DIET, CARDIOVASCULAR DISEASE RISK FACTORS, AND BONE HEALTH IN OLDER PUERTO RICANS

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ABSTRACT

Background: Cardiovascular diseases (CVD) and osteoporosis are two major public health problems in the aging population. Prior research has established an association between the two conditions. However, it remains unclear how risk factors for CVD, as opposed to incident CVD events, affect bone health. Puerto Rican adults living in Massachusetts have documented health disparities and have a disproportionate cardiometabolic risk burden compared to other Hispanic subgroups, but little is known about bone health in this population. It is therefore important to understand the association between CVD risk factors and bone health in this high risk group.
Objectives: The objectives of this dissertation work were to (1) Investigate the association between ten-year risk of coronary heart disease (CHD) and bone health; (2) Explore the association between central fat mass and bone health; (3) Examine the association between C-reactive protein (CRP), pro-inflammatory cytokines (interleukin-6 (IL-6) and tumor necrosis factor- α (TNF-α)), and bone health; (4) Develop a diet and lifestyle score based on the 2006 American Heart Association (AHA) Diet and Lifestyle recommendations (AHA-DLR) and examine its association (i) with available CVD risk factors, and (ii) bone health.
Study Design: Participants (n=636, with variation by objective based on data availability and exclusion criteria), aged 47-79 years, were enrolled in the Boston Puerto Rican Osteoporosis study, an ancillary study to the Boston Puerto Rican Health Study.
Results: Among women, the ten-year Framingham CHD risk score was inversely associated with bone mineral density (BMD) at the femoral neck (FN) (P=0.03). Borderline associations were seen at the trochanter (TR) and total hip (TH) (P>0.05). No associations were seen at the lumbar spine (LS). Central fat mass was inversely associated with BMD (P<0.008) at all four bone sites, among women. Among men, higher central fat mass was associated with lower BMD at all three hip sites (P<0.02) but not at the LS. Postmenopausal women in the second tertile of TNF-α (2.4-4.1 pg/mL) and intermediate concentration (1-3 mg/L) of CRP, compared to the lowest categories, had lower LS BMD (P<0.05). Women in the second tertile of TNF-α had lower FN BMD (P< 0.05). An inverse trend at the LS BMD (P for trend=0.04) was observed across tertiles of IL-6 (pg/mL). Postmenopausal women with multiple exposures to elevated inflammatory markers had a lower LS BMD (P for trend=0.04). In Puerto Rican men and women, greater adherence to the AHA-DLR was significantly associated with higher HDL cholesterol (P=0.001), lower waist circumference (P<0.0001), ten-year risk of CHD (P=0.01 in women), insulin (P=0.0003), glucose (P=0.01 in those with BMI<25), and CRP concentrations (P=0.02). Among men and women, the AHA diet and lifestyle score was associated with higher BMD at the FN, TR, and TH (P<0.05). No component of the AHA-DLR, alone, was responsible for the observed positive associations.
Conclusions: CVD risk factors are associated with poor bone health in the Puerto Rican population. Importantly, guidelines intended for CVD risk reduction were consistent with better bone health. This underscores the need to synchronize guidelines for general chronic disease prevention that provide a simpler public health message. The results of this dissertation provide foundational data guiding further research and policy development. They suggest that interventions yielding reductions in CVD risk factors may have significant impact on reducing both CVD and osteoporosis.

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CHAPTER ONE: INTRODUCTION

STATEMENT OF PROBLEM

Osteoporosis and cardiovascular disease (CVD), two multi-factorial and chronic diseases, are major public health concerns in the western world. While traditionally thought to be independent and unrelated disease entities, recent evidence from basic science and clinical work suggests a common etiology. Several epidemiological studies have established an association between fracture risk (1-6), low bone mineral density (BMD) (7-11), and incident cardiovascular events. A literature review on this topic has revealed several important gaps in knowledge. First, studies examining the association between CVD and bone health have reported both positive and null associations. Second, it remains unclear if risk factors for CVD, as opposed to CVD events, affect BMD and the odds of osteoporosis or osteopenia. Identifying an association with CVD risk factors will allow us to focus efforts on prevention of disease rather than treatment following the occurrence of an incident CVD event. Third, no studies have evaluated the effect of adherence to guidelines intended for CVD risk reduction on bone health. Finally, these associations have not been explored among Puerto Ricans, a group with documented health disparities and the highest prevalence of several chronic conditions, including central obesity, type 2 diabetes, and physical disability (12-13), all of which are important risk factors for both CVD and osteoporosis. Identifying these associations will not only provide us with an improved scientific understanding of the etiology of CVD and osteoporosis in Puerto Ricans but will also inform future public health practice. Recognizing key links between CVD and bone will allow us to synchronize and simplify

dietary and lifestyle guidelines for two conditions that have a considerable impact on the aging population.

SIGNIFICANCE OF THE PROBLEM

The Hispanic population is the largest and most rapidly growing minority group in the country. Reducing health disparities in minority populations remains a major public health challenge in the United States (US). Cardiovascular disease remains the leading cause of death among Hispanics. In fact, Hispanics have a disproportionate cardiometabolic risk burden compared to other groups. For example, Mensah et al (14), using national surveys, determined CVD and risk factor prevalence in adults, aged 18 years and older, by race/ethnicity. The authors found that among men, the highest prevalence of obesity (29.2%) was found in Mexican Americans who had completed a high school education. Likewise, prevalence of hypercholesterolemia was high among Mexican American men. Further, among women who completed a high school education, Mexican American women tended to have high prevalence of elevated concentrations of C-reactive protein and fibrinogen. The severity of these disparities in CVD risk factors challenges the notion of the Hispanic paradox, where Hispanic groups were thought to have lower CVD and mortality despite unfavorable conditions (15-16).

Traditionally, most research on Hispanics has focused on Mexican Americans, primarily due to their majority as a sub-group. However, Puerto Ricans are the second largest Hispanic subgroup in the US (17) and the largest in the Northeastern United States (US) (18). The Puerto Rican population is also one of the most economically disadvantaged groups in the country. Few studies have evaluated the health and health

behaviors of Puerto Rican adults. The Massachusetts Hispanic Elders Study (MAHES) was a statewide representative sample of Hispanic adults (Dominicans and Puerto Ricans), aged 60 years and older, with a neighborhood based comparison sample of non-Hispanic white adults. It was the first large study to highlight health disparities in older Puerto Rican adults in the US mainland. This study demonstrated that Puerto Rican elders were significantly more likely to have physical disability, depression, cognitive impairment, diabetes, and other chronic health conditions than non-Hispanic white elders living in the same neighborhoods (12-13,19-21). This suggests that the disparity is not due only to physical or neighborhood location, and that other factors must be influencing these differences.

Baseline data from the Boston Puerto Rican Health Study of 1357 Puerto Rican adults, ages 45-75 years, not only confirm the health disparities identified by MAHES, but alarmingly show even greater prevalence among those of comparable ages (22). Importantly, type 2 diabetes, systolic hypertension, low HDL cholesterol, and central obesity, among other well known risk factors for heart disease, were also highly prevalent even in those aged 45-60 years (22). Further, poor physical function in this age group was associated with obesity, diabetes, depression, history of heart attack, stroke, and arthritis, after adjusting for age, sex, education, income, and lifestyle ($P < 0.05$) (23). Mattei et al (24) demonstrated that Puerto Ricans living in Boston experienced physiological dysregulation that was associated with increased odds of chronic conditions such as abdominal obesity, hypertension, diabetes, self-reported CVD, and arthritis. Higher intake of the traditional diet of Puerto Ricans, with a foundation of rice and beans, was associated with lower HDL concentration (P for trend = 0.007) and higher likelihood of

metabolic syndrome (odds ratio = 1.7; 95% confidence interval: 1.04-2.7). Likewise, a meat and French fries pattern was found to be associated with higher blood pressure (systolic *P* for trend = 0.03 and diastolic *P* for trend < 0.001) and waist circumference (*P* for trend = 0.04) (25). The high prevalence of multiple CVD risk factors in Puerto Ricans coupled with unhealthy dietary practices across socio-ecological levels, show that this population is a high-risk group.

Given the biological plausibility for a connection between CVD and bone, it is imperative to understand how CVD risk factors affect bone health before the actual onset of an incident CVD event. To our knowledge, there is a paucity of data linking the high prevalence of CVD risk factors with bone health in this population. Results from this dissertation work may have implications for prevention beyond that of clinical CVD. Interventions yielding even modest reductions in CVD risk factors may have a dual impact on reducing both CVD and osteoporosis. The existence of a large and unique cohort of older Puerto Ricans provided a highly efficient framework to better understand CVD health disparities and osteoporosis risk in this understudied and high-risk population.

STATEMENT OF HYPOTHESES

Hypothesis 1: Higher 10-year risk of coronary heart disease (CHD) is associated with lower BMD and higher odds of osteoporosis.

Specific Aim 1: To investigate the association between 10-year risk of CHD (measured by the Framingham Risk Score (FRS)) and bone health (BMD and osteoporosis) of the femoral neck, trochanter, total hip, and lumbar spine (L2-L4).

Hypothesis 2: Central obesity is associated with lower BMD and higher odds of osteoporosis.

Specific Aim 2: To determine the association between abdominal fat mass and bone health (BMD and osteoporosis) of the femoral neck, trochanter, total hip, and lumbar spine (L2-L4).

Hypothesis 3: Lower circulating concentrations of inflammatory markers are associated with higher bone mass and lower odds of osteoporosis.

Specific Aim 3: To investigate the association between C-reactive protein (CRP), interleukin (IL)-6, and tumor necrosis factor (TNF)-α and bone health (BMD and osteoporosis) of the femoral neck, trochanter, total hip, and lumbar spine (L2-L4).

Hypothesis 4: Higher American Heart Association (AHA) diet and lifestyle score (indicating greater adherence to the AHA Diet and Lifestyle Recommendations) is associated with greater BMD and lower odds of osteoporosis.

Specific Aim 4a: To develop a unique AHA diet and lifestyle score based on the 2006 AHA Diet and Lifestyle Recommendations and validate it against selected CVD risk factors.

Specific Aim 4b: To determine the association between the AHA diet and lifestyle score and bone health (BMD and osteoporosis) of the femoral neck, trochanter, total hip, and lumbar spine (L2-L4).

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CHAPTER TWO: REVIEW OF LITERATURE

CARDIOVASCULAR DISEASE

A Public Health Problem

Cardiovascular diseases are the leading cause of death in the United States (US). It is estimated that more than one in three Americans have one or more types of cardiovascular disease (CVD). Of the 82.6 million American adults affected by CVD, 40.4 million are estimated to be age 60 or older. Mortality data show that CVD deaths accounted for 33.6% of all deaths in 2007. More than 2,200 Americans die from CVD each day, an average of one death every 39 seconds. While CVD death rates have declined in recent years, the burden of the disease remains high [1].

The prevalence and control of traditional CVD risk factors remains a challenge for many Americans. Nearly 66% of the US population is overweight or obese. Recent evidence indicate that a body mass index (BMI) of 25.0 to 29.9 is associated with a 13% greater risk (95% confidence interval [CI]: 1.09 - 1.17) of all-cause mortality relative to BMI<25 [2]. Data from the National Center for Health Statistics/National Health and Nutrition Examination Survey (NHANES) 2005-2008 show that an estimated 33.5 million adults, aged 20 years and older, had serum cholesterol concentrations ≥240 mg/dL, with a prevalence of 16.2% [1]. Significant disparities exist in the prevalence and control of these risk factors. Data from NHANES/National Center for Health Statistics (NCHS) 2005-06 indicate that rates of blood pressure control were lower in Mexican Americans (35.2%) than in non-Hispanic whites (46.1%) [3]. Among people aged 18 years and older, Hispanics or Latinos were less likely (27.8%) compared to non-Hispanic whites (38.1%), to engage in regular leisure-time physical activity, defined as light to

moderate activity for at least 30 minutes, 5 times per week, or vigorous activity for at least 20 minutes, 3 or more times per week. Similarly, 11.0% and 12.7% of Mexican American men and women, aged 20 years and older, compared to 6.8% and 6.5% of non-Hispanic white men and women were found to have physician-diagnosed diabetes [1].

Fortunately, a significant proportion of CVD can be prevented by adopting a healthy lifestyle. Recognizing this, one of the goals of Healthy People 2020 is to improve cardiovascular health and quality of life through prevention, detection, and treatment of risk factors for heart attack and stroke [4]. Yet, this remains an issue for many Americans. National data from the Behavioral Risk Factor Surveillance System indicate that the prevalence of individual healthy lifestyle characteristics was not high. This was particularly true for consuming 5 fruits and vegetables per day (23.3%, 95% CI: 22.9%-23.7%) and engaging in regular physical activity (22.2%, 95% CI: 21.8%-22.6%). It has been estimated that only 3% (95% CI: 2.8%-3.2%) of the US adheres to all four healthy lifestyle characteristics which include non-smoking, maintaining a healthy weight, consuming at least 5 servings of fruits and vegetables, and engaging in regular physical activity [5].

Cardiovascular Health in Puerto Ricans

CVD is the leading cause of death among both Hispanic males and females [6]. Recent studies have challenged the “Hispanic Paradox”, an observation that Hispanics have lower all-cause and cardiovascular mortality despite greater rates of risk factors and socioeconomic disadvantages [7-8]. The Hispanic paradox described in past research may derive from inconsistencies in counts of Hispanic-origin deaths and populations [8].

Among participants with diabetes in the San Antonio Heart Study, contrary to the prediction of the “Hispanic Paradox”, US born Mexican Americans were at a greater risk (hazard ratio (HR)=1.66, 95% CI: 1.04-2.65) and Mexican-born Mexican Americans had similar risk (HR=0.89, 95% CI: 0.40-2.01) for CVD mortality when compared to non-Hispanic whites [9]. In an expanded analysis of all the San Antonio Heart Study participants, aged 45-64 years at baseline, Hunt and researchers [10] confirmed the previous findings. Age and gender adjusted hazard ratios for CVD and coronary heart disease (CHD) mortality were 1.70 (95% CI: 1.30, 2.24) and 1.60 (95% CI: 1.09, 2.36) for Mexican Americans compared to non-Hispanic whites. The Corpus Christi Heart Project documented that validated CHD mortality in Mexican Americans exceeded that for non-Hispanic whites living in the same community [11]. Moreover, multiple co-morbidities and risk factors for CVD are reported more frequently in Hispanics than other ethnic groups.

Among Hispanic sub-groups, Puerto Ricans suffer from greater chronic disease burden than Dominicans or non-Hispanic whites living in the same neighborhood [12]. Not surprisingly, CVD is the leading cause of death in Puerto Rico [13]. Yet, compared to other minority populations, there has been a paucity of research on CVD risk in Puerto Ricans. Early evidence from the 1960s and 70s had suggested that Puerto Ricans were relatively protected from coronary disease and hypertension compared to the predominantly Caucasian Framingham population [14]. In fact, data from the International Atherosclerosis Project in the 1960’s demonstrated less severe athermoatous changes in the coronary arteries and aortas of Puerto Ricans compared to the US continentals [15]. Genetic factors, and healthy diet and lifestyle were suggested as

potential reasons for this relative protection from CHD [16]. However, with gradual transition from an agricultural to an industrial economy, Puerto Rico has witnessed an increase in CVD rates.

A significant amount of information on CVD in Puerto Ricans comes from the National Heart Lung and Blood Institute funded Puerto Rico Heart Health Program. The program was established in 1964 to determine the incidence of CHD in this population and to clarify etiologic factors. It is the only large-scale cohort in Puerto Rico that has focused on cardiovascular health. The population selected for this cohort included 10,000 males, aged 45-64 years, living in both urban and rural areas of the island [17]. The primary finding from this study was the presence of urban-rural differences in heart disease. The prevalence of definite myocardial infarction, established by electrocardiographic criteria, was significantly higher in the 45 to 54 year urban group. In addition, there were sizeable and statistically significant differences between urban and rural participants in several CVD risk factors. For example, cigarette smoking, fat consumption, blood pressure, heart rate, relative weight, serum cholesterol, serum triglycerides, prevalence of diabetes and hypertension were all lower in rural dwellers [18]. Additionally, mainland-island differences were recently described by Ho and colleagues [19], using data from the Behavioral Risk Factor Surveillance System and a survey of Puerto Ricans living in New York City. They found that, compared with islanders, mainland Puerto Ricans had higher prevalence of diabetes (standardized relative risk (RR) = 1.4, 95% CI: 1.01-2.0) and those with diabetes showed higher prevalence of smoking (standardized RR = 4.2, 95% CI: 2.3-7.7) and physical illness (standardized RR=1.5, 95% CI: 1.1-2.0) compared to Puerto Ricans living on the island.

Recent epidemiological data show that although mortality from CHD and stroke is steadily decreasing in the US, it is increasing in Puerto Rico [20]. An increase in CVD risk factors such as obesity, diabetes, social stress, and dietary changes may be contributing to this observation. We have previously shown that older Puerto Ricans living in the continental US suffer from higher prevalence of CVD risk factors such as systolic hypertension, type 2 diabetes, obesity, and depression, compared to neighborhood matched non-Hispanic white populations [21-22]. Several factors contribute to the disproportionate chronic disease burden of Puerto Ricans living in Massachusetts. First, large proportions of Puerto Ricans live below the poverty line, mainly in crowded, urban environments [23] thus limiting access to both health protective goods and health care. Second, as with many groups in such circumstances, their health-related behaviors tend to be characterized by low levels of physical activity and poor dietary habits [12, 24]. Given the high prevalence of CVD risk factors and the socio-economic disadvantage they face, this dissertation work focuses on the association between select CVD risk factors and bone health in a population of Puerto Rican adults living in the greater Boston area.

OSTEOPOROSIS

A Public Health Problem

Elderly people are the fastest growing population worldwide. The population aged 65 and over, will increase by 36% between 2010 and 2020, from 40 million to 55 million people. It is estimated that by the year 2030, the number of older persons will double from the number in 2007 to 72.1 million. The group >65 years of age is expected to

represent nearly 20% of the population by the year 2030. Of this group, the population of people >85 years is projected to increase from 5.8 million in 2010 to 6.6 million in 2020, an estimated 15% increase in one decade [25].

As the world's population ages, osteoporosis is threatening to become a major public health problem with severe economic and social consequences. Osteoporosis is defined as a "systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue leading to enhanced bone fragility and a consequent increase in fracture incidence [26]. The World Health Organization defines osteoporosis as a bone mineral density (BMD) less than or equal to 2.5 standard deviations (SD) below the mean for young adults and low bone mass (or osteopenia) as a BMD between 1 and 2.5 SD (also referred to as T-score) below the young adult group. A T-score less than 1 SD below the reference mean indicates normal BMD [27].

Osteoporosis and low bone mass affect more than half of the population aged 50 and older. Based on current trends, it is estimated that the prevalence of these conditions will continue to rise from 44 million in 2002 to 61 million by 2020 [28]. While BMD is conventionally used as a proxy for bone strength, fractures are the clinically relevant outcome. Globally, the number of hip fractures is estimated to approximately double from 1.96 million in 1990 to 2.6 million by the year 2025, and 4.5 million by the year 2050 [29]. However, more recent estimates from a small Midwestern community indicate that between 1980 and 2006, the incidence of a first-ever hip fracture declined by 1.37% per year for women in the US [30]. Other national data from NHANES corroborate these findings, indicating that osteoporosis reduction targets identified for Healthy People 2010 have been met.

Despite these declines in hip fracture incidence, the number of older US adults with low femoral BMD (5.3 million with osteoporosis and 34.5 million with osteopenia at the femoral neck) remains considerable [31]. The Surgeon General estimates that the number of hip fractures could double by the year 2040 due to the increase in the aging population [32]. This is especially important because mortality at 6 and 12 months following a hip fracture is 11-23% and 22-29%, respectively. Trends over the last 40 years have shown that, despite decreases in hip fracture incidence, mortality after injury remained essentially unchanged [33].

Osteoporosis related fractures impose a significant financial burden on the US health care system. For 2005, the cost to the healthcare system associated with osteoporosis-related fractures has been estimated at $17 billion and is projected to increase to $25 billion by 2025 [34]. Using both privately insured and Medicare claims data, Pike et al [35] noted that the direct (i.e., healthcare associated) costs and indirect (i.e., work loss) related costs of non-vertebral (e.g., pelvis, femur, lower leg, forearm, upper arm, and rib) fractures were substantial. Mean incremental healthcare costs per non-vertebral fracture in Medicare and privately insured claims were $13, 387 and $5,961, respectively. Among patients with available disability data, work loss accounted for 29.5% of total costs for each employee with a non-vertebral fracture. These patients are at substantial risk for subsequent non-vertebral fractures [36] and have substantial excess costs beyond the first year in which the fracture occurs [37].

Osteoporosis in Hispanics

While most of our knowledge about the treatment of osteoporosis comes from research in non-Hispanic white postmenopausal women, osteoporosis remains a

significant health problem in the Hispanic population. Differences in BMD between Hispanics and non-Hispanic whites have varied in different cross-sectional studies, based on the variables adjusted for. For example, in postmenopausal women, aged 50-69 years, from three ethnic groups (Caucasians, Filipinas, and Hispanics) in San Diego County, ethnic differences in BMD were minimized after accounting for body size using either BMI or fat and lean tissue mass along with height and other lifestyle variables [38]. Similarly, in a small study of young Puerto Rican and non-Hispanic white women, no differences in total body BMD existed after accounting for differences in age, weight, height, and fat [39]. In another study of 401 Hispanic and 451 non-Hispanic white men, aged 30-79 years, living in Boston, significant differences in BMD were restricted to the hip region, where non-Hispanic white men had lower BMD. Yet, Hispanic men had the steepest age-related declines in BMC and BMD [40] suggesting that this population is at high risk. Using data from NHANES 1999-2004, Looker et al [41] noted that non-Hispanic whites had significantly higher age-adjusted mean total body BMD than Mexican Americans, and that this difference persisted in both sexes and across each decade of age, except for women under 50 years of age.

Differences in the prevalence of osteoporosis and osteoporosis-related fractures also exist. Using Medicare data, Cheng et al. [42] demonstrated that the prevalence of osteoporosis among Medicare beneficiaries was two times higher for Hispanic Americans compared to African Americans and that this prevalence was substantially higher in women than in men. Likewise, the NHANES multiethnic data show that Mexican American and non-Hispanic white women had the highest prevalence of osteoporosis [43]. Rates of hip fracture over the past two decades have nearly doubled in Hispanic

women and increased by 80% in Hispanic men while they remained unchanged among African-Americans and non-Hispanic whites [44]. Among participants in the National Osteoporosis Risk Assessment study, non-Hispanic white and Hispanic postmenopausal women had the highest risk for fracture followed by Native Americans, blacks, and Asian Americans. These differences persisted even after adjusting for BMD, weight, and other covariates [45].

Disparities in fracture risk between Hispanics and Caucasians in the US may reflect the disparities in different risk factors, including activity levels, nutrition, risk of falling, obesity, frailty, and BMD. For example, Hispanic women have lower levels of physical activity [46] and lower 25-hydroxyvitamin D3 concentrations [47] compared to white women. Studies in minority populations need to consider other culture risk factors in addition to the traditional factors for osteoporosis risk. Using an ecological approach, Evans et al [48] predicted an increased prevalence of self-reported fractures among older Hispanic women who preferred Spanish, had decreased emotional support, had increased financial support, and had not smoked.

Disparities in the economic burden of fracture also exist. While the cost of osteoporosis related fractures is projected to rise by 50% in 2025 for Caucasians, this increase is estimated to be nearly 175% for Hispanics [34]. Racial and socioeconomic disparities in bone density testing remain before and after hip fracture. Hispanic women were 66% less likely to undergo bone density testing before a hip fracture. Although this decreased to 58% after a hip fracture, racial differences in osteoporosis evaluation continued to occur [49].

The majority of investigations in the Hispanic community have focused on Mexican Americans, with much less data available in other Hispanic groups such as Puerto Ricans. To our knowledge, this is the only large scale cohort with bone and body composition measurements in older Puerto Ricans, which provided us with a unique opportunity to study the proposed associations in this high-risk group.

CVD AND OSTEOPOROSIS – TWO RELATED DISEASE ENTITIES

As noted in the previous section, CVD and osteoporosis are two major public health problems, leading to increased morbidity and mortality in the aging population. Although historically thought to be independent conditions, elegant *in vitro* and *in vivo* studies indicate that there are several common underlying pathophysiological mechanisms linking both diseases. Several epidemiologic studies have reported an association between low bone mass and CVD. In one of the earliest studies, von der Recke et al. [50] noted that women in the lowest quartile of BMD had twice the risk of CVD death compared to those in the highest quartile. For every one SD decrease (0.4 g/cm), the relative risk of dying from CVD within 17 years of menopause was increased 2.3 fold (95% CI: 1.0-5.3, $P<0.05$). In 6,046 women, aged 65 years or older, taking part in the Study of Osteoporotic Fractures, hip BMD loss was associated with increased mortality from coronary heart disease (HR=1.3 per SD; 95% CI: 1.0-1.8). This association persisted even after adjustment for age, baseline BMD, diabetes, hypertension, incident fractures, smoking, physical activity, health status, weight loss, and calcium use suggesting that bone loss and coronary disease share common etiologies [51]. In the same study, among 9,704 ambulatory women aged 65 years or older,

diminished BMD at the proximal radius was strongly associated with deaths from stroke (RR=1.74; 95% CI: 1.12-2.70), an association that was not confounded by history of previous stroke, hypertension, postmenopausal use of estrogen, thiazide diuretic treatment, diabetes mellitus, and smoking. Similar associations were also seen in a Swedish population where risk of death for each SD decrease in femoral neck BMD was 1.41 (95% CI: 1.21-1.64) [52]. Interestingly, in both studies, low BMD and not fracture, per se, was associated with increased risk of mortality lending important information when assessing the cost-effectiveness of BMD screening [53]. Both research groups also noted that for each SD decrease in BMD at the calcaneus (0.09 g/cm^2) [54] or at the femoral neck [52], risk of stroke increased by 1.31 (95% CI: 1.03-1.65) and 1.23 (95% CI: 1.01-1.49), respectively. The similarity of risk estimates in both studies lends credibility to the association between bone and CVD. Other prospective studies have also demonstrated an association between bone health and incident CVD events. For example, in the MINOS study, men in the lowest quartile of whole body BMD had nearly a two-fold increase in risk for CVD events compared to men in the three upper quartiles (HR=1.78, 95% CI: 1.05-3.03) [55]. In a biracial cohort, spine volumetric BMD was inversely associated with incident CVD in white (HR (integral) =1.39, 95% CI: 1.03=1.84; HR (cortical) = 1.38, 95% CI: 1.03-1.87) but not black men. On the other hand, areal BMD measures of the total hip (HR = 1.36, 95% CI: 1.03-1.78), femoral neck (HR = 1.44, 95% CI: 1.10-1.90), and trochanter (HR = 1.34, 95% CI: 1.04-1.72) exhibited significant associations with incident CVD in blacks but not in whites [56].

While the evidence for the prospective relationship between bone and CVD is strong, the reverse relationship has also been reported. For instance, heart failure was

independently associated with a greater risk of any orthopedic fracture (odds ratio [OR]=4.0, 95% CI: 2.9-5.3) or hip fracture (OR=6.3, 95% CI: 3.4-11.8) [57]. In another elegant study of identical twins, Sennerby et al [58] noted that the multivariable-adjusted HR of hip fracture after a diagnosis of heart failure was 4.40 (95% CI: 3.43-5.63); after a stroke, the HR was 5.09 (95% CI: 4.18-6.20); after a diagnosis of peripheral atherosclerosis, the HR was 3.20 (95% CI: 2.28-4.50); and after an ischemic heart disease event, the HR was 2.32 (95% CI: 1.91-2.84). Interestingly, the identical twin without heart failure and stroke also had an increased rate of hip fracture after their co-twin had been exposed to these respective diseases. This indicates the presence of genetic factors in the association between CVD and fracture risk. Employing a population-based case-control study design of 1,327 incident hip fracture cases and 3,170 randomly selected population controls, Sennerby and others [59] found that among women, aged 50 to 81 years, in Sweden, the risk of hip fracture doubled after a CVD event (OR=2.38; 95% CI: 1.92-2.94) even after adjustment for variables including several chronic diseases. The authors noted a gradient increase in risk of hip fracture with increasing number of hospitalizations for CVD and highest fracture risk occurred the first year after the CVD event. Contrary to these findings, in the Cardiovascular Health Study, after adjustment for shared risk factors such as age, race, education, income, BMI, health status, smoking, estrogen use, menopause, diabetes, physical activity, cardiac medications, association between heart failure and hip fracture (HR for men=1.59, 95% CI: 0.93-2.72; HR for women=1.41, 95% CI: 0.98-2.03) was attenuated [60].

In addition to incident CVD events, several prospective and cross-sectional studies have established an association between sub-clinical measures of atherosclerosis

and bone. Aortic calcification, a surrogate measure of atherosclerosis, measured either by radiographs or computed tomography, was associated with lower BMD and accelerated bone loss at the proximal femur [61] and a higher odds of vertebral and hip fractures (OR=4.8, 95% CI: 3.6-6.5; OR=2.9, 95% CI: 1.8-4.8, respectively) [62]. Postmenopausal women with osteoporosis or osteopenia [63-64] have higher coronary calcium scores, indicating greater coronary calcification compared to their healthy counterparts.

Several studies in different ethnic groups have consistently shown that BMD at different sites were significantly and inversely associated with other sub-clinical measures of atherosclerosis, including intima media thickness [65-66] and ankle-brachial index [67-68]. Likewise, pulse wave velocity, a measure of arterial stiffness, was negatively correlated with femur [69-70] and lumbar spine [71] BMD in separate cohorts.

Few studies have evaluated the association between CVD and bone health in Hispanics, and none in Puerto Ricans. In the San Antonio Osteoporosis Study, a strong inverse association was demonstrated between BMD and intima media thickness in both Mexican American men and women, over 60 years of age. This association was independent of known environmental factors and risk factors of CVD [72]. Although the evidence for a link between CVD and bone health is substantial, less is known about how CVD risk factors affect bone health. Given the high prevalence of CVD risk factors in the Hispanic population, especially Puerto Ricans, it is critical to understand the link between CVD risk factors and bone. The first paper of this dissertation work examined the association between the ten-year risk of CHD, as assessed by the Framingham risk equations, and bone health.

Whilst several common mechanisms for CVD and osteoporosis have been postulated, few studies have explored the association between CVD risk factors such as central obesity and pro-inflammatory cytokines and bone health. The epidemiological evidence and the biological plausibility behind such associations will be discussed in the following sections.

POTENTIAL MECHANISMS LINKING CARDIOVASCULAR DISEASE AND OSTEOPOROSIS

Central Fat Mass

Rates of obesity and osteoporosis have increased dramatically in recent years. Paradoxically, there is extensive epidemiological and clinical evidence to show that a higher body weight is associated with lower bone loss [73-74]. In fact, studies in anorexic participants have shown that substantial bone loss occurs with a decrease in body weight [75-76]. A plethora of evidence shows that fat mass, a main component of body weight, is also associated with higher bone mass. In a cross-sectional study of 140 white postmenopausal women, aged 45-71 years, total body fat was the most significant predictor of BMD throughout the skeleton [77]. Similar results with lumbar spine BMD were seen in Japanese postmenopausal [78], but not pre-menopausal [78-79], women. Longitudinal analysis of postmenopausal Caucasian women revealed that annual changes in fat tissue mass and weight were significantly associated with annual changes in regional BMD after adjustment for initial bone mineral values ($P<0.05$) [80-81].

Reid [82] and Zhao et al [83] summarized the mechanisms of the fat-bone relationship. First, a higher fat mass exerts a greater mechanical stress on the bone. In

response, bone mass increases to accommodate this greater load on the skeleton. Second, several hormones link the two tissues. The hypothalamus centrally modulates fat and bone via the sympathetic nervous system by regulating appetite, insulin sensitivity, energy use, and skeletal remodeling [84]. Third, the adipose tissue is now recognized as a metabolically active tissue that secretes a variety of biologically active molecules including estrogen, leptin, resistin, and adiponectin. Further, the secretion of bone-active hormones from the pancreas, such as insulin and amylin, may also explain the relation between fat and bone. Finally, but most importantly, both adipocytes and osteoblasts have a common bone marrow-derived mesenchymal stem cell origin. Stromal cells are regulated by endocrine, paracrine, and autocrine signals, and enter bone, cartilage, or fat lineages depending on their mode of activation and the local environment [84]. However, this mechanism would suggest an inverse association between fat and bone, contrary to the traditionally held notion that fat is protective of bone. In fact, several recent studies have suggested that excessive fat mass may not be protective against bone loss. With the recognition of fat as an endocrine organ, the effect of fat mass on bone may extend beyond its mechanical load on the skeleton.

Comparing NHANES 1999-2002 data with NHANES III data, Looker et al [85] found a positive relationship between BMI and BMD but concluded that the increasing overweight rates among older women are not likely to lead to a significant reduction in osteoporosis prevalence. More recently, comparing NHANES III data with NHANES 2005-2006 data, Looker et al [31] found that the prevalence of osteoporosis at the femoral neck decreased but changes in BMI did not fully explain this decline. Previous studies that have demonstrated a positive association between fat and bone were

confounded by the mechanical loading effects of body weight. In both Chinese and Caucasian subjects, when the mechanical loading effect of body weight on bone mass was taken into account, the phenotypic correlation between fat mass or percentage of fat mass and bone was negative [86]. Similarly, in a study of 7,137 Chinese men, 4,585 premenopausal women, and 2,248 postmenopausal Chinese women, Hsu et al [87] found that across 5-kg strata of body weight, fat mass was significantly and inversely associated with bone mineral content (BMC) in the whole body and total hip.

While the evidence for a link between total fat mass and bone is considerable, little is known about the relationship between central fat mass and bone. A consistent body of literature suggests that central fat may be a stronger predictor of chronic disease risk than overall fat mass. Central obesity is a strong risk factor for CVD and diabetes in both men and women [88-90]. At the same time, abdominal or visceral fat mass is associated with inflammatory markers [91] and other cardiovascular risk markers, including impaired glucose tolerance, hypercholesterolemia, hypertriglyceridemia, and hypertension [92]. Visceral fat is considered to be more metabolically active as it increases hepatic free fatty acids delivery [93]. Cellular constituents of adipose tissue secrete cytokines and chemokines that may affect vascular disease [94]. A small body of literature has examined associations between measures of central adiposity and bone. Using magnetic resource imaging or computed tomography, four studies found that visceral fat was strongly and inversely associated with femoral bone phenotypes [95], BMD of lower limbs, trunk, and whole body [96], and spine BMD [97-98] in different populations and age groups.

Waist circumference is a relatively inexpensive and a surrogate measure for central adiposity. Abdominal obesity (defined as a waist circumference >102 cm for men and >88 cm for women) is a component of the metabolic syndrome and several studies have explored the relationship between metabolic syndrome and bone health. In the Rancho Bernardo Study, after adjusting for BMI, metabolic syndrome was associated with lower, not higher, BMD in 417 men and 671 women. At the same time, incidence of osteoporotic non-vertebral fractures was also higher in participants with metabolic syndrome [99]. On the other hand, in the MINOS study, a prospective study of male osteoporosis, men with metabolic syndrome had lower BMD but also a lower fracture risk. An examination of the components revealed that lower BMD was related to differences in abdominal obesity while the lower fracture risk was related to hypertriglyceridemia [100]. Finally, in two separate Korean cohorts, femoral neck BMD [101] and lumbar spine BMD [102] was significantly lower in subjects with metabolic syndrome. Among metabolic syndrome components, waist circumference was the most important factor in this negative association [101-102]. In contrast to these studies, Kinjo et al [103] noted that in NHANES III, femoral neck BMD was higher among subjects with metabolic syndrome (0.86 g/cm^2) than those without it (0.80 g/cm^2, P<0.0001). However, when stratified by BMI, these differences disappeared illustrating the importance of adjusting for the mechanical loading effect of fat mass. Other measures of central adiposity such as waist-to-hip ratio were also found to be negatively associated with BMD when adjusted for body weight [104-105].

While computed tomography is considered the gold standard in assessing centrally located fat, soft tissue analyses of DXA scans can also be used to estimate

visceral adipose tissue [106] by dividing the whole-body scan into sub-regions of interest [107]. In the second paper of this dissertation, we assessed abdominal fat mass using specialized regional body composition software (ENCORE v 12.2) on whole body DXA scans. We demonstrated the association between total fat mass (kg), also assessed from whole body scans, abdominal fat mass (g), and bone health before and after adjustment for body weight and height.

Pro-inflammatory cytokines

In the past decade, inflammation has been identified as the underlying biological mechanism for the development and progression of atherosclerosis [108]. More recently, the role of the immune system in the development of osteoporosis is slowly being unraveled. In this section, we will review both the molecular underpinnings of the role of inflammation in bone biology and the epidemiological evidence suggesting a role for pro-inflammatory cytokines in bone health.

Molecular mechanisms

During the bone remodeling cycle, bone resorption is coupled to bone formation. Multiple pro-inflammatory cytokines (interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α) and hormones (vitamin D and estrogen) not only regulate the coupling between these systems but also the differentiation of osteoclasts and osteoblasts [109]. Early cell culture work by Thomson et al [110-111] and McSheehy et al [112-113] showed that stimulation of osteoblastic precursor cells with IL-1, TNF-α, parathyroid hormone, and 1,25 $(OH)_2$ vitamin D_3 promoted the development of osteoclasts, primarily through their ability to stimulate the production of cytokines such as IL-6 and IL-11 [114]. A systemic

or local imbalance in these factors may result in increased osteoclast formation and eventually bone loss.

Inflammation modulates osteoclast formation and thereby bone resorption primarily through two mechanisms – 1) Pro-inflammatory cytokines have a final common mediator of osteoclast function, the receptor activator of nuclear factor-B (RANK) and its functional ligand (RANKL). RANKL is a membrane bound TNF receptor expressed on osteoblasts precursor cells that recognize RANK on the osteoblast surface through a direct cell-cell interaction. This process is essential for osteoclast differentiation, activation, and survival. 2) Osteoclastogenesis can be regulated through the modulation of macrophage colony stimulating factor (M-CSF). M-CSF is produced by bone marrow stromal cells and is essential for macrophage survival and proliferation. M-CSF binds to receptors on osteoclast precursors in the bone marrow and via the action of RANKL, stimulates the differentiation of these precursors to activated osteoclasts [115].

TNF-α, expressed by T-lymphocytes, increases osteoclast formation and bone resorption both directly and by augmenting the sensitivity of maturing osteoclasts to the essential osteoclastogenic factor RANKL. Further, TNF-α has potent anti-apoptotic effects on osteoclasts, prolonging their lifespan [116]. IL-6 is another key cytokine involved in osteoclastogenesis. One of the earliest evidence for a role of IL-6 in bone resorption came from the study of Ohsaki et al [117]. These researchers demonstrated that giant cell tumors of bone, which may have many features of osteoclasts, synthesize and secrete IL-6, suggesting that IL-6 plays an autocrine/paracrine role in bone resorption. T lymphocytes also secrete pro-resorptive cytokines, including IL-1, IL-6 and

IL-17, each of which can stimulate RANKL expression by osteoblasts and fibroblasts, permitting osteoclast formation by a contact-dependent process [118].

In recent years, there has been an expansion in our understanding of how estrogen withdrawal affects bone remodeling by modulating the production of pro-inflammatory cytokines and growth factors from the bone marrow. *In vivo* and *in vitro* models of postmenopausal osteoporosis demonstrate that estrogen deficiency leads to an increase in the adaptive immune function which ultimately leads to an increased production of TNF-α by activated T cells [119-120]. Further, it has been postulated that estrogen exerts its effect on bone, not by direct action per se, but by inhibiting IL-6 gene expression [121]. Therefore, it is important to consider potential interactions with either serum estradiol concentrations or postmenopausal status when considering the relationship between pro-inflammatory cytokines and bone health.

Epidemiologic evidence

C-reactive protein (CRP) is a member of the pentraxin family of innate immune recognition proteins. CRP is one of the acute phase proteins that increases during systemic inflammation and is produced mainly in the liver. High levels of high sensitivity CRP (hs-CRP) consistently predict recurrent coronary events in patients with unstable angina and acute myocardial infarction. Recent studies have shown that high levels of systemic inflammation, indicated by higher circulating concentrations of CRP, are associated with low BMD and increased fracture risk. In the population-based Bruneck study (n=919), the multivariate adjusted relative risk of non-traumatic fractures in the highest (median CRP=4.60 mg/L) vs lowest (median CRP=0.70 mg/L) tertile group of hs-CRP was 9.4 (95% CI: 3.6-24.8) [122]. In the Geelong Osteoporosis Study, hs-CRP

was not associated with serum C-telopeptide or BMD of the femur, spine, forearm, or total body. Still, risk of fractures increased from 16.3% (95% CI: 6.8%-25.8%) for ln-hs-CRP less than -1 SD (0.96 mg/L) to 28.9% (95% CI: 17.7%-40.1%) for ln-hs-CRP greater than 1 SD (6.35 mg/L) [123]. On the other hand, in the Health Aging and Body Composition Study, a population based cohort of 2,985 men and women, aged 70-79 years, borderline associations were noted between circulating concentrations of pro-inflammatory cytokines and fracture risk. The multivariate adjusted relative risk of fracture for subjects in the highest vs lowest quartile of the inflammatory marker was 1.34 (95% CI: 0.99-1.82) for CRP, 1.28 (95% CI: 0.95-1.74) for IL-6, and 1.28 (0.97-1.70) for TNF-α. Interestingly, in subjects with three or more (out of seven) high inflammatory markers, the relative risk of fracture was 2.65 (1.44-4.89) in comparison with subjects with no elevated markers (*P* for trend = 0.001). Because the relative importance of each individual cytokine is difficult to assess, the composite inflammatory index is a useful way to assess risk of cumulative exposure to elevated cytokine concentrations [124].

Other prospective studies have explored the association between pro-inflammatory cytokines and change in BMD primarily in postmenopausal women. In a longitudinal study of 137 postmenopausal women, aged 52-80 years, serum IL-6 was the single most important predictor of femoral, but not lumbar spine, bone loss in women up to 10 years of menopause [125]. Ding et al [126] found that change in BMD in 168 randomly selected participants (mean age=63 years), were significantly predicted by IL-6 (hip and spine) and TNF-α (spine). On the other hand, Shea et al [127] found no evidence for a role for IL-6 and CRP and BMD either cross-sectionally at baseline or during the 3-

year follow-up. The lack of an association may have to do with the older age of participants in this study. It has been suggested before that IL-6 is a predictor of bone loss only during the first decade of menopause [125].

Like prospective studies, evidence from cross-sectional reports has been conflicting. In an analysis of 11,732 Korean pre- and postmenopausal women, aged ≥30 years, women in the highest quintile of hs-CRP (≥1.2 mg/L) had a significantly lower femoral neck BMD, compared to women in the lowest quintile (≤0.4 mg/L). Effect sizes at the lumbar spine were similar (β=-0.014) but were not significant (*P*=0.06) [128]. In a smaller study of 24 postmenopausal women, Papadopoulos and others [129] found a negative correlation at the Ward's triangle (r=-0.42, *P*<0.01) and radius ultradistal (r=-0.42, *P*<0.01). Only marginal associations were noted at the femoral neck (r=-0.25, *P*=0.08) and the lumbar spine (L2-L4) (r=-0.26, *P*=0.07). The small sample size and the fact that the Ward's triangle is not a clinically relevant site calls into question the validity of this study.

Cytokine production of IL-6 and TNF-α by peripheral blood mononuclear cells has been shown to be associated with bone health [130-132]. Contrary to the evidence from these studies, Bhupathiraju et al [133] found no association between hs-CRP and trabecular bone at the distal tibia in a group of healthy postmenopausal women. Likewise, two studies in Caucasian women [134-135] and one Iranian postmenopausal women [136] found no associations between CRP, IL-6, femoral neck, proximal femur, and lumbar spine BMD. The contradicting evidence from these studies is hard to explain but can be attributed to differences in study populations. Further, it may be that circulating concentrations of the cytokines may not be a true reflection of the events in the

microenvironment of the bone. Cytokines have short half lives in circulation and the presence of soluble cytokine receptors may sequester cytokine proteins [130]. Thus, the usefulness of serum or plasma biomarkers of cytokine concentrations remains unclear.

In the third paper of this thesis, we attempted to understand the complex association of circulating concentrations of pro-inflammatory cytokines with bone health while taking into consideration the role of estrogen in modulating this association. Moreover, all models were carefully constructed considering the basic biology, the interplay between the bone tissue and the immune system, and adjusting for important confounders.

Diet

Dietary Patterns

The traditional approach in nutritional epidemiology has been to study the effects of single nutrients or foods on health outcomes. However, individuals do not consume single nutrients but eat meals consisting of a variety of foods, with complex combinations of nutrients that are likely to be interactive or synergistic [137-138]. Pattern analysis provides an additional dimension to examining the relationship between diet and disease risk and suggests a more comprehensive approach to disease prevention or treatment, because the focus is on the entire diet rather than on just one food or nutrient [139].

Dietary pattern analysis using score-based approaches (diet indexes) is an “a priori” approach that is based on published dietary recommendations. The development of the score itself is based on interpretation of current dietary guidelines. Points are given based on how the individual’s diet conforms to a given dietary recommendation. Points are summed across the different components to arrive at a summary score. Thus, the

score is only as good as the components that make up the score. There has also been some debate as to whether the components of the score should be weighted equally or differentially. Further, scores need to be updated constantly as new nutrition knowledge evolves. Nevertheless, diet scores are particularly advantageous because they summarize dietary behavior into a single score, are easy to interpret, and are hypothesis-generating.

Dietary Patterns: A link between heart health and bone health?

Diet is one of the most important modifiable factors for development and maintenance of bone and for the prevention of CVD. This duality provides the biological possibility for it being a key link in the relationship between CVD and bone. For example, certain dietary patterns termed as “Healthy” or “Prudent” and defined by high intakes of fruit, vegetables, and cereal are associated with a reduced incidence of CHD [139-141] and greater BMD [142-143] compared to those consuming a “Western” pattern, typically characterized by greater proportions of intake from meat, dairy, sweet baked products, and alcohol.

Certain foods benefit both the heart and the bone tissue either independently or through common biological mechanisms underlying both diseases. For example, foods such as fruits and vegetables have been repeatedly shown to have benefits for CVD and bone health. In a meta-analysis of 12 studies by He et al [144], those consuming 3-5 or >5 servings/day of fruits and vegetables had a CHD risk reduction of 7% (95% CI: 0.86-1.00, P=0.06) and 17% (0.77-0.89, P<0.0001), respectively compared with individuals who consumed less than 3 servings/day of fruit and vegetables. At the same time, data from the Framingham Osteoporosis Study showed that participants who typically ate fruit, vegetables, and cereal had greater BMD than those with greater proportions of

intake from meat, dairy, sweet baked products; and alcohol [143]. The mechanisms by which fruits and vegetables exert their protective effects on the heart and bone are not entirely clear but may be due to their antioxidant or anti-inflammatory effect. For instance, we recently demonstrated that a greater variety in fruit and vegetable intake was associated with lower inflammation [145], a plausible mechanism linking both conditions.

In 2006, Lichtenstein et al. [146] published dietary guidelines for CVD risk reduction in Americans >2 years of age. A dietary pattern that is consistent with these recommendations is the Dietary Approaches to Stop Hypertension (DASH) diet. This dietary pattern is rich in fruits, vegetables, and low-fat dairy products. It also includes whole grains, poultry, fish, and nuts and is reduced in fat, red meat, sweets, and sugar-containing beverages. Compared with the control diet, the DASH diet provided lower levels of total fat, saturated fat, and dietary cholesterol, and higher levels of potassium, magnesium, calcium, fiber, and protein. Sodium was held constant across the diets while alcohol was limited to two drinks per day [147].

Several prospective cohort studies have examined potential associations between adherence to a DASH dietary pattern and incident CVD events. Levitan et al. [148] tested the hypothesis that diets consistent with the DASH diet would be associated with a lower incidence of heart failure in separate cohorts. In the Swedish mammography cohort, after 7-years of follow-up, women with the greatest adherence scores to the DASH diet had a 37% lower risk of heart failure (95% CI: 0.48-0.81, *P* for trend <0.001). Similarly, in a cohort of Swedish men, aged 45-79 years, men in the greatest quartile of the DASH component score had a 22% lower rate of heart failure events than those in the lowest

quartile (95% CI: 5%-35%, *P* for trend = 0.006) [149]. Using a diet score to measure adherence to the DASH diet, Fung et al. [150] noted a 14% lower risk of CHD among female nurses in the highest quintile compared to nurses in the lowest quintile (95% CI: 0.67-0.85, *P* for trend <0.001). Most interestingly, cross-sectional analysis in a subgroup of women with blood samples showed that the DASH score was significantly associated with lower plasma levels of CRP (*P*=0.008 for trend) and IL-6 (*P*=0.04 for trend), both of which are markers of inflammation. Because inflammation is a pathophysiological mechanism linking heart and bone health, these results imply that a DASH dietary pattern not only influences heart health but also bone health. In fact, data from an ancillary study to the DASH-Sodium trial suggest that the DASH diet (over 30 days) significantly reduced markers of bone turnover. Thus, the DASH diet, if sustained, may improve bone mineral status [151].

Improving diet and lifestyle is a critical component of the AHA's strategy for CVD risk reduction. Recognizing that diet is a critical component of lifestyle, in 2006, the AHA released Diet and Lifestyle Recommendations for CVD risk reduction. These recommendations are part of a comprehensive plan to achieve specific goals for CVD risk reduction [146]. As part of this dissertation work, we have developed a unique diet and lifestyle score to measure adherence to these recommendations. Further, we validated our score by examining cross-sectional associations with selected CVD risk factors [152]. Finally, in the fifth paper, we examined if the AHA diet and lifestyle score was associated with higher BMD and a lower odds of osteoporosis.

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CHAPTER THREE

Ten-Year Risk of Coronary Heart Disease and Bone Health in older Puerto Rican women[1-5]

(To be submitted to Osteoporosis International)

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Running head: Framingham risk score and bone health

ABSTRACT

Summary: In Puerto Rican women, ten-year risk of coronary heart disease (CHD) was associated with lower bone mineral density (BMD) and odds of osteoporosis or osteopenia at the femoral neck. This suggests the possibility that a tool for assessing CHD risk may also be valuable for assessing bone health at the femoral neck.

Purpose: Epidemiological data have shown an association between CHD and fracture risk. The purpose of this study was to examine if a CHD risk assessment tool is associated with BMD and risk of osteoporosis or osteopenia in a Hispanic population.

Methods: We used data on 354 Puerto Rican women, aged 47-79 years, enrolled in the Boston Puerto Rican Osteoporosis Study. General linear models were used to test for cross-sectional associations between ten-year risk of CHD, assessed using the Framingham risk score (FRS), and BMD (measured using DXA).

Results: After adjustment for alcohol use, BMI, height, physical activity, plasma vitamin D, income, and season of BMD measurement, higher FRS was associated with lower femoral neck, trochanter, and total hip BMD (β=-0.013 to -0.015, p <0.006). However, after adjustment for energy-adjusted calcium intake, these associations were attenuated (β=-0.009 to -0.011) and remained significant only at the femoral neck. No associations were seen at the lumbar spine (p>0.05). For every unit increase in the FRS, the odds of osteoporosis or osteopenia at the femoral neck increased by 3% (95% CI: 1.00-1.07).

Conclusions: The Framingham risk equations may be a useful tool for identifying low femoral neck BMD in a population of older Puerto Rican women.

Keywords: Framingham risk score, bone mineral density, osteoporosis, epidemiology, Puerto Ricans, Boston Puerto Rican Health Study

INTRODUCTION

Cardiovascular disease (CVD) and osteoporosis are two major public health problems in the aging population that have severe physical, economic, and social consequences. It is estimated that nearly 1 in 3 Americans have one or more types of CVD (1). At the same time, osteoporosis and low bone mass affect more than half of the population, aged 50 years and older (2). In 2006, health care spending and lost productivity from CVD exceeded $400 billion (3). Likewise, for 2005, the cost to the healthcare system associated with osteoporosis-related fractures has been estimated at $17 billion and is projected to increase to $25 billion by 2025 (4). Although historically thought to be independent diseases of the aging process, several prospective studies have shown that those with CVD have a greater risk of fracture or low BMD later in life (5-7).

The National Osteoporosis Foundation recommends BMD testing of all women age 65 and older (8). Yet, racial disparities in dual energy X-ray absorptiometry (DXA) screening rates exist (9-10). Compared to non-Hispanic white women, Hispanic women are 66% less likely to undergo bone density testing prior to a hip fracture. While this decreased to 58% after a hip fracture, racial differences in osteoporosis evaluation remain in Hispanics (10). Given this, development of risk prediction scores based on traditional risk factors may be useful for identifying high-risk groups. The World Health Organization Fracture Risk Assessment tool is one such score that evaluates the 10-year fracture risk of patients, but is based on BMD at the femoral neck (11). To our knowledge, no universal risk prediction scores exist for identifying those at low BMD.

The Framingham risk equations, developed from the experience of the Framingham Heart Study, are risk assessment tools that allow physicians to predict 10-

year multivariate coronary heart disease (CHD) risk in those without overt CVD. These risk equations are based on recommended guidelines of blood pressure, total cholesterol, and LDL cholesterol. Given the biological plausibility for a link between osteoporosis and CVD, it is possible that risk equations developed for identification of individuals at high risk for CHD can also identify those at high risk for low BMD or osteoporosis. The aim of this study was to identify an association between 10-year risk of CHD, as assessed by the Framingham risk score (FRS), and BMD in a population of older Puerto Rican women living in the greater Boston area.

MATERIALS AND METHODS

Study Population

The study population consisted of 517 women enrolled in the Boston Puerto Rican Osteoporosis Study, an ancillary study to the Boston Puerto Rican Health Study. The Boston Puerto Rican Health Study is a two-year prospective longitudinal study of 45-75 year old Puerto Ricans living in the greater Boston area. Details of the study design, eligibility criteria, and baseline characteristics of the cohort have been published elsewhere (12). Briefly, at baseline and two-years, bilingual interviewers administered a series of questionnaires to capture information at the participant's home. At the end of the two year follow-up, participants were re-consented for the Osteoporosis study. Consenting participants (n=540 of 799 eligible women) visited the Metabolic Research Unit at the Human Nutrition Research Center on Aging at Tufts University to obtain bone density measurements, provide a fasted blood draw, and answer additional questions on sun exposure and osteoporosis medication use. Every attempt was made to complete this

visit within one month of completion of the two-year follow-up. Primary reasons for non-participation in the Osteoporosis study included not being interested (n=108), scheduling problems (n=105), loss-to-follow up (n=23), and relocation out of Massachusetts (n=11). Another 12 participants died since their two-year follow-up interview. No significant differences were found for other socio-demographic characteristics, dietary intakes, lipid concentrations, or health measures between those who consented and those who did not. All participants signed informed consent forms that were approved by the Institutional Review Board at Tufts Medical Center.

At the time of analysis, complete information from the two-year follow-up and the Osteoporosis study visit was available on 517 women. The FRS was only calculated on those free of CVD (n=374) and those with complete information on components making up the FRS (n=354).

Assessment of exposure

We used the Framingham risk equations developed by Wilson et al (13) to calculate the estimated 10-year risk of CHD for each participant free of CVD at the two-year follow-up (from self report). Total CHD disease risk was defined as risk of angina pectoris, unstable angina, myocardial infarction and sudden death. The risk factors considered part of the FRS are sex, age, diabetes, smoking, systolic and diastolic blood pressure, total cholesterol, LDL, and HDL cholesterol. Diabetes status was defined as fasting plasma glucose $\geq$ 126 mg/dL or use of diabetes medication.

Assessment of outcome

For all analyses, we made an *a-priori* decision to only include BMD measurements at the femoral neck, total hip, and posterior-anterior lumbar spine (L2-L4) based on recommendations from the International Society for Clinical Densitometry (14). We also measured BMD at the trochanter, as inclusion of this anatomical site provides a complete picture of the hip. BMD (g/cm^2) at these four sites was measured by DXA (GE-Lunar model Prodigy scanner, Madison, WI) using standard procedures. The root mean square precision of these measurements was 0.65% for total hip BMD, 1.03% for the trochanter, 1.31% for the femoral neck, and 1.04% for the lumbar spine. For femur measurements, the right hip was scanned unless there was a history of hip fracture or joint replacement. Using the WHO definition, osteoporosis and osteopenia were defined as T-score thresholds of equal to or more than 2.5 or 1.0 SD, respectively, below the healthy young adult mean at the respective bone site (15). All scans with T-scores > 4.0 were reviewed by an academic endocrinologist (BDH) for extra-skeletal calcification or for presence of non-anatomical parts in the DXA scan region. One poor quality femur scan was excluded from the current analyses.

Blood measures

At the two-year and osteoporosis study visits, participants provided fasting blood samples. To extract plasma, blood was collected into vacutainers containing EDTA and immediately centrifuged at 3421 x *g* at 4^oC for 15 minutes. Plasma 25-hydroxy vitamin D (ng/mL) was measured from blood collected at the Osteoporosis study visit, using a ^{125}I radioimmunoassay kit procedure (DiaSorin Inc, Stillwater, MN) as specified by the manufacturer's procedural documentation (68100E). The intra- and inter-assay CV% are

10.8% and 9.4% respectively. From plasma collected at the two-year visit, total cholesterol, HDL cholesterol, and triglyceride concentrations were analyzed using an enzymatic endpoint reaction on the Olympus AU400e with Olympus cholesterol (OSR6116), HDL (OSR6156), and triglyceride (OSR6033) reagents, respectively. The intra- and inter-assay CV% for total cholesterol, HDL cholesterol, and triglycerides are 1.8, 3.0, 2.8, and 2.2, 7.0, 2.7, respectively. LDL cholesterol was calculated using the Friedewald formula unless triglyceride concentrations exceed 400 mg/dL in which case LDL cholesterol was calculated directly (16).

Other measures

Age (in years) was calculated by subtracting the date of the interview from the participant's date of birth. At the Osteoporosis study visit, we administered a short questionnaire to assess osteoporosis prescription medication use (y/n), which includes use of bisphosphonates, calcitonin, calcium, vitamin D, and cod liver oil. Standing height was measured with a stadiometer (Seca, Germany). Weight was measured with a digital scale (Seca, Model Alpha, Germany). Body mass index (BMI) was calculated as weight (in kg) divided by height (in m^2). At the two-year follow-up interview, information on sex, current smoking status, and total household income were collected through questionnaire. Alcohol use was assessed as none, moderate (defined as >0 and ≤1 drink/day for women and >0 and ≤2 drinks/day for men), or heavy (>1 drink/day for women and >2 drinks/day for men). Physical activity was assessed using a modified Paffenbarger questionnaire of the Harvard Alumni Activity Survey (17-18). Detailed information on medication use was collected by asking participants to show the bottles for medications they currently

take. Blood pressure was measured using an electronic sphygmomanometer (Dinamap™ Model 8260, Critikon, Tampa, FL) at three time points during the interview. An average of the second and third readings was used for each systolic and diastolic blood pressure. Usual calcium intake (mg/d) was assessed using a semi-quantitative food frequency questionnaire that was developed and validated for the Puerto Rican population (19). Energy-adjusted calcium intakes were calculated by regressing usual dietary calcium intake on total energy (20). Because season is known to affect BMD measurements in the New England area (21), we adjusted for season of BMD measurement as follows: July to September was coded as summer, October to December as fall, January to March as winter, and April to June as spring.

Statistical Analyses

All statistical analyses were performed using SAS 9.2 (Cary, NC). Formal hypothesis testing was two-sided with a nominal type I error rate of 0.05. Women were divided into tertiles of FRS. We calculated age adjusted means for lifestyle, socio-economic, anthropometric, and health characteristics across tertiles of FRS with general linear models (GLM). We assessed significance across categories of FRS using linear (for continuous variables) or logistic (for categorical variables) regression. Tests for linear trend were conducted by assigning each participant the median FRS for each tertile category and treating this value as a continuous measure in a regression model.

We used the GLM procedure to model associations between FRS (continuous and categorical) and BMD (continuous) of the femoral neck, trochanter, total hip, and lumbar spine. In our first (risk factor) model, we adjusted for alcohol use (none, moderate,

heavy), BMI (kg/m^2), height (m), physical activity score, plasma vitamin D (ng/mL), total household income ($/y), and season of BMD measurement. To adjust for confounding due to indication due to medication use, we adjusted for osteoporosis and cardiovascular medication use in the second (medication) model. In our final (nutrient) model, we included energy-adjusted dietary calcium intakes (mg/d) as a covariate. Because age, current smoking status, and diabetes are part of the FRS, these were not adjusted for in any of the models. For all linear models, we checked the assumptions of linearity and homogeneity by examining the residuals of the outcome versus the exposure. Final models were checked for outliers and influential points using scatterplots. All analyses were adjusted for multiple comparisons using Dunnett's adjustment with the lowest quartile as the reference group. We used logistic regression to model the odds of either osteoporosis or osteopenia for every unit increase in FRS.

RESULTS

Median FRS in the highest tertile was about 3 times higher compared to the lowest tertile (**Table 1**). Women in the highest tertile, compared to women in the lowest tertile, were older, and had higher BMI, higher systolic and diastolic blood pressure, and lower HDL concentration. They were also more likely to have type 2 diabetes, be current smokers, and to use osteoporosis and cardiovascular medications.

Before inclusion of energy-adjusted calcium intake, BMD at the three hip sites was lower by 0.013-0.015 g/cm^2 for every 5 units higher FRS (**Table 2**). However, after adjustment for energy-adjusted calcium intakes, significance was retained only at the femoral neck. No significant associations were seen at the lumbar spine. At the femoral

neck, women in the highest tertile of the FRS had lower BMD compared to women in the lowest tertile, before adjustment for calcium intake ($p < 0.05$). However, adjustment for this variable, attenuated associations (p for trend = 0.09) (**Figure 1A**). At the trochanter and the total hip, significant linear trend was observed before inclusion of calcium intake in the model (p for trend = 0.02 and 0.03, respectively). However, after accounting for differences in calcium intake, linear trend across tertiles was attenuated at both hip sites (**Figures 1B and 1C**). As observed with the continuous FRS, no association was seen across tertiles of FRS for lumbar spine BMD (p for trend = 0.89) (**Figure 1D**).

The odds of osteoporosis or osteopenia at the femoral neck was 3% higher (95% CI: 1.00 – 1.07) for every unit increase in the FRS. At both the trochanter and the total hip, the odds of osteoporosis or osteopenia were higher by 4% for every unit increase in the FRS (95% CI: 1.00-1.07 and 1.00-1.08, respectively) before adjustment for calcium. Adjustment for calcium intake attenuated these associations (trochanter OR= 1.03, 95% CI: 0.99-1.07; total hip OR = 1.03, 95% CI: 0.98-1.07, respectively). The FRS was not associated with higher odds of osteoporosis/osteopenia at the lumbar spine (OR=0.98, 95% CI: 0.94-1.01).

DISCUSSION

In this cross-sectional analysis of older Puerto Rican women, consistent with our stated hypothesis, ten-year risk of CHD was inversely associated with femoral neck BMD. We found similar effect sizes at the trochanter and total hip but these associations were attenuated and became non-significant after adjustment for calcium intake. Although this limits our conclusions about these associations, there is some evidence to

show that calcium intake is associated with CVD risk (22), thereby making it a potential confounder. We also documented that for every unit increase in ten-year risk of CHD, the odds of osteoporosis or osteopenia at the femoral neck were higher by 3%. No significant associations were seen at the lumbar spine with either BMD or osteoporosis risk.

Our findings are consistent with those reported previously in the literature. Applying the Framingham risk equations to a multi-ethnic population, Broussard and Magnus (23) found that women with a 10%–19% CHD risk were 45% more likely and those with a ≥20% CHD risk were 73% more likely to have low BMD compared to women who had a <10% CHD risk. Our results may not be directly comparable to those of Broussard and Magnus (23) as we were unable to classify our participants into risk categories. Using much earlier data from men participating in the Puerto Rican Heart Health Program in the mid 1960's, D'Agostino et al (24) found that the FRS systematically overestimated CHD events. However, recent data indicate that the prevalence of risk factors in Puerto Ricans has increased since then (25-26). By treating the FRS as a continuous measure, as opposed to a categorical measure, we avoided potential misclassification and participants in our cohort were ranked appropriately according to their risk estimates. Nevertheless, our results are in accordance with Broussard and Magnus (23) who found inverse associations between the FRS and BMD.

While we did not have incident data, findings from our study are in general agreement with those studies that showed that incident CHD events like heart failure (6-7) or sub-clinical measures of atherosclerosis (27-30) were independently associated with greater risk of orthopedic fracture and low BMD. On the other hand, in the Cardiovascular Health Study, after adjustment for shared risk factors such as age, race,

education, income, BMI, health status, smoking, estrogen use, menopause, diabetes, physical activity, and cardiac medications, an association between heart failure and hip fracture disappeared (31).

Several factors that comprise the FRS may be responsible for the inverse association with bone health. Previous reports from the Framingham Osteoporosis Study have shown that age and smoking are two important risk factors for bone loss (32-33). Both factors are associated with a pro-inflammatory state (34-35), a mechanism that is common to the development of both CVD and osteoporosis. The lipid components of the FRS include LDL cholesterol, HDL cholesterol, and total cholesterol concentrations. Evidence surrounding the role of lipids in BMD has been conflicting and inconclusive. Most recently, in Korean postmenopausal women, HDL cholesterol was positively associated with BMD at the lumbar spine, but not at hip sites (36). Likewise, Orozoco (37) found that early postmenopausal women with an atherogenic lipid profile, defined as cholesterol ≥240 mg/dl or LDL cholesterol ≥160 mg/dl or Lp(a) ≥25 mg/dl, had lower lumbar and femoral BMD and increased risk of osteopenia, relative to those with normal lipid profile. The cut-points used by Orozoco (37) for defining hypercholesterolemia are consistent with those used in the Framingham risk equations. Similarly, in another Italian study, women with plasma LDL cholesterol concentrations ≥160 mg/dL had 48% (95% CI: 1.04-2.34) and 74% (95% CI: 1.18-2.60) greater probability of being classified as osteopenic than women with plasma LDL of 130-159 mg/dL and <130 mg/dL, respectively (38). Contrary to these findings, Buizert et al (39) found no association between total cholesterol and BMD at the calcaneus. Surprisingly, men and women in the highest quartile of HDL cholesterol concentration had significantly lower BMD, but

participants in the Boston Puerto Rican Osteoporosis Study had serum HDL cholesterol and total cholesterol/HDL cholesterol ratios in the normal range. Despite the conflicting literature, biological plausibility for the association between lipids and BMD exists. In vitro and animal data show that oxidized lipids enhance production of receptor activator of nuclear factor kappaB ligand by T lymphocytes ultimately resulting in bone loss (40).

Blood pressure, another important risk factor for CHD and a component of the FRS, has been shown to be inversely associated with bone mineral content in overweight and obese Hispanic women (41). These inverse associations may be due to abnormalities in calcium metabolism and sustained hypercalciuria and hyperparathyroidism that accompany hypertension (42). The final component of the FRS is diabetes status. In a recent review, Yaturu (43) summarized that compared with individuals without diabetes, men and women with diabetes have a higher risk of fractures, particularly at the hip. Further, while those with type 2 diabetes may or may not have lower BMD, there is substantial evidence of altered bone quality in diabetes. The underlying biological mechanisms may include the effects of pro-inflammatory cytokines, advanced glycation end products, and negative calcium balance (43-44).

While most literature on the link between CVD and osteoporosis has focused on incident CVD events, we were able to demonstrate that a constellation of CVD risk factors (in the form of the FRS), in a cohort free of CVD, also affect bone health. Our findings of a significant association at the femoral neck are important to consider. The most common fractures of the hip are those of the femoral neck. In fact, mortality following a fracture of the neck of the femur is ~33% (45), making this a clinically relevant site. The Puerto Rican population living in Massachusetts has been shown to

have established health disparities and to carry a disproportionately large burden of cardiometabolic risk (46). The Puerto Rican population is also one of the most economically disadvantaged groups in the country. Previous studies have shown that Hispanics as a group are less likely than non-Hispanic whites to undergo bone density testing prior to and after a hip fracture (10). Although no screening tool can replace DXA for assessing osteoporosis risk, given disparities in bone density testing, the FRS may be an alternative tool to target those at a high risk for poor bone health.

Our results need to be interpreted in the context of a few limitations. First, we were unable to examine associations in Puerto Rican men due to lack of statistical power and, thus, our results may not be directly applicable to them. Using NHANES III data, Broussard and Magnus (23) noted that the FRS was a strong predictor for low BMD in women but not men. It may be that factors conferring greater risk of osteoporosis are different in men. Second, we used cross-sectional data and, therefore, our findings do not allow us to make statements regarding causation. Third, as with any epidemiological study, residual confounding remains a strong possibility. Still, covariates chosen for inclusion in all our statistical models were based on previous knowledge of risk factors for bone health and underlying biological mechanisms.

In conclusion, in the absence of BMD screening by DXA, the FRS may serve as a useful tool to identify women at risk of low femoral neck BMD. Our findings need to be replicated in men and other ethnic populations. Finally, longitudinal data are required to clarify the direction of association between the FRS and bone health.

CONFLICT OF INTEREST

No Disclosures

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Table 1: Descriptive characteristics of Puerto Rican women across tertiles of the Framingham risk score

Characteristic[1]	Women			P-trend
	1	2	3	
	6 (1-7)	11 (8-13)	20 (15-32)	
n	107	143	115	
Age (y)	55.9 (0.6)	60.1 (0.6)†	63.4 (0.6)†	<0.0001
BMI (kg/m^2)	31.7 (0.7)	32.7 (0.6)	33.9 (0.7)	0.04
Physical activity score	31.8 (0.4)	31.1 (0.4)	31.1 (0.4)	0.37
Current smoking (y) (%)	10.2	13.5	29.5***	0.0001
Alcohol use[3] (%)				
Non-drinker	67.6	71.8	66.7	0.74
Moderate drinker	29.7	25.4	31.4	0.62
Heavy drinker	1.5	2.8	1.2	0.69
Total household income ($/y)	18530 (2299)	19558 (1875)	16283 (2172)	0.38
LDL cholesterol (mg/dl)	110 (4)	119 (3)	113 (3)	0.91
HDL cholesterol (mg/dl)	55 (1)	50 (1)**	40 (1)†	<0.0001
Total cholesterol (mg/dl)	190 (4)	198 (4)	188 (4)	0.39
Systolic blood pressure (mmHg)	123 (2)	137 (1)†	147 (2)†	<0.0001
Diastolic blood pressure (mmHg)	75 (1)	81 (1)†	85 (1)†	<0.0001
Diabetes (y) (%)	7.6	31.9†	74.9†	<0.0001

Medication use (%)				
Osteoporosis prescription				
Calcium and vitamin D	50.0	43.5	35.5*	0.05
Other[4]	29.1	17.0*	15.5*	0.05
Cardiovascular	53.0	62.5	75.8**	0.0007
Lipid lowering	37.7	38.0	57.1**	0.003
Hypertension	44.4	48.0	68.1***	0.0002
Plasma vitamin D (ng/ml)	19.7 (0.7)	20.2 (0.6)	18.6 (0.7)	0.18
Dietary calcium intake (mg/d)[2]	809 (36)	749 (29)	805 (32)	0.75

[1]Adjusted for age (y)

[2]Adjusted for age (y), energy intake (kcal/d)

[3]Non-drinkers were those consuming <12 drinks/year; Moderate drinkers were those consuming up to 1 drink/d; Heavy drinkers were women consuming >1 drink/d

[4]Other osteoporosis prescription medications include calcitonin, alendronate, etidronate, cod liver oil, and other

*P<0.05, **P<0.01, ***P<0.001, † P<0.0001 compared to tertile 1 (Dunnett's adjustment)

Table 2: Cross-sectional associations between 10-y risk of coronary heart disease and bone mineral density (g/cm^2)

Bone mineral density	Women		
	n	β (SE)	P-value
Femoral neck			
Model 1	335	-0.013 (0.005)	0.003
Model 2	335	-0.015 (0.005)	0.002
Model 3	283	-0.011 (0.005)	0.03
Trochanter			
Model 1	335	-0.009 (0.004)	0.04
Model 2	335	-0.013 (0.005)	0.006
Model 3	283	-0.009 (0.005)	0.09
Total hip			
Model 1	331	-0.012 (0.005)	0.02
Model 2	331	-0.015 (0.005)	0.005
Model 3	279	-0.011 (0.006)	0.06
Lumbar spine (L2-L4)			
Model 1	336	-0.008 (0.006)	0.18
Model 2	336	-0.010 (0.006)	0.12
Model 3	284	-0.003 (0.007)	0.67

β coefficients (SE) for every 5 unit increase in 10-year risk of coronary heart disease

Model 1 (Risk factor model): Adjusted for alcohol use (none, moderate, heavy), BMI (kg/m^2), height (m), physical activity score, plasma vitamin D (ng/mL), total household income ($/y), season of BMD measurement (winter, spring, summer, fall)

Model 2 (Medication model): Model 1 + osteoporosis medication use (y/n), cardiovascular medication use (y/n)

Model 3 (Nutrient model): Model 2 + energy adjusted dietary calcium (mg/d)

FIGURE LEGEND

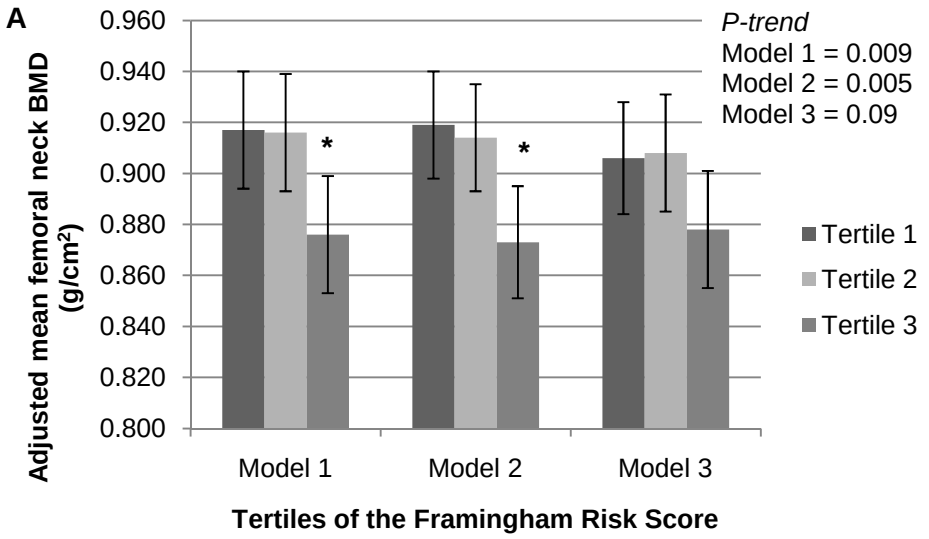
Figures 1A – 1D: Adjusted mean BMD (g/cm^2) of older Puerto Rican women across tertiles of the Framingham Risk Score

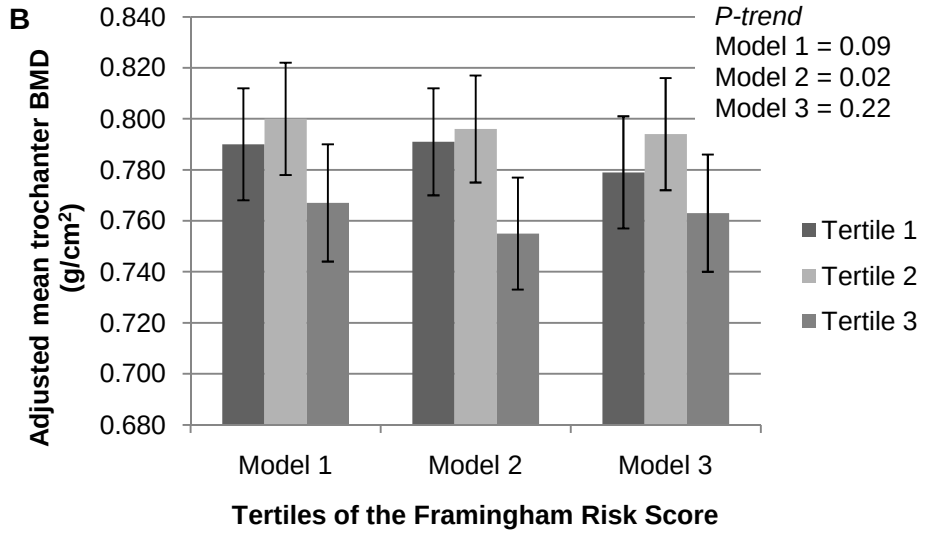
Model 1 (Risk factor model): Adjusted for alcohol use (none, moderate, heavy), BMI (kg/m^2), height (m), physical activity score, plasma vitamin D (ng/mL), total household income ($/y), season of BMD measurement (winter, spring, summer, fall)

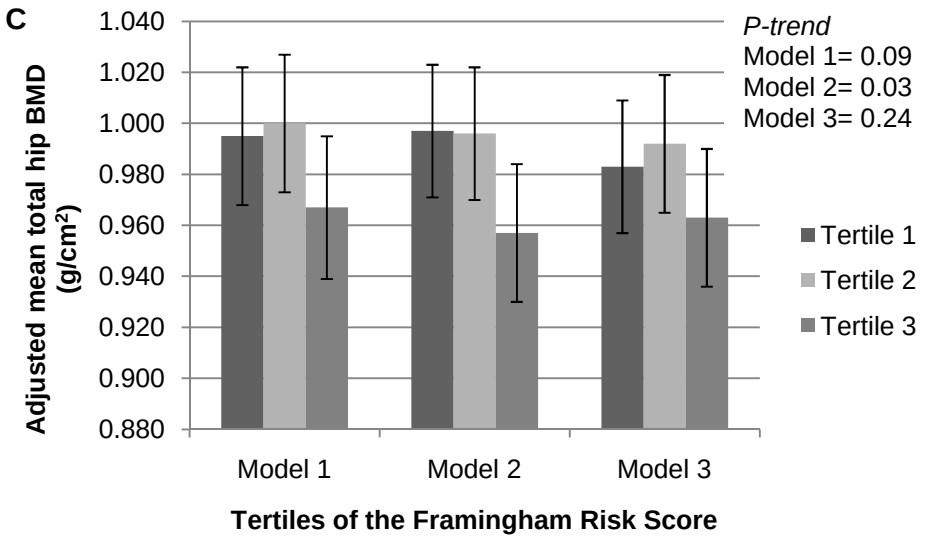
Model 2 (Medication model): Model 1 + osteoporosis medication use (y/n), cardiovascular medication use (y/n)

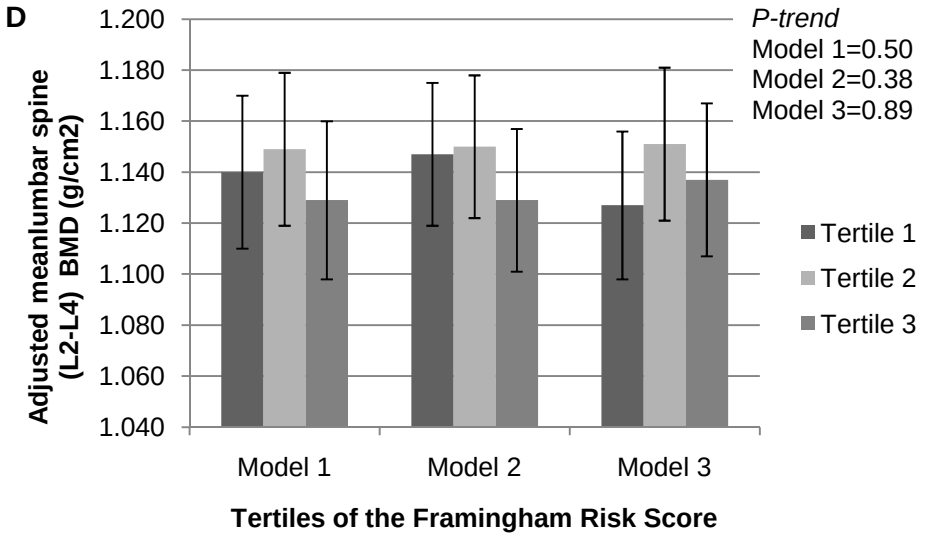
Model 3 (Nutrient model): Model 2 + energy adjusted dietary calcium (mg/d)

Figures 1A – 1D






C
1.040
1.020
1.000
0.980
0.960
0.940
0.920
0.900
0.880
Adjusted mean total hip BMD (g/cm²)
P-trend
Model 1= 0.09
Model 2= 0.03
Model 3= 0.24
Tertile 1
Tertile 2
Tertile 3
Model 1
Model 2
Model 3
Tertiles of the Framingham Risk Score


D
1.200
1.180
1.160
1.140
1.120
1.100
1.080
1.060
1.040
Adjusted meanlumbar spine (L2-L4) BMD (g/cm2)
P-trend
Model 1=0.50
Model 2=0.38
Model 3=0.89
Tertile 1
Tertile 2
Tertile 3
Model 1
Model 2
Model 3
Tertiles of the Framingham Risk Score

CHAPTER FOUR

Centrally located body fat is associated with lower bone mineral density: The Boston Puerto Rican Health Study[1-5]

(To be submitted to the American Journal of Clinical Nutrition)

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Running head: Abdominal fat mass and bone mineral density

Keywords: abdominal fat mass, central obesity, bone mineral density, osteoporosis, epidemiology, Puerto Ricans

ABSTRACT

Background: Few studies have evaluated the association between abdominal obesity and bone health.

Objective: We tested the hypothesis that higher abdominal fat mass (AFM) is associated with poor bone health.

Design: A cross-sectional study was conducted in 636 Puerto Rican adults, aged 45–75 y. Bone mineral density (BMD) of the femoral neck, trochanter, total femur, and lumbar spine (L2-L4) were measured using dual energy x-ray absorptiometry (DXA). AFM and total fat mass (TFM) were assessed using regional body composition software from whole body DXA scans. Osteoporosis or osteopenia were defined as T-score ≤ -2.5 SD and -1.0 to -2.5 SD, respectively, at the respective bone site.

Results: After adjustment for weight, height, and other covariates, AFM was inversely associated with BMD at the three hip sites in both women and men, and lumbar spine in women but not men. The odds for osteoporosis/osteopenia at each of the femoral sites increased for every 100 g higher AFM. The highest odds were observed at the femoral neck (women OR=1.25, 95% CI: 1.11, 1.42; men OR=1.37, 95% CI: 1.04, 1.28). For TFM, modest associations were seen at the femoral neck and trochanter among women. Among men, similar modest associations were seen at the three hip sites.

Conclusions: Higher AFM is associated with poor bone health in this Puerto Rican sample. Efforts to reduce abdominal obesity will not only reduce the risk of chronic disease but may also improve bone health.

INTRODUCTION

Obesity and osteoporosis are two major public health concerns with high prevalence rates, the later disproportionally affecting older adults. Osteoporosis and low bone mass affect nearly 44 million US adults, aged 50 and older (1). By 2025, annual fractures and costs are expected to rise by 50% from $17 billion in 2005. The greatest increase in costs is estimated to be 175% for Hispanics (2), suggesting that this is a high risk group. Likewise, the prevalence of obesity, especially abdominal obesity, remains disturbingly high among adults in the United States. Recent estimates from the National Health and Nutrition Examination Survey (NHANES) indicate that the prevalence of abdominal obesity among men and women has increased from 37.8% and 55.8% during 1999-2000 to 43.7% and 61.8% during 2007-2008 (3).

The prevailing view regarding the relationship between fat and bone mass is that body fat is a protective factor against osteoporosis primarily due to its weight bearing effect on the skeleton. With the recognition of fat as an endocrine organ, the effect of fat mass on bone may extend beyond its mechanical load on the skeleton. Comparing NHANES 1999-2002 data with NHANES III data, Looker et al (4) found a positive relationship between body mass index (BMI) and bone mineral density (BMD) but concluded that the increasing overweight rates among older women are not likely to lead to a significant reduction in osteoporosis prevalence. More recently, comparing NHANES III data with NHANES 2005-2006 data, Looker et al (5) found that the prevalence of osteoporosis at the femoral neck decreased but changes in BMI did not fully explain this decline. While most of the research on the association between fat mass and BMD focused on total fat mass (TFM), it is not clear how abdominal fat mass (AFM)

is associated with bone mass. Abdominal obesity, assessed using waist circumference, is associated with higher mortality independent of BMI (6). Further, AFM is known to contribute to inflammation (7, 8), insulin resistance (9), dyslipidemia (10), metabolic syndrome (11), and hypertension (12). Given the established risks associated with AFM, it is not clear how AFM is associated with bone mass after controlling for its mechanical loading effect, especially in an ethnic population.

Most research in Hispanics has focused on Mexican Americans, owing to their majority as a Hispanic sub-group. However, Puerto Ricans are the largest Hispanic sub-group in the northeastern U.S. and prior research indicates that they have established health disparities and a greater burden of chronic disease (13). Metabolic syndrome, characterized by abdominal obesity, is also high in this group (14). Yet, there is paucity of research on bone health in this population. Because the health care costs of both osteoporosis and abdominal obesity, and the associated increase in chronic disease risk, are considerable, it is crucial to understand how AFM affects BMD independent of body weight. We, therefore, studied this important association in a group of 636 older Puerto Rican adults (men=167, women=469), aged 47-79 years, living in the greater Boston area.

PARTICIPANTS AND METHODS

Participants

Participants in the Boston Puerto Rican Health Study, a prospective cohort study of older Puerto Ricans, aged 47-79 years, living in the greater Boston area, were asked to attend an examination at the USDA Human Nutrition Research Center for Aging

(HNRCA) at Tufts University to assess bone mineral density and body composition obtained by a dual-energy X-ray (DXA) absorptiometry scan. The design of the Boston Puerto Rican Health Study has been described in detail elsewhere (14). Briefly, at baseline and two-years, bilingual interviewers visited the participants' home and administered questionnaires to collect information on socioeconomic status, health and health behaviors, acculturation, depressive symptomatology, stress, social support, usual diet, and cognitive functioning. In addition, anthropometric, blood pressure, and physical performance measures were collected. Biological samples, including saliva, urine, and 12-h fasting blood, were collected by the phlebotomist in the participants' homes on a day following the interview or as soon as possible thereafter. At the completion of the two-year follow-up, participants were re-consented for the Osteoporosis study. An appointment was made for consenting participants to visit the Metabolic Research Unit at the HNRCA at Tufts University to obtain bone density and body composition measurements, for an additional blood draw, and to complete additional questionnaires on osteoporosis medication use and sunlight exposure. Multiple attempts were made to complete this visit within one month of the two-year follow-up visit for the parent study. All questionnaires were administered by trained bilingual interviewers. By September 2010, of a total of 1123 participants who completed two-year follow-up visits, 756 consented to the Osteoporosis study. Primary reasons for non-participation included not being interested in the Osteoporosis study (n=163), scheduling problems (n=139), loss-to-follow up (n=33), and moves out of Massachusetts (n=15). Further, 17 participants died since their two-year follow-up interview. Women who declined participation were more likely to be older (61.3 vs 59.3 y, P=0.001) and have higher energy-adjusted intakes

of alcohol (4.5 vs 1.5 g/d, *P*=0.05). Men who declined participation in the Osteoporosis study were more likely to be older (61.6 vs 58.4 y, *P*=0.003), have lower BMI (28.6 vs 30.2, *P*=0.03), and lower waist circumference (100 vs 105 cm, *P*=0.02). No other significant differences were found for socio-demographic or dietary variables. For analyses with femoral BMD measures as the outcome, we excluded one participant with a poor quality hip scan. At the time of analysis, complete and cleaned data were available for 636 participants (167 men and 469 women). All study protocols were approved by the Institutional Review Board of Tufts Medical Center.

Methods

Outcome assessment

Based on recommendations from the International Society for Clinical Densitometry (15), we made an *a-priori* decision to only include BMD measurements at the femoral neck, total hip, and posterior-anterior lumbar spine (L2-L4) in all our analyses. In addition, we also included the trochanter, as inclusion of this anatomical site provides a complete picture of the hip. We measured BMD (g/cm^2) of the femoral neck, trochanter, total hip, and lumbar spine by DXA (GE-Lunar model Prodigy scanner, Madison, WI) using standard procedures. The root mean square precision of these measurements were 0.65% for total hip BMD, 1.03% for the trochanter, 1.31% for the femoral neck, and 1.04% for the lumbar spine (16). For femur measurements, the right hip was scanned unless there was a history of hip fracture or joint replacement. During the study, stability of DXA measurements was determined by scanning an external standard (aluminum spine phantom, Lunar Radiation Corp.) every week. Using the WHO

definitions, osteoporosis and osteopenia were defined as T-score thresholds of ≥ 2.5 or 1.0 SD, respectively, below the healthy young adult mean at the respective bone site. We reviewed all scans with T-scores > 4.0 to check for extra-skeletal calcification or for presence of non-anatomical parts in the DXA scan region.

Exposure assessment

Total fat mass (kg) was assessed from whole body scans. Abdominal fat mass (g) was measured using specialized regional body composition software (ENCORE v 12.2) from whole body DXA scans.

Assessment of covariates

At the two-year follow-up visit, information on age, sex, and smoking status was collected by questionnaire. Physical activity was assessed using a modified Paffenbarger questionnaire from the Harvard Alumni Activity Survey (17, 18). Usual intakes of calcium (mg/d), alcohol (g/d) and total energy (kcal/d) were assessed using a semi-quantitative food frequency questionnaire, specifically developed and validated for the Puerto Rican population (19). At the Osteoporosis study visit, we administered a short questionnaire to assess osteoporosis prescription medication use (y/n) including use of bisphosphonates, calcitonin, calcium, vitamin D, and cod liver oil. Because BMD is known to vary by season in the New England area (20, 21), we created a four level categorical variable for season of BMD measurement as follows: July, August, and September were coded as summer; October, November, and December as fall; January, February, and March as winter; and April, May, and June as spring. Standing height was

measured with a stadiometer (Seca, Germany). Weight was measured with a digital scale (Seca, Model Alpha, Germany). Fasting blood samples (12 h) were drawn from participants by a certified phlebotomist during the morning of the Osteoporosis study visit. Blood was collected into vacutainers containing EDTA and plasma separated by immediately centrifuging at 3421 x *g* at 4^{o}C for 15 minutes. Plasma 25-hydroxy vitamin D (ng/mL) was measured using a ^{125}I radioimmunoassay kit procedure (DiaSorin Inc, Stillwater, MN) as specified by the manufacturer's procedural documentation (68100E). The intra- and inter-assay CV% are 10.8% and 9.4% respectively.

Statistical analyses

All statistical analyses were performed in SAS 9.2 (SAS Institute Inc., Cary, NC). Formal hypothesis testing was two-sided with a nominal type I error rate of 0.05. Because distribution of central (abdominal) fat mass is sex-specific, we stratified all analyses by sex. Participants were divided into quartiles of AFM, separately for men and women. We calculated age adjusted means for lifestyle, socio-economic, anthropometric, and health characteristics across increasing quartiles of AFM by using PROC GLM. Similarly, dietary intakes were examined across quartiles, using analysis of variance and adjusting for age and energy intake. We assessed significance across categories of abdominal fat mass using linear (for continuous variables) or logistic (for categorical variables) regression. Tests for linear trend were conducted by assigning each participant the median grams of AFM for each quartile category and treating this value as a continuous measure in a regression model.

We used the general linear models procedure to model associations between AFM (continuous and categorical) and BMD (continuous) of the femoral neck, trochanter, total hip, and lumbar spine. In our first model, we adjusted for age (y), current smoking status (y/n), intakes of alcohol (g/d), calcium (mg/d), and total energy (kcal/d), season of BMD measurement (spring, summer, fall, winter), physical activity score, plasma 25(OH) D (ng/mL) concentration, and osteoporosis medication use (y/n). To adjust for confounding due to skeletal size and the mechanical loading of body weight, we included height and body weight, respectively in the second model. For all linear models, we checked the assumptions of linearity and homogeneity by examining the residuals of the outcome versus the exposure. Final models were checked for outliers and influential points using scatterplots. All analyses were adjusted for multiple comparisons using Dunnett's adjustment with the lowest quartile as the reference group. We used logistic regression to model the odds of either osteoporosis or osteopenia for each 100 g higher abdominal fat mass. To test if AFM has different effects than TFM on bone, we repeated all our analyses by replacing AFM with TFM as the main exposure variable.

RESULTS

There was nearly 3-times greater fat mass around the abdominal area in Puerto Rican women in the highest versus lowest quartile of AFM (**Table 1**). Median grams of AFM in quartiles 1, 2, 3, and 4 were 1.7, 2.5, 3.2, and 4.7 kg, respectively. Women in the highest compared to those in the lowest quartile of AFM were more likely to be older, and to have higher BMI, body weight, skeletal size, and waist circumference. They were less likely to be current smokers or to be physically active, and had lower educational

status compared to women with the least AFM. Puerto Rican men in the highest quartile of AFM had nearly 2.5 times higher AFM compared to men in the lowest quartile (**Table 2**). Median values of AFM in increasing higher quartiles were 2.1, 2.9, 3.6, and 5.0 kg, respectively. Similar to their female counterparts, these men were more likely to have higher BMI and waist circumference compared to men in the lowest quartile of AFM. These men were also less likely to be current smokers or, to be physically active, and had lower educational status, compared to men with the lowest AFM.

As expected, AFM was positively associated with BMD before adjustment for weight and height in both men and women (**Table 3**). After differences in body weight and skeletal size were taken into account (model 2), the direction of association between AFM and BMD changed. These associations were significant at all four bone sites in women. In men, significant negative associations were observed at all bone sites except the lumbar spine. Among women, TFM (kg) was significantly and positively associated with BMD before adjustment for weight and height. After adjustment for body weight and height, associations changed direction and remained significant at only two bone sites (trochanter and total femur). In men, TFM was positively, but not significantly, associated with BMD. However, after controlling for weight and height, all associations changed direction, with significant associations at the hip sites but not at the lumbar spine. Effect sizes for TFM were much lower compared to effect sizes for AFM.

Among Puerto Rican women, before adjustment for weight and height, BMD at the trochanter, total femur, and lumbar spine was higher across increasing quartiles of AFM (**Figure 1A**). At the femoral neck, BMD was higher across quartiles but this difference did not reach significance (P for trend = 0.07). After adjustment for the

mechanical loading effect of body weight and skeletal size, BMD in the highest quartile of AFM for the three hip sites was significantly lower compared to BMD in the lowest quartile. While the differences in lumbar spine BMD across quartiles of AFM did not reach significance, there was a marginal trend (*P* for trend=0.08) (**Figure 1B**). When AFM was replaced by TFM as the primary exposure variable, BMD at all four bone sites was greater across increasing quartiles of TFM, before adjustment for weight and height (**Figure 1C**). After controlling for these variables, no significant differences in BMD remained across quartiles of TFM for any of the four bone sites (**Figure 1D**). Among Puerto Rican men, BMD of the trochanter and total femur was higher across increasing quartiles of AFM (*P* for trend=0.03) before adjustment for weight and height (**Figure 2A**). Once height and weight were adjusted, the association between AFM and BMD became inverse, but was only significant at the trochanter (*P* for trend = 0.04) (**Figure 2B**). Prior to adjustment for weight and height, TFM was again positively associated with BMD, but the association was significant only at the total femur (*P* for trend = 0.04) (**Figure 2C**). As noted previously, when weight and height were included as covariates in the model, TFM was inversely associated with BMD at all four bone sites, and was significant at the trochanter (*P* for trend=0.02) and total femur (*P* for trend=0.05) (**Figure 2D**).

Among women, before adjustment for weight and height, AFM appeared to be protective against osteoporosis or osteopenia (**Figure 3A**). The multiple adjusted odds ratios (OR) of osteoporosis or osteopenia for every 100 g higher AFM were 0.94 (0.89-0.98), 0.97 (0.95-0.98), 0.98 (0.95-0.99), 0.97 (0.95-0.99) for the femoral neck, trochanter, total femur, and lumbar spine, respectively. Conversely, after statistical

adjustment for weight and height, higher AFM was associated with higher likelihood of osteoporosis and osteopenia at each bone site. The strongest associations were noted at the femoral neck. For every 100 g higher AFM, the multiple-adjusted odds for osteoporosis or osteopenia increased by 25% (OR=1.25, 95% CI: 1.11, 1.42). More modest, but significant, associations were seen at the trochanter (OR=1.13, 95% CI: 1.07, 1.19) and total hip (OR=1.11, 95% CI: 1.05, 1.18) but not at the lumbar spine (OR=1.00, 95% CI: 0.95, 1.05) (Figure 3A). Among men, before adjustment for weight and height (model 1), AFM was not associated with the likelihood of osteoporosis/osteopenia at any of the four bone sites (**Figure 3B**). As observed previously in women, among men, higher AFM was associated with a higher likelihood of osteoporosis or osteopenia after adjustment for weight and height (model 2). Among the four bone sites, AFM had the strongest association at the femoral neck. The odds for osteoporosis or osteopenia at the femoral neck increased by 37% (95% CI: 1.11, 1.74) for every 100 g higher AFM. Similar effect sizes were noted at the trochanter (OR=1.16, 95% CI: 1.06, 1.28) and total femur (OR=1.15, 95% CI: 1.04, 1.28). No significant associations were observed at the lumbar spine (OR=1.02, 95% CI: 0.94, 1.11) (Figure 3B).

DISCUSSION

In this cross-sectional study of Puerto Rican older men and women, both AFM and TFM were inversely associated with BMD. Yet, effect sizes were much smaller for TFM compared to those with AFM. After controlling for confounding due to body weight and skeletal size, higher AFM, but not TFM, was associated with lower BMD at all four sites in women and at the three hip sites in men. In both sexes, the strongest associations

were seen at the femoral neck. Among both men and women, after adjustments for weight and height, the likelihood of osteoporosis or osteopenia at all three hip sites increased with every 100 g increase in AFM. Thus, AFM appears to have a strong, inverse association with bone mass beyond the mechanical loading effect of body weight and differences in skeletal size. To our knowledge, the current study is the first to demonstrate the inverse association between AFM, measured using DXA, and bone mass specifically in a Hispanic population.

The direction of associations observed before and after adjustment for body weight and height are particularly noteworthy. Fat mass is a major component of body weight. Obesity, a condition characterized by excessive fat mass, has been traditionally thought to be protective for bone mass. In fact, low body weight, especially among older adults, is an established risk factor for osteoporosis. Moreover, in the WHO fracture risk assessment tool (22), a higher body weight is associated with a lower 10-year risk of fracture. The primary mechanism for the positive relationship between fat and bone is due to the load on the skeleton by body weight. However, a few studies (23, 24) have demonstrated that when the mechanical loading effect of body weight is statistically removed by including body weight as a covariate, fat mass is, in fact, negatively associated with bone. Most recently, Reid (25) contested that fat mass should not be adjusted for body weight due to the potential collinearity between the two variables. However, our hypothesis was that central fat mass is negatively associated with BMD after accounting for the mechanical loading effect of body weight. Formal statistical tests revealed that there was not significant collinearity between AFM or TFM and body weight, suggesting that our statistical methods are valid.

The differences in the effect sizes of AFM and TFM with BMD are particularly striking. AFM is known to be more metabolically and biologically active and produces a variety of autocrine and paracrine hormones, chemokines, and cytokines that affect bone metabolism. Among individuals with excess visceral fat, there is a greater flux of free fatty acids to the liver via the portal vein. An increase in the delivery of free fatty to the liver signals a greater production of glucose output by the liver, eventually leading to an insulin-resistant state (26). Insulin resistance, an essential feature of type 2 diabetes, has been shown to increase risk of fracture (27, 28). A second potential mechanism for the negative association between AFM and BMD may have to do with the production of pro-inflammatory molecules such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). Recent research has established that the release of many inflammatory adipokines by adipose tissue is enhanced in obese individuals although these cytokines are primarily released by the nonfat cells of human adipose tissue (29). Visceral adipose tissue is known to release greater amounts of cytokines compared to abdominal subcutaneous tissue (30). In addition to these pro-inflammatory cytokines, high-sensitivity C-reactive protein (CRP), a marker of systemic inflammation, concentrations are also elevated among individuals with abdominal obesity (31, 32) independent of BMI. Both prospective and cross-sectional analyses have indicated that higher circulating concentrations of pro-inflammatory cytokines, including CRP (33-35), IL-6 (36), and TNF-α (33) are associated with lower BMD and greater fracture risk (37). In addition to greater production of pro-inflammatory cytokines by abdominal adipose tissue, it is also known that production of adiponectin is reduced in obese individuals. Adiponectin, an adipose-derived hormone, is inversely associated with visceral fat (38, 39) and other

measures of central obesity, such as waist circumference (40). Elegant *in vitro* and animal studies have elucidated the role of adiponectin on the skeleton. Adiponectin exerts an activity to increase bone mass by suppressing osteoclastogenesis and by activating osteoblastogenesis (41). Further, adiponectin receptors, AdipoR1 and AdipoR2, are expressed in bone forming cells (42). However, most recently, adiponectin knock-out mice were shown to have increased bone mass, suggesting that adiponectin may have other indirect effects on bone (43). Finally, serum ostecalcin, a bone-derived protein that regulates bone formation, has been recently found to be inversely associated with visceral adiposity (44). The modest effect sizes noted for associations of TFM and BMD may indicate that TFM may have small or negligible effects on BMD beyond its weight bearing effect.

Our results are consistent with those from other studies that used computed tomography, magnetic resonance imaging, or anthropometry to measure abdominal obesity. Gilsanz et al (45) noted that visceral, but not subcutaneous, fat was negatively associated with the structure and strength of the femur in young women. Similarly, in a group of obese adolescent girls, visceral adipose tissue was a negative predictor of both hip and spine BMD (46). Likewise, Huang et al (47) demonstrated that lumbar spine BMD is reduced in association with greater visceral fat in HIV infected men with lipodystrophy. Using waist-to-hip ratio as a marker for visceral fat, two independent studies in Korean men (48) and post-menopausal women (49) found that BMD of the calcaneus (48) and the lumbar spine (49) were negatively correlated with waist-to-hip ratio, after adjustment for BMI or body weight. Unlike the study populations of Huang (47), Russell (46), and Kim (49), we did not find any associations at the lumbar spine,

among men, possibly due to the presence of osteophytes, disc space narrowing, and end-plate sclerosis and the presence of other structural artifacts such as extra-skeletal calcifications. Lumbar spine BMD measurements can be confounded by these structural artifacts that can artificially increase the BMD measurement (50, 51). Nevertheless, our finding of a strong association of AFM with femoral neck BMD is of public health importance because death rate within one-year of fractured neck of femur is between 20%-35% (52).

The results of the current study should be interpreted in the context of a few limitations. First, because we used DXA to measure AFM, we are unable to differentiate between visceral and subcutaneous fat. While visceral fat is thought to be more strongly associated with disease risk, a recent study showed that measures of central obesity were better associated with coronary artery calcium than direct measures of visceral adiposity (53). Still, future studies should evaluate the independent roles of visceral versus subcutaneous fat depots on bone. Second, as with any observational study, residual confounding is still a possibility. However, covariates included in our models were carefully selected based on underlying biological mechanisms. Finally, our study is cross-sectional in nature and hence we are unable to make inferences of causality.

In conclusion, our findings of a negative association between AFM and bone mass in a Hispanic population provide compelling evidence to the existing literature that AFM is a significant risk factor for osteoporosis. While our results should be replicated in other populations, our finding support the urgent need for development of public health programs tailored to specific ethnic groups that focus on prevention and treatment of abdominal obesity.

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Table 1: Characteristics of Puerto Rican women across quartiles of abdominal fat mass

	Quartiles of Abdominal Fat Mass (g)‡				
	1 1669 (251–2080)	2 2519 (2100–2880)	3 3199 (2884–3984)	4 4719 (3988–7178)	*P* for trend
n	116	116	117	116	
Age[a] (y)	62.3 (0.7)	60.9 (0.7)	60.0 (0.7)*	59.3 (0.7)**	0.002
Current smoker (y/n)	23.9	15.8	14.2	12.2*	0.03
Alcohol intake[b] (g/d)	3.09 (0.65)	1.47 (0.63)	1.16 (0.63)	1.34 (0.65)	0.09
Calcium intake[b] (g/d)	997 (48)	1011 (47)	1013 (47)	891 (48)	0.09
Plasma 25(OH) D [c] (ng/mL)	20.5 (0.7)	18.6 (0.7)	19.0 (0.7)	18.7 (0.7)	0.13
BMI[c] (kg/m^2)	25.6 (0.4)	30.6 (0.3)†	34.0 (0.3)†	41.9 (0.4)†	<0.0001
Weight (kg)	60.3 (0.8)	72.1 (0.8)†	81.9 (0.8)†	101.9 (0.8)†	<0.0001
Height (m)	1.53 (0.01)	1.54 (0.01)	1.55 (0.01)*	1.56 (0.01)**	0.0001
Waist circumference [c] (cms)	87.0 (0.9)	98.8 (0.8)†	105.6 (0.8)†	121.7 (0.8)†	<0.0001
Physical activity score [c]	31.7 (0.4)	31.7 (0.4)	30.7 (0.4)	29.9 (0.4)**	<0.0001
Education (%)					
<9th grade	39.7	49.0	57.4*	60.9**	0.001
9th-12th grade/GED	42.2	31.7	29.1	26.6*	0.02
College/some graduate school	18.2	19.2	13.5	12.6	0.16
Osteoporosis medication use (yes) (%)	57.3	53.7	44.6	47.9	0.11
Total household income [c] ($/y)	19318 (1858)	18655 (1879)	14287 (1837)	15665 (1828)	0.11

‡ Median (range), [a]Adjusted for age, [b] Adjusted for age, sex, energy intake, [c] Adjusted for age and sex

*P<0.05, **P<0.01, ***P<0.001, †P<0.0001 compared to the first quartile

Table 2: Characteristics of Puerto Rican men across quartiles of abdominal fat mass

	Quartiles of Abdominal Fat Mass (g)‡				
	1 2074 (614–2412)	2 2853 (2413–3235)	3 3554 (3237–4140)	4 5000 (4152–7821)	*P* for trend
n	41	41	41	41	
Age[a] (y)	59.1 (1.2)	60.5 (1.2)	59.7 (1.2)	60.2 (1.2)	0.62
Current smoker (yes/no)	53.1	24.9**	26.7*	20.3**	0.004
Alcohol intake[b] (g/d)	19.4 (5.1)	5.6 (5.3)	6.6 (5.5)	2.9 (5.1)	0.04
Calcium intake[b] (g/d)	966 (67)	878 (69)	945 (71)	997 (66)	0.53
Plasma 25(OH) D [c] (ng/mL)	18.1 (1.0)	17.1 (1.0)	16.9 (1.0)	17.2 (1.0)	0.61
BMI[c] (kg/m^2)	24.4 (0.4)	27.8 (0.4)†	30.7 (0.4)†	37.3 (0.4)†	<0.0001
Weight (kg)	66.8 (1.4)	78.5 (1.4)†	86.2 (1.4)†	105.8 (1.4)†	<0.0001
Height (m)	1.65 (0.01)	1.68 (0.01)	1.68 (0.01)	1.69 (0.01)*	0.03
Waist circumference [c] (cm)	89.9 (1.2)	100.7 (1.2)†	105.5 (1.2)†	121.1 (1.2)†	<0.0001
Physical activity score [c]	34.1 (0.8)	31.7 (0.8)	30.7 (0.8)**	31.0 (0.8)*	0.01
Education (%)					
<9th grade	56.9	43.3	53.8	34.7	0.08
9th-12th grade/GED	28.9	41.7	36.5	47.7	0.12
College/some graduate school	14.3	14.9	9.7	15.1	0.99
Osteoporosis medication use (yes) (%)	31.9	7.2**	19.5	27.4	0.85
Total household income[c] ($/y)	16688 (3391)	21161 (3390)	21245 (3525)	16181 (3478)	0.77

‡ Median (range), [a]Adjusted for age, [b] Adjusted for age, sex, energy intake, [c] Adjusted for age and sex

*P<0.05, **P<0.01, ***P<0.001, †P<0.0001 compared to the first quartile

Table 3: Association between abdominal fat mass (kg) and bone mineral density (g/cm^2)

		Abdominal Fat Mass (kg)							
		Femoral Neck		Trochanter		Total Femur		Lumbar Spine	
		β ± SE	*P*	β ± SE	*P*	β ± SE	*P*	β ± SE	*P*
Female	Model 1	0.010 ± 0.005	0.07	0.027 ± 0.005	<0.0001	0.029 ± 0.006	<0.0001	0.024 ± 0.007	0.0007
	Model 2	-0.059 ± 0.012	<0.0001	-0.043 ± 0.012	0.0005	-0.048 ± 0.014	0.0005	-0.044 ± 0.017	0.008
Male	Model 1	0.010 ± 0.011	0.33	0.023 ± 0.010	0.03	0.025 ± 0.011	0.03	0.012 ± 0.013	0.37
	Model 2	-0.068 ± 0.025	0.008	-0.057 ± 0.024	0.02	-0.059 ± 0.026	0.02	-0.002 ± 0.032	0.96
		Total Fat Mass (kg)							
		Femoral Neck		**Trochanter**		**Total Femur**		**Lumbar Spine**	
		β ± SE	***P***	**β ± SE**	***P***	**β ± SE**	***P***	**β ± SE**	***P***
Female	Model 1	0.0002 ± 0.0001	0.0003	0.0003 ± 0.0001	<0.0001	0.0004 ± 0.0001	<0.0001	0.0003 ± 0.0001	<0.0001
	Model 2	-0.0002 ± 0.0002	0.21	-0.0006 ± 0.0002	0.001	-0.0007 ± 0.0002	0.0005	-0.0004 ± 0.0003	0.12
Male	Model 1	0.0001 ± 0.0001	0.42	0.0002 ± 0.0001	0.11	0.0003 ± 0.0001	0.08	0.0001 ± 0.0002	0.42
	Model 2	-0.001 ± 0.0003	0.001	-0.001 ± 0.0003	<0.0001	-0.001 ± 0.0003	0.0004	-0.0002 ± 0.0004	0.64

Model 1: Adjusted for age (y), current smoking status (y/n), alcohol intake (g/d), energy intake (kcal/d), season of BMD measurement (spring, summer, fall, winter), osteoporosis prescription medications use (y/n), physical activity score, calcium intake (mg/d), plasma 25(OH) D status (ng/mL)

Model 2: Model 1 + weight (kg), height (m)

FIGURE LEGEND

Figure 1A: Adjusted mean bone mineral density (BMD) (g/cm^2), before adjustment for weight and height, across quartiles of abdominal fat mass among women

Adjusted for age (y), current smoking status (y/n), alcohol intake (g/d), energy intake (kcal/d), season of BMD measurement (spring, summer, fall, winter), osteoporosis prescription medications use (y/n), physical activity score, calcium intake (mg/d), plasma 25(OH) D status (ng/mL)

Figure 1B: Adjusted mean bone mineral density (BMD) (g/cm^2), after adjustment for weight and height, across quartiles of abdominal fat mass among women

Adjusted for age (y), current smoking status (y/n), alcohol intake (g/d), energy intake (kcal/d), season of BMD measurement (spring, summer, fall, winter), osteoporosis prescription medications use (y/n), physical activity score, calcium intake (mg/d), plasma 25(OH) D status (ng/mL), body weight (kg), height (m)

Figure 1C: Adjusted mean bone mineral density (BMD) (g/cm^2), before adjustment for weight and height, across quartiles of total fat mass among women

Adjusted for age (y), current smoking status (y/n), alcohol intake (g/d), energy intake (kcal/d), season of BMD measurement (spring, summer, fall, winter), osteoporosis prescription medications use (y/n), physical activity score, calcium intake (mg/d), plasma 25(OH) D status (ng/mL)

Figure 1D: Adjusted mean bone mineral density (BMD) (g/cm^2), after adjustment for weight and height, across quartiles of total fat mass among women

Adjusted for age (y), current smoking status (y/n), alcohol intake (g/d), energy intake (kcal/d), season of BMD measurement (spring, summer, fall, winter), osteoporosis

prescription medications use (y/n), physical activity score, calcium intake (mg/d), plasma 25(OH) D status (ng/mL), body weight (kg), height (m)

Figure 2A: Adjusted mean bone mineral density (BMD) (g/cm^2), before adjustment for weight and height, across quartiles of abdominal fat mass among men
Adjusted for age (y), current smoking status (y/n), alcohol intake (g/d), energy intake (kcal/d), season of BMD measurement (spring, summer, fall, winter), osteoporosis prescription medications use (y/n), physical activity score, calcium intake (mg/d), plasma 25(OH) D status (ng/mL)

Figure 2B: Adjusted mean bone mineral density (BMD) (g/cm^2), after adjustment for weight and height, across quartiles of abdominal fat mass among men
Adjusted for age (y), current smoking status (y/n), alcohol intake (g/d), energy intake (kcal/d), season of BMD measurement (spring, summer, fall, winter), osteoporosis prescription medications use (y/n), physical activity score, calcium intake (mg/d), plasma 25(OH) D status (ng/mL), body weight (kg), height (m)

Figure 2C: Adjusted mean bone mineral density (BMD) (g/cm^2), before adjustment for weight and height, across quartiles of total fat mass among men
Adjusted for age (y), current smoking status (y/n), alcohol intake (g/d), energy intake (kcal/d), season of BMD measurement (spring, summer, fall, winter), osteoporosis prescription medications use (y/n), physical activity score, calcium intake (mg/d), plasma 25(OH) D status (ng/mL)

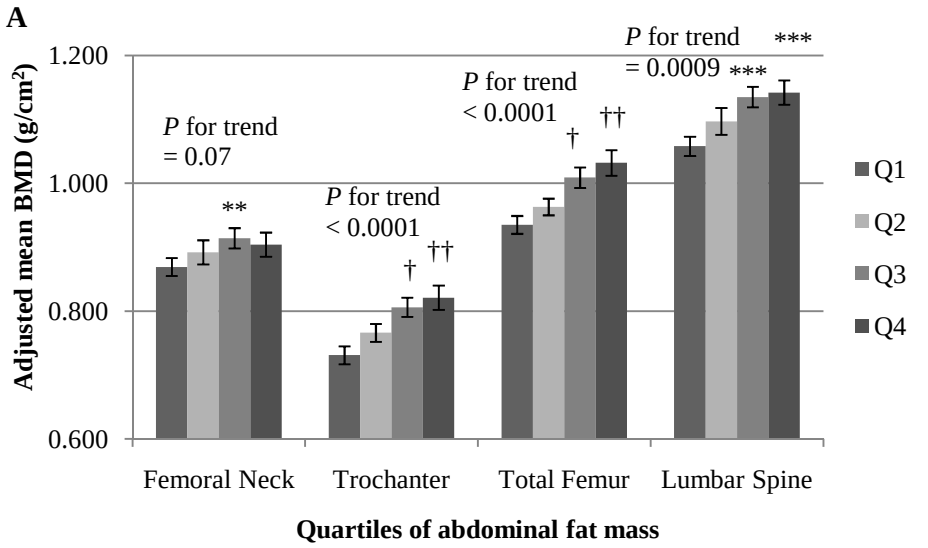
Figure 2D: Adjusted mean bone mineral density (BMD) (g/cm^2), after adjustment for weight and height, across quartiles of total fat mass among men

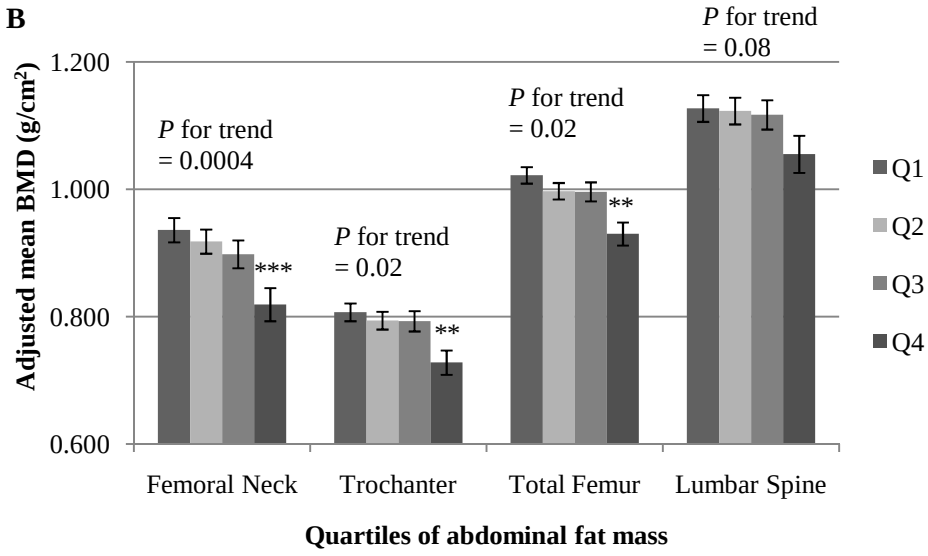
Adjusted for age (y), current smoking status (y/n), alcohol intake (g/d), energy intake (kcal/d), season of BMD measurement (spring, summer, fall, winter), osteoporosis prescription medications use (y/n), physical activity score, calcium intake (mg/d), plasma 25(OH) D status (ng/mL), body weight (kg), height (m)

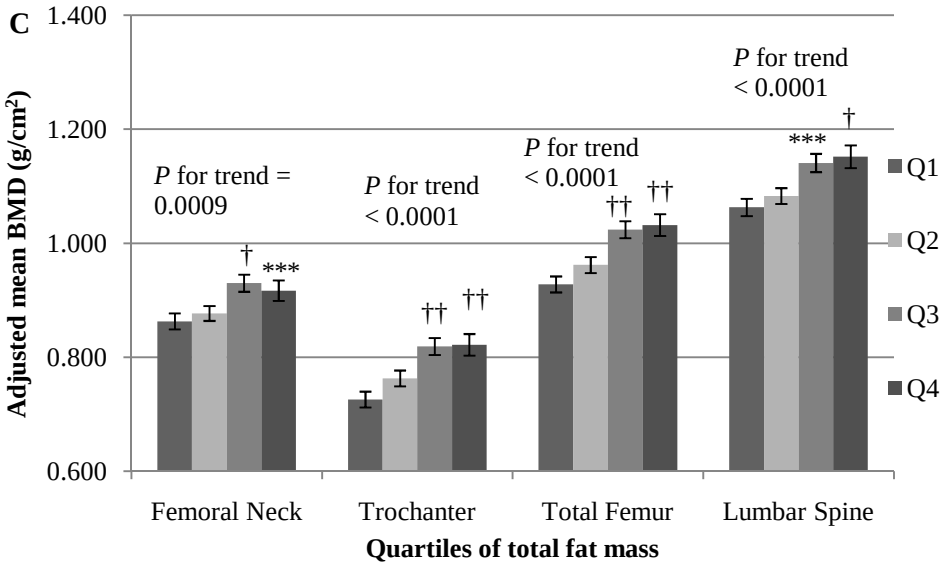
Figure 3A: Odds ratio (95% confidence interval) of osteoporosis or osteopenia for every 100g higher abdominal fat mass or 1 kg increase in total fat mass among women
Adjusted for age (y), current smoking status (y/n), alcohol intake (g/d), energy intake (kcal/d), season of BMD measurement (spring, summer, fall, winter), osteoporosis prescription medications use (y/n), physical activity score, calcium intake (mg/d), plasma 25(OH) D status (ng/mL), body weight (kg), height (m)

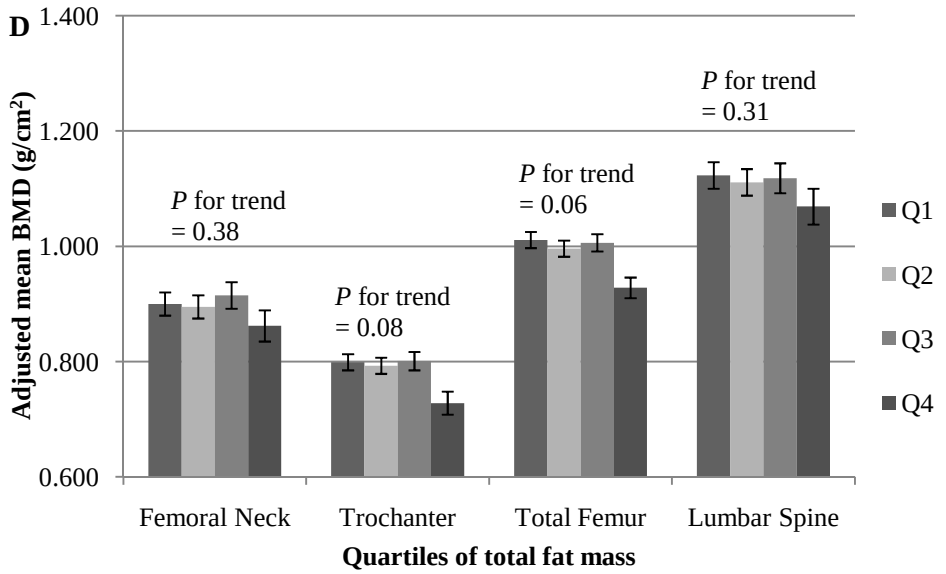
Figure 3B: Odds ratio (95% confidence interval) of osteoporosis or osteopenia for every 100g higher abdominal fat mass or 1 kg increase in total fat mass among men
Adjusted for age (y), current smoking status (y/n), alcohol intake (g/d), energy intake (kcal/d), season of BMD measurement (spring, summer, fall, winter), osteoporosis prescription medications use (y/n), physical activity score, calcium intake (mg/d), plasma 25(OH) D status (ng/mL), body weight (kg), height (m)

Figures 1A-1D



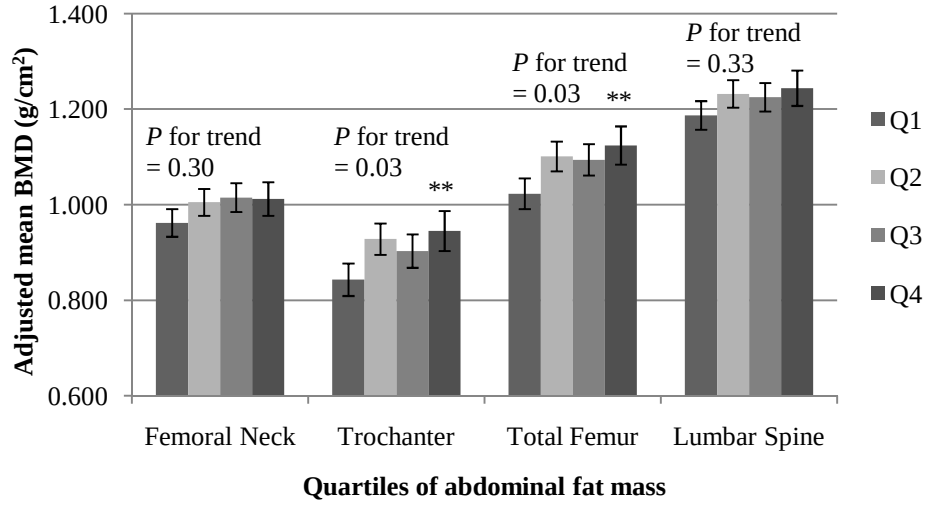



C
1.400
1.200
1.000
0.800
0.600
Adjusted mean BMD (g/cm²)
P for trend = 0.0009
P for trend < 0.0001
P for trend < 0.0001
P for trend < 0.0001
†
***
††
††
††
***
†
Q1
Q2
Q3
Q4
Femoral Neck
Trochanter
Total Femur
Lumbar Spine
Quartiles of total fat mass

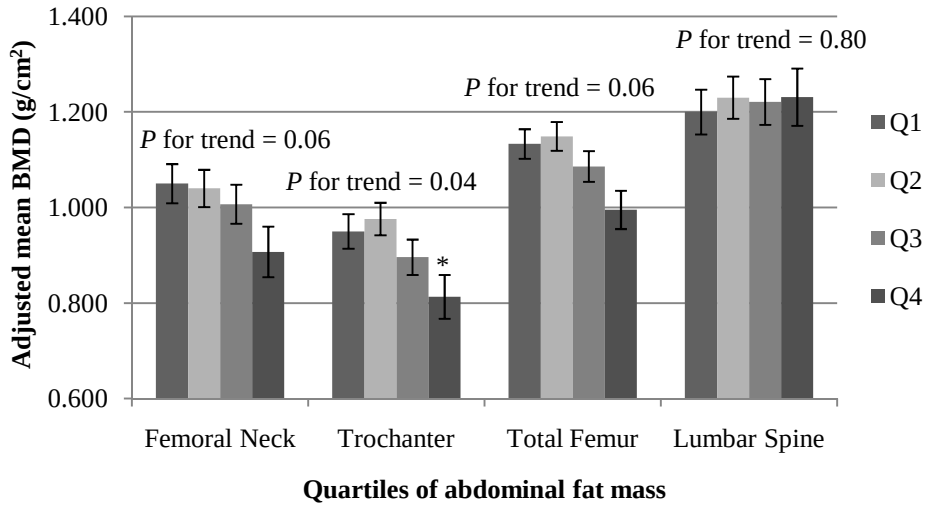

D
1.400
1.200
1.000
0.800
0.600
Adjusted mean BMD (g/cm²)
P for trend = 0.38
P for trend = 0.08
P for trend = 0.06
P for trend = 0.31
Q1
Q2
Q3
Q4
Femoral Neck
Trochanter
Total Femur
Lumbar Spine
Quartiles of total fat mass

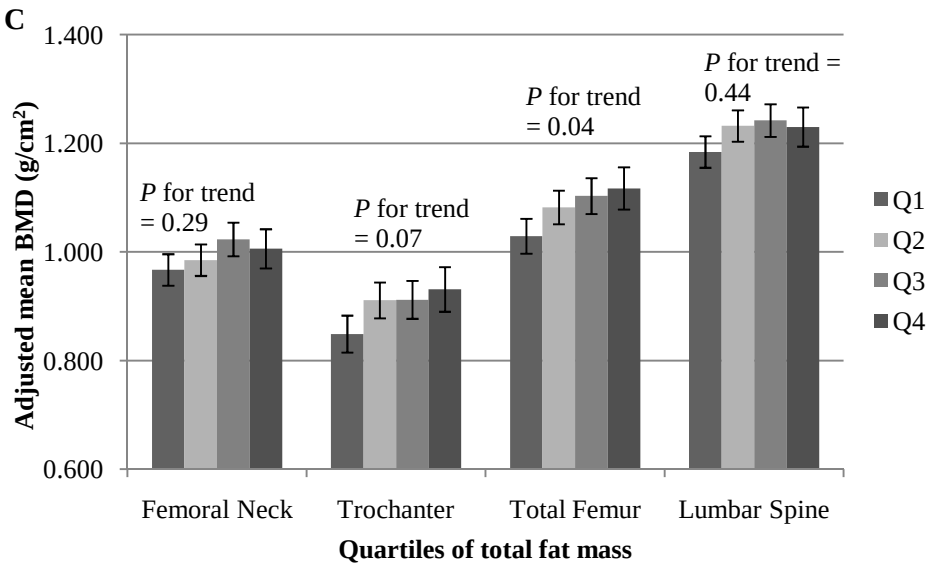
Figures 2A-2D

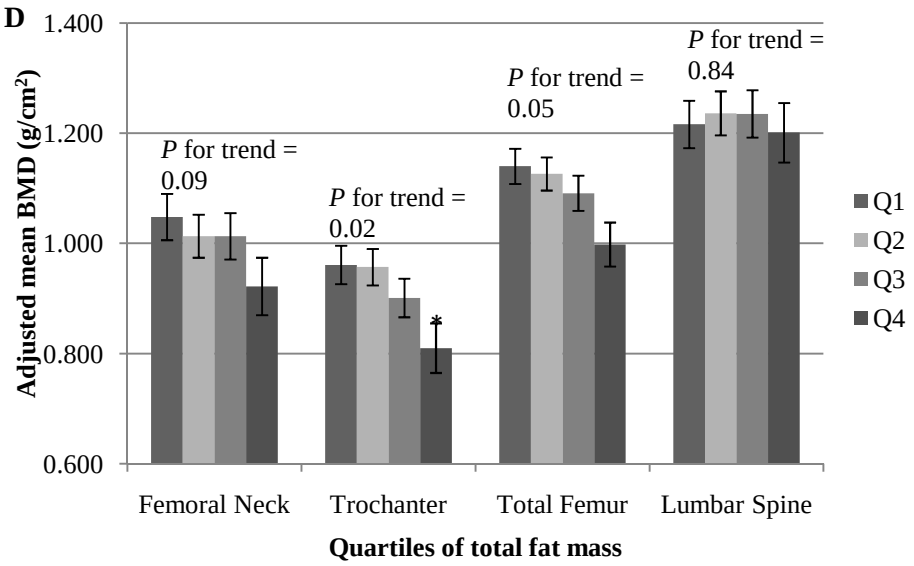
A



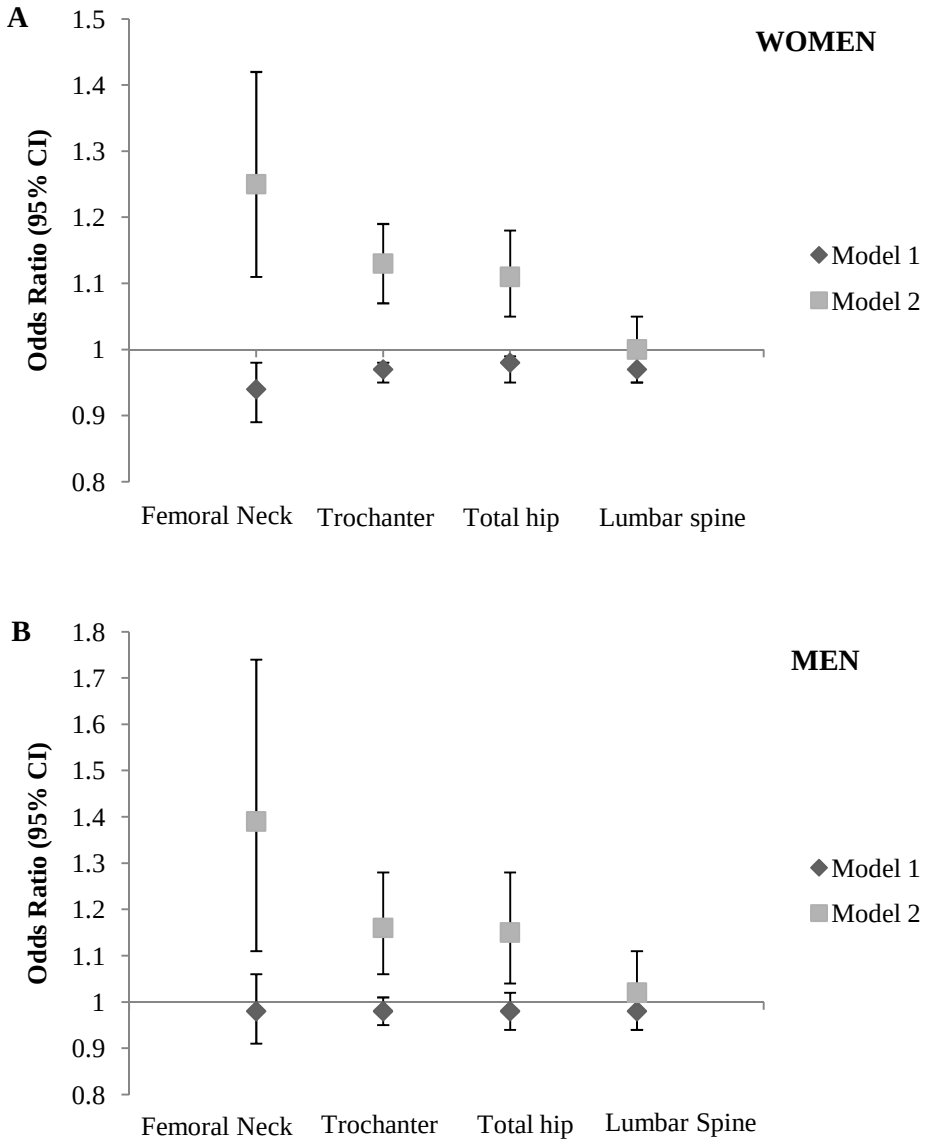
B




C
1.400
1.200
1.000
0.800
0.600
Adjusted mean BMD (g/cm²)
P for trend = 0.29
P for trend = 0.07
P for trend = 0.04
P for trend = 0.44
Q1
Q2
Q3
Q4
Femoral Neck
Trochanter
Total Femur
Lumbar Spine
Quartiles of total fat mass


D
1.400
1.200
1.000
0.800
0.600
Adjusted mean BMD (g/cm²)
P for trend = 0.09
P for trend = 0.02
P for trend = 0.05
P for trend = 0.84
*
Q1
Q2
Q3
Q4
Femoral Neck
Trochanter
Total Femur
Lumbar Spine
Quartiles of total fat mass

Figures 3A-3B



CHAPTER FIVE

Associations between inflammatory markers and bone health in Puerto Rican women[1-5]

(To be submitted to the Journal of Bone and Mineral Research)

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All authors have no conflicts of interest

ABSTRACT

Inflammatory markers have been shown to influence bone health. We evaluated cross-sectional associations of inflammatory markers (C-reactive protein (CRP), interleukin (IL)-6, and tumor necrosis factor alpha (TNF-α) with bone mineral density (BMD) at the femoral neck, trochanter, total hip, and lumbar spine (L2-L4) in 403 postmenopausal Puerto Rican women. Participants were divided into normal (<1 mg/L), intermediate (1-3 mg/L), and high (>3 mg/L) CRP categories and also into tertiles of IL-6 and TNF-α. We calculated adjusted mean BMD at each bone site across categories or tertiles of cytokines adjusting for age, current smoking, BMI, height, white blood cell count, physical activity score, season of BMD measurement, non-steroidal anti-inflammatory medication use, osteoporosis medication use, estradiol, plasma vitamin D, and intakes of calcium and energy. Women in the second tertile of TNF-α (2.4-4.1 pg/mL) and intermediate concentration of CRP (1–3 mg/L) had lower lumbar spine BMD compared to women in the lowest groups ($p<0.05$). Those in the second (vs. lowest) tertile of TNF-α had lower femoral neck BMD ($p<0.05$). Inverse trends at the trochanter ($p=0.07$) and lumbar spine BMD ($p = 0.04$) were observed across tertiles of IL-6 (pg/mL). Those with multiple exposures to elevated cytokines had lower lumbar spine BMD (p for trend = 0.04). Participants with intermediate (vs. low) CRP had 2.25 higher odds for osteoporosis/osteopenia (95% CI: 1.00-5.10) at the lumbar spine. No associations were observed at other bone sites or with IL-6 or TNF-α. These results suggest a potential role of inflammation in postmenopausal bone health.

Keywords: inflammation, pro-inflammatory cytokines, postmenopausal, bone, osteoporosis, population studies

INTRODUCTION

Osteoporosis, a disease characterized by low bone mass and microarchitectural deterioration of bone tissue (1), is a major public health threat to more than 44 million Americans, aged 50 or older. Prevalence data from the third National Health and Nutrition Examination Survey (NHANES III, 1988-94) indicate that 49% of Hispanic (mostly Mexican-American) women, aged 50 years and older, had low bone mass, that 10% had osteoporosis, and that 23% of Hispanic men, aged 50 years and older, had low bone mass and 3% had osteoporosis (2-3). While several advances in the treatment of osteoporosis have been made, the cost to the healthcare system due to osteoporosis related fractures remains high and is projected to rise by nearly 175% for Hispanics by the year 2020 (4), suggesting that this is a high risk group and that there is a need for more research.

In the past decade, inflammation has emerged as an important risk factor in the development of osteoporosis (5-6). The immune system modifies bone resorption and possibly bone formation through complex and multi-faceted interactions that involve T and B lymphocytes, dendritic cells, cytokines, and cell to cell interactions (5). In fact, an important underlying mechanism for the protective effect of estrogen on the skeleton is due to its regulatory effect on pro-inflammatory cytokines (7). Elegant *in vitro* and ovariectomized animal models of menopause have elucidated complex mechanisms between estrogen deficiency, the immune system, and the skeleton (8-9). Previous observational studies have shown that elevated levels of C-reactive protein (CRP), a marker of systemic inflammation and an established risk factor for cardiovascular

disease, are associated with an increased risk of fracture (10-11), low bone mineral density (BMD) (12), and greater bone turnover (13).

It is important to understand the potential association between systemic inflammation and bone health in the Puerto Rican population. Puerto Ricans, the second largest Hispanic subgroup in the US and the largest in the northeastern US, have documented health disparities and bear a disproportionate amount of chronic disease burden compared to their neighborhood Hispanic and non-Hispanic counterparts (14). Results from our earlier survey (14-15) suggest considerable stress in the Puerto Rican population that manifests in depression, abdominal obesity, and metabolic syndrome, conditions known to raise markers of systemic inflammation such as CRP and pro-inflammatory cytokines. Establishment of an association between inflammatory markers and bone will provide insights into possible mechanisms by which BMD declines in this high risk population and may serve as an important target for prevention of osteoporosis in this and other populations with high prevalence of inflammation. Against this background, our hypothesis was that higher circulating concentrations of CRP and pro-inflammatory cytokines are associated with low BMD.

MATERIALS AND METHODS

Participants

The Boston Puerto Rican Health Study is a longitudinal investigation of the association between physiological dysregulation, nutrition, and chronic health outcomes in Puerto Rican adults, aged 45-75 years (y), living in the greater Boston area. The design of the Boston Puerto Rican Health Study has been described in detail elsewhere (16).

At baseline and two-year follow-up interview, participants completed a home interview in the language of their preference (Spanish or English). The interview consisted of questionnaires that collected information on socioeconomic status, health history and behaviors, acculturation, and dietary intake. Anthropometric and blood pressure measures were also obtained. On the day following the interview, or as soon as possible thereafter, fasting blood samples were collected by the study phlebotomist. At the end of the two-year follow-up interview, participants were re-consented for participation in the Osteoporosis study. Consenting participants visited the Metabolic Research Unit at the USDA Jean Mayer Human Nutrition Research Center on Aging at Tufts University for bone measurements, to answer additional questionnaires on osteoporosis prescription medication use, and to provide an additional fasted blood sample. Every effort was made to complete the Osteoporosis study visit within one month of the two-year interview. Written informed consent was obtained for all participants before participation in the study in accordance with the guidelines established by the Institutional Review Board at Tufts University/Tufts Medical Center.

Because estrogen withdrawal is known to modify the association between pro-inflammatory cytokines and bone (7), we made an *a-priori* decision to examine associations by menopausal status. For the current study, we restricted our analyses to postmenopausal women (n=403). The numbers of men (n=191) and pre-menopausal women (n=117) in our cohort were too few across cytokine categories, resulting in insufficient statistical power to detect associations.

Assessment of exposure

Inflammatory markers were measured in fasting blood obtained at the Osteoporosis study visit. Serum high sensitivity CRP was measured using a solid-phase, two-site chemiluminescent immunometric assay with a commercial kit (IMMULITE 1000, Diagnostics Products Corporation, Los Angeles, CA). Serum high-sensitivity interleukin (IL)-6 and tumor necrosis factor (TNF)-α were measured using a quantitative sandwich enzyme immunoassay commercial kit (R&D systems Inc., Minneapolis, MN). The minimum detectable dose for IL-6 and TNF-α is less than 0.70 pg/mL and 0.12 pg/mL, respectively. The intra-assay CV% for CRP, IL-6, and TNF-α are 6.0, 1.6-4.2, and 5.3-8.8, respectively. The inter-assay CV% for CRP, IL-6, and TNF-α are 7.3, 3.3-6.4, and 10.8-16.7, respectively.

Assessment of outcome

According to recommendations of the International Society for Clinical Densitometry (17), we made an *a-priori* decision to only include BMD measurements at the femoral neck, total hip, and posterior-anterior lumbar spine (L2-L4) in our analyses. In addition, we included the trochanter, as inclusion of this bone site provides a complete picture of the hip. BMD (g/cm^2) at all four bone sites was measured by dual energy x-ray absorptiometry (GE-Lunar model Prodigy scanner, WI) using standard procedures. The root mean square precision of these measurements were 0.65% for total hip BMD, 1.03% for the trochanter, 1.31% for the femoral neck, and 1.04% for the lumbar spine (18). For femur measurements, the right hip was scanned unless there was a history of hip fracture or joint replacement.

Assessment of menopausal status

Women reported if they had already gone through menopause or are currently going through menopause. Because we noted significant misclassification of self-reported menopausal status, we created a decision tree to identify women who were post-menopausal. Women on estrogen replacement therapy were automatically coded as post-menopausal. It is estimated that the average age of menopause in the United States is 51 years, and that by age 55, more than 90% of women have experienced menopause (19). Based on this, we used a conservative age cut-off of 60 y to classify women as post-menopausal. Further, because normal estradiol concentrations for postmenopausal women are between 0-30 pg/mL (20), women with an estradiol concentration <30pg/mL were also classified as postmenopausal.

Potential confounding factors

At the two-year home interview (the visit just prior to the DXA scan visit), participants reported current smoking status, household income, and non-steroidal anti-inflammatory medication use. Poverty was calculated for each participant by comparing annual household income to the poverty guidelines provided by the Department of Health and Human Services, taking into account the year of the interview and the subject's family size. A modified Paffenbarger questionnaire of the Harvard Alumni Activity Survey (21-22) was used to create a physical activity score, calculated by summing the amount of time spent in each activity, multiplied by weighting factors that correspond with oxygen consumption by physical activity intensity for that activity. Waist circumference was measured using a non-elastic tape on the smallest area of the waist,

and was recorded to the nearest one-tenth of a centimeter. Usual intakes of total energy and calcium were assessed using a semi-quantitative food frequency questionnaire (FFQ) developed and validated for this population (23). White blood cell (WBC) count was measured in whole blood.

Age at the Osteoporosis study visit, was calculated by subtracting date of birth from date of visit. Participants reported information on osteoporosis prescription medication use (y/n). Standing height was measured with a stadiometer (Seca, Germany). Weight was measured with a digital scale (Seca, Model Alpha, Germany). Body mass index (BMI) was calculated as weight (in kg) divided by height (m^2). Plasma 25-hydroxy vitamin D (ng/mL) was measured using a ^{125}I radioimmunoassay kit procedure (DiaSorin Inc, Stillwater, MN). The intra- and inter-assay CV% are 10.8% and 9.4% respectively. Serum estradiol (pg/mL) was also measured using a radioimmunoassay kit procedure (Diagnostic Systems Laboratories, Inc., Webster, Texas). The intra- and inter-assay CV% are 6.6% and 7.2%, respectively. Because BMD is known to vary by season in the New England area (24), we created a four level categorical variable for season of BMD measurement as follows: July, August, and September were coded as summer; October, November, and December as fall; January, February, and March as winter; and April, May, and June as spring.

Statistical Analyses

All statistical analyses were performed using SAS (version 9.2, SAS Institute, Cary, NC). Hypothesis testing was two-sided, with a significance level of $p \leq 0.05$. Histograms and scatter plots were used to assess distribution of data and identify

potential outliers. Because distributions of inflammatory markers were skewed, a logarithmic transformation was applied to improve normality. Descriptive characteristics were calculated for a sample of postmenopausal women. As acute infection is known to raise cytokine concentrations, we excluded participants with a WBC count >10.8 mm^3 (n=10) in all analyses. Participants with high WBC count were more likely to be smokers (59.2% vs 14.1%, $p<0.0001$) and to use non-steroidal anti-inflammatory medications (69.2% vs 33.3%, $p=0.02$).

Participants were categorized into normal (<1 mg/L), intermediate (1-3 mg/L), high (>3 mg/L) CRP concentration (25). Because quantitative clinical cut-points are not available for IL-6 and TNF, participants were divided into tertile categories. We used the general linear models procedure to model the associations of cytokine concentrations (categorical) and BMD (continuous). In our first model, we adjusted for known osteoporosis risk factors. These included age (y), BMI (kg/m^2), current smoking status (y/n), physical activity score, and season of BMD measurement. To correct for differences due to skeletal size, we included height (m) as a covariate. To adjust for elevations in cytokine concentrations due to acute infections, WBC (mm^3) was included as a covariate. Finally, to account for confounding due to indication, non-steroidal anti-inflammatory and osteoporosis prescription medication use (y/n) were added to model 1. In model 2, we further adjusted for serum estradiol concentration (pg/mL). In our final model, we adjusted for differences in intakes of total energy (kcal/d) and calcium (mg/d), and for plasma vitamin D concentration (ng/mL).

All linear models were checked for assumption of linearity and homogeneity by examining the distribution of the residuals. To estimate the influence of a data point,

observations with a high Cook's distance were examined for validity of the observed values. If the value of the observation was consistent with the data, the observation was retained in the regression analysis. We used logistic regression to model the odds of osteoporosis or osteopenia for each unit increase in logarithmic IL-6, TNF-α, and across categories of CRP concentrations. Models were sequentially adjusted for potential confounding variables as described above.

For models with CRP as the main exposure, we performed sensitivity analyses to exclude participants with CRP concentration >10mg/L. We repeated analyses to examine adjusted BMD across four categories of CRP – normal (<1 mg/L), intermediate (1-3 mg/L), high (>3 and <10 mg/L), and very high (>10 mg/L) CRP. We conducted a post-hoc analysis to assess the effect of cumulative exposure to cytokines on LS BMD. We adapted an approach used previously in the Health Aging, and Body Composition Study (26), to create a cumulative inflammatory index for each participant, by summing the category (1, 2, or 3) for each cytokine. Thus, for all 3 cytokines together, participants received a score between 0 and 6. Adjusted lumbar spine BMD was compared across categories of 0-2, >2 and ≤4, and >4 and ≤6 of the cumulative inflammatory index.

For all analyses, we adjusted for multiple comparisons using Dunnett's adjustment, with the lowest category as the reference group. Tests for linear trend were conducted, assigning each participant the median cytokine concentration of the corresponding category, and treating this variable as a continuous measure. The current analyses include data from 393 postmenopausal women who had normal WBC counts (<10.8 mm^3). For those with missing data on age (n=3), CRP (n=5), and BMI (n=2) at the

Osteoporosis study visit, we used data from their 2-year visit, as this was collected just prior to the Osteoporosis study visit.

RESULTS

The mean age of post-menopausal women in our sample was 62.3 y (range: 47.1-79.1 y). The majority engaged in sedentary or light physical activity, while nearly 75% lived below the poverty line. Women in the highest CRP, compared to the lowest, category had a higher BMI, higher waist circumference, higher serum estradiol, and higher circulating IL-6 concentration. Few women used estrogen replacement therapy (**Table 1**). Women in our sample had high circulating concentrations of inflammatory markers. Only 18% had CRP concentration <1 mg/L. Nearly 37% and 18% had high (>3 mg/L) and very high (>10 mg/L) CRP. We compared the CRP concentration at the Osteoporosis study visit with CRP concentration at the baseline follow-up visit for all women with CRP >10 mg/L. Nearly 93% and 54% of participants with CRP>10 mg/L at the Osteoporosis study visit had consistently high (>3 mg/L) or very high (>10 mg/L) CRP at baseline.

Women with intermediate CRP (1-3 mg/L) concentration had lower lumbar spine BMD compared to those with normal CRP (<1 mg/L) (p<0.05) (**Table 2**). No significant differences were noted between those with high and normal CRP at any of the bone sites. Results did not change substantially when participants with CRP concentration >10 mg/L were excluded (data not shown).

No significant associations in BMD were seen across tertiles of IL-6. However, there was a decreasing trend in trochanter and lumbar spine BMD (p for trend=0.07 and

0.04, respectively). Like CRP, participants in the second tertile of TNF-α had significantly lower BMD at the femoral neck ($p \leq 0.05$) and lumbar spine ($p \leq 0.01$). No significant differences in BMD were seen between participants in the extreme tertiles of TNF-α (Table 2).

Participants in the intermediate and high CRP categories did not have significantly higher odds of osteoporosis or osteopenia at the three hip sites. At the lumbar spine, those in the intermediate CRP category had more than two-fold higher odds of osteoporosis or osteopenia compared to those with normal CRP. However, adjustment for intakes of total energy, calcium, and plasma vitamin D attenuated these results (OR=2.25, 95% CI: 1.00-5.10) (**Table 3**). No associations were noted between odds of osteoporosis or osteopenia, log IL-6 or log TNF-α.

Because we noted an effect, or trend toward an effect, for each cytokine on lumbar spine BMD, we evaluated the effect of cumulative exposure to elevated cytokine concentrations on this bone site. Participants with a cumulative inflammatory index >4, compared to those with ≤2, had significantly lower lumbar spine BMD ($p \leq 0.05$) but this difference was attenuated after adjustment for intakes of calcium and total energy, and plasma vitamin D ($p < 0.06$). However, there was a decreasing linear trend in lumbar spine BMD across the cumulative inflammatory index (p for trend = 0.04) (**Figure 1**).

DISCUSSION

To our knowledge, this is the first study to examine the associations between pro-inflammatory cytokines and bone health in a Hispanic postmenopausal population. The most consistent association was observed at the lumbar spine BMD, suggesting that

trabecular bone may be more susceptible to turnover by the action of cytokines. We found that intermediate concentrations of CRP and TNF-α (tertile 2), but not IL-6, were associated with lower lumbar spine BMD. Further, intermediate concentration of TNF-α was also associated with lower femoral neck BMD. These associations were independent of several important osteoporosis risk factors.

During the bone remodeling cycle, bone resorption is coupled to bone formation. Multiple cytokines (IL-1, IL-6, TNF-α) and hormones (vitamin D and estrogen) not only regulate the coupling between these systems but also the differentiation of osteoclasts and osteoblasts (5). CRP is a member of the pentraxin family of innate immune response proteins (27) and is an acute phase protein produced by hepatocytes. As a marker of systemic inflammation, CRP is a strong risk factor for CVD and may be the underlying link between osteoporosis and CVD. While evidence in recent years has indicated that elevated CRP concentration is associated with lower BMD (12) and greater risk for fracture (10-11), other studies have not been able to establish an association (28-29). We found that concentrations of CRP that are associated with average risk of CVD (1-3 mg/L) were associated with lower BMD at the lumbar spine. Interestingly, there were no differences in BMD between those with high and low CRP. Because it is known that considerable within-person variability exists in the measurement of CRP, possible variation is higher among participants with CRP concentration on the higher end at any single point in time and thus these participants may be more likely to be misclassified. However, we found that participants with CRP concentration >10 mg/L at the bone visit, were also likely to have had consistently high values at their baseline visit. While the recommendation for public health practice is to measure CRP in a metabolically stable

person without obvious inflammatory conditions (25), it is important to note that a considerable proportion of our population are overweight or obese (89%) and have metabolic syndrome (~80%) both of which are conditions of systemic inflammation. While participants with high CRP had higher IL-6 and lower estradiol concentrations, adjustment for these variables did not change results. No other differences were noted between those with high and intermediate CRP. Although we adjusted for potential confounders, it is always possible that residual confounding exists through unknown variables.

IL-6, a pluripotent protein that is stimulated by parathyroid hormone, is known to stimulate bone resorbing osteoclasts. IL-6 exerts its actions on bone by binding to a specific cell-surface IL-6 receptor. *In vitro* and animal studies have shown that estrogen withdrawal results in increased osteoclast development that is mediated by IL-6 (30). It has been suggested that, in humans, IL-6 is a major predictor of bone loss only during the first decade of menopause (31). While we cannot accurately estimate the years since menopause for our participants, it can be reasonably assumed that a considerable proportion of our sample is 10 years since menopause. This may account for the weak trends observed across tertiles. However, our results are in agreement with those of Ding et al. (32) and Zheng et al. (33) who have shown that, in women more than 10 years past the menopause, serum IL-6 (32) or IL-6 produced by stimulated whole blood cells (33) were associated with lower lumbar spine BMD.

TNF-α is another pro-inflammatory cytokine that is expressed by T-lymphocytes (34). Bone marrow stromal cells express TNF-α receptors and when exposed to TNF-α, these cells produce Receptor Activator for Nuclear Factor κ B Ligand (RANKL),

Monocyte Colony Stimulating Factor (M-CSF), and IL-1, all of which stimulate osteoclast formation and activation. TNF-α may directly stimulate osteoclast formation, independent of RANKL, perhaps through the action of transforming growth factor (TGF-β) (35). Estrogen regulates TNF-α by suppressing its production by T-cells and by regulating T-cell differentiation and activity. Thus, estrogen withdrawal during menopause is accompanied by increased production of inflammatory cytokines, including TNF-α (36). The bulk of the evidence for a role of TNF-α in bone loss has emerged from *in vitro* and animal models; results from human studies have been limited and inconsistent. Our results are in agreement with those of Zheng et al (33) who reported that TNF-α production by stimulated whole blood cells was negatively associated with lumbar spine BMD in postmenopausal women. Similarly, in a population of 163 participants, aged 50-79 y in Southern Tasmania, baseline TNF-α was associated with 2.9 y change in total body BMD (β=-0.31% per quartile, p=0.04) and lumbar spine BMD (β=-0.29% per quartile, p=0.02) (32). On the other hand, TNF-α production by peripheral blood mononuclear cells was not associated with lumbar spine BMD in 50 healthy premenopausal women (37), potentially due to the protective effect of estrogen. As observed with CRP, we found that participants in the second, but not third, tertile had significantly lower femoral neck and lumbar spine BMD. Although reasons for these results are not clear, it is possible that variation in TNF-α concentration could be high with higher values. At the same time, we did not observe any significant differences in socio-demographic or health characteristics in participants between the second and third tertile (data not shown).

It is interesting to note that all three pro-inflammatory cytokines were associated, to some extent, with lumbar spine BMD suggesting that trabecular bone may be the primary tissue affected by systemic inflammation. However, it is uncertain if circulating concentrations of pro-inflammatory cytokines are an indication of events in the bone microenvironment. Measuring cytokine production through stimulation of peripheral blood mononuclear cells may provide a precise picture of the biology in the bone microenvironment. Further, soluble receptors for TNF-α and IL-6 may better represent chronic inflammation than measurement of the cytokines. It is thought that these receptors are released into circulation in an effort to protect against excessive elevations in their ligands (38) and in response to infection (39).

The strength of the present study lies in our ability to examine multiple markers of systemic inflammation in older Puerto Rican women. Further, we were able to assess the association between cytokines and BMD independent of several biological and lifestyle factors that contribute to age-associated bone loss. However, our findings need to be interpreted in the context of a few limitations. First, while there is considerable evidence for biological plausibility for an effect of pro-inflammatory cytokines on bone, our results are cross-sectional and thus we cannot make inferences about causality. Second, the limited sample size prevented us from observing significant associations, if any, between cytokines and odds of osteoporosis or osteopenia. Third, we did not have objective criteria to assess menopausal status. However, we used established cut-offs and guidelines from national agencies to determine a women's menopausal status. Our estimates were likely conservative and we may have classified some postmenopausal women as pre- or peri-menopausal. This would attenuate observed results as such

misclassification would result in smaller sample size. We did not have sufficient power to examine these associations in premenopausal women and men. Finally, concentrations of inflammatory markers, especially of CRP, were very high in our population, possibly due to the high prevalence of abdominal obesity and other chronic diseases. Hence, our results may not be directly generalized to a healthy population.

In summary, this study demonstrates that pro-inflammatory cytokines are associated with lumbar spine BMD in postmenopausal Puerto Rican women. These findings are interesting, as they provide a possible patho-physiological link between cardiovascular disease and osteoporosis. While BMD is a good measure of bone health, fracture is the clinically relevant outcome of interest. Further investigation is needed to confirm these findings in relation to fracture risk.

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Table 1
Descriptive characteristics of Puerto Rican postmenopausal women by CRP categories[*]

Characteristic[a]	CRP (mg/L)		
	<1	**1-3**	**>3**
n	69	109	215
Age (y)	62.6 ± 0.8	62.0 ± 0.7	62.5 ± 0.5
Current smoker (y) (%)	17.5	14.6	11.2
BMI (kg/m^2)	29.3 ± 0.7	30.6 ± 0.6	34.2 ± 0.4†
Waist circumference (cm)	96.8 ± 1.7	98.5 ± 1.3	106 ± 1.0†
Physical activity score	31.3 ± 0.5	31.7 ± 0.4	30.5 ± 0.3
Below poverty line (%)	72.6	78.7	68.9
Dietary calcium[b] (g/d)	777 ± 44	765 ± 34	787 ± 25
Plasma vitamin D (ng/mL)	20.3 ± 0.9	19.8 ± 0.7	19.4 ± 0.5
Serum estradiol (pg/mL)	18.5 ± 1.2	18.5 ± 0.9	23.2 ± 0.7***
Current estrogen use (y) (%)	1.5	2.8	3.3
Bone mineral density (g/cm^2)			
Femoral neck	0.896 ± 0.016	0.860 ± 0.013	0.897 ± 0.009
Trochanter	0.765 ± 0.016	0.749 ± 0.013	0.794 ± 0.009
Total hip	0.971 ± 0.018	0.943 ± 0.014	1.010 ± 0.010
Lumbar spine (L2-L4)	1.118 ± 0.020	1.067 ± 0.016	1.122 ± 0.012
Pro-inflammatory cytokines[c]			
IL-6 (pg/mL)	2.23 ± 1.08	2.48 ± 1.06	4.17 ± 1.04†
TNF-α (pg/mL)	1.95 ± 1.06	2.01 ± 1.05	2.10 ± 1.03
Medication use (%)			
Non-steroidal anti-inflammatory	32.0	27.6	35.6
Osteoporosis medications	57.7	53.6	50.6

Data are least square means ± SD (all such values) or %. BMI = body mass index; IL-6 = interleukin-6; TNF-α = tumor necrosis factor alpha

[a]Data are adjusted for age

[b]Data are adjusted for age and energy intake

[c]Geometric mean ± SD

$^{***}p$ <0.001, $^{\dagger}p$ <0.0001

Table 2
Adjusted mean BMD (g/cm^2) by cytokine categories in Puerto Rican postmenopausal women

BONE SITE	MODEL	n	CRP (mg/L) <1 (n=69)	CRP (mg/L) 1-3 (n=109)	CRP (mg/L) >3 (n=215)	*p* for trend
Femoral neck	1	336	0.900 (0.017)	0.858 (0.015)	0.884 (0.012)	0.71
	2	332	0.901 (0.017)	0.860 (0.015)	0.884 (0.012)	0.82
	3	276	0.882 (0.020)	0.857 (0.017)	0.874 (0.013)	0.77
Trochanter	1	336	0.776 (0.017)	0.746 (0.015)	0.768 (0.012)	0.61
	2	332	0.776 (0.017)	0.745 (0.015)	0.769 (0.012)	0.57
	3	276	0.760 (0.019)	0.745 (0.016)	0.760 (0.013)	0.64
Total hip	1	330	0.984 (0.019)	0.938 (0.017)	0.968 (0.013)	0.63
	2	326	0.983 (0.020)	0.937 (0.017)	0.969 (0.014)	0.59
	3	270	0.965 (0.022)	0.936 (0.019)	0.960 (0.015)	0.60
Lumbar spine (L2-L4)	1	336	1.120 (0.023)	1.054 (0.020)*	1.080 (0.015)	0.62
	2	332	1.120 (0.023)	1.055 (0.020)*	1.081 (0.016)	0.58
	3	276	1.111 (0.025)	1.041 (0.021)*	1.076 (0.016)	0.58
BONE SITE	**MODEL**	**n**	**IL-6 (pg/mL) Tertile 1 1.65 (0.68-2.40) n=130**	**IL-6 (pg/mL) Tertile 2 3.18 (2.42-4.12) n=131**	**IL-6 (pg/mL) Tertile 3 5.83 (4.14-19.33) n=129**	***p* for trend**
Femoral neck	1	325	0.878 (0.015)	0.894 (0.014)	0.866 (0.014)	0.40
	2	325	0.880 (0.015)	0.895 (0.014)	0.865 (0.014)	0.31
	3	270	0.873 (0.017)	0.886 (0.015)	0.856 (0.015)	0.30
Trochanter	1	325	0.772 (0.015)	0.775 (0.014)	0.745 (0.014)	0.11
	2	325	0.773 (0.015)	0.775 (0.014)	0.744 (0.014)	0.10
	3	270	0.770 (0.017)	0.769 (0.015)	0.736 (0.014)	0.07
Total hip	1	320	0.964 (0.017)	0.972 (0.016)	0.949 (0.016)	0.39
	2	320	0.965 (0.018)	0.972 (0.016)	0.948 (0.016)	0.38
	3	265	0.960 (0.020)	0.963 (0.017)	0.941 (0.017)	0.36
Lumbar spine (L2-L4)	1	325	1.102 (0.020)	1.102 (0.019)	1.048 (0.018)	0.02
	2	325	1.106 (0.020)	1.103 (0.019)	1.047 (0.018)*	0.01
	3	270	1.097 (0.022)	1.089 (0.020)	1.044 (0.019)	0.04
BONE SITE	**MODEL**	**n**	**TNF-α (pg/mL) Tertile 1 1.34 (0.52-1.72) n=132**	**TNF-α (pg/mL) Tertile 2 2.01 (1.73-2.40) n=128**	**TNF-α (pg/mL) Tertile 3 2.97 (2.41-10.83) n=130**	***p* for trend**
Femoral neck	1	326	0.902 (0.014)	0.857 (0.014)*	0.879 (0.013)	0.32
	2	326	0.903 (0.014)	0.857 (0.014)**	0.879 (0.013)	0.31
	3	271	0.897 (0.017)	0.848 (0.015)*	0.875 (0.014)	0.61
Trochanter	1	326	0.777 (0.014)	0.744 (0.014)	0.767 (0.013)	0.75
	2	326	0.777 (0.014)	0.744 (0.014)	0.767 (0.013)	0.75
	3	271	0.771 (0.016)	0.732 (0.014)	0.767 (0.014)	0.74

Total hip	1	321	0.974 (0.016)	0.939 (0.016)	0.970 (0.015)	0.91
	2	321	0.974 (0.016)	0.939 (0.016)	0.970 (0.015)	0.90
	3	266	0.969 (0.019)	0.927 (0.017)	0.968 (0.016)	0.61
Lumbar spine (L2-L4)	1	326	1.116 (0.019)	1.051 (0.018)**	1.077 (0.018)	0.16
	2	326	1.117 (0.019)	1.052 (0.018)**	1.078 (0.018)	0.15
	3	271	1.106 (0.021)	1.041 (0.019)**	1.079 (0.018)	0.54

**$p \leq 0.01$, *$p \leq 0.05$

Model 1: Adjusted for age (y), current smoking (y/n), BMI (kg/m^2), height (m), white blood cell count (mm^3), physical activity score, season of BMD measurement (summer, fall, winter, spring), non-steroidal anti-inflammatory medication use (y/n), osteoporosis medication use (y/n)

Model 2: Model 1 + estradiol (pg/mL)

Model 3: Model 2 + calcium intake (mg/d), energy intake (kcal/d), plasma vitamin D status (ng/mL)

Table 3
Odds ratio (95% confidence interval) for osteoporosis/osteopenia by CRP categories (mg/L)

BONE SITE	MODEL	CRP (mg/L)		
		<1	1-3	>3
Femoral neck	1	Ref	1.45 (0.72-3.14)	0.94 (0.48-1.87)
	2	Ref	1.47 (0.70-3.08)	0.95 (0.47-1.90)
	3	Ref	1.33 (0.58-3.07)	0.93 (0.42-2.06)
Trochanter	1	Ref	1.26 (0.62-2.55)	0.91 (0.47-1.76)
	2	Ref	1.26 (0.62-2.56)	0.91 (0.46-1.79)
	3	Ref	1.50 (0.67-3.35)	1.06 (0.49-2.27)
Total hip	1	Ref	1.73 (0.80-3.74)	0.92 (0.44-1.94)
	2	Ref	1.71 (0.79-3.71)	0.84 (0.39-1.81)
	3	Ref	1.75 (0.74-4.18)	0.94 (0.40-2.22)
Lumbar spine (L2-L4)	1	Ref	2.27 (1.11-4.63)	1.14 (0.59-2.21)
	2	Ref	2.27 (1.11-4.64)	1.15 (0.59-2.25)
	3	Ref	2.25 (1.00-5.10)	1.03 (0.48-2.23)

Model 1: Adjusted for age (y), current smoking (y/n), BMI (kg/m^2), height (m), white blood cell count (mm^3), physical activity score, season of BMD measurement (summer, fall, winter, spring), non-steroidal anti-inflammatory medication use (y/n), osteoporosis medication use (y/n)
Model 2: Model 1 + estradiol (pg/mL)
Model 3: Model 2 + calcium intake (mg/d), energy intake (kcal/d), plasma vitamin D status (ng/mL)

FIGURE LEGEND

Figure 1: Adjusted mean lumbar spine BMD (g/cm^2) by the sum of tertile/category numbers for pro-inflammatory cytokines

*$p \leq 0.05$

Adjusted for age (y), current smoking (y/n), BMI (kg/m^2), height (m), white blood cell count (mm^3), physical activity score, season of BMD measurement (summer, fall, winter, spring), non-steroidal anti-inflammatory medication use (y/n), osteoporosis medication use (y/n), estradiol (pg/mL), calcium intake (mg/d), energy intake (kcal/d), plasma vitamin D status (ng/mL).

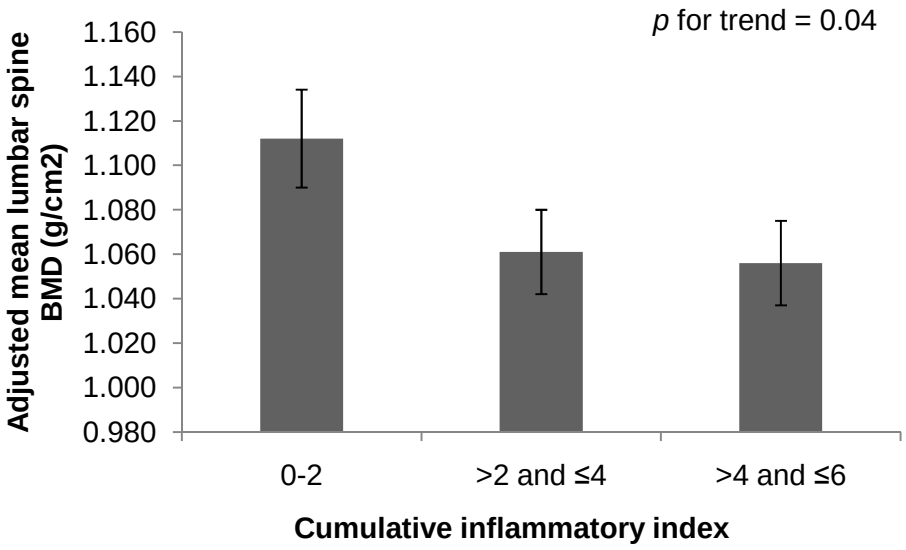

Figure 1: Adjusted mean lumbar spine BMD (g/cm^2) by the sum of tertile/category numbers for pro-inflammatory cytokines

CHAPTER SIX

Adherence index based on the AHA 2006 Diet and Lifestyle Recommendations is associated with select cardiovascular disease risk factors in older Puerto Ricans[1-5]

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Running head: AHA diet and lifestyle score and CVD risk factors

[7]Abbreviations used: AHA-DLS, AHA Diet and Lifestyle Score; AHA-DLR, AHA Diet and Lifestyle Recommendations; CHD, coronary heart disease; CRP, C-reactive protein; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; FRS, Framingham risk score; HHANES, Hispanic Health and Nutrition Examination Survey; PSS, Perceived stress score; UL, upper limit; WC, waist circumference

ABSTRACT

In 2006, the AHA released diet and lifestyle recommendations (AHA-DLR) for cardiovascular disease (CVD) risk reduction. The effect of adherence to these recommendations on CVD risk is unknown. Our objective was to develop a unique diet and lifestyle score based on the AHA-DLR, and to evaluate this score in relation to available CVD risk factors. In a cross-sectional study of Puerto Rican adults, aged 45-75 y, living in the greater Boston area, information was available for the following variables; diet (semi-quantitative FFQ), blood pressure, waist circumference (WC), ten-year risk of coronary heart disease (CHD) (Framingham risk score), and fasting plasma lipids, serum glucose, insulin, and C-reactive protein (CRP) concentrations. We developed a diet and lifestyle score (AHA-DLS) based on the AHA-DLR. The AHA-DLS had both internal consistency and content validity. It was significantly associated with plasma HDL cholesterol (P=0.001), serum insulin (P=0.0003) and CRP concentrations (P=0.02), WC (P<0.0001), and ten-year risk of CHD score (P=0.01 in women). The AHA-DLS was inversely associated with serum glucose among those with a BMI<25 (P=0.01). Women and men in the highest quartile of the AHA-DLS had significantly lower serum insulin (P-trend=0.0003) and CRP concentrations (P-trend=0.002), WC (P-trend=0.0003), and higher HDL cholesterol (P-trend=0.008). The AHA-DLS is a useful tool to measure adherence to the AHA-DLR and may be used to examine associations between diet and lifestyle behaviors and CVD risk.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death among Hispanics residing in the U.S., contributing to nearly one in every four deaths (1). Hispanics report more multiple co-morbidities and CVD risk factors than non-Hispanic whites (2). The "Hispanic Paradox", an observation that Hispanics have lower all-cause and cardiovascular mortality despite greater prevalence of risk factors and socioeconomic disadvantage (3) has been recently challenged (4). The paradox concept was primarily based on data from Mexican Americans (5, 6). Other Hispanic groups, such as Puerto Ricans, differ in ancestral genetic history and exposures to known risk factors.

Puerto Ricans have unique dietary intake patterns, as well as social, cultural and environmental exposures that may contribute to CVD risk. For example, data from the Hispanic Health and Nutrition Examination Survey (HHANES, 1986-89) showed that Puerto Ricans living on the U.S. mainland reported lower consumption of vegetables, cereals, and protein rich foods than other Hispanic groups (7). Data from the Massachusetts Hispanic Elders Study (MAHES) showed that Puerto Rican elders consumed diets high in refined carbohydrates and low in fiber, and that diets had greater variety with higher level of acculturation (8). Little is known about how the Puerto Rican diet is associated with CVD risk.

While the relationship between diet and disease has been traditionally studied using single foods or nutrients as the exposure, individuals consume meals consisting of a variety of foods, with complex combinations of nutrients that are likely to be interactive or synergistic (9, 10). Pattern analysis provides an additional dimension to analyses of diet and disease risk, and provides a more realistic approach to disease prevention or

treatment, because the focus is on the entire diet rather than a single food or nutrient (11). Dietary pattern analysis using score-based approaches is an "*a priori*" approach based on published dietary recommendations. Diet indexes have been constructed based on national recommendations to evaluate their effect on disease risk (12, 13). In 2006, the AHA released Diet and Lifestyle Recommendations (AHA-DLR) for CVD risk reduction (14). While these are aimed at decreasing CVD risk in the general population, we know of no studies that assessed the effect of adherence to AHA-DLR on CVD risk factors.

Our aim was to characterize dietary patterns of the Puerto Rican population by creating a unique diet and lifestyle score based on the principles of the AHA-DLR. We tested both the content and predictive validity of the American Heart Association-Diet and Lifestyle score (AHA-DLS) by assessing cross-sectional associations between the AHA-DLS, nutrient intakes, available CVD risk factors, and a risk assessment tool, the Framingham risk score (FRS).

METHODS

Study Participants. The Boston Puerto Rican Health Study is an ongoing population-based longitudinal cohort study of 1500 Puerto Rican adults, aged 45 – 75 y, living in the greater Boston area. The study design and methods of the Boston Puerto Rican Health Study have been described in detail elsewhere (15). Briefly, self-identified Puerto Ricans were recruited primarily through door-to-door enumeration from high Hispanic density blocks based on year 2000 Census data. Other forms of recruitment included participation in community events/fairs, referrals from participants, and calls to the study office from flyers distributed at community locations. At baseline, bilingual interviewers visited the

participant's home to complete a comprehensive set of questionnaires. In addition, fasting blood samples were collected by a certified phlebotomist on the day following the home interview, or soon thereafter. Only those participants who were unable to answer questions due to serious health conditions, those who planned to move away from the greater Boston area within 2 y, and those with a low mini-mental state examination score (<10) were excluded from the study.

For the current analyses, we excluded participants reporting implausible energy intakes (<2510 or >20,083 kJ) (*n*=67) at baseline. We also excluded participants with missing information on variables needed for computing the AHA-DLS (*n*=67). There were no significant differences in baseline socio-demographic characteristics between those with complete and incomplete information. However, participants with missing data had higher fat intake (92.2 vs 77.3 g/d, *P*=0.02) and greater percent of energy from total fat (35.2% vs 31.9%, *P*=0.0002) compared to those with complete data. The present study includes 1203 participants with complete baseline data available at the time of analysis. All study protocols were approved by the Institutional Review Board of Tufts University/Tufts Medical Center.

Dietary Assessment. Habitual food consumption and nutrient intakes were captured using a semi-quantitative FFQ designed and validated for the Puerto Rican population (16). The FFQ was based on the format of the National Cancer Institute (NCI)/Block FFQ. Foods that contributed to nutrient intake of Puerto Rican adults in the HHANES were ranked to identify foods to be added to the food list. These included plantains, avocado, mango,

cassava, empanadas, and custard. Compared to the NCI/Block FFQ, the revised FFQ captured intakes reported in the 24-hour recalls more accurately.

Reported food intakes were converted into gram amounts. To reflect the food groups in the AHA-DLR, we created four food groups (fruit, vegetables, fish, and alcohol) based on the USDA food grouping system. Self-reported mixed dishes were disaggregated and intake amounts were added to the appropriate food group. Nutrient intakes were calculated using the Nutrition Data System for Research software (version 2007, Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN).

AHA-DLS components and scoring. We constructed a new score based on AHA-DLR for Americans (14) (**Table 1**). Foods, nutrients, and lifestyle variables were used to calculate the AHA-DLS, adapting from approaches used previously in the development of the USDA Healthy Eating Index (17), but based directly on 8 of 9 AHA-DLR recommendations for CVD risk reduction. A total possible score of 110 is calculated from scores for each of the sub-components (Table 1).

We used two components to represent adherence to the recommendation for balancing calorie intake and physical activity to achieve or maintain a healthy body weight. Given potential mis-reporting of energy intake using FFQ (18) and because energy imbalance is reflected in body weight, we assigned scores to participants based on their BMI status. A second component for physical activity was dichotomous. Participants engaging in moderate or vigorous activity were allotted 10 points, while those engaging in sedentary or light activity were not assigned any points.

Both quantity and variety in fruit and vegetable intake were used to measure adherence to the recommendation to consume a diet rich in these components. For each participant, we calculated the total servings of fruit and vegetables (excluding starchy vegetables) consumed per day. Because the AHA-DLR do not consider fruit juice as equivalent to whole fruit, we did not count fruit juice. Because a quantitative guideline for adequacy in fruit and vegetable intake was not provided by the AHA-DLR, we used the CDC recommendation of at least 5 servings of fruit and vegetables per day (19). One serving of fruit equals ½ cup of dried fruit or 1 cup of fruit or 100% fruit juice. A serving of vegetable equals 1 cup of non-leafy vegetables, 1 cup of vegetable juice, or 2 cups of raw leafy vegetables. Variety in fruit and vegetable intake was defined as the number of unique types of fruit and vegetables consumed at least once per month. Based on the category assigned, participants received a score of 0, 5, or 10.

The AHA recommends that at least half of grain intake come from whole-grains. Detailed methodology for creation of a whole-grain database is described elsewhere (20). The percentage of whole-grain intake was determined by dividing grams of whole-grain by grams of total grain intake. Participants consuming at least half of total grain intake as whole-grains were assigned 10 points. Because many Americans do not meet this recommendation (21), scores were prorated linearly between 0 and 10 for intakes between 0 and 50%. Because cereal, but not fruit, fiber has been reported to be associated with reduced CVD risk (22, 23), we did not assign a score for total fiber intake. However, by measuring whole-grain intake, we were able to capture cereal fiber intake.

One component measured adherence to fish intake. Because the recommendation is based on both oily and non-oily fish, we measured servings of total fish intake per week, but excluded intake of deep fried fish (24, 25). One serving of fish is ~227 grams.

The AHA-DLR include consuming <7% of energy as saturated fat, <1% of energy as *trans* fat, and <300 mg cholesterol/d. In addition, the AHA-DLR also state that a range of 25% - 35% of energy from total fat is appropriate in a healthy dietary pattern. Therefore, we created four components – one each for saturated fat, *trans* fat, dietary cholesterol, and percentage of energy from total fat. Dietary cholesterol is known to raise blood cholesterol in only approximately one-third of people. However, intakes of saturated and *trans* fatty acids are known to result in dyslipidemia (26). Thus, intakes of both saturated and *trans* fat received greater weight (6 points) than did dietary cholesterol or percent of energy from total fat (4 points). Intakes below the cut-points provided by the AHA-DLR were given maximum credit. Intakes at the recommendation level received half the total points. Intakes between these ranges were prorated linearly. No points were awarded for intakes over the recommendations. Sensitivity analyses included repeating analyses by providing greater weight to saturated and *trans* fat (8 points each) and lower weight to dietary cholesterol and percent of energy from fat (2 points each).

Scores for added sugars were based on the most recent scientific statement issued by the AHA (27). This statement proposes a specific upper limit (UL) for added sugars. Accordingly, a prudent UL is half of the discretionary calorie allowance for each individual. However, if an individual consumes alcohol, this is reduced to accommodate the additional energy from alcohol intake. We first determined the suggested energy intake for each age/sex group using tables provided by Britten et al. (28) for development

of food intake patterns for the MyPyramid system. To prevent overestimation of discretionary calories, we assumed each participant to be sedentary. Based on the suggested energy intake, we then determined the discretionary calorie allowance for each energy level using the MyPyramid food intake patterns. The UL was set as ½ the discretionary calories for non-drinkers and 1/3 the discretionary calories for alcohol consumers. Those exceeding the UL were awarded no points. Participants with intakes at the UL received only partial credit. Those with added sugar intake below the UL received higher scores, prorated linearly for intakes between the UL and no added sugar.

The salt recommendation is represented by one component. Participants with intakes less than the desirable standard of 1.5 g/d were awarded 10 points. The AHA recognizes that reducing sodium intake to 1.5 g/d may not be easily achievable due to the high-sodium food supply. Thus, they propose an achievable recommendation of 2.3 g/d. Participants meeting this recommendation received a score of 5 with scores prorated linearly between 1.5 and 2.3 g/d.

The AHA-DLR for alcohol consumption provides cut-off points of ≤2 drinks/d for men and ≤1 drink/d for women. One drink equals 355 ml of regular beer, 148 ml of wine, or 44 ml of spirits. Scores were prorated linearly for intakes between 0 - 2 drinks/d for men and 0-1 drink/d for women. Those consuming more than this received no points. Based on documented protective effects of moderate alcohol consumption (29, 30), no points were awarded for non-drinkers, as well.

Lifestyle assessment. Standing height and weight were measured in duplicate. Weight was measured using a quality clinical scale (Toledo Weight Plate, Model I5S, Bay State

and Systems Inc., Burlington, MA), which was calibrated regularly with known weights. Height was measured using a Harpenden pocket stadiometer. BMI was calculated as weight (kg) divided by height (m^2). Physical activity was assessed using a modified Paffenbarger questionnaire from the Harvard Alumni Activity Survey (31, 32).

Biologic measures. A 12-h fasting blood sample was drawn by a certified phlebotomist on the day following the home interview, or as soon as possible thereafter, in the participant's home. For plasma, blood was collected into vacutainers containing EDTA, inverted gently prior to processing and centrifuged at 3421x *g* at 4^{o}C for 15 minutes, and kept cold. For serum, blood was collected into vacutainers containing no anticoagulant, allowed to clot at room temperature for approximately 15 minutes, centrifuged at 3421x *g* at 4^{o}C for 15 minutes and placed upright in a cooler but not directly on ice. Whole blood was collected into a separate vacutainer and kept on a rocker at room temperature until analyzed for hematology measures. All vacutainers were shielded from light during specimen collection, processing and handling. All samples were kept cold and brought back to the Nutrition Evaluation Laboratory at the Jean Mayer USDA Human Nutrition Research Center for further processing and storage. Aliquots were stored in cryogenic tubes at -80^{o}C prior to analysis.

Other covariates. Information on age, education, household income, and family size were collected using a questionnaire based on questions from the third NHANES, the HHANES, and the National Health Interview Survey Supplement on Aging. Information on health behaviors includes smoking and frequency, history, and type of alcohol

consumption. Diabetes status was defined as fasting serum glucose ≥6.99 mmol/L or use of diabetes medication (33). Hypertension was defined as blood pressure ≥140/90 mmHg or use of anti-hypertensive medication (34). Detailed information on prescription and over-the-counter medication use was collected. Acculturation was captured using the bi-dimensional Acculturation Scale for Hispanics. The scale yields two scores which rank acculturation in the Hispanic and the non-Hispanic domains (35). A score of 100 indicates full acculturation with fluent English language use. We also administered the Spanish version of the Perceived Stress Scale (36).

Outcome measures. We used the Framingham risk equations (37) to calculate the estimated FRS for each participant free of heart attack, heart disease, and stroke (self report) at baseline (*n*=254 men and 688 women). Risk factors considered include sex, age, diabetes, smoking, systolic and diastolic blood pressure, total cholesterol, and LDL and HDL cholesterol. CRP was measured in serum using a solid-phase, two-site chemiluminescent immunometric assay with a commercial kit (IMMULITE 1000, Diagnostics Products Corporation, Los Angeles, CA). The intra- and inter-assay CV% for this assay are 4.2-6.4% and 4.8-10.0% respectively. Plasma cholesterol, TG, and HDL cholesterol concentrations were analyzed with an enzymatic endpoint reaction in an Olympus AU400, using standard operating procedures. The intra- and inter-assay CV% for plasma cholesterol, TG, and HDL cholesterol concentrations are 1.8% and 2.2%, 2.8% and 2.7%, and 3.0 and 7.0%, respectively. LDL cholesterol was calculated using the Friedewald formula, unless TG concentrations exceeded 4.52 mmol/L (38). Serum glucose was measured using an enzymatic kinetic reaction on the Olympus AU400 with

Olympus Glucose reagents (OSCR6121). Serum insulin was measured using a solid-phase, two-site chemiluminescent immunometric assay using a commercial kit (IMMULITE 1000, Diagnostic Products Corporation, Los Angeles, CA). The intra- and inter-assay CV% for serum glucose and insulin are 2.0% and 3.4%, 5.2-6.4% and 5.9-8.0%, respectively. Blood pressure was measured using an electronic sphygmomanometer (Model HEM-71, OMRON Healthcare, Vernon Hills, IL) at three different time points during the interview. An average of the second and third readings was used to obtain systolic and diastolic blood pressure. WC was measured using a non-elastic tape on the smallest area of the waist and was recorded to the nearest one-tenth of a centimeter.

Statistical analyses. Statistical analyses were conducted using SAS 9.2 (Cary, NC). The AHA-DLS was used as a continuous measure and was also divided into quartile categories. We calculated the age and sex adjusted means for socio-demographic characteristics, health behaviors, and biological measures across quartiles of AHA-DLS with ANCOVA. We tested the content validity of the AHA-DLS by calculating age, sex, and energy adjusted intakes of nutrients known to be associated with a diet based on the AHA-DLR, across quartiles of the AHA-DLS. We assessed the significance across quartiles of AHA-DLS using linear (for continuous variables) or logistic regression (for categorical variables). All analyses were adjusted for multiple comparisons using Tukey's HSD. Internal consistency was determined using inter-item correlation matrixes. Spearman rank correlations were used to examine associations between individual component scores and the total AHA-DLS, as well as among the individual component

scores. Bonferroni adjustment was applied for multiple testing. For all other analyses, a *P*-value of 0.05 was considered statistically significant.

Because the AHA-DLR were formulated for CVD risk reduction, we tested the association of the AHA-DLS with plasma lipoprotein measures, the FRS, and CVD risk factors, including systolic and diastolic blood pressure, serum glucose, insulin, CRP, and WC. A logarithmic transformation was applied to plasma TG, serum glucose, insulin, and CRP to improve normality. Log transformed values were back transformed and results were expressed as geometric means. To test the association between the AHA-DLS and plasma lipoproteins, we adjusted for age, sex, smoking status, diabetes, hypertension, and WC. Models with LDL cholesterol as the outcome were adjusted for lipid lowering medication use and HDL cholesterol (model 2). Models with HDL cholesterol as the outcome variable were adjusted for LDL cholesterol, TG, and cardiovascular medication use. Models with TG as the outcome were adjusted for LDL cholesterol, cardiovascular medication use, and total carbohydrate intake (model 2). In our final model (model 3), we further adjusted for acculturation and PSS. Because risk equations for calculating the FRS differ for men and women, we constructed sex-specific models to test the associations between FRS and AHA-DLS with the following adjustments - 1) age, supplement use, and cardiovascular medication use, 2) model 1 + WC, and income, 3) model 2 + acculturation and PSS. We used ANCOVA to test associations between the AHA-DLS, systolic and diastolic blood pressure, serum glucose, and serum insulin. In our base model, we adjusted for age, sex, and smoking status. Because diabetes is known to affect blood pressure (39), models with systolic and diastolic blood pressure were also adjusted for diabetes status. Models 2 and 3 were further adjusted for supplement use,

medication use, WC, income, acculturation, and PSS. Models with WC as the outcome variable were sequentially adjusted for the following covariates: 1) age, sex, smoking status, diabetes, hypertension, and BMI, 2) model 1 + CRP, insulin medication use, and income, 3) model 2 + acculturation and PSS. We used a similar approach to test associations between AHA-DLS and log CRP. Model 1 was adjusted for age, sex, smoking status, diabetes, and hypertension. Model 2 further adjusted for WC, white blood cell count, and income. In our final model, we adjusted for acculturation and PSS. We tested for effect modification by sex, BMI, and diabetes status by including a cross-product term in the regression model. Tests for linear trend were conducted across quartile categories by including the median score for each quartile as a continuous measure in the regression model.

RESULTS

The American Heart Association Diet and Lifestyle Score. The AHA-DLS was normally distributed. The mean AHA-DLS for participants in our cohort was 32.1 (range 5.1-72.2) out of a total possible score of 110. Fewer than 3% of the population scored more than half of the maximum possible score (**Table 2**). Median intake of fruit and vegetables was below the CDC recommendation. Nearly 75% of participants did not meet recommendations for added sugars and sodium intake. Nearly all (~98%) had intake of whole grains below the recommendation. Spearman rank correlation coefficients between sub-components and the total AHA-DLS were all positive and significant (P<0.0001), and ranged from 0.13 for dietary cholesterol to 0.56 for variety in fruit and vegetable intake (Table 2). Adjustment for age, sex, and energy intake slightly

strengthened, but did not significantly change, these correlations (data not shown). Correlation coefficients between the sub-components ranged from -0.32 for fish and dietary cholesterol to 0.51 for saturated fat and *trans* fat intakes (**Supplemental Table 1**).

Participant characteristics and Content validity. There was more than a 2-fold difference in the median scores of the extreme quartiles of the AHA-DLS (**Table 3**). Those in the highest quartile, compared to the lowest, were more likely to be physically active, alcohol consumers, acculturated, supplement users, to report less perceived stress, and to have lower BMI, and greater education and household income.

There was no significant difference in energy intake across quartiles of the AHA-DLS **(Table 4)**. The AHA-DLS was positively associated with intakes of protein, total carbohydrate, fiber, n-3 fatty acids, alcohol, β-carotene, lycopene, folate, vitamin C, potassium, and magnesium across quartiles (*P*-trend <0.0001). Conversely, participants in the highest quartile of the AHA-DLS had lower intakes of added sugar, total fat, and percent of energy from total fat (*P*-trend <0.0001).

CVD Risk Factors. The AHA-DLS was positively associated with HDL cholesterol, but no significant associations were noted with LDL cholesterol or TG concentrations. Likewise, no significant interactions were noted with sex, BMI, or diabetes status (*P*>0.10). The AHA-DLS was inversely associated with the FRS in women (*P*=0.01) but not men (*P*=0.32). There was no evidence for effect modification by BMI or diabetes (*P*>0.10). However, there was a significant interaction between BMI and log glucose (*P*=0.002). The AHA-DLS was inversely associated with log glucose among those with a

BMI <25 (*P*=0.01) but not in those participants who were overweight or obese (*P*=0.58). Serum insulin, WC, and CRP were each inversely associated with the AHA-DLS after multivariate adjustment (*P*=0.0003, *P*<0.0001, and *P*=0.02 respectively). No significant associations were noted with blood pressure. HDL cholesterol increased across quartiles of the AHA-DLS (*P*-trend=0.008). There were decreasing trends in adjusted mean insulin (*P*-trend=0.0003) and CRP (*P*-trend=0.002) concentrations, and WC (*P*-trend=0.0003) across quartiles of the AHA-DLS. Among those with BMI <25, there was an inverse trend in geometric mean glucose concentration across AHA-DLS quartiles (*P*-trend=0.004) **(Figure 1).** There were no substantial differences in results when saturated and *trans* fat received greater weight (8 vs 6 points) compared to dietary cholesterol and percent of energy from fat (4 vs 2 points) (data not shown).

DISCUSSION

We developed the AHA-DLS to assess the relationship between adherence to the AHA-DLR and CVD risk. During the process of development, we attempted to limit subjectivity in the creation of food groups and interpretation of the recommendations. The decision to include lifestyle factors in the AHA-DLS was based on the premise that the AHA-DLR were intended to provide a foundation for a public health approach to CVD risk reduction through both diet and lifestyle modifications. Thus, a unique feature of the AHA-DLS is that it includes foods, nutrients, health, and lifestyle factors. To our knowledge, this is the first diet and lifestyle score developed from the AHA-DLR.

Due to the nature of the recommendations, scales for scoring are both categorical and continual. For foods and nutrients, scoring was on a continuous scale. This is

advantageous because it does not assume a linear relationship but allows for U-shaped correlations with health outcomes, when appropriate (13). We interpreted ideal body weight as within the recommended range for BMI. Because BMI is used to estimate healthy body weight based on a person's height (40), this variable represents the recommendation to balance energy intake and expenditure. The AHA-DLR restrict intakes of saturated fat, *trans* fat and cholesterol, which tend to be highly inter-correlated and thus contribute a large proportion to the total score (41). Further, increased consumption of foods such as red meat, which is high in both saturated fat and dietary cholesterol may be associated with reduced consumption of foods such as fish, further contributing to the cumulative effect of scoring components (12).

Two examples of dietary patterns which appear to be generally consistent with the AHA-DLR are the Dietary Approaches to Stop Hypertension (DASH) diet (42) and Therapeutic Lifestyle Changes provided by the National Heart Lung and Blood Institute (43). Previous studies have shown that a score based on the DASH diet was associated with CVD endpoints, such as non-fatal and fatal CHD (44), incident heart failure (45, 46), and CHD and CVD mortality (47). Our results are consistent with those of Nettleton et al (48) who observed that a Comprehensive Healthy Dietary Pattern score was associated with lower WC and lower fasting CRP and insulin, but not with fasting glucose, or blood pressure, in a multi-ethnic population, aged 45-84 y. Our finding of a positive association between the AHA-DLS and HDL cholesterol is important in the Puerto Rican population, where a high prevalence of low HDL cholesterol concentration has been identified, both previously (49) and in this study. Unlike the study populations of Dauchet et al (50) and Schulze et al (51) who noted that a diet consistent with the

DASH principles was associated with lower blood pressure, participants in our study were older, and nearly 70% had hypertension at study enrollment. This may have limited our ability to observe an association between the AHA-DLS and blood pressure.

Our findings need to be interpreted in the context of a few limitations. First, we characterized diet using a semi-quantitative FFQ, which by its nature has limited precision and some misclassification. However, while FFQ cannot measure absolute intakes, they have been shown to rank usual intakes well (52). A second limitation is that we categorized the BMI, physical activity, and variety components. This categorization may limit the range of possible scores. However, we did not find substantial evidence that, for example, a BMI of 18.5 should receive a different score than a BMI of 24.9. Similarly, there were no national guidelines or recommendations for variety in fruit and vegetable intake. We thus based our scoring criteria on the distribution of our data.

A third limitation is the subjectivity involved in the differential scoring of the fat components. While it is known that saturated and *trans* fats contribute to greater CVD risk than dietary cholesterol or percent of energy intake from fat (26), our decision to assign 6 vs 4 points was ultimately subjective. However, when we repeated our analyses providing greater weight to saturated and *trans* fat and lower weight to dietary cholesterol and percent of energy from fat did not change the results substantially. Additionally, our associations between the AHA-DLS and CVD risk factors are cross-sectional in nature. It is possible that those with these CVD risk factors may have modified their diet to a healthier pattern, thus attenuating relationships between the AHA-DLS and CVD risk factors.

Lastly, we used the FRS to estimate the 10-y risk of CHD among participants in our study. The FRS was developed primarily from the experience of the Framingham Heart Study, a predominantly Caucasian population (37). Portability of the FRS to the Puerto Rican population has been previously determined and the score was found to systematically overestimate CHD events in much older data with Puerto Ricans living in Puerto Rico (53). However, risk levels have changed considerably since the Puerto Rico Heart Health Program, conducted in 1965 (54). By using the FRS as a continuous measure, participants in our study will be ranked appropriately according to their risk estimates and the linear associations between the AHA-DLS and FRS are, thus, valid.

In the present study, we observed that only approximately 3% of the population had a score $\geq$50%, indicating poor adherence to the AHA-DLR. An important observation is that even relatively modest adherence to the AHA-DLR was associated with significantly lower FRS, serum insulin concentration, WC and CRP concentration, and higher HDL cholesterol concentration in this high risk minority group. In conclusion, the AHA-DLS appears to be a useful instrument for assessing adherence to the AHA-DLR in this group of Puerto Rican adults, and to assess relationships with diet and lifestyle behaviors and health outcomes. While this study is limited to Puerto Ricans living in the Boston area, there is no reason to expect that the AHA-DLS cannot be generalized to Puerto Ricans elsewhere and to other ethnic groups. Results from our study provide important information to public policy for an understudied population with documented health disparities. The Puerto Rican population appears to be at high risk for chronic disease, and diet and lifestyle interventions based on the AHA-DLR may provide significant benefit.

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Table 1: Components and scoring system of the AHA-DLS

Component	Scores for sub-components (possible values/range)	Maximum points (range)	Scoring system	Score
Balance calorie intake and physical activity to achieve or maintain a healthy body weight		20 (0-20)		
BMI, *kg/m*^{2}	10 (0, 5, or 10)		BMI < 18.5	5
			BMI ≥18.5 and <25	10
			BMI 25-29.9	5
			BMI >30	0
Physical Activity	10 (0 or 10)		Moderate/Vigorous	10
			Sedentary	0
Consume a diet rich in fruits and vegetables		20 (0-20)		
Fruit and Vegetable Intake[1], *servings/d*	10 (0-10)		≥ 5	10
			< 2 – 5	0 – 10
Fruit and Vegetable Variety, *percentile of distribution*	10 (0, 5, or 10)		<25th	0
			25th – 75th	5
			>75th	10
Choose whole-grain, high-fiber foods		10 (0-10)		
% of total grain that is whole grain[1]			≥50	10
			<50	0 – 10
Consume fish, especially oily fish, at least twice a wk		10 (0-10)		
Total fish intake (excluding fried) [1], *servings/wk*			≥ 2	10
			0 – ≤ 2	0 – 10
Limit your intake of saturated and *trans* fat and cholesterol		20 (0-20)		
Saturated fat[1], *% energy*	6 (0-6)		≤ 3.5	6

			>3.5 – ≤ 7	3 – 6
			≥ 7 - ≥15	0 – 3
			> 15	0
Trans fat[1], *% energy*	6 (0-6)		≤ 0.5	6
			>0.5 – ≤1	3 – 6
			>1 – ≤3	0 – 3
			> 3	0
Dietary cholesterol[1], *mg/d*	4 (0-4)		≤150	4
			>150 - ≤300	0 – 4
			>300	0
Total fat, *%energy*	4 (0, 2, 4)		25-35	4
			<25	2
			>35	0
Minimize your intake of beverages and foods with added sugars[2]		10 (0-10)		
Added sugars[1], *g/d*			> UL of discretionary calories	0
			UL	5
			0 - ≤ UL	5 – 10
Choose and prepare foods with little or no salt		10 (0-10)		
Sodium[1], *g/d*			≤ 1.5	10
			>1.5 - ≤ 2.3	5 – 10
			> 2.3	0
If you consume alcohol, do so in moderation		10 (0-10)		
Alcohol[1], *servings/d*			>0 to ≤2 drinks/d for men and >0 to ≤ 1 drink/d for women	0 – 10
			Non-drinkers	0
			>2 drinks/d for men and >1 drink/d for women	0

[1] Scores were prorated linearly for intakes between ranges; [2] For participants who consume alcohol, the UL for added sugars is 1/3rd the discretionary calories. For participants who don't consume alcohol, the UL is ½ the discretionary calories

Table 2: Component Values, Score Distributions, and Correlations of the AHA-DLS in older Puerto Ricans

Component	%[1]	Intake distribution[2]	Score distribution[2,3]	% with minimum score	% with maximum score	Spearman rank 'r'
Balance calorie intake and physical activity to achieve or maintain a healthy body weight						
BMI[3], *kg/m²*				57.7	12.6	0.29
BMI < 18.5	0.3	NA	0			
BMI ≥18.5 and <25	12.6	NA	10			
BMI 25-29.9	29.7	NA	5			
BMI >30	57.4	NA	0			
Physical Activity[3]				95.6	4.4	0.21
Moderate/Vigorous	4.4	NA	10			
Sedentary	95.6	NA	0			
Consume a diet rich in fruits and vegetables						
Fruit and Vegetable Intake, *servings/d*	NA	2.70 (0.83 – 6.46)	2.32 (0 – 10)	32.8	12.2	0.46
Fruit and Vegetable Variety[3,4], *percentile of distribution*				25.0	25.0	0.56
<25th	25.0	17.0 (8 – 21)[4]	0			
25th – 75th	50.0	27.0 (22 – 31)[4]	5			
>75th	25.0	36.0 (32 – 43)[4]	10			

Choose whole-grain, high-fiber foods, *% of total grain that is whole grain*	NA	10.3 (0.6 – 36.4)	2.05 (0.12 – 7.3)	0	1.7	0.39
Consume fish, especially oily fish, at least twice a wk, *servings/d*	NA	0.83 (0.04 – 3.72)	4.15 (0.21 – 10)	0	18.1	0.41
Limit your intake of saturated and trans fat and cholesterol						
Saturated fat, *% energy*	NA	9.49 (5.9 – 13.3)	2.1 (0.6 – 3.9)	0	0	0.34
Trans fat, *% energy*	NA	1.2 (0.6 – 1.8)	2.8 (1.7 – 5.2)	0	1.5	0.28
Dietary cholesterol, *mg/d*	NA	271 (96 – 634)	0.8 (0 – 4)	43.1	17.5	0.13
Total fat[3], *% energy*		32.2 (22.6 – 40.5)		29.6	59.6	0.24
25-35	29.6		4			
<25	10.8		2			
>35	59.6		0			
Minimize your intake of beverages and foods with added sugars, *g/d of added sugars*	NA	47.8 (9.9 – 147)	0 (0 – 7.3)	84.2	0	0.17
Choose and prepare foods with little or no salt, *mg/d of sodium*	NA	4414 (1950 – 9390)	0 (0 – 7.2)	89.5	1.3	0.25
If you consume alcohol, do so in moderation[5], *servings/d*	NA	0.2 (0.02 – 3.1)	5.5 (0 – 9.3)	67.5	<0.01	0.31
TOTAL SCORE			31.4 (14.9 – 50.5)			

NA = Not applicable
[1]Percentage of participants falling under each categorical component
[2]Median (5^{th} – 95^{th} percentile) (all such values)
[3]Points for each categorical component
[4]Variety defined as the total number of unique fruits and vegetables consumed at least once per month in the last 12 months. Median variety (5^{th} – 95^{th} percentile); [5]Values only for current drinkers

Table 3: Participant characteristics across quartiles of the AHA-DLS in the Boston Puerto Rican Health Study

Characteristic[2]	Quartiles of AHA-DLS[1]				*P*-trend
	Q1 19.0 (4.8 – 23.1)	Q2 26.7 (23.1 – 30.4)	Q3 33.8 (30.4 – 38.5)	Q4 43.0 (38.6 – 72.2)	
n	300	301	301	301	
Age, *y*	56.4 ± 0.5	57.5 ± 0.5	57.5 ± 0.5	57.7 ± 0.5	0.18
Female, *%*	75.1	70.4	73.4	70.0	0.28
BMI, kg/m^2	33.2 ± 0.4	31.6 ± 0.4*	30.9 ± 0.4†	29.2 ±0.4†	<0.0001
Physical activity, *%*					
Sedentary/Light	98.7	96.0	95.8	86.3†	<0.0001
Moderate/Vigorous	1.3	4.0	4.2	13.7†	<0.0001
Smoking, *%*					
Never	39.9	46.0	34.7	43.3	0.63
Past	30.4	29.9	33.9	31.1	0.99
Current	28.8	22.6	30.3	24.7	0.63
Alcohol, *%*					
Nondrinker	76.1	65.5**	54.1†	36.1†	<0.0001
Moderate	15.7	25.2**	35.9†	52.9†	<0.0001
Heavy	5.5	7.3	8.2	9.3	0.03
Diabetes (y/n), *%*	43.6	40.0	41.0	38.0	0.20
Hypertension (y/n), *%*	69.7	70.9	71.6	67.8	0.62
Total household income, *$/y*	14518 ± 2054	16723 ± 2017	21389 ± 2032	23535 ± 1994**	0.0003
Education, *%*					
<8th grade	51.5	50.3	46.8	39.0**	0.0007
9th-12th grade/GED	39.9	36.3	38.2	38.5	0.84
College/some graduate school	8.4	13.4	14.6**	22.5†	<0.0001
Supplement use (y/n), *%*	53.5	53.5	59.5	63.1*	0.006
Acculturation, *%*	22.0 ± 1.2	24.4 ± 1.2	25.2 ± 1.2	30.1 ± 1.2†	<0.0001
PSS	25.2 ± 0.6	23.0 ± 0.5	22.3 ± 0.6***	21.3 ± 0.5†	<0.0001

[1]Median (range)

[2] Values are mean ± SEM or %, adjusted for age/sex and calculated using ANCOVA

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{\dagger}P<0.0001$ compared to quartile 1, adjusting for age/sex using ANCOVA for linear variables and logistic regression for categorical variables. Adjustments were made for multiple comparisons using Tukey's Honestly Significant Difference.

Table 4: Selected daily intake of nutrients known to be available in food groups that constitute the AHA-DLS

Nutrient intake[2]	Quartiles of AHA-DLS[1]				*P*-trend
	Q1 19.0 (4.8 – 23.1)	Q2 26.7 (23.1 – 30.4)	Q3 33.8 (30.4 – 38.5)	Q4 43.0 (38.6 – 72.2)	
n	300	301	301	301	
Energy[3], *kJ/d*	9425 ± 217	9166 ± 213	9852 ± 215	9174 ± 213	0.85
Protein, *g/d*	87.8 ± 1.1	89.7 ± 1.1	91.6 ± 1.1*	93.8 ± 1.0***	<0.0001
Total carbohydrate, *g/d*	265 ± 3	267 ± 3	271 ± 3	275 ± 3	0.003
Added sugar, *g/d*	64.1 ± 2.4	58.0 ± 2.3	54.1 ± 2.3**	52.9 ± 2.3**	0.0002
Total fiber, *g/d*	16.9 ± 0.3	18.1 ± 0.3*	19.8 ± 0.3†	21.2 ± 0.3†	<0.0001
Total fat, *g/d*	81.9 ± 0.8	79.1 ± 0.8*	76.4 ± 0.8†	72.8 ± 0.8†	<0.0001
Total fat, *% energy*	33.9 ± 0.3	32.7 ± 0.3*	31.6 ± 0.3†	29.8 ± 0.3†	<0.0001
Total n-3 fatty acids, *g/d*	1.58 ± 0.03	1.66 ± 0.03	1.74 ± 0.03***	1.75 ± 0.03***	<0.0001
Trans fatty acids, *g/d*	3.19 ± 0.06	2.97 ± 0.06**	2.75 ± 0.06†	2.58 ± 0.06†	<0.0001
Alcohol, *g/d*	2.9 ± 0.8	4.7 ± 0.8	5.3 ± 0.8	7.0 ± 0.8**	0.0003
β-carotene, *µg/d*	2519 ± 176	2768 ± 173	3079 ± 176	3750 ± 173†	<0.0001
Lycopene, *µg/d*	6383 ± 236	6440 ± 232	7532 ± 235**	7734 ± 232***	<0.0001
Folic acid, *µg/d*	465 ± 10	487 ± 10	535 ± 10†	557 ± 10†	<0.0001
Vitamin C, *mg/d*	113 ± 5	124 ± 5	143 ± 5†	165 ± 5†	<0.0001
Potassium, *mg/d*	2899 ± 36	3111 ± 36***	3240 ± 36†	3465 ± 35†	<0.0001
Magnesium, *mg/d*	301 ± 6	332 ± 5***	350 ± 6†	380 ± 5†	<0.0001

[1]Median (range)

[2] Values are mean ± SEM, adjusted for age, sex, and energy intake using ANCOVA

[3] Values are mean ± SEM, adjusted for age and sex using ANCOVA.

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{\dagger}P<0.0001$ compared to quartile 1 using ANCOVA. Adjustments were made for multiple comparisons using Tukey's Honestly Significant Difference.

Table 5: Cross-sectional associations between the AHA-DLS and CVD Risk Factors in older Puerto Ricans[1]

CVD Risk Factor	*n*	β ± SE	*P*-value
Plasma lipids	1069		
LDL cholesterol[2], *mmol/L*			
Model 1		0.014 ± 0.024	0.56
Model 2		-0.012 ± 0.026	0.63
Model 3		-0.011 ± 0.026	0.67
HDL cholesterol[3], *mmol/L*			
Model 1		0.034 ± 0.009	<0.0001
Model 2		0.031 ± 0.009	0.0004
Model 3		0.029 ± 0.009	0.001
Log TG[4], *mmol/L*			
Model 1		0.003 ± 0.006	0.59
Model 2		0.005 ± 0.006	0.41
Model 3		0.003 ± 0.006	0.57
FRS[5]			
Men	219		
Model 1		0.092 ± 0.450	0.84
Model 2		0.423 ± 0.491	0.39
Model 3		0.495 ± 0.499	0.32
Women	628		
Model 1		-0.804 ± 0.250	0.001
Model 2		-0.679 ± 0.272	0.01
Model 3		-0.717 ± 0.279	0.01
Other CVD risk factors			
Systolic blood pressure[6], *mmHg*	1078		
Model 1		-0.402 ± 0.519	0.43
Model 2		-0.035 ± 0.272	0.95
Model 3		-0.184 ± 0.559	0.74
Diastolic blood pressure[6], *mmHg*	1077		
Model 1		-0.585 ± 0.291	0.05
Model 2		-0.315 ± 0.308	0.31
Model 3		-0.278 ± 0.315	0.37
Log serum glucose[7], *mmol/L*			
BMI <25, *kg/m^2*	138		
Model 1		-0.014 ± 0.010	0.16
Model 2		-0.025 ± 0.011	0.02
Model 3		-0.029 ± 0.011	0.01

BMI ≥25, *kg/m*^{2}	958		
Model 1		-0.008 ± 0.004	0.07
Model 2		-0.002 ± 0.004	0.60
Model 3		-0.002 ± 0.004	0.58
Log serum insulin[7], *pmol/L*	1093		
Model 1		-0.059 ± 0.008	<0.0001
Model 2		-0.030 ± 0.008	0.0003
Model 3		-0.030 ± 0.008	0.0003
WC[8], *cm*	1083		
Model 1		-0.10 ± 0.03	<0.0001
Model 2		-0.10 ± 0.03	<0.0001
Model 3		-0.10 ± 0.03	<0.0001
Inflammatory marker	1083		
C-reactive protein[9], *mg/L*			
Model 1		-0.008 ± 0.001	<0.0001
Model 2		-0.003 ± 0.001	0.02
Model 3		-0.003 ± 0.001	0.02

[1]Beta-coefficients ± SE for every 10 unit increase in the AHA-DLS were calculated using ANCOVA

[2]Model 1: Adjusted for age (y), sex, energy intake (kcal/d), smoking status (former, current, never), diabetes (y/n), hypertension (y/n). Model 2: Model 1 + lipid medication use (y/n), HDL cholesterol (mmol/L), income ($/y), WC (cm). Model 3: Model 2 + acculturation (%), PSS

[3]Model 1: Adjusted for age (y), sex, energy intake (kcal/d), smoking status (former, current, never), diabetes (y/n), hypertension (y/n). Model 2: Model 1 + LDL cholesterol (mmol/L), TG (mmol/L), cardiovascular medication use (y/n), income ($/y), WC (cm). Model 3: Model 2 + acculturation (%), PSS

[4]Model 1: Adjusted for age (y), sex, energy intake (kcal/d), smoking status (former, current, never), diabetes (y/n), hypertension (y/n). Model 2: Model 1 + LDL cholesterol (mmol/L), HDL cholesterol (mmol/L), total carbohydrate intake (g/d), cardiovascular

medication use (y/n), income ($/y), WC (cm). Model 3: Model 2 + acculturation (%), PSS

[5]Model 1: adjusted for age (y), energy intake (kcal/d), supplement use (y/n), cardiovascular medication use (y/n). Model 2: Model 1 + WC (cm), income ($/y). Model 3 adjusted for model 2 + acculturation (%), PSS.

[6]Model 1: Adjusted for age (y), sex, energy intake (kcal/d), smoking status (former, current, never), diabetes (y/n). Model 2: Model 1 + supplement use (y/n), hypertension medication use (y/n), WC (cm), income ($/y). Model 3: Model 2 + acculturation (%), PSS

[7]Model 1: Adjusted for age (y), sex, energy intake (kcal/d), smoking status (former, current, never). Model 2: Model 1 + supplement use (y/n), diabetes medication use (y/n), WC (cm), income ($/y). Model 3: Model 2 + acculturation (%), PSS

[8]Model 1: Adjusted for age (y), sex, energy intake (kcal/d), smoking status (former, current, never), diabetes (y/n), hypertension (y/n), BMI (kg/m^2). Model 2: Model 1 + CRP (mg/L), income ($/y), insulin medication use (y/n). Model 3: Model 2 + acculturation (%), PSS

[9]Model 1: Adjusted for age (y), sex, energy intake (kcal/d), smoking status (former, current, never), diabetes (y/n), hypertension (y/n). Model 2: Model 1 + white blood cell count (mm^3), income ($/y), WC (cm). Model 3: Model 2 + acculturation (%), PSS

FIGURE LEGEND

Figure 1. Adjusted means for select CVD risk markers across quartiles of the AHA-DLS in older Puerto Ricans.

Values are adjusted mean±SE, *n* per quartile: Q1=300, Q2=301, Q3=301, Q4=301, **P<0.01, *P<0.05 compared to quartile 1 using ANCOVA. Adjustments for multiple comparisons were made using Tukey's Honestly Significant Difference.

A – Adjusted for age (y), sex, energy intake (kcal/d), smoking status (current, former, never), diabetes (y/n), hypertension (y/n), lipid medication use (y/n), HDL cholesterol (mmol/L), income ($/y), WC (cm), acculturation (%), PSS

B – Adjusted for age (y), sex, energy intake (kcal/d), smoking status (current, former, never), diabetes (y/n), hypertension (y/n), LDL cholesterol (mmol/L), TG (mmol/L), cardiovascular medication use (y/n), income ($/y), WC (cm), acculturation (%), PSS

C – Adjusted for age (y), sex, energy intake (kcal/d), smoking status (current, former, never), diabetes (y/n), hypertension (y/n), LDL cholesterol (mmol/L), HDL cholesterol (mmol/L), total carbohydrate intake (g/d), cardiovascular medication use (y/n), income ($/y), WC (cm), acculturation (%), PSS

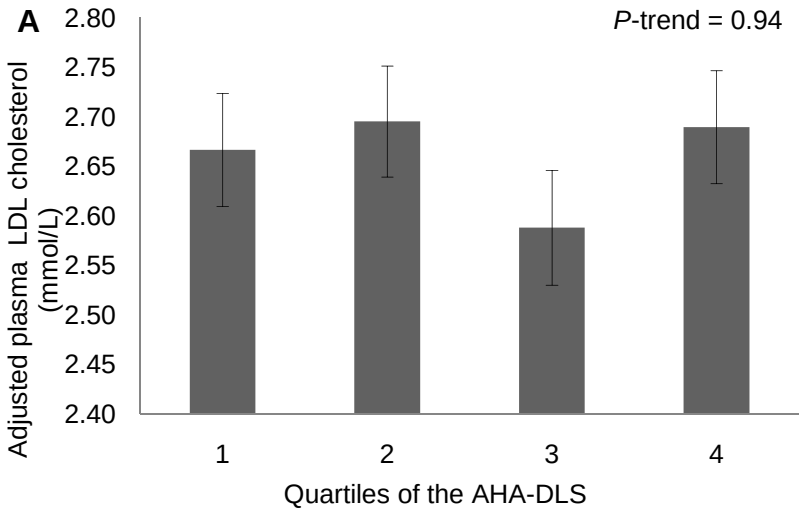
D – Adjusted for age (y), energy intake (kcal/d), supplement use (y/n), cardiovascular medication use (y/n), income ($/y), WC (cm), acculturation (%), PSS

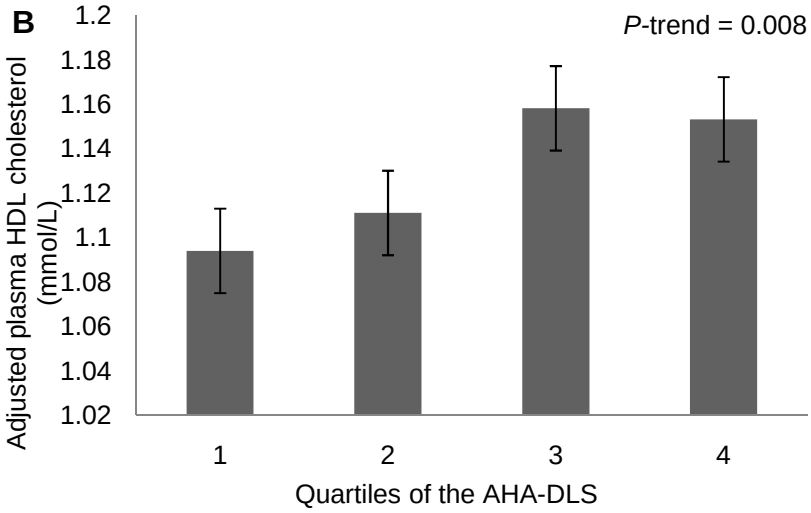
E, F – Adjusted for age (y), sex, energy intake (kcal/d), smoking status (current, former, never), diabetes (y/n), supplement use (y/n), hypertension medication use (y/n), income ($/y), WC (cm), acculturation (%), PSS

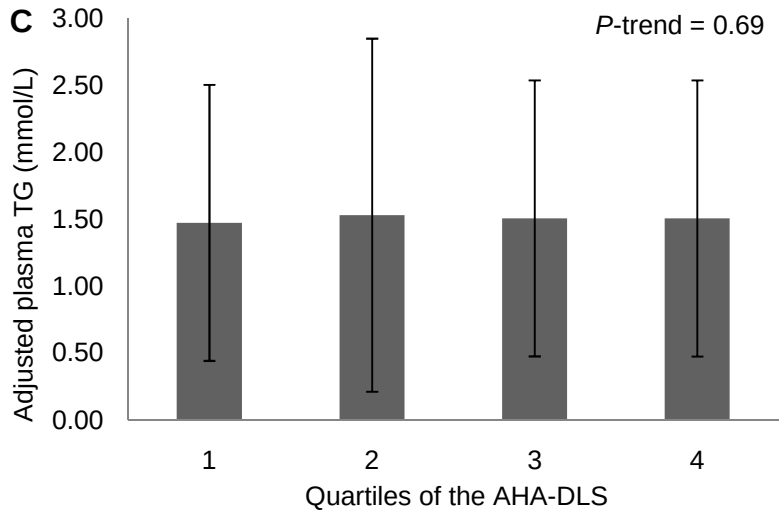
G, H – Adjusted for age (y), sex, energy intake (kcal/d), smoking status (current, former, never), supplement use (y/n), diabetes medication use (y/n), income ($/y), WC (cm), acculturation (%), PSS

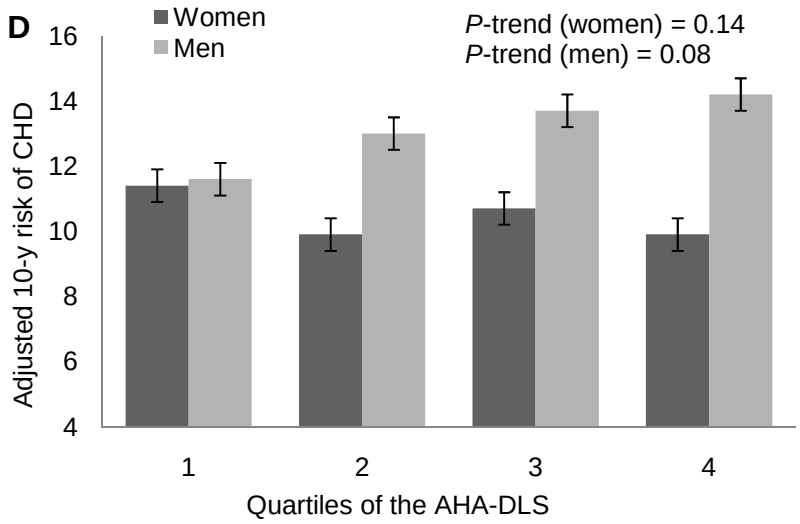
I – Adjusted for age (y), sex, energy intake (kcal/d), smoking status (current, former, never), diabetes (y/n), hypertension (y/n), BMI (kg/m^2), CRP (mg/L), insulin medication use (y/n), income ($/y), acculturation (%), PSS

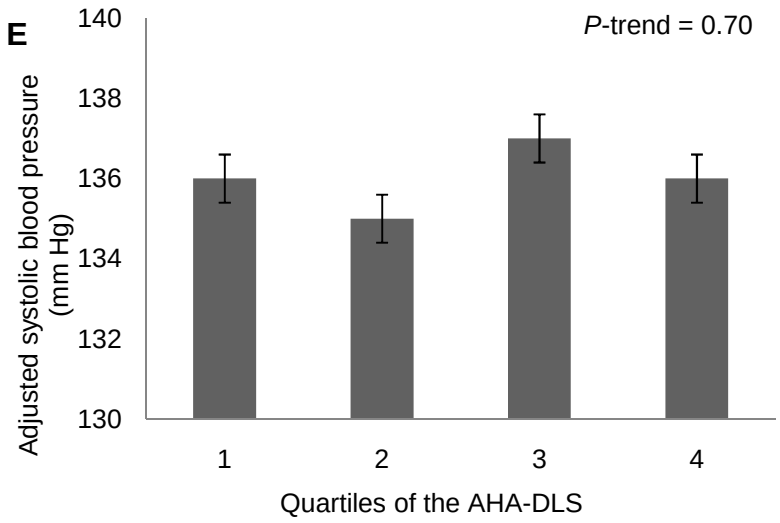
J – Adjusted for age (y), sex, energy intake (kcal/d), smoking status (current, former, never), diabetes (y/n), hypertension (y/n), white blood cell count (mm^3), income ($/y), WC (cm), acculturation (%), PSS

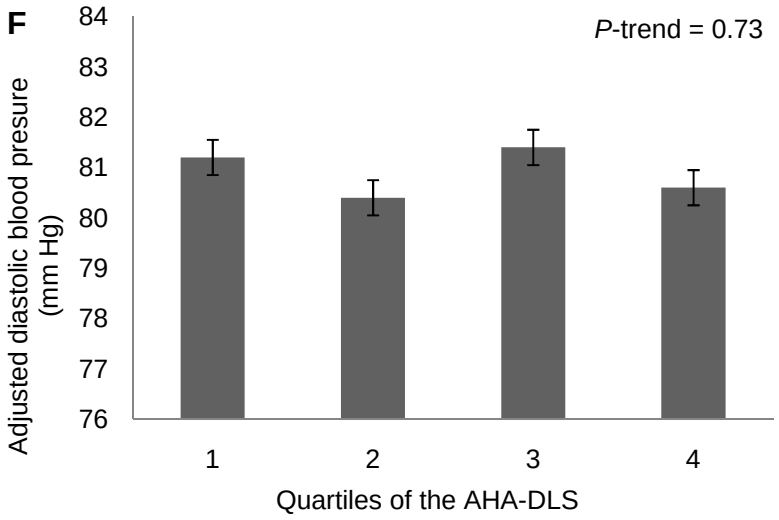

A
P-trend = 0.94
Adjusted plasma LDL cholesterol (mmol/L)
2.80
2.75
2.70
2.65
2.60
2.55
2.50
2.45
2.40
1
2
3
4
Quartiles of the AHA-DLS

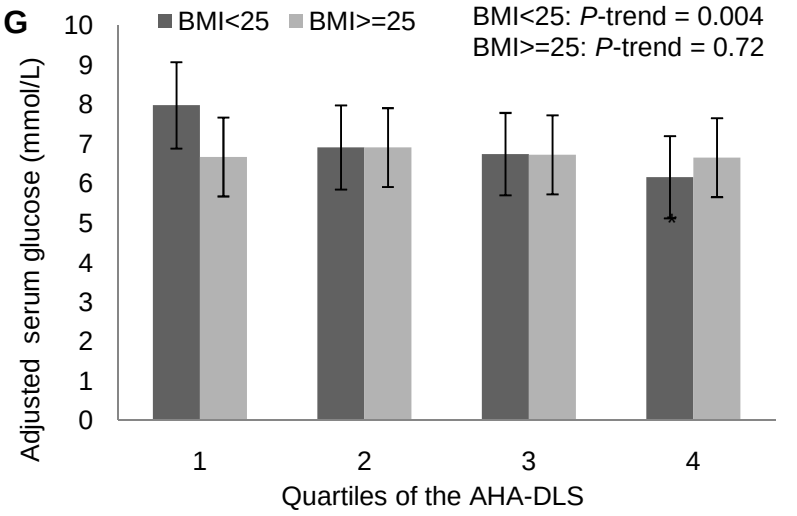

B
P-trend = 0.008
Adjusted plasma HDL cholesterol (mmol/L)
1.2
1.18
1.16
1.14
1.12
1.1
1.08
1.06
1.04
1.02
1
2
3
4
Quartiles of the AHA-DLS

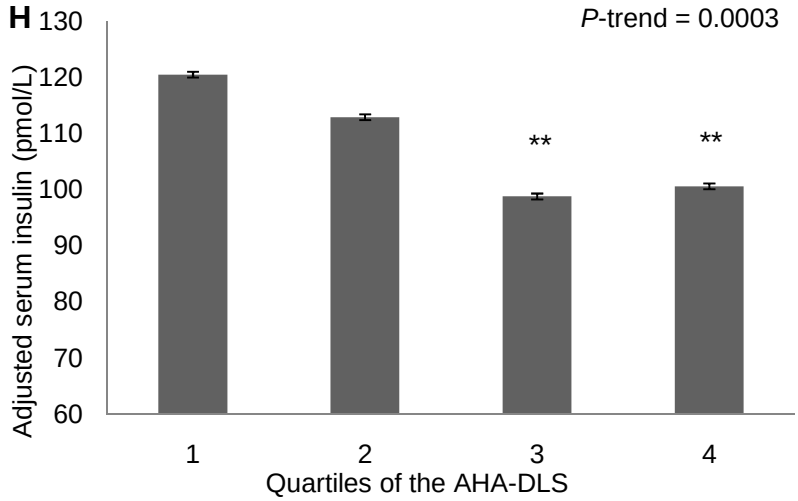

C
P-trend = 0.69
Adjusted plasma TG (mmol/L)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
1
2
3
4
Quartiles of the AHA-DLS

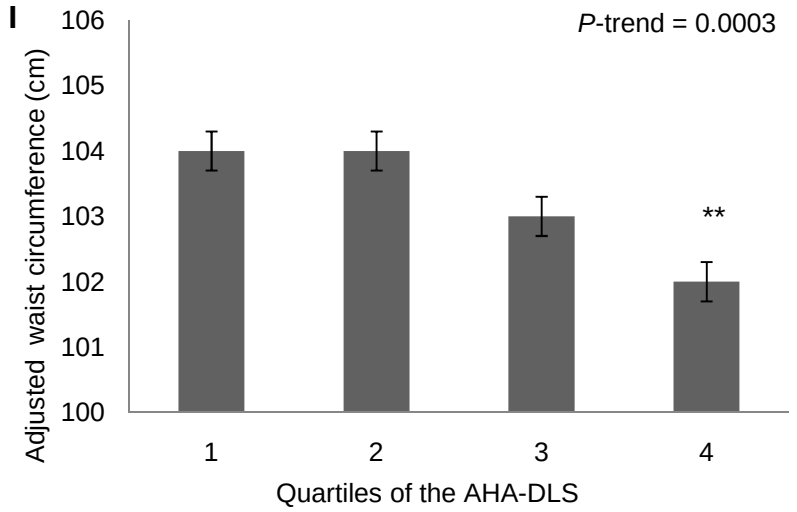

D
Women
Men
P-trend (women) = 0.14
P-trend (men) = 0.08
Adjusted 10-y risk of CHD
16
14
12
10
8
6
4
1
2
3
4
Quartiles of the AHA-DLS

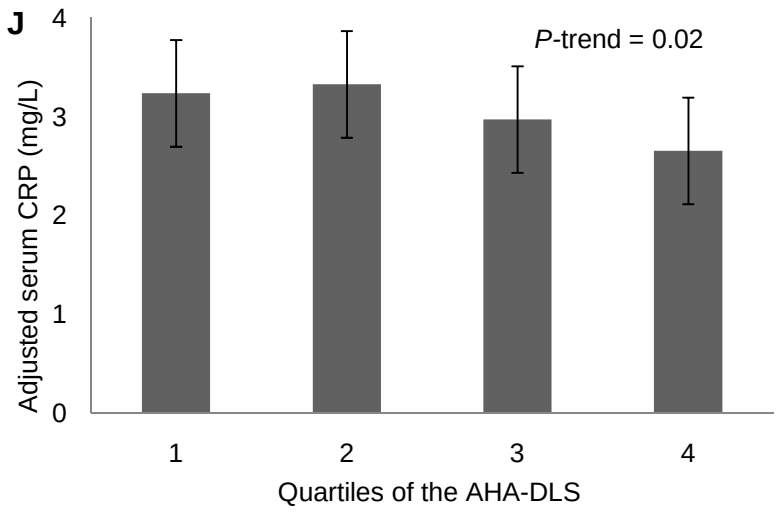

E
P-trend = 0.70
Adjusted systolic blood pressure (mm Hg)
140
138
136
134
132
130
1
2
3
4
Quartiles of the AHA-DLS


F
P-trend = 0.73
Adjusted diastolic blood presure (mm Hg)
84
83
82
81
80
79
78
77
76
1
2
3
4
Quartiles of the AHA-DLS


G
BMI<25
BMI>=25
BMI<25: P-trend = 0.004
BMI>=25: P-trend = 0.72
Adjusted serum glucose (mmol/L)
10
9
8
7
6
5
4
3
2
1
0
*
1
2
3
4
Quartiles of the AHA-DLS


H
P-trend = 0.0003
Adjusted serum insulin (pmol/L)
130
120
110
100
90
80
70
60
**
**
1
2
3
4
Quartiles of the AHA-DLS


I
P-trend = 0.0003
Adjusted waist circumference (cm)
106
105
104
103
102
101
100
**
1
2
3
4
Quartiles of the AHA-DLS


J
4
3
2
1
0
Adjusted serum CRP (mg/L)
P-trend = 0.02
1
2
3
4
Quartiles of the AHA-DLS

ONLINE SUPPLEMENTAL MATERIAL

Supplemental Table 1: Spearman rank correlation coefficients (rho) among the sub-components of the AHA-DLS in the Boston Puerto Rican Health Study

	AHA-DLS components												
AHA-DLS components	BMI	Physical activity	Fruit & vegetable intake	Variety in fruit & vegetable intake	Whole grains	Fish	Saturated fat	*Trans* fat	Dietary cholesterol	% of energy from total fat	Added sugars	Sodium	Alcohol
BMI		0.10*	-0.08	-0.04	-0.03	-0.08	0.05	0.03	0.03	0.04	-0.04	0.05	0.003
Physical Activity			0.02	0.05	-0.01	0.04	0.003	0.003	-0.07	-0.01	0.003	-0.05	-0.01
Fruit & vegetable intake				0.30*	0.14*	0.26*	0.05	0.15*	-0.24*	-0.03	-0.14*	-0.18*	0.11*
Variety in fruit & vegetable intake					0.24*	0.28*	-0.05	0.01	-0.10*	0.02	-0.05	-0.02	0.11*
Whole grains						0.10*	0.09*	0.09*	0.13*	0.06	0.04	0.13*	-0.02

Fish	-0.04	-0.01	-0.32*	-0.05	-0.05	-0.17*	0.11*
Saturated fat		0.51*	0.45*	0.39*	0.04	0.21*	-0.06
Trans fat			0.24*	0.19*	-0.02	0.09*	-0.06
Dietary cholesterol				0.20*	0.20*	0.41*	-0.16*
% of energy from total fat					-0.04	0.04	-0.008
Added sugars						0.19*	-0.11*
Sodium							-0.08
Alcohol							

*$P \leq 0.004$ after Bonferroni adjustment for multiple comparisons

CHAPTER SEVEN

Adherence to the 2006 American Heart Association Diet and Lifestyle Recommendations for cardiovascular disease risk reduction is associated with better bone health in older Puerto Ricans[1-5]

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Running head: AHA diet and lifestyle score and bone mineral density

Keywords: dietary patterns, AHA diet and lifestyle score, diet score, bone mineral density, osteoporosis, epidemiology, Puerto Ricans

ABSTRACT

Background: Cardiovascular disease and osteoporosis are two major public health problems that share common pathophysiological mechanisms. It is possible that strategies that reduce CVD risk may also benefit bone health.

Objective: We tested the hypothesis that adherence to the American Heart Association 2006 Diet and Lifestyle Recommendations (AHA-DLR) is associated with bone health.

Design: A unique diet and lifestyle score (American Heart Association Diet and Lifestyle Score, AHA-DLS) was developed to assess adherence to the guidelines in a cross-sectional study of 467 Puerto Ricans, aged 47-79 years. Bone mineral density (BMD) at the femoral neck, trochanter, total hip, and lumbar spine (L2-L4) was measured using dual energy X-ray absorptiometry.

Results: Adherence to the AHA-DLR was poor. The mean (SD) AHA-DLS was 32.8 (11.0) out of a total possible 110. For every 5 unit increase in the AHA-DLS, BMD at the femoral neck, trochanter, and total hip was associated with a 0.007-0.009 g/cm^2 (P<0.05) higher value. We observed positive linear associations between higher trochanter and total hip BMD across increasing tertiles of AHA-DLS (P for trend≤0.05). No component of the AHA-DLR, alone, was responsible for the observed positive associations. For every 5 unit increase in the AHA-DLS, the odds for osteoporosis or osteopenia at the total hip was 14% (OR=0.86, 95% CI: 0.75-0.97) lower. No significant associations with AHA-DLS were observed at the other BMD sites.Conclusions: Dietary guidelines for CVD risk reduction are associated with better bone health at the total hip in this Hispanic cohort. Synchronizing dietary guidelines for these two common diseases may provide a simplified public health message.

INTRODUCTION

Cardiovascular disease (CVD) and osteoporosis are chronic conditions which result in major public health burden, but have remained understudied in minority populations. CVD is the leading cause of death among Hispanics. Among Mexican American adults aged 20 years and older, 28.5% of men and 34.5% of women have CVD (1). Among individuals aged ≥65 years, the prevalence of osteoporosis and osteoporosis related fractures is two times higher for Hispanic Americans than African Americans (2). Further, the prevalence of risk factors for CVD and osteoporosis, such as smoking, physical inactivity, and metabolic syndrome, remain high in the Hispanic population (1). While CVD and osteoporosis have long been thought to be independent chronic diseases coexisting in the aging population, recent evidence from basic science and epidemiological research has demonstrated that these two chronic conditions are linked by several biological mechanisms such as inflammation. This implies that strategies for CVD risk reduction may have a potential impact not only in reducing CVD, but also osteoporosis.

The most basic components for chronic disease prevention and risk reduction are diet and lifestyle. Recognizing this, the American Heart Association (AHA) periodically releases guidelines intended to reduce CVD risk in Americans >2 years of age. The most recent diet and lifestyle recommendations released in 2006 deserve special emphasis, as these guidelines are based on an overall healthy dietary pattern, making positive lifestyle choices, and provide multiple benefits for reduction of CVD risk factors (3). It has been suggested that these guidelines may also protect from other chronic diseases, such as type 2 diabetes and osteoporosis (4). However, no studies have evaluated the effect of adherence to CVD risk reduction recommendations on bone health. This is particularly

important in the Puerto Rican population, a group with documented health disparities (5). Puerto Ricans, the second largest Hispanic group in the US and the largest in northeastern US, have traditionally been underrepresented in research. Yet, they present with multiple co-morbidities and have a high prevalence of CVD risk factors (6).

Understanding the potential benefit of dietary and lifestyle recommendations, originally intended for CVD risk reduction, on bone health may help synchronize guidelines for two major chronic conditions and provide foundational data for public health practice. Thus, the objective of the present study was to examine if adherence to the AHA 2006 Diet and Lifestyle recommendations is associated with better bone health in a population of older Puerto Ricans living in the greater Boston area.

PARTICIPANTS AND METHODS

Participants

Participants from this study were part of the Boston Puerto Rican Osteoporosis study, an ancillary study to the Boston Puerto Rican Health Study. The design of the Boston Puerto Rican Health Study has been described in detail elsewhere (7). Recruitment began in 2004 by enrolling self-identified Puerto Ricans, aged 45-75 years, living in the greater Boston area. At baseline and two-years, home-interviews were conducted by bilingual interviewers who administered questionnaires to obtain information on socioeconomic status, health and health behaviors, acculturation, stress, and usual diet. In addition, anthropometric and blood pressure measures were recorded. Biological samples, including saliva, urine, and 12-h fasting blood, were collected by the phlebotomist in the participants' homes on the day following the interview or as soon as

possible thereafter. At the end of the 2-year home visit, participants were re-consented to be enrolled into the osteoporosis study. Between December 2006 and September 2010, 756 of the 1123 participants who completed the 2-year follow-up interview, had visited the Metabolic Research Unit at the USDA Human Nutrition Research Center for Aging (HNRCA) at Tufts University for measurement of bone mineral density (BMD), 12-h fasted blood draw, and administration of a questionnaire to assess sun exposure, estrogen use, and history of falls and fracture. Every attempt was made to schedule the osteoporosis study visit within one month of completion of the two-year follow-up. Primary reasons for non-participation in the current study included not being interested (n=163), scheduling problems (n=139), loss-to-follow up (n=33), and moved out of Massachusetts (n=15). Another 17 participants had died since their two-year follow-up interview. Participants who declined participation were more likely to be older (61.5 vs 59.2 years, P=0.0001) and to report higher levels of perceived stress (21.0 vs 22.4, P=0.05). No other significant differences were found for other socio-demographic or dietary variables. At the time of analysis, complete bone density information was available on 713 participants (192 men and 521 women). All study protocols were approved by the Institutional Review Board of Tufts Medical Center.

Methods

Measurement of bone mineral density and osteoporosis

BMD of the hip and lumbar spine (L2-L4) was measured by a dual-X ray absorptiometry (DXA) using a GE-Lunar model Prodigy scanner in the Bone Metabolism Laboratory at the HNRCA. The root mean square precision of these measurements in our

laboratory is 1.31% for femoral neck BMD, 1.03% for BMD of the trochanter 0.65% for total hip BMD, and 1.04% for lumbar spine BMD (8). In all our analyses, we made an *a-priori* decision to include BMD measurements at the femoral neck, total hip, and posterior-anterior lumbar spine (L2-L4), based on recommendations from the International Society for Clinical Densitometry (9) and trochanter to provide a complete picture of the total hip. We used the WHO definition of osteoporosis and osteopenia as T-score thresholds of equal to or more than 2.5 or 1.0 SD, respectively, below the healthy young adult mean at the respective bone site. All scans with T-scores >4.0 were reviewed by an academic endocrinologist (BDH) to check for extra-skeletal calcification or for presence of non-anatomical parts in the DXA scan region. For the current analyses, we excluded one poor quality femur scan.

Dietary assessment and American Heart Association Diet and Lifestyle score

At the baseline and two-year follow-up interview, usual dietary intake was assessed using a semi-quantitative FFQ that was designed for and validated with the Puerto Rican population (10). Reported food intakes were converted into gram amounts. Serving sizes were calculated by dividing the gram amount of the food with the reference serving amounts from the USDA Food Guide Pyramid. Nutrient intakes were calculated using the Nutrition Data System for Research software (version 2007), developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN.

We constructed a unique diet and lifestyle score based on the 2006 AHA Diet and Lifestyle Recommendations (AHA-DLR) for CVD risk reduction (3). A higher score indicates greater adherence to the AHA-DLR. The development and validation of the

AHA-DLS has been described in detail elsewhere (11). Briefly, a total of nine recommendations have been put forth by the AHA for CVD risk reduction. These include: balance calorie intake and physical activity to achieve or maintain a healthy body weight; consume a diet rich in fruits and vegetables; choose whole-grain, high-fiber foods; consume fish, especially oily-fish, at least twice a week; limit your intake of saturated and *trans* fat and cholesterol; minimize your intake of beverages and foods with added sugars; choose and prepare foods with little or no salt; if you consume alcohol, do so in moderation; and when you eat food that is prepared outside of the home, follow the AHA-DLR. The AHA-DLS was based on the first eight recommendations. Participants received scores for each of the eight components with scores ranging from a minimum of zero to maximums of four, six, or ten for the various sub-components. The total possible score on the AHA-DLS was 110. Foods, nutrients, and lifestyle variables (BMI and physical activity) were included in the development of the AHA-DLS. Greater adherence to the AHA-DLR was associated with higher HDL concentration, and lower serum glucose (only in those with BMI <25), insulin and CRP concentrations, waist circumference, and ten-year coronary heart disease risk (assessed by the Framingham risk score, among women) (11).

We used two sub-components, BMI and physical activity, to represent adherence to the first recommendation to balance calorie intake and physical activity to achieve or maintain a healthy body weight. Because greater body weight is associated with higher BMD (12), we modified the AHA-DLS by removing the BMI sub-component. Because the total possible score for the BMI sub-component was 10 points, total points possible on the modified AHA-DLS were reduced to 100. To control for potential confounding

due to BMI, this variable was adjusted for in all statistical analyses. The two-year modified AHA-DLS was not computed for those with missing data on any of the sub-components that make up the score (n=35). Those with missing data on sub-components were more likely to report higher perceived stress (mean perceived stress score: 22.0 vs 27.6, *P*=0.0003) and were less likely to be physically active (mean physical activity score: 31.3 vs 29.4, *P*=0.01). No other differences were noted in other socio-demographic variables. For the current analyses, we used the 2-year modified AHA-DLS as our main exposure variable. Because diet is known to be relatively stable (13), we used the baseline modified AHA-DLS as our main exposure variable when information on the two-year modified AHA-DLS was missing (n=35). Complete information on bone health and the two-year modified AHA-DLS was available on 467 participants (male=115, female=352).

Assessment of covariates

We calculated age by subtracting the date of the participant's Osteoporosis study visit from the participant's date of birth. Standing height was measured with a stadiometer (Seca, Germany). Weight was measured with a digital scale (Seca, Model Alpha, Germany). BMI was calculated by dividing weight (kg) by height (m^2). At the Osteoporosis study visit, we administered a short questionnaire to collect information on prescription medication use for treatment of osteoporosis (y/n), defined as current use of bisphosphonates, calcitonin, calcium, vitamin D, and cod liver oil. Because season is known to affect BMD in the New England area (14-15), we created a four level categorical variable for season of BMD measurement as follows: July to September was

coded as summer; October to December as fall; January to March as winter; and April to June as spring. Plasma 25-hydroxy vitamin D (ng/mL) was measured using a ^{125}I radioimmunoassay kit procedure (DiaSorin Inc, Stillwater, MN). The intra- and inter-assay CV% for this analyte are 10.8% and 9.4%, respectively.

Information on educational status of the participant was captured at the baseline home visit and was categorized as $<5^{th}$ grade, 5^{th}-12^{th} grade/GED, college or some graduate school. At the two-year follow-up interview, we collected information on total household income ($/year) and current smoking (y/n) status. Vitamin supplement use was identified by asking participants to show us the containers for any prescription or over-the-counter supplements used. This was treated as a binary variable (y/n) in the current analysis. Acculturation was assessed as reported preference of language use in various everyday activities (16) and the Spanish version of the Perceived stress scale was administered (17). Usual intakes of calcium (mg/d) and total energy (kcal/d) were assessed by FFQ (10).

Statistical analyses

All statistical analyses were completed using SAS statistical software (version 9.2; SAS Institute, Cary, NC). Formal hypothesis testing was two-sided with a nominal type I error rate of 0.05. We examined the distributions of the primary analytic variables and other descriptive data. The modified AHA-DLS was treated as both a continuous variable and was also divided into energy-adjusted tertiles using the residual method (18). We assessed the stability of the diet over two years by comparing baseline measures of the modified AHA-DLS with the two-year follow-up visit values. We calculated the age- and

sex-adjusted least square means using general linear models for the socio-demographic characteristics, lifestyle behaviors, and biologic measures across energy-adjusted tertiles of the modified AHA-DLS. Dietary characteristics were additionally adjusted for energy intake. We assessed statistical significance across categories of the modified AHA-DLS using linear (for continuous variables) and logistic (for categorical variables) regression.

Associations between the modified AHA-DLS, both as a continuous (5 unit increase) and categorical measure (energy-adjusted tertiles), and BMD (continuous) of the femoral neck, trochanter, total femur, and lumbar spine were examined using the general linear models procedure. For each association, three multivariable models were constructed. First, we adjusted for socio-demographic characteristics and osteoporosis risk factors, including age (years), sex (male/female), BMI (kg/m^2), height (m), current smoking status (y/n), educational status ($<5^{th}$ grade, 5^{th}-12^{th} grade/GED, college/some graduate school), season of BMD measurement (summer, fall, winter, spring), plasma vitamin D concentration (ng/mL), and intakes of total energy (kcal/d) and calcium (mg/d). In our second model, we controlled for confounding by indication by including current use of prescription osteoporosis medications (y/n) and vitamin supplements (y/n). Because the diet of Hispanic elders is known to be associated with acculturation (19), we included this variable in model 3. Finally, to consider the effect of potential confounding due to stress, we also adjusted for perceived stress score in model 3. Adjusted mean BMD at each bone site was calculated across energy-adjusted tertiles of the modified AHA-DLS. All analyses were adjusted for multiple comparisons using Dunnett's adjustment with the lowest tertile as the reference group.

To determine the importance of each of the AHA recommendations on BMD, we included scores for each sub-component as an independent variable one at a time in the regression model. The association with BMD was calculated by 5 unit increase in each sub-component. Each regression model was adjusted for covariates and the total modified AHA-DLS minus the sub-component being investigated. The recommendation for limiting intake of saturated fat, *trans* fat, and cholesterol is composed of 4 sub-components – saturated fat, *trans* fat, dietary cholesterol, and percent energy from total fat. Because the range of scores possible for the last two sub-components is 0-4, it is not possible to calculate a 5-unit increase in these sub-components. Thus, we combined the scores for all these sub-components under the fat recommendation to arrive at a composite score (range 0-20) for this recommendation. We applied a Bonferroni adjustment by multiplying the alpha with the total number of comparisons at each bone site (P=0.006). For all linear regression models, we tested the assumptions of linearity and homogeneity by examining the residuals of the outcome against the modified AHA-DLS.

We used logistic regression to model the odds of osteoporosis or osteopenia for a 5% increase in the modified AHA-DLS sequentially adjusting for the covariates used in the linear regression models. In all our analyses, we tested for potential effect modification due to sex by including a cross-product term with the modified AHA-DLS score. Tests for linear trend were conducted by assigning each participant the median value of the modified AHA-DLS for each tertile category and treating this as a continuous variable in regression analyses.

RESULTS

The AHA-DLS was normally distributed in our sample. The means (SD) of the modified AHA-DLS at baseline and two-year follow-up visit were 32.9 (11.2) and 32.8 (11.0), respectively. Only 4.7% (n=22) of study participants had a baseline AHA-DLS that was 2.0 SD away from their two-year AHA-DLS. Adherence to the AHA-DLS was low. Fewer than 3% (n=12) of the participants received a score of more than half of the total possible score. No significant interaction was observed with sex (P>0.05) in any of our analyses. Thus, data from men and women were analyzed together and sex was included as a covariate in regression models.

The median modified AHA-DLS score in the highest tertile (40.9) was about twice that of the median score in the lowest tertile (20.0). As seen in Table 1, those in the highest tertile, compared to the lowest, were more likely to be older, to have higher calcium intake, higher total household income, and a higher educational status, and were less likely to be current smokers. Highest (vs. lowest) tertile participants were also more likely to have higher acculturation values and lower perceived stress (**Table 1**). After multivariate adjustment, for every 5-unit increase in the modified AHA-DLS, BMD at the femoral neck, trochanter, and total hip was higher by 0.007 to 0.009 g/cm^2 (P<0.05 for each). While the point estimate was similar for the lumbar spine (β=0.007, 95% CI: -0.001, 0.015), the association at this bone site did not reach statistical significance (P=0.14) (**Table 2**).

At the trochanter and the total hip, a significant linear trend was observed for BMD across increasing tertiles of the modified AHA-DLS (P for trend ≤ 0.05). At the femoral neck and lumbar spine, BMD was higher across tertiles, but this difference did

not reach significance (*P* for trend=0.18) (**Figure 1**). The contribution of each sub-component of the modified AHA-DLS to BMD was assessed by adjusting for the remaining portion of the AHA-DLS and for covariates included in model 3 (**Table 3**). After applying a Bonferroni adjustment for multiple comparisons, none of the individual sub-components were significantly associated with BMD at all of the four bone sites (*P*>0.004). After multivariate adjustment, the modified AHA-DLS was associated with decreased risk of osteoporosis or osteopenia at the total hip (**Figure 2**). For each 5-unit increase in the modified AHA-DLS, the multiple adjusted odds ratios (OR, 95% CI) for osteoporosis/osteopenia were 0.98 (0.88, 1.09), 0.91 (0.81, 1.01), 0.86 (0.75-0.97), and 1.07 (0.96-1.19) for the femoral neck, trochanter, total hip, and lumbar spine, respectively.

DISCUSSION

In this cross-sectional study of older Puerto Rican adults, adherence to the American Heart Association 2006 Diet and Lifestyle Recommendations was associated with higher BMD at the femoral neck, trochanter, and total hip. The similarity of effect sizes at all three hip sites suggests that diet and lifestyle do not have differential effects at the various types of bone within these femoral sites. However, at the lumbar spine, while effect sizes were similar in magnitude to those at the hip, the association did not reach statistical significance. An interesting observation is that no single recommendation alone was responsible for the positive associations between the modified AHA-DLS and BMD at the hip sites, reinforcing the importance of patterns of healthy behavior, rather than single dietary or lifestyle choices to reducing chronic disease risk.

Accumulating evidence supports a biological association between CVD and osteoporosis. In addition to age, other risk factors for CVD including inflammation (20-21), oxidative stress (22), and hypercholesterolemia (23) have all been associated with osteoporosis. The presence of these unifying mechanisms makes it plausible that strategies that reduce CVD risk also prevent bone loss. Components in the AHA-DLR may target these common mechanisms or directly influence bone health. We recently demonstrated that greater variety in fruit and vegetable intake is associated with lower CRP concentration (24). Further, diets high in nutrients associated with fruit and vegetable intake, such as vitamin C (25) and carotenoids (26) were associated with better bone health (27). Dietary antioxidants prevent inflammation and oxidative stress induced by reactive oxygen species (28). Vitamin C is known to have an important role in the vascular bed by inhibiting endothelial dysfunction and decreasing oxidative stress related to the activation of macrophages (29). In vitro experiments demonstrated that β-cryptoxanthin, a carotenoid, has a unique anabolic effect on bone calcification by increasing calcium content and alkaline phosphatase activity in the femoral-diaphyseal and femoral-metaphyseal tissues of young rats (30). Phytonutrients, especially polyphenols, present in whole grains and a variety of fruits and vegetables affect osteoblasts at different stages including proliferation, differentiation and mineralization potentially by modulating the expression of transcription factors and by affecting cell signaling (31). Greater intakes of whole grain (32) and fish (33) have also been associated with lower inflammation. However, most recently, in the Cardiovascular Health Study, fish consumption was associated with very small differences in BMD and no associations were noted with hip fracture risk (34).

An important element in the AHA-DLR is limiting saturated fat to <7% of energy, *trans* fat <1% of energy, and cholesterol to <300 mg/d. While there are few data on the effect of *trans* fat on bone, *trans* fatty acid intake adversely affects circulating lipid concentrations, triggers systemic inflammation, and induces endothelial dysfunction (35) all of which are mechanisms common to the development of disease in vascular cells and bone tissue. Likewise, in vitro studies have shown that saturated fats not only generate reactive oxygen species (36) but also increase osteoclast survival (37) both of which decrease bone mass. Most recently, a population-based analysis of National Health and Nutrition Examination Survey (NHANES) III data showed that dietary saturated fat intake was inversely associated with femoral neck BMD (38).

The AHA-DLR to limit beverages and foods with added sugars and sodium intake to <1500 mg/d may benefit bone directly. High sodium consumption is detrimental to bone as it increases urinary calcium excretion (39). Correspondingly, added sugars are known to affect mineral balance and may decrease femur strength (40). The recommendation to consume alcohol in moderation has been established as a protective factor for vascular and bone tissue. The increase in HDL concentration induced by moderate alcohol consumption (41) may be another shared mechanism between CVD and bone health. Further, moderate ingestion of alcohol is associated with an ethanol specific suppression of serum carboxyterminal telopeptide of type I collagen, a marker of bone resorption, in a non-calcitonin- and a non-PTH-dependent fashion (42). Finally, physical activity, an important lifestyle factor that is part of the modified AHA-DLS, may provide both primary and secondary effects against CVD and osteoporosis. While a meta-analysis has shown that physical activity has an effect on the lumbar spine, but not the femur (43),

the positive effects of physical activity on bone health are thought to be mediated by suppression of bone turnover (44).

Although there are no data directly assessing the effect of adhering to the AHA-DLR on bone health, our findings are in agreement with several studies that have tested associations between dietary patterns based on the principles of the AHA-DLR, or individual components of the AHA-DLR, and BMD. An example of an eating pattern consistent with the AHA-DLR is the Dietary Approaches to Stop Hypertension (DASH) diet. Consumption of the DASH diet has been reported to reduce bone turnover (45). Our data support earlier observations that a dietary pattern based on fruit, vegetables, and cereal or whole grain is associated with higher BMD (46-47). Likewise, consuming a "Healthy" pattern defined by high intakes of green and dark yellow vegetables, mushrooms, fish, and shellfish, fruit, and processed fish by premenopausal Japanese farm women was reported to be positively correlated with forearm BMD. Consistent with the AHA recommendations for dietary fat type and cholesterol, these authors noted that a "Western" pattern tended to be inversely associated with BMD (48). In contrast to that previously reported (49), we did not find a significant association between BMD at the lumbar spine with adherence to a dietary pattern characterized by high consumption of fish and olive oil and low red meat.

The current study is unique in that it is the first to investigate the association between a validated diet and lifestyle score, originally intended for CVD risk reduction, on bone health. However, our study has several limitations. First, the cross-sectional nature of our associations limits our ability to draw inferences about causality. Longitudinal data are needed to assess the effect of diet and lifestyle on bone loss. It is

possible that participants diagnosed with osteoporosis may make healthier lifestyle and dietary choices. However, if this were true, our associations would be biased toward the null. Second, while we adjusted for important potential confounders, residual confounding always remains a possibility. However, our data allowed for adjustment of a comprehensive set of covariates, based on current knowledge of potential confounders and underlying biological mechanisms. The relatively smaller sample size and greater variability in spine BMD values may have limited our ability to observe potential associations at the lumbar spine. Finally, our study was limited to the Puerto Rican population, and although it is likely these findings can be applied to the general population, they should be replicated in other groups.

In conclusion, dietary and lifestyle recommendations for CVD risk reduction appear to be associated with better bone health. Adherence to these AHA recommendations had a similar effect at all sites, although associations at the lumbar spine were non-significant. This may be due to the presence of structural artifacts in spine scans such as osteophytes that can artificially increase and confound BMD measurements (50). Our findings have important public health implications. Synchronizing guidelines, both dietary and lifestyle, provides a holistic approach to the prevention of two major diseases that contribute significantly to morbidity and mortality of the aging population.

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Table 1: Characteristics of Puerto Ricans by energy-adjusted tertiles of the modified American Heart Association Diet and Lifestyle score

Characteristic	Tertiles of AHA-DLS[1]			
	1 20.0 (6.8 – 25.0)	2 29.8 (25.0 – 33.7)	3 40.9 (33.8 – 60.4)	***P*-trend**
n	155	156	156	
Age (y)[2]	59.8 ± 0.6	60.1 ± 0.6	62.2 ± 0.6**	0.003
BMI (kg/m^2)[2]	31.5 ± 0.6	31.8 ±0.6	30.9 ± 0.6	0.48
Height (m)[2]	1.61 ± 0.01	1.61 ± 0.01	1.62 ± 0.01	0.31
Current smoker (%)[3]	26.7	28.0	18.0*	0.05
Total energy intake (kcal/d)[2]	1932 ± 64	1898 ± 64	1937 ± 62	0.95
Calcium intake (mg/d)[4]	809 ± 40	915 ± 40	1007 ± 38***	0.0002
Plasma vitamin D (ng/mL)[2]	18.1 ± 0.6	18.1 ± 0.6	18.0 ± 0.6	0.89
Diabetes (y/n) (%)[3]	43.8	47.0	46.5	0.66
Total household income ($/y)[2]	13520 ± 1512	14605 ± 1533	21304 ± 1469***	0.0001
Education (%)[3]				
<5th grade	28.7	23.6	15.2*	0.004
5th-12th grade/GED	67.3	62.5	59.2	0.15
College/some graduate school	4.0	13.9**	25.5†	<0.0001
Vitamin supplement use (y/n) (%)[3]	34.6	31.4	41.0	0.23
Osteoporosis medication use (y/n) (%)[3]	32.9	33.1	39.4	0.23
Season of BMD measurement (%)[3]				
Summer	28.6	30.2	31.5	0.60
Winter	21.8	24.4	18.2	0.42
Fall	22.6	25.2	22.4	0.94
Spring	26.9	20.2	27.9	0.80
Acculturation[2]	20.5 ± 1.7	21.2 ± 1.7	29.2 ± 1.6***	<0.0001
Perceived Stress Score[2]	23.8 ± 0.8	22.5 ± 0.8	20.6 ± 0.7**	0.002

[1]Median (range)
[2]Values are mean ± SEM, adjusted for age/sex
[3]%, adjusted for age and sex
[4]Values are mean ± SEM, adjusted for age, sex, and energy intake
*P<0.05, **P<0.01, ***P<0.001, †P<0.0001 compared to tertile 1 (Dunnett's adjustment), adjusting for age/sex using analysis of covariance (PROC GLM)

Table 2: Cross-sectional associations between energy-adjusted modified American Heart Association Diet and Lifestyle score and bone mineral density (g/cm^2)

	Femoral Neck	*P*	Trochanter	*P*	Total Femur	*P*	Lumbar Spine (L2-L4)	*P*
Model 1	0.007 (0.001, 0.012)	0.03	0.009 (0.003, 0.015)	0.01	0.009 (0.003, 0.015)	0.02	0.006 (-0.002, 0.014)	0.20
Model 2	0.007 (0.001, 0.013)	0.02	0.009 (0.003, 0.015)	0.009	0.009 (0.003, 0.016)	0.01	0.006 (-0.002, 0.014)	0.19
Model 3	0.007 (0.001, 0.013)	0.03	0.008 (0.002, 0.014)	0.02	0.008 (0.002, 0.015)	0.02	0.007 (-0.001, 0.015)	0.14

β (SE) for every 5 unit increase in AHA-DLS score

Model 1: Adjusted for age (y), sex, BMI (kg/m^2), height (m), current smoking (y/n), educational status, season of BMD measurement (summer, spring, fall, winter), plasma vitamin D status (ng/mL), intakes of total energy (kcal/d) and calcium (mg/d)

Model 2: Model 1 + osteoporosis medication use (y/n), multivitamin use (y/n)

Model 3: Model 2 + acculturation, perceived stress score

Table 3: Associations between sub-components of energy-adjusted modified American Heart Association Diet and Lifestyle score and bone mineral density (g/cm^2)

Sub-component	β (99.4% CI)[1]			
	Femoral Neck	Trochanter	Total hip	Lumbar Spine (L2-L4)
Physical activity	-0.002 (-0.044, 0.040)	-0.007 (-0.048, 0.034)	-0.006 (-0.051, 0.039)	-0.012 (-0.068, 0.044)
Fruit and vegetable intake	-0.0002 (-0.029, 0.029)	-0.004 (-0.032, 0.024)	-0.001 (-0.032, 0.031)	-0.003 (-0.041, 0.036)
Fruit and vegetable variety	0.013 (-0.013, 0.038)	0.017 (-0.008, 0.042)	0.014 (-0.013, 0.041)	-0.007 (-0.041, 0.027)
Whole grain intake	0.002 (-0.034, 0.038)	0.014 (-0.021, 0.049)	0.018 (-0.035, 0.073)	0.025 (-0.023, 0.073)
Fish intake	0.016 (-0.012, 0.043)	0.013 (-0.014, 0.040)	0.013 (-0.017, 0.043)	0.025 (-0.012, 0.062)
Fat component	-0.008 (-0.033, 0.017)	-0.006 (-0.031, 0.019)	-0.008 (-0.035, 0.019)	-0.009 (-0.043, 0.025)
Sodium intake	0.013 (-0.019, 0.046)	0.014 (-0.018, 0.046)	0.018 (-0.018, 0.053)	0.009 (-0.035, 0.052)
Added sugar intake	0.020 (-0.016, 0.056)	0.021 (-0.014, 0.056)	0.027 (-0.012, 0.066)	0.032 (-0.016, 0.080)
Alcohol intake	0.009 (-0.021, 0.039)	0.017 (-0.012, 0.047)	0.016 (-0.017, 0.048)	0.019 (-0.021, 0.059)

[1]Association for a 5-unit increase in score of each sub-component

Adjusted for age (y), sex, BMI (kg/m^2), height (m), current smoking (y/n), educational status (<5th grade, 5th – 12th grade, some college/grad school), season of BMD measurement (summer, spring, fall, winter), plasma vitamin D status (ng/mL), intakes of total energy (kcal/d) and calcium (mg/d), osteoporosis medication use (y/n), multivitamin use (y/n), acculturation (%), perceived stress score, and total modified AHA-DLS minus the component being studied

FIGURE LEGEND

Figure 1: Adjusted mean bone mineral density (g/cm^2) across energy-adjusted tertiles of modified American Heart Association Diet and Lifestyle score.

Adjusted for age (years), sex, BMI (kg/m^2), height (m), current smoking (y/n), educational status ($<5^{th}$ grade, 5^{th} – 12^{th} grade, some college/grad school), season of BMD measurement (summer, spring, fall, winter), plasma vitamin D status (ng/mL), intakes of total energy (kcal/d) and calcium (mg/d), osteoporosis medication use (y/n), vitamin supplement use (y/n), acculturation (%), and perceived stress score.

$^{*}P<0.05$ compared to tertile 1 using Dunnett's adjustment

Figure 2: Odds ratio (95% confidence interval) of osteoporosis or osteopenia for every 5 unit increase in the modified American Heart Association Diet and Lifestyle score.

Adjusted for age (years), sex, BMI (kg/m^2), height (m), current smoking (y/n), educational status ($<5^{th}$ grade, 5^{th} – 12^{th} grade, some college/grad school), season of BMD measurement (summer, spring, fall, winter), plasma vitamin D status (ng/mL), intakes of total energy (kcal/d) and calcium (mg/d), osteoporosis medication use (y/n), vitamin supplement use (y/n), acculturation (%), and perceived stress score.

Figure 1

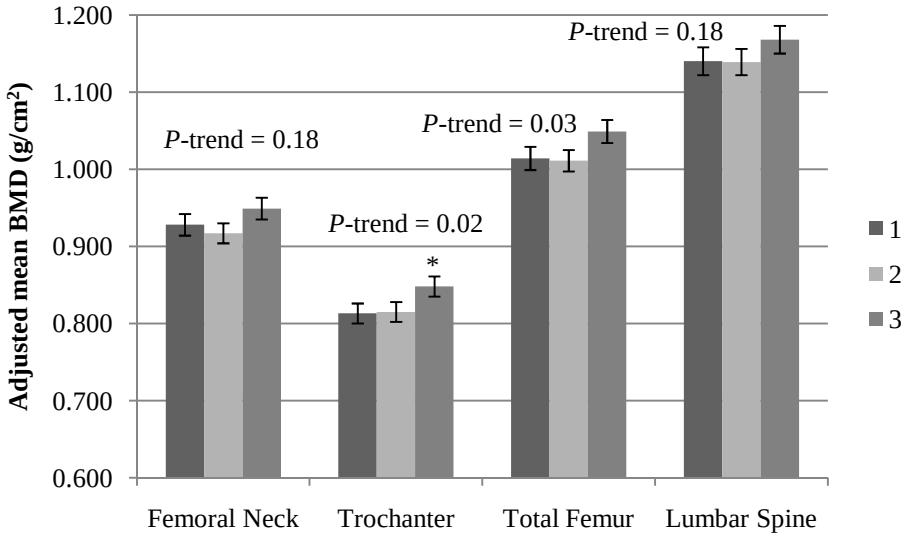
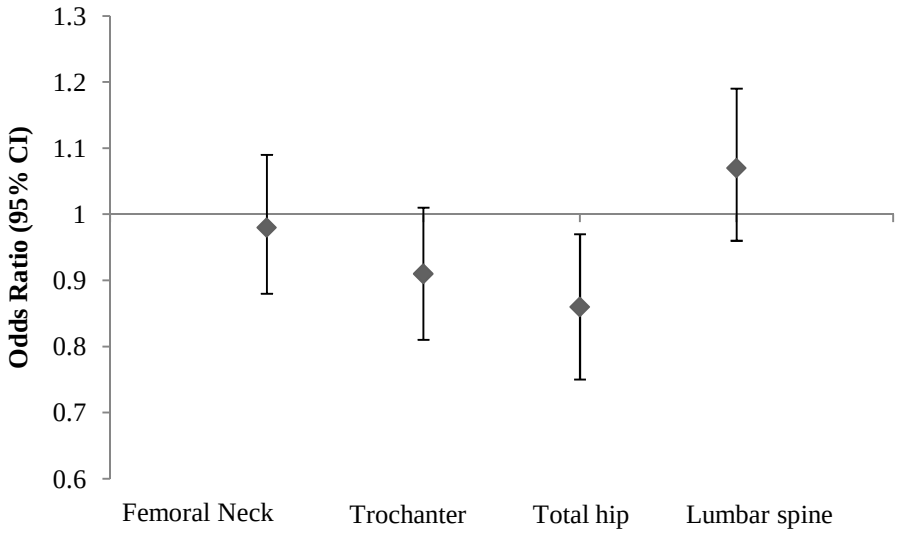


Figure 2



CHAPTER EIGHT: SUMMARY AND DISCUSSION

This thesis was designed to examine the intersection between diet, cardiovascular disease (CVD) risk factors, and bone health in a population of older Puerto Ricans living in the greater Boston area. As the world's population is aging, osteoporosis and CVD threaten to remain major public health problems with enormous social, psychological, and economic consequences. Several studies have demonstrated that incident CVD events are associated with reduced bone mineral density (BMD) [1-5] and greater risk of bone fractures [6-11] later in life. However, few studies have evaluated how CVD risk factors, as opposed to incident CVD events, are associated with bone health, especially in a high risk minority population. The results of this dissertation show that among older Puerto Ricans, certain CVD risk factors are associated with lower BMD and a higher risk of osteoporosis or osteopenia at several skeletal sites.

In the first report of this doctoral work, we examined how a constellation of CVD risk factors, including age, total, LDL, and HDL cholesterol concentrations, systolic and diastolic blood pressure, diabetes status, and smoking were associated with bone health. These risk factors comprise the Framingham risk score (FRS) which is an assessment tool used to estimate 10-year risk of developing coronary heart disease (CHD) outcomes for adults, aged 30 and older, without preexisting heart disease [12]. To our knowledge this is the first report to assess the FRS and relate it to bone health in a Puerto Rican population living on the United States (US) mainland. This was an important research aim as this group presents with important disparities in many of the risk factors assessed with the tool, including low HDL-C concentration [13], high prevalence of smoking [14], and high blood pressure [15]. Concern exists regarding the portability of the FRS to other

populations as the tool was developed using Framingham Heart Study data, a predominantly non-Hispanic white cohort. Using earlier data from Puerto Rico, D'Agostino et al. [16] demonstrated that the FRS systematically overestimated 5-year CHD events in Puerto Ricans. Recalibration of the Framingham Cox regression models by replacing Framingham mean values of the risk factors with the cohort's own mean values and the Framingham average incidence rate with the cohort's own average incidence rate was found to substantially improve the performance of the FRS [16]. However, these data were derived from men participating in the Puerto Rican Heart Health Program in the mid 1960's; and it is important to note that the prevalence of risk factors in Puerto Ricans has increased since that time [15, 17]. Further, instead of categorizing our participants into low, intermediate, and high risk categories, we used the FRS as a continuous variable. This minimized potential exposure misclassification and participants were ranked appropriately according to their risk estimates. Among women, after adjustment for alcohol use, body mass index (BMI), height, physical activity, plasma vitamin D concentration, income, and season of BMD measurement, higher FRS was associated with lower femoral neck, trochanter, and total hip BMD (β=-0.013 to -0.015, P<0.006). Once we corrected for differences in energy-adjusted calcium intake, these associations were attenuated (β=-0.009 to -0.011) and significance was retained only at the femoral neck. We did not find any associations at the lumbar spine (P>0.05). Corollary to the BMD analysis, we noted that for every unit increase in the FRS, the odds of osteoporosis or osteopenia at the femoral neck increased by 3% (95% CI: 1.00-1.07). The small number of men in our study limited our ability to observe similar associations in this group due to lack of statistical power. Findings from this report can directly inform

policy. This report underscores previous findings that those who are at high risk for CHD also have a higher likelihood of osteoporosis or osteopenia. Because racial disparities in BMD testing exist [18-19], the FRS may be a simple tool to identify high risk women in need of BMD screening.

Given that abdominal obesity is a prevalent in the Puerto Rican population, in the second report of this doctoral thesis, we examined the association between central fat mass and bone health in this population. Contrary to the traditionally held viewpoint that fat mass is protective of bone, we demonstrated that greater central fat mass was actually a very strong risk factor for poor bone health. Abdominal fat mass is known to contribute to inflammation [20-21], insulin resistance [22], dyslipidemia [23], metabolic syndrome [24], and hypertension [25]. Previous studies that have examined the role of central fat on bone have failed to correct for the mechanical loading effect of body weight on bone. As part of this dissertation, we showed how the association between central fat and bone switches between a positive and negative effect before and after controlling for body weight. Using novel regional body composition software, we were able to quantify the amount of fat mass around the abdomen from whole body DXA scans.

As expected, abdominal fat mass was positively associated with BMD before adjustment for weight and height in both men and women. However, after differences in body weight and skeletal size were taken into account, the direction of association changed to a negative one. These associations were significant at all four skeletal sites in women (β=-0.043 to -0.059, P<0.008). In men, significant negative associations were observed at all hip sites (β=-0.057 to -0.068, P<0.02) but not at the lumbar spine (β=-0.002, P=0.96). We also tested the hypothesis that total fat mass will have weaker

associations, compared to abdominal fat mass, with bone. Among women, total fat mass (kg) was significantly and positively associated with BMD before adjustment for weight and height. After adjustment for body weight and height, associations changed direction and remained significant at the trochanter (β=-0.0006, *P*=0.001) and total femur (β=-0.0007, *P*=0.0005). In men, total fat mass was positively, but not significantly, associated with BMD. However, after controlling for weight and height, all associations changed direction, with significant associations at the hip sites (β=-0.001, *P*<0.001) but not at the lumbar spine (β=-0.0002, *P*=0.64). Supporting the notion that abdominal fat mass has different metabolic effects than total fat mass per se, we found that effect sizes for total fat mass were much lower compared to effect sizes for abdominal fat mass, suggesting that centrally located body fat has a greater effect on bone. Consistent with the effects on BMD, we found parallel associations between abdominal fat mass and odds of osteoporosis or osteopenia at the various skeletal sites. Among women, before adjustment for weight and height, abdominal fat mass appeared to be protective against osteoporosis or osteopenia. Conversely, after statistical adjustment for weight and height, higher abdominal fat mass was associated with higher likelihood of osteoporosis and osteopenia at each bone site. The strongest associations were noted at the femoral neck. For every 100 g higher abdominal fat mass, the multiple-adjusted odds for osteoporosis or osteopenia increased by 25% (95% CI: 1.11-1.42) and 37% (95% CI: 1.11-1.74) in women and men, respectively. The direction of associations observed before and after adjustment for body weight and height are particularly noteworthy. Our results suggest that central fat mass is detrimental to bone beyond its weight bearing effects. These findings are disconcerting for several reasons. First, trends data don’t predict decreases in

the prevalence of abdominal obesity. In fact, NHANES data indicate that the largest increases in mean waist circumference between 1999 and 2008 occurred among Mexican American women (+3.6 cm) [26]. While prevalence data on Puerto Ricans is limited, data from the Massachusetts Hispanic Elders Study (MAHES) [27] and baseline data from the Boston Puerto Rican Health Study [28] show that the prevalence of abdominal obesity in Puerto Ricans is much higher (60% in MAHES, 73% in BPRHS) than national estimates for the US, in general, and for Mexican Americans, in particular. Our findings of a negative association between abdominal fat mass and bone mass in a Hispanic population provide compelling evidence that abdominal fat mass is a significant risk factor not only for CVD but also for osteoporosis. Results from this work carry important policy implications. Given that trends in abdominal obesity increased by 43.4% for men and 30.5% for women between the periods of 1988-1994 and 1999-2000 [29], there is an urgent need for effective strategies to reduce central and overall obesity in our growing Hispanic population. It has been suggested that either a reduction of 500 kcal/d or large increases in physical activity (eg: 110-150 min of walking/day) are required to reverse mean body weights to those in the 1970's [30]. Given the difficulty in achieving these goals, development of public health programs tailored to specific ethnic groups that focus on prevention of body weight gain with increasing age are required.

With the high prevalence of abdominal obesity in this group and the knowledge that higher abdominal fat mass results in a greater production of pro-inflammatory cytokines [20-21], the third analysis of this dissertation work evaluated the role of three inflammatory markers, namely C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α), on bone health. Postmenopausal women in the second

tertile of TNF-α (pg/mL) and intermediate concentration (1-3 mg/L) of CRP had lower lumbar spine BMD compared to women in the lowest groups ($P< 0.05$). Likewise, those in the second (vs. lowest) tertile of TNF-α had lower femoral neck BMD ($P< 0.05$). It is not completely evident why such differences were not seen among women with the highest concentrations of TNF-α and CRP. One possible explanation is that because cytokine concentrations are highly elevated in this group, considerable variation may occur at such high values. Inverse trends at the trochanter ($P=0.07$) and lumbar spine BMD ($P = 0.04$) were observed across tertiles of IL-6 (pg/mL). Because cytokines don't exert their effects in isolation [31], in secondary analyses, we evaluated the effect of cumulative exposure to multiple cytokines on BMD. Those with multiple exposures to elevated cytokines had lower lumbar spine BMD (P for trend = 0.04). We also found that women with intermediate (vs. low) CRP had 2.25 higher odds for osteoporosis or osteopenia (95% CI: 1.00-5.10) at the lumbar spine. We found no associations at other bone sites or with IL-6 or TNF-α. Findings from this analysis have provided valuable insights into possible mechanisms by which BMD declines in this high risk population. We demonstrated that inflammatory markers are associated with lumbar spine BMD in postmenopausal Puerto Rican women. These findings are interesting, as they provide a possible pathophysiological link between CVD and osteoporosis. Future studies should evaluate whether reduction in circulating concentrations of cytokines decreases risk of CVD and osteoporosis. This knowledge may serve as an important target for prevention of osteoporosis in this and other populations with a high prevalence of inflammation.

When examining the link between CVD and osteoporosis, the role of diet cannot be ruled out. Dietary pattern analysis offers a novel method to represent overall dietary

intake and provides an additional dimension to examining the relationship between diet and disease risk and suggests a more comprehensive approach to disease prevention or treatment, because the focus is on the entire diet rather than on just one food or nutrient [32]. Dietary pattern analysis using score-based approaches (diet indexes) is an “a priori” approach that is based on published dietary recommendations. Several diet indexes have been constructed based on these recommendations to evaluate their effect on markers of disease risk [33-38].

Recognizing that diet is an important factor in the prevention of chronic disease, in the final set of analyses of this doctoral work, we tested the hypothesis that a dietary pattern that was originally intended for CVD risk reduction is beneficial to bone. To this end, we first developed a unique diet and lifestyle score (AHA-DLS) based on the American Heart Association (AHA) 2006 Diet and Lifestyle Recommendations (AHA-DLR) [39]. Because these recommendations were intended for CVD risk reduction, we validated the AHA-DLS by examining associations with select CVD risk factors, including blood pressure, waist circumference, ten-year risk of CHD as assessed by the FRS, fasting plasma lipids, serum glucose, serum insulin, and CRP concentrations. We found that adherence to the AHA-DLR was poor. Fewer than 3% of the cohort scored more than half of the maximum possible score of 110. Findings from our study bring into focus the inadequacies of the Puerto Rican diet in meeting national recommendations. Median intake of fruit and vegetables was below the CDC recommendation for consuming at least 5 fruits and vegetables per day [40]. Nearly 75% of participants did not meet recommendations for added sugars and sodium intake. Nearly all (~98%) had intake of whole grains below the recommendation. Yet, even relatively modest adherence

to the AHA recommendations was associated with significantly lower FRS, serum insulin concentration, WC and CRP concentration, and higher HDL cholesterol concentration in this high risk minority group. Given the scope for improvement in both dietary and lifestyle practices, policy efforts should not focus solely on reinforcing the guidelines in this population but should rather understand predictors of dietary change. Puerto Ricans have unique dietary intake patterns, as well as social, cultural and environmental exposures that may contribute to CVD risk. There is a crucial need to understand attitudes, motivators, and other characteristics that influence certain dietary and lifestyle choices and to develop culturally sensitive policy initiatives that will help this group meet national dietary recommendations.

In the final report of this dissertation work, we further evaluated the role of the AHA-DLS on bone health. We found that for every 5 unit increase in the AHA-DLS, BMD at the femoral neck, trochanter, and total hip was associated with a 0.007-0.009 g/cm^2 ($P<0.05$) higher value. We observed positive linear associations between higher trochanter and total hip BMD across increasing tertiles of AHA-DLS (P for trend ≤ 0.05). An interesting observation is that no single recommendation alone was responsible for the positive associations between the modified AHA-DLS and BMD at the hip sites. This alone is an important policy message which reinforces the importance of patterns of healthy behavior, rather than single dietary or lifestyle choices to reducing chronic disease risk. Given that translation of nutrient-based targets to the public has proven difficult, future policy efforts should continue to focus on food-based targets as these are more easily understood by the general population. Our data showed that for every 5 unit increase in the AHA-DLS, the odds for osteoporosis or osteopenia in the total hip was

14% (OR=0.86, 95% CI: 0.75-0.97) lower. We did not find significant associations between the AHA-DLS and other BMD sites. Consequently, we conclude that dietary patterns for CVD risk reduction also benefit bone health in Puerto Ricans living in the US. Our findings have important public health implications. Synchronizing guidelines, both dietary and lifestyle, provides a holistic approach to the prevention of two major diseases that contribute significantly to morbidity and mortality of the aging population and provide foundational data for public health practice.

DIRECTIONS FOR FUTURE RESEARCH

Our study has several implications for future public health practice and research. First, we have conducted a cross-sectional study on diet, CVD risk factors, and bone health in Puerto Rican adults living in the greater Boston area. The cross-sectional nature of this dissertation work impedes our ability to clarify the direction of associations and to make inferences about causality. While the results of this dissertation work have shed light on important and interesting associations, longitudinal studies are warranted. Studies relating change in exposure variables to change in BMD will provide important foundational data for implementing intervention studies and will inform clinical practice. Second, while BMD is a valuable measure of bone health, fracture is the clinically relevant outcome. Future studies should evaluate the association between exposures in our study and fracture risk. We were unable to examine associations of the FRS and inflammatory markers with bone in men and pre-menopausal women due to lack of statistical power. Studies need to confirm our findings in these groups as it is possible that sex steroids may modify associations between these exposures and bone [41]. At the same time, the Puerto Rican population differs from other Hispanic groups in ancestral

genetic history [42], as well as in exposures to known risk factors for CVD. Differing risk factors include unique dietary intake and physical activity patterns, as well as social, cultural and environmental structures that contribute to and affect CVD risk factors. Thus, findings in our study need to be replicated in the general population.

Positive changes in morbidity and mortality of heart disease and osteoporosis and associated risk factors are largely attributed to dietary and lifestyle practices. Thus, in addition to longitudinal studies, intervention programs are needed in this group. However, existing health promotion strategies that positively influence behavioral changes have focused merely on the individual with limited long-term success. To influence behavior change and promote sustainable healthful behaviors, a socio-ecologic approach to health promotion is needed to influence such behaviors at the individual, social, cultural, and environmental levels. They must take into account the importance of social network in promoting changes in behaviors. Intervention programs need to be evidence-based and must address psychosocial aspects of motivation and efficacy. It is also important to design programs that address environmental influences on health behaviors [43]. Effective community-based heart disease prevention programs that emphasize risk factor screening and risk reduction through heart healthy eating are needed. Further, intervention studies to increase physical activity and improve dietary habits in minority populations should be culturally sensitive and should consider the community's beliefs, practices, and value systems. A recent scientific statement from the American Heart Association states the interventions in minority population groups should consider several important factors [44]. These include considering the setting in which healthcare is delivered, the importance of having interventions led by peers rather than

professionals, assessing acculturation levels before program implementation, being sensitive to literacy levels of the group, and finally understanding the barriers to behavior change [44].

In summary, our findings suggest that risk factors for CVD influence BMD and the likelihood for osteoporosis and osteopenia at several skeletal sites. Results from this dissertation work guide the way for future interventional studies in an understudied population with documented health disparities. These intervention studies are critical to clarify the causal role of CVD risk factors in bone health. The Puerto Rican population appears to be at high risk for chronic disease, and diet and lifestyle interventions based on the AHA-DLR may provide significant benefit to not only CVD risk reduction but also to improvements in bone health. Implementation of interventions that yield even modest reductions in CVD risk factors may have a huge impact on reducing both CVD and osteoporosis. Such studies will in turn allow for targeted efforts in the primary prevention of these diseases.

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