8 oz. glass)		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cream, e.g. coffee, whipped (Tbs)		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sour Cream (Tbs)		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Non-dairy coffee whitener (tsp.)		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sherbet or ice milk (1/2 cup)		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ice cream (1/2 cup)		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Yogurt (1 cup)		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cottage or ricotta cheese (1/2 cup)		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cream cheese (1 oz.)		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other cheese, e.g., American, cheddar, etc., plain or as part of a dish (1 slice or 1 oz. serving)		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Margarine (pat), added to food or bread; exclude use in cooking		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Butter (pat), added to food or bread; exclude use in cooking		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Please turn to page 2

3. (Continued) Please fill in your average use, during the past year, of each specified food.

Please try to average your seasonal use of foods over the entire year. For example, if a food such as cantaloupe is eaten 4 times a week during the approximate 3 months that it is in season, then the average use would be once per week.

FRUITS	Never, or less than once per month	1-3 per mo.	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day
Raisins (1 oz. or small pack) or grapes	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Prunes (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Bananas (1)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Cantaloupe (1/4 melon)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Watermelon (1 slice)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Fresh apples or pears (1)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Apple juice or cider (small glass)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Oranges (1)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Orange juice (small glass)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Grapefruit (1/2)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Grapefruit juice (small glass)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Other fruit juices (small glass)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Strawberries, fresh, frozen or canned (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Blueberries, fresh, frozen or canned (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Peaches, apricots or plums (1 fresh, or 1/2 cup canned)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐

VEGETABLES	Never, or less than once per month	1-3 per mo.	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day
Tomatoes (1)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Tomato juice (small glass)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Tomato sauce (1/2 cup) e.g., spaghetti sauce	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Red chili sauce (1 Tbs)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Tofu or soybeans (3-4 oz.)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
String beans (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Broccoli (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Cabbage or cole slaw (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Cauliflower (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Brussels sprouts (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Carrots, raw (1/2 carrot or 2-4 sticks)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Carrots, cooked (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Corn (1 ear or 1/2 cup frozen or canned)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Peas, or lima beans (1/2 cup fresh, frozen, canned)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Mixed vegetables (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Beans or lentils, baked or dried (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Yellow (winter) squash (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Eggplant, zucchini, or other summer squash (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Yams or sweet potatoes (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Spinach, cooked (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Spinach, raw as in salad	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Kale, mustard or chard greens (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Iceberg or head lettuce (serving)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Romaine or leaf lettuce (serving)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Celery (4" stick)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Beets (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Alfalfa sprouts (1/2 cup)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Garlic, fresh or powdered (1 clove or shake)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐

EGGS, MEAT, ETC.	Never, or less than once per month	1-3 per mo.	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day
Eggs (1)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Chicken or turkey, with skin (4-6 oz.)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Chicken or turkey, without skin (4-6 oz.)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Bacon (2 slices)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐
Hot dogs (1)	☐	☐	☐ (W)	☐	☐	☐ (D)	☐	☐	☐

Please go to page 3

3. (Continued) Please fill in your average use,
during the past year, of each specified food.

		Never, or less than once per month	1-3 per mo.	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day	
MEATS (CONTINUED)											
Processed meats, e.g., sausage, salami, bologna, etc. (piece or slice)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Liver (3-4 oz.)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Hamburger (1 patty)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Beef, pork, or lamb as a sandwich or mixed dish, e.g., stew, casserole, lasagna, etc.		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Beef, pork, or lamb as a main dish, e.g., steak, roast, ham, etc. (4-6 oz.)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Canned tuna fish (3-4 oz.)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Dark meat fish, e.g., mackerel, salmon, sardines, bluefish, swordfish (3-5 oz.)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Other fish (3-5 oz.)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Shrimp, lobster, scallops as a main dish		☐	☐	☐	☐	☐	☐	☐	☐	☐	
BREADS, CEREALS, STARCHES											
Cold breakfast cereal (1 cup)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Cooked oatmeal (1 cup)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Other cooked breakfast cereal (1 cup)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
White bread (slice), including pita bread		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Dark bread (slice)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
English muffins, bagels, or rolls (1)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Muffins or biscuits (1)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Brown rice (1 cup)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
White rice (1 cup)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Pasta, e.g., spaghetti, noodles, etc. (1 cup)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Other grains, e.g., bulgur, kasha, couscous, etc. (1 cup)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Pancakes or waffles (serving)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
French fried potatoes (4 oz.)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Potatoes, baked, boiled (1) or mashed (1 cup)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Potato chips or corn chips (small bag or 1 oz.)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Crackers, Triskets, Wheat Thins (1)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
Pizza (2 slices)		☐	☐	☐	☐	☐	☐	☐	☐	☐	
BEVERAGES											
CARBONATED BEVERAGES	Low Calorie (sugar-free) types										
	Low calorie cola, e.g., Tab with caffeine	☐	☐	☐	☐	☐	☐	☐	☐	☐	
	Low calorie caffeine-free cola, e.g., Pepsi Free	☐	☐	☐	☐	☐	☐	☐	☐	☐	
	Other low calorie carbonated beverage, e.g., Fresca, Diet 7-Up, diet ginger ale	☐	☐	☐	☐	☐	☐	☐	☐	☐	
Regular types (not sugar-free)	Coke, Pepsi, or other cola with sugar	☐	☐	☐	☐	☐	☐	☐	☐	☐	
	Caffeine Free Coke, Pepsi, or other cola with sugar	☐	☐	☐	☐	☐	☐	☐	☐	☐	
OTHER BEVERAGES	Other carbonated beverage with sugar, e.g., 7-Up, ginger ale	☐	☐	☐	☐	☐	☐	☐	☐	☐	
	Hawaiian Punch, lemonade, or other non-carbonated fruit drinks (1 glass, bottle, can)	☐	☐	☐	☐	☐	☐	☐	☐	☐	
	Decaffeinated coffee (1 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐	
	Coffee (1 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐	
	Tea (1 cup), not herbal teas	☐	☐	☐	☐	☐	☐	☐	☐	☐	
	Beer (1 glass, bottle, can)	☐	☐	☐	☐	☐	☐	☐	☐	☐	
	Red wine (4 oz. glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	
	White wine (4 oz. glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐	
	Liquor, e.g., whiskey, gin, etc. (1 drink or shot)	☐	☐	☐	☐	☐	☐	☐	☐	☐	

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Guidelines for Review of Willet Food Frequency Questionnaire

The purpose of the Willet Food Frequency Questionnaire is to obtain information about what the participant usually eats and drinks. The questions review specific foods and portion sizes, to find out how often, on average, the specified amount was eaten or drunk during the past year. The Willet Food Frequency Form is completed prior to the participant's clinic visit.

Special arrangements may be made if the participant is illiterate, has problems reading, cannot read English, or is unable to answer the questions accurately due to physical or cognitive disabilities. This may be evident for example, the answer sheet has all circles filled out in the first column or is not filled out at all.

1. Check that there are no staples, rips, tears, or writing other than where indicated. If so, the form must be redone.
2. Make sure that the form is completed with a #2 pencil.
3. Check that circles are filled in completely - no Xs, checkmarks, etc.
4. Check that a response has been filled in for every line. If never used, fill in that circle.
5. Check that there is only one response for every line.
6. For vitamins, make sure the brand, the dose and how long taken is written in the spaces provided.
7. Make sure that all extra foods are written in the numbered spaces (up to 4 items) with complete information.
8. Make sure that what is written in the extra foods section is not something that is already in another part of the questionnaire.
9. Make sure to check for completeness of I.D. number.
10. Make sure to stamp the date on top when the participant brings in the form.

APPENDIX III: PHYSICAL ACTIVITY QUESTIONNAIRE

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health-related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an *International Physical Activity Prevalence Study* is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous and moderate activities that you did in the **last 7 days**. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ Yes

☐ No

Skip to PART 2: TRANSPORTATION

The next questions are about all the physical activity you did in the last 7 days as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, heavy construction, or climbing up stairs as part of your work? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ days per week

☐

No vigorous job-related physical activity

Skip to question 4

3. How much time did you usually spend on one of those days doing vigorous physical activities as part of your work?

_____ hours per day

_____ minutes per day

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads as part of your work? Please do not include walking.

_____ days per week

☐

No moderate job-related physical activity

Skip to question 6

LONG LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised October 2002.

5. How much time did you usually spend on one of those days doing moderate physical activities as part of your work?
- _____ hours per day
_____ minutes per day
6. During the last 7 days, on how many days did you walk for at least 10 minutes at a time as part of your work? Please do not count any walking you did to travel to or from work.
- _____ days per week
- ☐ No job-related walking → Skip to *PART 2: TRANSPORTATION*
7. How much time did you usually spend on one of those days walking as part of your work?
- _____ hours per day
_____ minutes per day

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the last 7 days, on how many days did you travel in a motor vehicle like a train, bus, car, or tram?
- _____ days per week
- ☐ No traveling in a motor vehicle → Skip to question 10
9. How much time did you usually spend on one of those days traveling in a train, bus, car, tram, or other kind of motor vehicle?
- _____ hours per day
_____ minutes per day

Now think only about the bicycling and walking you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the last 7 days, on how many days did you bicycle for at least 10 minutes at a time to go from place to place?
- _____ days per week
- ☐ No bicycling from place to place → Skip to question 12

11. How much time did you usually spend on one of those days to bicycle from place to place?
- _____ hours per day
_____ minutes per day
12. During the last 7 days, on how many days did you walk for at least 10 minutes at a time to go from place to place?
- _____ days per week
- ☐ No walking from place to place → *Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY*
13. How much time did you usually spend on one of those days walking from place to place?
- _____ hours per day
_____ minutes per day

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the last 7 days in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, chopping wood, shoveling snow, or digging in the garden or yard?
- _____ days per week
- ☐ No vigorous activity in garden or yard → *Skip to question 16*
15. How much time did you usually spend on one of those days doing vigorous physical activities in the garden or yard?
- _____ hours per day
_____ minutes per day
16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, sweeping, washing windows, and raking in the garden or yard?
- _____ days per week
- ☐ No moderate activity in garden or yard → *Skip to question 18*

17. How much time did you usually spend on one of those days doing moderate physical activities in the garden or yard?
- _____ hours per day
_____ minutes per day
18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, washing windows, scrubbing floors and sweeping inside your home?
- _____ days per week
- ☐ No moderate activity inside home → *Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY*
19. How much time did you usually spend on one of those days doing moderate physical activities inside your home?
- _____ hours per day
_____ minutes per day

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the last 7 days solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the last 7 days, on how many days did you walk for at least 10 minutes at a time in your leisure time?
- _____ days per week
- ☐ No walking in leisure time → *Skip to question 22*
21. How much time did you usually spend on one of those days walking in your leisure time?
- _____ hours per day
_____ minutes per day
22. Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like aerobics, running, fast bicycling, or fast swimming in your leisure time?
- _____ days per week
- ☐ No vigorous activity in leisure time → *Skip to question 24*

23. How much time did you usually spend on one of those days doing vigorous physical activities in your leisure time?
- _____ hours per day
_____ minutes per day
24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis in your leisure time?
- _____ days per week
- ☐ No moderate activity in leisure time ➔ *Skip to PART 5: TIME SPENT SITTING*
25. How much time did you usually spend on one of those days doing moderate physical activities in your leisure time?
- _____ hours per day
_____ minutes per day

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the last 7 days, how much time did you usually spend sitting on a weekday?
- _____ hours per day
_____ minutes per day
27. During the last 7 days, how much time did you usually spend sitting on a weekend day?
- _____ hours per day
_____ minutes per day

This is the end of the questionnaire, thank you for participating.

APPENDIX IV: DESCRIPTIVE STATISTICS FOR ALL SUBJECTS

Participant	Gender	25(OH)D nmol/L	GF-1 µg/L	Systolic	Diastolic	Waist Cir.	Glucose	BUN	Creatinine µmol/L	Calcium	Total Protein	Albumin	Globulin	A/G Ratio	Gamma-GT	Alk Phos	AST	ALT	Sodium	Potassium	Oxalate	CO2	e GFR mL		
1	F	165.9	109.5	110	68	29	77	22	0.88	25	9.4	7.7	4.9	2.8	1.8	0.8	57	29	24	138	4.3	103	24	104	90
2	M	54.4	69.2	122	84	34.5	86	15	1.13	13.3	9.4	7	4.5	2.5	1.8	0.8	57	25	19	137	4.3	103	26	92	80
3	M	104.6	47.1	116	68	34	83	20	1.26	15.9	9.3	7.2	4.6	2.6	1.8	0.7	27	46	72	136	4.5	104	23	87	75
4	F	170.1	90.2	108	54	25.75	84	14	0.9	15.6	9.5	7.2	4.3	2.7	1.7	0.6	80	16	7	136	4.2	107	20	101	87
6	F	135.9	53.7	114	72	26	74	12	0.82	14.6	9.4	7.1	4.5	2.6	1.7	0.8	51	15	9	138	4.5	106	22	111	95
7	F	102.9	57.2	98	48	31.25	82	11	0.77	14.3	9.4	6.8	4.3	2.5	1.7	0.5	45	13	10	137	4.1	104	24	117	101
10	F	297.5	41.5	142	98	33.5	81	9	0.65	13.8	9.5	6.9	4.4	2.5	1.8	0.3	61	15	7	139	3.9	104	23	140	121
11	F	216.5	78.1	104	68	30.5	83	12	0.67	17.9	9.3	7.6	4.3	3.3	1.3	0.8	68	22	15	138	4.4	103	24	142	122
12	F	239.5	69.1	106	62	28.5	86	16	0.66	24.2	9	6.8	4.1	2.7	1.5	0.4	49	11	12	137	4	103	25	135	117
A	F	97.8	53.7	114	66	29.5	85	8	0.75	10.7	9.5	6.8	4.2	2.6	1.6	0.8	117	16	13	137	4.6	104	24	121	104
C	F	115.7	50.9	114	64	29	94	13	0.9	14.4	9.5	7.1	4.8	2.3	2.1	0.7	60	20	16	136	4.3	102	26	101	87
E	M	150.8	68.8	116	64	31	97	15	1.11	15.5	9.9	7.6	4.6	3	1.5	0.6	47	28	28	140	4.2	104	26	106	91
H	M	147.1	48.3	114	58	36	89	22	0.94	23.4	9.7	7.4	4.3	3.1	1.4	1.3	94	28	36	138	4.1	103	25	130	112
J	F	144.1	75.6	116	66	26.5	95	10	0.76	13.2	8.9	6.7	4.3	2.4	1.8	0.4	45	14	8	139	4.2	106	25	126	109
K	F	140.2	79.4	132	72	28.5	94	16	1.05	15.2	9.1	6.9	4.3	2.6	1.7	0.4	46	14	13	140	4.1	105	25	85	74
L	M	301.3	59.3	122	74	30.5	91	22	1.24	17.7	9.8	7.1	4.7	2.4	2	0.5	49	26	22	141	4.5	107	27	89	76
MEAN		161.36975	65.25	115.5	67.875	30.25	86.1875	14.80125	0.905625	16.48975	9.40125	7.11875	4.45625	2.6625	1.7	0.65	59.25	21.3025	19.4375	138.1875	4.2625	104.375	24.3025	111.8375	96.3025
STDV		68.95381551	17.9406	10.5956	11.53475	3.05856	6.65448	4.63856	0.210764	4.23664	0.65518	0.314576	0.222017	0.270485	0.206559	0.244949	9.56186	16.2192	13.7792	0.195835	1.5	1.74045	16.08475	16.45061	
Participant		25(OH)D nmol/L	GF-1 µg/L	Systolic	Diastolic	Waist Cir.	Glucose	BUN	Creatinine µmol/L	Calcium	Total Protein	Albumin	Globulin	A/G Ratio	Gamma-GT	Alk Phos	AST	ALT	Sodium	Potassium	Oxalate	CO2	e GFR mL		
5	M	268.9	40.6	126	76	48	90	14	1.06	13.2	9.8	7.5	4.8	2.7	1.8	0.7	109	13	38	135	4.6	102	23	112	96
8	F	67.3	78	108	64	39	83	12	0.67	17.9	9.3	7.6	4.3	3.3	1.3	0.8	68	22	15	138	4.4	103	24	142	122
9	F	138.9	68.6	108	66	38	95	12	0.74	16.2	9.5	7.4	4.4	3	1.5	0.6	53	15	11	137	4	106	23	131	113
13	M	154.9	63.8	122	72	48	89	11	0.94	11.7	9	6.7	4.5	2.2	2	0.4	65	20	16	141	4.1	106	25	126	108
B	F	65.6	36.6	122	78	40.5	305	9	0.56	15.1	9.7	7.7	4.5	3.2	1.4	0.5	57	20	27	137	3.9	101	25	147	127
D	F	85.2	46.6	116	62	35.5	86	19	0.66	28.8	9.3	6.6	4.3	2.5	1.9	0.3	80	19	23	136	4.3	103	25	122	105
F	F	157.1	41.2	128	66	45	90	12	0.98	12.2	8.9	6.8	3.9	2.9	1.3	0.3	75	11	15	140	4.3	107	24	89	77
G	F	144.8	54.7	98	62	36.5	82	15	1.06	14.2	9.3	6.8	3.9	2.9	1.3	0.4	65	23	20	139	4.9	105	25	82	71
I	F	45	75.5	132	86	37	91	8	0.57	14	10.5	8.2	5.2	3	1.7	0.9	57	31	70	139	4.5	102	26	149	129
M	F	258	46.2	134	90	39	82	9	0.64	14.1	9.5	7.5	4.4	3.1	1.4	0.4	108	16	10	137	4.5	103	24	142	122
MEAN		140.37	55.18	119.4	71.2	40.65	110.3	12.1	0.788	15.84	9.48	7.28	4.42	2.86	1.56	0.55	73.3	19	24.5	138.1	4.35	103.8	24.4	124.2	107
STDV		77.826521	15.25586	11.70185	10.04115	4.672812	68.59227	3.28126	0.210544	4.931351	0.454117	0.257499	0.195789	0.360706	0.267499	0.195789	20.30355	5.754884	18.03238	1.72884	0.196073	2.03861	0.966092	23.55042	20.25394

APPENDIX V: IGF-1 ELISA RESULTS

OD 1	OD 2	MEAN	LOGMEAN	CONC
0.615	0.61	0.6125	2.039568	109.5
0.415	0.39	0.4025	1.800796	63.2
0.295	0.286	0.2905	1.673451	47.1
0.497	0.58	0.5385	1.955429	90.2
0.234	0.233	0.2335	1.608641	40.6
0.335	0.345	0.34	1.729733	53.7
0.367	0.362	0.3645	1.75759	57.2
0.476	0.49	0.483	1.892325	78
0.423	0.444	0.4335	1.836043	68.6
0.237	0.246	0.2415	1.617737	41.5
0.482	0.485	0.4835	1.892894	78.1
0.445	0.428	0.4365	1.839454	69.1
0.444	0.368	0.406	1.804775	63.8
0.329	0.351	0.34	1.729733	53.7
0.19	0.197	0.1935	1.563161	36.6
0.317	0.324	0.3205	1.707561	50.9
0.289	0.283	0.286	1.668334	46.6
0.458	0.389	0.4235	1.824673	66.8
0.236	0.242	0.239	1.614895	41.2
0.36	0.334	0.347	1.737692	54.7
0.279	0.32	0.2995	1.683684	48.3
0.5	0.441	0.4705	1.878113	75.5
0.489	0.455	0.472	1.879818	75.6
0.493	0.486	0.4895	1.899716	79.4
0.384	0.372	0.378	1.772939	59.3
0.274	0.291	0.2825	1.664355	46.2

APPENDIX VI: IGF-1 ELISA VALIDATION

APPENDIX VII: 25(OH)D ELISA RESULTS

OD 1	OD 2	MEAN	LOGMEAN	CONC
1.431	0.896	1.1635	2.214529	163.9
1.763	1.688	1.7255	1.735905	54.4
1.784	1.001	1.3925	2.019503	104.6
1.422	0.867	1.1445	2.23071	170.1
0.939	0.883	0.911	2.429569	268.9
1.738	0.795	1.2665	2.12681	133.9
1.874	0.928	1.401	2.012264	102.9
1.726	1.508	1.617	1.828309	67.3
1.201	1.157	1.179	2.201329	158.9
0.903	0.816	0.8595	2.473429	297.5
1.047	0.996	1.0215	2.335462	216.5
1.171	0.769	0.97	2.379322	239.5
1.398	0.986	1.192	2.190257	154.9
2.005	0.848	1.4265	1.990547	97.8
1.631	1.63	1.6305	1.816811	65.6
1.726	0.956	1.341	2.063362	115.7
1.201	1.817	1.509	1.920286	83.2
0.903	1.509	1.206	2.178334	150.8
1.047	1.323	1.185	2.196219	157.1
1.171	1.282	1.2265	2.160875	144.8
1.398	1.039	1.2185	2.167689	147.1
2.005	1.64	1.8225	1.653296	45
1.631	0.827	1.229	2.158746	144.1
1.088	1.398	1.243	2.146823	140.2
0.923	0.783	0.853	2.478964	301.3
0.914	0.95	0.932	2.411685	258

APPENDIX VIII: 25(OH)D ELISA VALIDATION

APPENDIX IX: t-TEST ASSUMING EQUAL VARIANCES: WAIST
CIRCUMFERENCE

t-Test: Two-Sample Assuming Equal Variances	Waist Circumference	
	<i>Variable 1</i>	<i>Variable 2</i>
Mean	30.25	40.65
Variance	9.308333	21.83611
Observations	16	10
Pooled Variance	14.00625	
Hypothesized Mean Difference	0	
df	24	
t Stat	-6.89359	
P(T<=t) one-tail	1.98E-07	
t Critical one-tail	1.710882	
P(T<=t) two-tail	3.96E-07	
t Critical two-tail	2.063899	

APPENDIX X: t-TEST ASSUMING EQUAL VARIANCES: IGF-1

t-Test: Two-Sample Assuming Equal Variances	IGF-1	
	<i>Variable 1</i>	<i>Variable 2</i>
Mean	65.225	55.18
Variance	321.878	232.864
Observations	16	10
Pooled Variance	288.4978	
Hypothesized Mean Difference	0	
df	24	
t Stat	1.467074	
P(T<=t) one-tail	0.077669	
t Critical one-tail	1.710882	
P(T<=t) two-tail	0.155339	
t Critical two-tail	2.063899	

APPENDIX XI: t-TEST ASSUMING EQUAL VARIANCES: 25(OH)D

t-Test: Two-Sample Assuming Equal Variances			Vitamin D
	Variable 1	Variable 2	
Mean	161.2688	140.37	
Variance	4891.018	6056.044556	
Observations	16	10	
Pooled Variance	5327.903		
Hypothesized Mean Difference	0		
df	24		
t Stat	0.710257		
P(T<=t) one-tail	0.242195		
t Critical one-tail	1.710882		
P(T<=t) two-tail	0.48439		
t Critical two-tail	2.063899		

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