SERUM 25(OH)D CONCENTRATION & CARDIOVASCULAR DISEASE RISK ASSOCIATIONS AMONG OLDER PUERTO RICANS

Dissertation Presented

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ABSTRACT

Cardiovascular disease (CVD) is the number one single cause of mortality and morbidity, accounting for approximately 30% of deaths worldwide. Numerous risk factors for CVD are well established, but novel ones continue to emerge. Several reports have suggested that there is an association between plasma 25-hydroxyvitamin D concentration (25(OH)D) and CVD. Vitamin D deficiency is prevalent, especially among ethnic minorities.

The objective of this dissertation was to investigate, cross-sectionally and longitudinally, the relation between serum 25(OH)D concentration and CVD risk factors in 970 Puerto Rican adults, 270 men and 700 women, aged 45-75 years: (1) to investigate, longitudinally, the relation between serum 25(OH)D concentration and lipid profile, specifically, high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), total cholesterol (TC) and triglycerides (TG). (2) to investigate, cross-sectionally, the relation between serum 25(OH)D concentration and serum inflammatory markers, specifically, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and high-sensitivity C-reactive protein (hsCRP). (3) to investigate the longitudinal effect of body mass index (BMI), and waist circumference (WC) as measures of adiposity on serum 25(OH)D concentration. (4) to investigate, cross-sectionally, the association of regional and total adiposity, specifically, total body fat (TBF), percent body fat (%TBF), android fat-mass (AFM) and gynoid fat-mass (GFM), using dual-energy x-ray absorptiometry (DXA), on serum 25(OH)D concentration.

The cross sectional analyses from the two year time point showed that among all participants, HDL-C had a significant positive association with plasma 25(OH)D
(P<0.01) while TG had significant negative association; but not LDL-C, or TC. The longitudinal analyses showed that when predicting 2.5-year change in plasma HDL-C, baseline-25(OH)D had a significant inverse association (β = -0.045 ± 0.02, P =0.007) controlling for age, sex, baseline-HDL-C concentration, BMI, and follow-up time. Similarly, after controlling for potential confounders, the baseline-25(OH)D was a significantly positive predictor of the change in plasma TG concentration over time (β = 0.40 ± 0.02, P =0.03). With regard to inflammatory markers, after adjusting for age, sex, and seasonality, the multivariate regression analyses showed that serum 25(OH)D had a significantly negative association with TNF-α and IL-6, (P<0.01 and P<0.04), respectively, but not with hsCRP. Further adjustment for numerous identified confounders did not alter the significant associations between serum 25(OH)D concentration with TNF-α and IL-6. The adiposity analyses showed that BMI and WC were both negatively correlated, at both time points, with serum 25(OH)D concentration (r = -0.1, P =0.02) and (r = -0.1, P =0.009), respectively. The multivariate analysis showed that when predicting the change of serum 25(OH)D concentration, only baseline-BMI had significantly inverse association (β = -0.22, P =0.01) controlling for age, sex, and baseline-BMI. This association remained significant after adjusting for numerous recognized confounders, while baseline-WC was not significantly associated with serum 25(OH)D concentration. When investigating regional adiposity, our analyses showed that neither TBF in grams nor %TBF were significantly associated with serum 25(OH)D. However, AFM seems to have an inverse significant association with serum 25(OH)D, whereas GFM showed a positive significant association with serum 25(OH)D.
In conclusion, even though 25(OH)D serum concentration was positively associated with HDL-C in our *cross-sectional* analyses, the *longitudinal* analyses showed that higher 25(OH)D serum concentration did not translate into an increase of the atheroprotective HDL-C, nor a decrease in the not-atheroprotective plasma TG in this population, which seems contradictory to the some of the literature. When we investigated the association of serum 25(OH)D and inflammation, lower serum 25(OH)D concentration was significantly associated with higher inflammatory concentration in this population. The third major finding of the present dissertation work is that obesity has an opposing effect on serum 25(OH)D concentration in this population of older Puerto Rican adults living in the Boston area, which might accelerate vitamin D deficiency status. Longitudinal studies are needed to clarify causal pathways.
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DEDICATION

To my lifelong partner, my husband Ashhal, and my rock and amazing children, Hamza and Mawada, for the love, the devotion, the support, the persistence and the encouragement needed to keep me going,

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CHAPTER ONE

INTRODUCTION

Cardiovascular disease (CVD) is the number one single cause of mortality and morbidity, accounting for approximately 30% of deaths worldwide in 2008. Numerous risk factors for CVD are well established, but novel ones continue to emerge. Atherosclerosis underlies various adverse vascular events that contribute to the development of CVD. It is considered a chronic inflammatory disease of the muscular arterial walls that is characterized by the accumulation of lipoproteins, immune response activation, and increased expression of growth factors, all of which leads to endothelial dysfunction and induces proliferation and migration of smooth muscle cells within the arterial wall. Despite substantial advances in drug discovery and development for treatment of cardiovascular disease over the last decade, the use of regimens such as lipid-lowering agents, anti-platelet drugs, and anti-inflammatory drugs has not solved the complex, multifactorial interplay of the pathophysiological drivers of CVD.

We proposed an interdisciplinary study of the association of various CVD risk factors and serum 25-hydroxyvitamin D [25(OH)D] concentration of the participants of Boston Puerto Rican Health Study (BPRHS) and the Boston Puerto Rican Osteoporosis Study (BPROS). We examined the association between serum 25(OH)D concentration and pro-inflammatory markers such interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and high-sensitivity C-reactive protein (hsCRP); plasma lipid profile, including total
cholesterol (TC), low-density lipoproteins (LDL-C), high-density lipoproteins (HDL-C), and triglycerides (TG); and adiposity measures including: body mass index (BMI), waist circumference (WC), and total body fat (TBF). The Boston Puerto Rican Health Project is an ongoing longitudinal study with two time-points and a sample size of 1502 participants.
STATEMENT OF THE PROBLEM

Cardiovascular disease (CVD) is a major public health concern worldwide [1]. The list of traditional risk factors for CVD published by the American Heart Association (AHA) includes family history, age, sex, behavioral/lifestyle, smoking, obesity, diabetes, hyperlipidemia and hypertension [2]. Atherosclerosis is one of major causes of CVD and it is characterized by lipid accumulation and systematic inflammation, and endothelial dysfunction. The expression of inflammatory cytokines is a critical factor influencing CVD progression [3]. Vitamin D (vitamin D) deficiency is now believed to be associated with CVD and immune response: vitamin D appears to have a regulatory influence on the inflammatory cytokines involved in the progression of systematic inflammation and endothelial dysfunction [4].

The aims of our study: Puerto Rican men and women, aged 45-75 years, will be evaluated both cross-sectionally and longitudinally, at baseline and at 2 years.

Aim One: To evaluate longitudinal associations between serum 25(OH)D concentration and plasma lipid profile, precisely, high-density lipoproteins (HDL-C), low-density lipoproteins (LDL-C), and total cholesterol (TC) and triglycerides (TG).

Aim Two: To evaluate cross-sectional associations between serum 25(OH)D concentration and inflammatory markers, precisely, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and high sensitivity C-reactive protein (hsCRP).

Aim Three: To evaluate the longitudinal associations between serum 25(OH)D concentration and adiposity phenotype, specifically, body mass index (BMI) and waist circumference (WC) among 970 participants of the BPRHS and BPROS studies.
**Aim Four:** To evaluate *cross-sectional* associations between serum 25(OH)D concentration and adiposity measures, specifically, total body fat mass (TBF) measured by dual-energy x-ray absorptiometry (DEXA).

The relationship between serum vitamin D concentration and CVD risk factors in general, and atherosclerosis, in particular, is well documented [5, 6]. The continued high incidence of CVD-related mortality and morbidity emphasizes the need to distinguish not only the contributors to CVD risk, but also to identify new therapeutic modalities for the treatment of atherosclerosis as a primary cause of CVD. We believe that the proposed work will contribute to the current knowledge of vitamin D and CVD risk. We know of no studies that have reported on associations between serum vitamin D concentration and CVD risk factors among older Puerto Ricans. Thus, results from our cohort project will be the first to assess this association, *longitudinally*, within this particular ethnicity.
The Epidemiology of Cardiovascular Disease and Atherosclerosis

Despite significant progress in scientific research of CVD, it remains the leading cause of morbidity and mortality worldwide [1, 7, 8]. According to the World Health Organization (WHO), in 2010, CVD accounted for 17.3 million deaths, which is about 29% of all global deaths; it is projected that CVD will be responsible for ~24 million deaths annually by the year 2030 [1]. In the USA, the mortality data for 2007 showed that CVD remained the primary cause of death in the USA; approximately 34% of deaths were CVD related [7, 9]. CVD risk factors are diverse, ranging from traditional factors such as age, sex, smoking, inactivity, obesity, family history, hypertension, and hyperlipidemia [10, 11]. However, traditional factors cannot fully explain the increase in CVD incidence, and the emergence of more novel pathophysiological factors such as inflammation, oxidative stress and adipocyte dysfunction might provide more insights on the development of CVD [9]. Atherosclerosis is a pathological condition that underlies various adverse vascular events that contribute to CVD development [12]. It is characterized by accelerated accumulation macrophages and oxidized LDL (oxLDL) within arterial blood vessels [3, 13]. The early stages of atherosclerosis are characterized by endothelial dysfunction—impairment of endothelium-dependent vasodilatation, which can be measured by brachial artery flow-mediated dilation (FMD) [14, 15].
The Progression of Atherosclerosis

The walls of large muscular arteries consist of three distinct, well-differentiated layers. The outermost layer relative to the vessel lumen is the *tunica adventitia*, which is composed of connective tissue; inside this is the *tunica media*, which consists of smooth muscle cells and elastic tissue; and the innermost layer, called the *intima*, consists of the internal elastic lamina and a monolayer of endothelial cells, which is continuously and directly exposed to blood and its constituents, along with the hemodynamic shear forces associated with the flowing blood [3, 13]. The endothelial layer of the intima is a critical controller of vascular homeostasis and functions as an anti-thrombotic vessel lining [16]. Vascular endothelial cells synthesize and release a wide array of mediators that act in autocrine and/or paracrine modes to modulate the structure of the artery, smooth muscle quiescence, endothelial passivity as well as blood constituents, vessel tone, and hemodynamic reactivity.

The development of atherosclerosis involves early endothelial activation generally believed to be triggered by various local factors such as oxidative stress and increased expression of pro-inflammatory mediators, all of which lead to increases of the adhesiveness of leukocytes or platelets through the increased expression of intracellular adhesion molecule (ICAM). Another hallmark of endothelial activation is the increased permeability of lipoproteins such as LDL-C and TG [3, 12, 13] and a defect in the production of nitric oxide (NO) from L-arginine by endothelial NO synthase (eNOS) [14].
NO is the most potent vasodilator yet identified and further acts to inhibit platelet activity and smooth-muscle cell proliferation. In addition to mediating endothelium-dependent vasodilatation, NO is critical for preventing platelet and leukocyte aggregation as well as leukocyte adhesion and infiltration [12, 14]. Further, trapped LDL-C undergoes modification including oxidation, lipolysis, and proteolysis, generating oxLDL-C, which has the ability to inhibit the production of NO as well as to stimulate the endothelium to produce pro-inflammatory molecules including adhesion molecules and growth factors. Subsequently, monocytes and lymphocytes migrate across the endothelial layer into the intima, where they proliferate and differentiate into macrophages. This migration is triggered by the accumulation of oxLDL-C. Moreover, increased expression of ICAM induces the adhesion of these monocytes along with LDL-C. Later, the extensive modification of the oxLDL-C allows their recognition by the scavenger receptors within the macrophage, and, hence, their uptake leads to foam-cell formation. Such receptor expression is mediated by TNF-α and interferon-γ (IFN-γ) cytokines. The progressive accumulation of foam cells, oxLDL-C, and monocytes leads to fatty streak formation.

As fatty streaks progress to advanced lesions, smooth muscle cells (SMC) migrate to the intima along with T cells, subsequently leading to the development of a necrotic core. This advanced lesion is characterized by dead foam cells, accelerated lipoprotein and SMC macrophage accumulation, and enhanced inflammatory response, all of which are covered by a fibrous cap that extends into the lumen. Rupture of the fibrous cap exposes the content of the necrotic core to the blood, leading to coagulation and recruitment of platelets, resulting in thrombosis [3, 12, 13].
Inflammation plays a pivotal role in all stages of atherogenesis, from foam cell to plaque formation, and all the way to rupture and ultimately to thrombosis [17, 18]. In fact, atherosclerotic lesions are characterized by the release of various pro-inflammatory cytokines, such as hsCRP, IL-1β, IL-6 and TNF-α. The binding of LDL-C to the endothelium is mediated by both IL-1β and TNF-α. Inflammatory cytokines such as IFN-γ activate the macrophages to undergo programmed cell death, apoptosis, causing a mass of extracellular lipids and debris. Thus, this cycle that starts with inflammation, modification of lipoprotein systems and further inflammation is an illustration of how the inflammatory response has a profound effect not only the development of atherosclerosis, but in some cases it is considered a healing and repair response.

Emerging evidence suggests that serum vitamin D concentration are associated with a wide spectrum of CVD risk factors, generally, and with atherosclerosis development, specifically [19-25]. It has been hypothesized that higher serum vitamin D concentration may play a major role in the attenuation of atherosclerosis development and improvement of endothelial function. However, due to the complexity of CVD, with its wide range of risk factors as well as its clinical biomarkers, and complexity of vitamin D with its limited dietary sources vs. synthesis from sunlight, the supporting evidence is mixed.
VITAMIN D METABOLISM

Overview of Synthesis and Metabolism

Vitamin D is a secosteroid compound with 27 carbons, there are two forms of vitamin D, D$_2$ or ergocalciferol (Figure 1), and D$_3$ cholecalciferol (Figure 2). It is an essential nutrient, but is unlike most other nutrients in terms of its limited food sources [26]. Only a few food sources, such as fatty fish and cod liver oil, naturally contain vitamin D, making it difficult to achieve optimal vitamin D concentration through food intake alone. Since the 1930s, dietary fortification with vitamin D has been established in a number of different products, including milk, soymilk, orange juice and selected cereal grains [27, 28]. In most cases, achieving optimum vitamin D only with dietary intake is not feasible [26, 29-31]; rather, a relatively efficient way of obtaining vitamin D is endogenously, via the ultraviolet-β activation of 7-dehydrocholestrol to pro-vitamin D$_3$, within the epidermal basal layer, which then undergoes isomerization producing the D$_3$ form, cholecalciferol (Figure 2). Afterward, cholecalciferol binds to the vitamin D-binding protein (VDBP) and is transported to the liver, where it gets its first hydroxylation at the 25 position by 25-hydorxylase, producing calcidiol or 25(OH)D (Figure 3) [32].

Afterward, cholecalciferol binds to the vitamin D-binding protein (VDBP) and is
transported to the liver, where it gets its first hydroxylation at the 25 position by 25-
hydroxylase, producing calcidiol or 25(OH)D (Figure 3) [32].

Several cytochrome-P450 enzymes (CYP-450s), such as CYP2J2, and CYP3A4, also exhibit hydroxylase activity, which metabolizes the less potent D₂ form (Figure 1) [33]. 25(OH)D is the blood biomarker for determining the status of vitamin D: it is characterized by a relatively long half-life of about 3 weeks. Mainly in the kidney, a second hydroxylation occurs at the 1-α position by CYP27B1, a 1α-hydroxylase, converting 25(OH)D to 1α,25(OH)₂D, calcitriol (Figure 4), which is the active metabolite, but which has a very short half-life, up to only 12-15 hours [32, 34-36]. This pathway is exquisitely regulated by parathyroid hormone (PTH), serum calcium concentration (Ca²⁺), and phosphate (P₁) concentration, and also by negative feedback inhibition from 1α,25(OH)₂D concentration [37]. CYP-P450 regulates the catabolism of vitamin D through the actions of CYP24A1, 24-hydroxylase, which hydroxylates both 25(OH)D and 1α,25(OH)₂D at the 24 position, leading to the inactive hydrophilic metabolites 24,25(OH)₂D and 1α,24,25(OH)₃D, which are readily excreted in the urine; consequently, this catabolic pathway is a limiting step [34].

**Vitamin D Receptor:**

Vitamin D and its analogs exert their differential effects on target cells through two main pathways: genomic and non-genomic pathways. The genomic pathway is mediated through the binding of 1α,25(OH)₂D and/or its analogs to the nuclear vitamin D
receptor (VDR) to exert transcriptional activation and gene expression repression. VDR is classified as a nuclear hormone receptor and is expressed and localized in various tissues where vitamin D has regulatory the pancreatic β cells, monocytes, dendritic cells, and peripheral T cells. The binding of 1α,25(OH)₂D to VDR requires a specific ligand, retinoid-X receptor (RXR). Thus, this later compound binds to response elements (VDREs) in the promoter region of targeted genes, which is followed by a cascade of binding of transcription factors, co-activators, and co-repressors to increase the transcription and/or suppress of target genes. The non-genomic pathway is through the binding of 1α,25(OH)₂D and/or its analogs to cytosolic and membrane VDR, which initiates a phosphorylation cascade of mitogen-activated protein kinases (MAPK) and extracellular signal-regulated kinase (ERK), which might participate in a cross-talk with the genomic pathway to modulate gene expression [37-39]. In conclusion, VDR is considered a ligand-induced receptor that controls the expression of more than a thousand genes, many of whom are involved in cell division, differentiation, and growth.

The literature is rich with considerable data for the role for vitamin D in bone and mineral homeostasis [40, 41], however; in the late 1970’s, the discovery of VDR among various types of tissues, other than bone and small intestine, has provided new insights to the non-traditional roles of vitamin D in the immune system regulation [42], cancer [43-45], CVD [46, 47] and diabetes [48, 49]. Moreover, the cloning of CYP27B1 in various tissues has provided more evidence that vitamin D activation is not only limited to the functions including: intestine, bone tissues and kidneys, as well as some immune cells, namely, kidney, but rather 1α-hydroxylase is locally expressed where vitamin D is needed including: (1) regulating the proliferation of normal cells such as the
keratinocytes; (2) regulating hormone secretion such as PTH, insulin, and growth hormone; (3) regulating both adaptive and innate immunity [50].

**The Association between Vitamin D Deficiency and CVD Risk Factors**

Vitamin D deficiency is considered a global epidemic. It has been estimated that more than one billion people have vitamin D concentration that are considered, *minimally*, insufficient [26, 29, 51-53]. Thus far, there is no agreement within the medical and/or scientific area on defining optimal levels of 25(OH)D. However, most scientists define *sufficient* serum 25(OH)D concentration as above 75 nmol/L; *insufficient* as 50-75 nmol/L; *deficient* as below 50 nmol/L; and *severely deficient* as below 25 nmol/L [26, 54-56]. In the US, 48% of young white girls in Maine and 52% of Hispanic adolescents in Boston were found to be vitamin D deficient [57, 58]. Another report indicated that among Hispanics, Puerto Ricans had the highest prevalence of vitamin D deficiency, 26% [59]. Interestingly, another report investigated the levels of vitamin D concentration among Puerto Ricans in Puerto Rico and found that about 55% of their sample had vitamin D concentration >70 nmol/L, 31% had levels between 50 and 70 nmol/L, and 14% had levels below 50 nmol/L [60]. In the Middle East, vitamin D deficiency ranges between 30 to 50% among adults [26]. A recent report from Scotland indicated that high percentages of healthy adults were vitamin D deficient, 29%, or severely deficient, 34.5% [61].

Aside from increased risk of osteoporosis and lower bone mineral density [40], vitamin D deficiency has been associated with increased risk of several illness including CVD and hypertension [20, 23, 62]; cancer, including breast and prostate [45, 63];
obesity and related metabolic disorders such as type-2-diabetes [48, 64]; sarcopenia and muscle weakness [65, 66]; and multiple sclerosis [67]. In the last decade, a wide range of scientific evidence has linked vitamin D deficiency to increased risk of CVD. First, a number of large, nonrandomized, prospective studies have documented that vitamin D deficiency was associated with CVD morbidity and mortality. The Framingham Offspring Study showed that the incidence of CVD was higher among individuals whose serum 25(OH)D concentration was below 37 nmol/L [68]. The Luric study showed that participants whose 25(OH)D concentration was in the lowest quartile (median=19 nmol/L) or the second lowest (median=33 nmol/L) had high adjusted risk for CVD mortality, relative to those in the highest quartile (median=71 nmol/L), 122% and 82% risk, respectively [69]. A meta-analysis showed that vitamin D supplementation, ranging between 300 and 2000 IU/day, was associated with lower total mortality, relative risk (RR) = 0.93 (95% CI:0.87-0.99) [70].

**Proposed Mechanisms of Anti-Inflammatory Actions of Vitamin D**

Several studies have documented that vitamin D exerts anti-proliferative and pro-differentiating effects on many types of normal and malignant cells [71, 72]. One of the proposed mechanisms for the association of calcitriol with atherosclerosis is through the increased expression of MAP kinase phosphatase-5 (MKP5) which, in turn, dephosphorylates and, thereby, inactivates MAPKs, such as p38 and the stress-activated protein kinase Jun-N-terminal kinase (JNK). This action leads to a decrease in the production of pro-inflammatory cytokines such as IL-6 [4]. Another proposed mechanism
is through inhibition of nuclear-factor-κB (NF-κB) activation and signaling, which is a major modulator of inflammatory and immune response, through increasing the expression of IκB protein and activating IκB kinase (IKKB) [73]. The activation of NFκB is accompanied with increased expression of a wide range of inflammatory cytokines, such as IL-6, IL-8, and TNFα [73, 74]. One recent study, by Talmor and collaborators, showed that in vitro calcitriol lowered the activity of NF-κB and p38, and the expression of ICAM and IL-6 [75]. Table 1 illustrates several in vivo experimental murine studies using ApoE-/- mice that investigated the effects of different vitamin D analogs on atherosclerosis.

The association between vitamin D and adiposity, in general, and body fat, in particular, was explained by Lumb and colleagues in 1971. They were the first to introduce the hypothesis of vitamin D sequestration in adipose tissue and muscle [76]. Later, they were able to substantiate that prior hypothesis and confirm the link between adiposity and vitamin D [77]. Subsequently, Wortsman and colleague confirmed the aforementioned premise, stating that due to vitamin D deposition within adipose tissue, the bioavailability of serum 25(OH)D and 1,25(OH)2D is lowered, leading to vitamin D insufficiency/deficiency and higher PTH. They showed that obese people evidence, lower [48].
Table 1: List of studies of calcitriol and analogs on atherosclerosis using ApoE\(^{-/-}\) mice

<table>
<thead>
<tr>
<th>Author</th>
<th>Hypothesis</th>
<th>Treatment</th>
<th>Animals</th>
<th>Treatment</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Becker et al. 2011 [78]</td>
<td>aortic wall</td>
<td>*19-nor</td>
<td>ApoE(^{-/-}) (n=16/trmt)</td>
<td>IP 5x/wk for 10 wks</td>
<td>*19-norc (\rightarrow) 0.1ug/kg *1.25D(_3) (\rightarrow) 0.03µg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*1.25D(_3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Becker et al. 2011 [78]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Husain et al. 2010 [79]</td>
<td>atherosclerosis</td>
<td>*9-nor</td>
<td>ApoE(^{-/-}) females n=12/trmt</td>
<td>IP 3x/wk for 16 wks</td>
<td>*19-norc (\rightarrow) 200 ng *Enlp (\rightarrow) 30mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*Enalapril</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Takeda et al. 2010 [80]</td>
<td>atherosclerosis</td>
<td>1,25D(_3) only</td>
<td>ApoE(^{-/-}) n=6-9/trmt</td>
<td>Oral 2x/wk for 12 wks</td>
<td>20 ng 200 ng (10 µg/kg)</td>
</tr>
<tr>
<td>Wang et al, 2011</td>
<td>Oxidative stress and proinflamm cytokines</td>
<td>1αD(_2)</td>
<td>NON mice (males) n=12/trmt</td>
<td>IP for 28 wks</td>
<td>1αD(_2) (\rightarrow) 125ng/kg</td>
</tr>
<tr>
<td>Wang et al, 2011 [81]</td>
<td>Oxidative stress and proinflamm cytokines</td>
<td>1αD(_2)</td>
<td>NON mice males n=12/trmt</td>
<td>IP for 28 wks</td>
<td>1αD(_2) (\rightarrow) 125ng/kg</td>
</tr>
</tbody>
</table>
PRELIMINARY STUDIES

The BPRHS is an ongoing longitudinal study that follows 1505 older Puerto Ricans in the Greater Boston Area. For the analyses we are presenting here, we used the baseline and 2-year data time points. Complete baseline serum 25(OH)D concentration and dietary vitamin D intake assessments were available for 1432 participants, 1004 women (mean age = 57.3 years) and 428 men (mean age = 56.6 years). Figure 13 shows the prevalence of serum 25(OH)D concentration among the participants of BPRHS. The majority of these Puerto Rican men and women (~75%) were considered vitamin D deficient, with concentration below 50 nmol/L, whereas only 4.2-3% had sufficient serum 25(OH)D concentration of vitamin D, with levels above 75 nmol/L.

Women had significantly lower scores for serum albumin, serum creatinine, systolic and diastolic blood pressure, HCY, VLDL-C, TG, and physical activity, but significantly higher scores for BMI, HDL-C, TC, LDL-C, and poverty rate. Compared to men, more women are taking supplements with a higher content of vitamin D -above 200 international units (IU) as well higher dietary intake of energy-adjusted total vitamin D relative to men. No differences were observed between men and women for glycosylated hemoglobin, fasting glucose, waist circumferences or Homa-IR (Table 2).

Analysis of dietary data shows that Puerto Rican women have significantly higher carbohydrate intake, with more of their calories deriving from carbohydrates compared to Puerto Rican men. On the other hand, the women tend to have significantly lower total energy and dietary cholesterol intakes than do Puerto Rican men; also, a lower portion of the women’s energy derives from fat sources compared to that for Puerto Rican men.
However, no significant differences were found by sex when dietary fiber and total fat were compared (Table. 3). Figures 6 and 7 show the baseline association between serum concentration and deciles of median intake of vitamin D from food and supplements among all participants (n=1416). There was a wide range of vitamin D intake, 201 to 599 IU/day, when vitamin D supplements are included with food sources (Figure 6). On the other hand, the range was very narrow when considering only the food sources of vitamin D, 208 to 217 IU/day (Figure 7). Food products made from whole milk, 2% milk, and 1% milk constituted 34% of vitamin D sources due to fortification with vitamin D. Fish ranked second, constituting 16.2% of the vitamin D source. Eggs and ready-to-eat cereal were fourth and fifth respectively, contributing about 4.5% and 4.3% of dietary vitamin D intake (Table 3).

![Figure 5](image.png)

**Figure 5:** The percent of BPRHS women and men in two age groups, with serum 25(OH)D concentration (n=1495): deficient (<50 nmol/L), insufficient (50-75 nmol/L) or sufficient (>75 nmol/L).
Table 4 shows the breakdown of age-adjusted means of serum 25(OH)D concentration by different population factors. In general, the use of vitamin D-containing supplements was associated with higher overall serum 25(OH)D concentration: our results show that both Puerto Rican men and women who consume minimal daily supplements (200 IU) vitamin D had significantly greater serum 25(OH)D concentration compared to non-users: 50.1 and 47.0 nmol/L, respectively (P for trend <0.0001). We observed a significant decreasing trend concentration across the four seasons, starting from the summer toward spring season (P for trend <0.0001). Puerto Rican women aged 60-75 years had significantly higher serum 25(OH)D concentration compared to those aged 45-59 yr: 45 and 42.1 nmol/L, respectively (P-value 0.009). On the other hand, women with higher BMI had significantly lower serum 25(OH)D concentration (P for trend 0.05). In addition, past and current female smokers had significantly lower 25(OH)D concentration (P for trend 0.04) compared to those who have never smoked.
Moreover, women, but not men, showed a significant increasing trend in serum 25(OH)D concentration with increased physical activity score (P for trend 0.02). Among men, lower concentration of serum albumin was associated with significantly lower serum 25(OH)D concentration: 32 and 44 nmol/L, respectively (P-value 0.05). Although higher alcohol intake appeared to correlate with lower serum 25(OH)D among men as well as women, this did not reach statistical significance. Finally, there was no significant relationship between serum 25(OH)D concentration, and activities of daily living (ADL) for either men or women.

Known and potential factors that influence serum 25(OH)D concentration were used to construct linear regression models (Table 5). The multiplicative interaction of sex with age was highly significant (P<0.0001), thus models are presented separately for men and women. In the fully adjusted model for women, serum 25(OH)D concentration was significantly lower, by 0.15 nmol/L, for each year of age (P = 0.03). Lower concentration were observed in winter and spring, -5.0 and -8.4 nmol/L, respectively (P = 0.0006 and < 0.0001) relative to summer. Higher BMI was associated with significantly lower concentration, - 0.21 nmol/L (P< 0.0001 for each BMI unit). Serum 25(OH)D concentration was 0.8 nmol/L greater for each IU difference in dietary vitamin D (P<0.0001). Use of vitamin D-containing supplements was independently associated with higher serum concentration (P<0.0001). Supplements containing vitamin D in the range of 200-400 IU/day or > 400 IU/day, were associated with significant higher serum 25(OH)D concentration—to 5.0 and 11.1 nmol/L respectively—vs. no supplement use (P<0.0001). Serum albumin was also associated with higher serum vitamin D concentration (P = 0.008). Women who never smoked had significantly higher serum
concentration, 3.4 nmol/L compared to currently smoking women ($P=0.01$). Serum creatinine, physical activity score, and alcohol intake were not significantly associated with serum 25(OH)D concentration in women.

For men, seasonality was associated with similar decreasing serum 25(OH)D concentration as seasons moved away from the summer season, but the reduction was more pronounced than in women: the winter and spring levels were -11.6 and -12.3 nmol/L lower, respectively, ($P<0.0001$ for each difference). Supplements with 200-400 IU/day of vitamin D (but not those $>400$ IU/day) were associated with serum 25(OH)D concentration 9.0 nmol/L higher than non-users concentration ($P<0.0001$). In addition, as reported above for women, serum albumin was independently associated with higher serum 25(OH)D concentration ($P=0.003$). In men, age, BMI, dietary intake of vitamin D, and smoking did not contribute to serum 25(OH)D concentration, unlike changes seen in women; nor was there any associations with the serum creatinine, physical activity score, or alcohol intake.
**TABLE 2** Descriptive characteristics of the BPRHS participants

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Women (n=1048)</th>
<th>Men (n=447)</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>57.3 ± 7.5</td>
<td>56.6 ± 8.0</td>
<td>0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Education (below 8th grade %)</strong></td>
<td>48.0</td>
<td>44.0</td>
<td>0.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Poverty (%)</strong></td>
<td>62.0</td>
<td>51.0</td>
<td>0.0002&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>BMI (kg/m^2)</strong></td>
<td>33.0 ± 7.0</td>
<td>29.6 ± 5.2</td>
<td>&lt;0.0001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Waist (cm)</strong></td>
<td>102.0</td>
<td>101.5</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Serum Albumin</strong>&lt;sup&gt;c&lt;/sup&gt; (g/L)</td>
<td>42.5 ± 7.0</td>
<td>43.2 ± 3.6</td>
<td>&lt;0.0001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Serum Creatinine</strong>&lt;sup&gt;c&lt;/sup&gt; (nmol/L)</td>
<td>68.0 ± 27.5</td>
<td>87.5 ± 45.1</td>
<td>&lt;0.0001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Number of Walking Miles/day</strong></td>
<td>1.0 ± 1.4</td>
<td>1.3 ± 1.6</td>
<td>&lt;0.0001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Serum 25-(OH)D</strong>&lt;sup&gt;c&lt;/sup&gt; (nmol/L)</td>
<td>43.1 ± 16.0</td>
<td>43.5 ± 16.5</td>
<td>0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Vitamin D Supplement use (&gt; 200 IU %)</strong></td>
<td>30.0</td>
<td>24.0</td>
<td>0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Dietary vitamin D</strong>&lt;sup&gt;ef&lt;/sup&gt; (IU/day)</td>
<td>212 ± 3.0</td>
<td>212 ± 3.4</td>
<td>0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Total vitamin D</strong>&lt;sup&gt;ef&lt;/sup&gt; (IU/day)</td>
<td>285 ± 4.5</td>
<td>285 ± 4.3</td>
<td>0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Glycosylated Hemoglobin (%)</strong></td>
<td>0.07 ± 2</td>
<td>0.07 ± 2</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>Fasting Glucose (mg/dL)</strong></td>
<td>120 ± 53.0</td>
<td>121 ± 52.5</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Homa-IR</strong></td>
<td>6.03 ± 17</td>
<td>6.0 ± 10.2</td>
<td>0.004&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>LDL-C (mg/dL)</strong></td>
<td>110.4 ± 34</td>
<td>101.0 ± 35.2</td>
<td>&lt;.0001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>HDL-C (mg/dL)</strong></td>
<td>47.02 ± 12.1</td>
<td>40.5 ± 12.4</td>
<td>&lt;.0001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>VLDL-C (mg/dL)</strong></td>
<td>29.3 ± 15.0</td>
<td>32.0 ± 19</td>
<td>0.007&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>TG (mg/dL)</strong></td>
<td>157.0 ± 99</td>
<td>174.0 ± 140</td>
<td>0.007&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Total Cholesterol (mg/dL)</strong></td>
<td>188.2 ± 41</td>
<td>174.2 ± 43</td>
<td>&lt;.0001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Homocysteine (µmol/L)</strong></td>
<td>8.45 ± 3.7</td>
<td>10.37 ± 5.7</td>
<td>&lt;.0001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mm Hg)</strong></td>
<td>134.1 ± 19.2</td>
<td>137.1 ± 19</td>
<td>&lt;.0001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mm Hg)</strong></td>
<td>80.11 ± 10.4</td>
<td>83.0 ± 11.2</td>
<td>&lt;.0001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are presented as mean ± SD unless indicated
<sup>b</sup> P-value are based on T-test, <sup>c</sup> P-value are based on chi-square test
<sup>d</sup> Range = 38.3
<sup>e</sup> Total vitamin D= vitamin D from supplement + vitamin D from diet.
<sup>f</sup> The recommended dietary allowance (RDA) for vitamin D for both men and women aged 19-50 years, and 51-70 years = 600 IU (15 ug /day) [82]
<sup>i</sup> Adjusted for total energy
TABLE 3: Selected dietary intake data for the participants of the BPRHS

<table>
<thead>
<tr>
<th></th>
<th>Women (n=1048)</th>
<th>Men (n=447)</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Energy (kcal)</td>
<td>2021± 32</td>
<td>2367 ± 52</td>
<td>&lt; 0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>273 ± 2</td>
<td>264 ± 3</td>
<td>&lt; 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Dietary fiber (g)</td>
<td>18.5 ± 0.2</td>
<td>18.3 ± 0.3</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>75.9 ± 0.5</td>
<td>77.0 ± 0.8</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>Saturated fat (g)</td>
<td>22.9 ± 0.2</td>
<td>23.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Monounsaturated fat (g)</td>
<td>26.2 ± 0.2</td>
<td>26.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Polyunsaturated fat (g)</td>
<td>17.7 ± 0.2</td>
<td>18.1 ± 0.3</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>91.8 ± 0.7</td>
<td>91.2 ± 1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>298± 5</td>
<td>334 ± 7</td>
<td>&lt; 0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>% Energy from Carbohydrate</td>
<td>52.2 ± 0.3</td>
<td>50.1 ± 0.5</td>
<td>&lt; 0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>% Energy from Total Fat</td>
<td>32.0 ± 0.2</td>
<td>32.8 ± 0.3</td>
<td>&lt; 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>% Energy from Protein</td>
<td>17.4 ± 0.1</td>
<td>17.2 ± 0.2</td>
<td>0.07</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± standard error adjusted for age and total energy.

<sup>b</sup>Significant differences by sex (t-tests)
**TABLE 4:** Major Dietary Sources of Vitamin D intake among Puerto Rican adults\(^a\)

<table>
<thead>
<tr>
<th>Rank</th>
<th>Food Groups</th>
<th>% of dietary vitamin D intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Milk (whole, 2% &amp; 1% milk products)</td>
<td>32.4</td>
</tr>
<tr>
<td>2</td>
<td>Supplements</td>
<td>28.7</td>
</tr>
<tr>
<td>3</td>
<td>Fish</td>
<td>16.2</td>
</tr>
<tr>
<td>4</td>
<td>Eggs</td>
<td>4.5</td>
</tr>
<tr>
<td>5</td>
<td>Cereal, Ready-to-eat, Cold</td>
<td>4.3</td>
</tr>
<tr>
<td>6</td>
<td>Beef</td>
<td>2.3</td>
</tr>
<tr>
<td>7</td>
<td>Processed Meat, Sausage, Frank</td>
<td>2.1</td>
</tr>
<tr>
<td>8</td>
<td>Chicken/Turkey</td>
<td>2.0</td>
</tr>
<tr>
<td>9</td>
<td>Pork</td>
<td>1.3</td>
</tr>
<tr>
<td>10</td>
<td>Ice cream, Sherbet, Frozen Yogurt</td>
<td>1.2</td>
</tr>
<tr>
<td>11</td>
<td>Yogurt</td>
<td>1.1</td>
</tr>
<tr>
<td>12</td>
<td>Cheese</td>
<td>0.9</td>
</tr>
</tbody>
</table>

\(^a\) Only food groups that contributed more than 0.8% of dietary vitamin D intake
| Variables                      | Women          |-Men |    |    |    | | n (%) | n (%) |    |    |    |    |
|-------------------------------|----------------|-----|----|----|----|----|
|                               | Women          | Men | LSM | SEM | P value | Women | Men | LSM | SEM | P value |
| Age (y)                       |                |     |     |     |         |       |     |     |     |         |
| 45-59                         | 651 (61.5%)    | 281 | 44.4 | 1.00 |         | 63.0% | 37.0% |     |     |         |
| 60-75                         | 407 (38.5%)    | 164 | 42.0 | 1.33 | 0.1     | 37.0% | 37.0% |     |     |         |
| Vitamin D Supplement IU/day   |                |     |     |     |         |       |     |     |     |         |
| ≤ 200                         | 738 (70.0%)    | 338 | 41.5 | 0.8  |         | 77.3% | 77.3% |     |     |         |
| 200 - 400                     | 224 (21.5%)    | 90  | 46.5 | 1.6  | <0.0001 | 20.5% | 20.5% |     |     |         |
| ≥ 400                         | 77 (8.5%)      | 9   | 53.1 | 2.0  | <0.0001 | 2.1%  | 2.1%  |     |     | <0.0001 |
| Season of measurement         |                |     |     |     |         |       |     |     |     |         |
| Summer (July-Sept)            | 356 (34.5%)    | 137 | 44.5 | 0.9  |         | 32.0% | 32.0% |     |     |         |
| Fall (October-Dec)            | 264 (26.0%)    | 124 | 46.0 | 0.8  | <0.0001 | 29.0% | 29.0% |     |     |         |
| Winter (Jan-March)            | 184 (18.0%)    | 78  | 41.1 | 1.2  | <0.0001 | 18.0% | 18.0% |     |     | <0.0001 |
| Spring (April-June)           | 224 (21.5%)    | 91  | 38.2 | 1.1  | <0.0001 | 21.3% | 21.3% |     |     | <0.0001 |
| Body Mass Index (kg/m²)       |                |     |     |     |         |       |     |     |     |         |
| ≤ 25                          | 114 (11.0%)    | 80  | 44.0 | 1.5  |         | 18.5% | 18.5% |     |     |         |
| 25-30                         | 282 (27.3%)    | 164 | 45.5 | 0.9  | 0.8     | 37.5% | 37.5% |     |     |         |
| 30-35                         | 307 (29.3%)    | 130 | 44.5 | 0.9  | 0.9     | 30.2% | 30.2% |     |     |         |
| ≥ 35                          | 307 (29.3%)    | 130 | 44.5 | 0.9  | 0.08    | 30.2% | 30.2% |     |     | 0.001 |
| Smoking Status                |                |     |     |     |         |       |     |     |     | 0.9 |

TABLE 5 Age-adjusted means for all variables
### Alcohol Intake

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Yes</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>832 (79.5%)</td>
<td>217 (20.5%)</td>
<td>291 (66.0%)</td>
</tr>
<tr>
<td></td>
<td>44.0</td>
<td>41.0</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>43.0</td>
<td>44.0</td>
<td>1.4</td>
</tr>
</tbody>
</table>

### Serum Albumin (g/L)

<table>
<thead>
<tr>
<th></th>
<th>&lt; 38</th>
<th>≥ 38</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>49 (5%)</td>
<td>964 (95%)</td>
</tr>
<tr>
<td></td>
<td>42.0</td>
<td>43.1</td>
</tr>
</tbody>
</table>

### Serum Creatinine (nmol/L)

<table>
<thead>
<tr>
<th></th>
<th>35.5 - 61.0</th>
<th>62.0 -70.0</th>
<th>71.0 -79.5</th>
<th>80.0 - 698</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>280 (27.5%)</td>
<td>298 (29.0%)</td>
<td>223 (22.0%)</td>
<td>221 (22.0%)</td>
</tr>
<tr>
<td></td>
<td>41.5</td>
<td>42.5</td>
<td>45.3</td>
<td>44.1</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>1.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

### Miles Walked/day

<table>
<thead>
<tr>
<th></th>
<th>0 mile/day</th>
<th>0- 0.5 mile/day</th>
<th>&gt; 0.5 mile/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>263 (25.0%)</td>
<td>395 (38.0%)</td>
<td>367 (37.0%)</td>
</tr>
<tr>
<td></td>
<td>41.3</td>
<td>42.5</td>
<td>45.0</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>0.8</td>
<td>1.0</td>
</tr>
</tbody>
</table>

---

1 *P-value* for individual comparisons to the referent group that is based on Dunnett multiple testing. 2 *P-value* for Trend.*Quintiles; **Quartiles

3 Reference group, P-values are calculated with Dunnett adjustment for multiple comparisons.
## TABLE 6 Multiple linear regression, stratified by sex (n= 1542)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women (n=966)</th>
<th>Men (n=404)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td>Age</td>
<td>0.15</td>
<td>0.07</td>
</tr>
<tr>
<td>Seasons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fall vs. summer</td>
<td>-1.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Winter vs. Summer</td>
<td>-5.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Spring vs. Summer</td>
<td>-8.40</td>
<td>1.3</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.21</td>
<td>0.07</td>
</tr>
<tr>
<td>vitamin D supplement use</td>
<td>5.0</td>
<td>1.1</td>
</tr>
<tr>
<td>200-400 IU vs. None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 400 IU vs. None</td>
<td>11.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Energy-adjusted dietary vitamin D (IU/day)</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Serum Creatinine (nmol/L)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum Albumin (g/L)</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Physical activity score</td>
<td>0.15</td>
<td>0.12</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never vs. Current</td>
<td>3.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Past vs. Current</td>
<td>2.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never vs. Heavy</td>
<td>3.8</td>
<td>2.5</td>
</tr>
<tr>
<td>Moderate vs. Heavy</td>
<td>4.6</td>
<td>2.5</td>
</tr>
</tbody>
</table>
RESEARCH DESIGN AND METHODOLOGY

The work presented in this dissertation involves collaboration among investigators with expertise in nutritional epidemiology and biochemistry, and statistical methodologies for clinical research. Using epidemiologic methods, we aimed to understand the association of between vitamin D status and CVD risk factors among Puerto Rican adults in Boston.

The parent study for the epidemiologic analyses is the Boston Puerto Rican Health Study, an ongoing longitudinal study to examine the associations among social and psychological stress, nutrition, allostatic load, and measures of depression, cognitive function, and physical disability in a cohort of older Puerto Ricans, aged 45-75 years, living in the Greater Boston, MA. Consenting participants returned to the Human Nutrition Research Center on Aging (HNRCA) at Tufts University at the end of 2 years for measurements of bone and body composition. For this analysis there were 1505 observations from baseline, 1276 observations from a 2-year time point, and 970 bone and body composition measurements.

**Study Population and Data Collection**

Participants were identified from areas of high Hispanic density in the Boston metropolitan area, as specified in the year 2000 Census. Blocks with 10 or more Hispanic persons aged 45 and older were selected for enumeration from census tracks that contain at least 25 Puerto Rican adults in the specified age range. After complete block enumeration (blocks were visited up to 5 or 6 times, and on weekends and evenings to
maximize identification of all relevant households), households with at least one Puerto Rican adult between the ages of 45-75 years were identified; one qualified individual was randomly selected from each household, and invited to participate. Only those participants who were unable to answer questions due to serious health conditions or who were not able to provide reliable information for other reasons such as dementia or alcoholism, were excluded from participation [83].

Data Collection

Baseline and 2-year visit: Informed consent was obtained during the baseline interview, and neuropsychological tests were conducted to identify participants who might require assistance (e.g., from a proxy); participants were excluded if their mini mental state examination score was <10. Enrolled participants completed additional questionnaires to capture information on general background and socioeconomic status, dietary intake, food security, health history, health behaviors, health insurance, medical conditions and use of medication, activities of daily living instrument, migration status, stress, depressive symptomology, and cognitive function. Moreover, anthropometric and blood pressure measures, and physical function tests, were completed. Participants were provided with urine collection containers, two saliva tubes with procedures for collection, and instructions for the next day’s 12-hour-fasting blood draw. The next day, a phlebotomist collected 12-hour fasting blood, urine, and saliva samples. Samples were analyzed at the Nutrition Evaluation Laboratory (NEL) at Tufts University; blood samples were immediately cooled to 4°C and plasma separated within 4 hours in a refrigerated centrifuge.
Bone visit protocol: During the 2-year follow-up visit, an appointment was made for participants to visit the HNRCA to obtain bone density and body composition measurements and an additional blood draw, and to complete additional questionnaires on sun exposure, family history of osteoporosis, and history of fracture. Participants were provided with written instructions for the urine collection, taking PABA tablets, and for fasting. Two days before their scheduled visit, participants received a reminder phone call and reinforced instructions for urine collection and overnight fasting. They were asked to complete the 24-hour urine collection in two containers, one for each 12 hours. All laboratory measurements for this study were performed by the NEL.

Covariates Assessments and Definition

Serum 25(OH)D concentration was measured with an $^{125}$I radioimmunoassay kit (DiaSorin Inc, Stillwater, MN 55082) as specified by the manufacturer (68100E). Overall adiposity was assessed from whole-body DXA scans (DXA; Lunar Prodigy; GE Healthcare, Madison, WI) measured during the bone visit. Regional body composition measures (android fat mass (grams and %), gynoid fat mass (grams and %), and android/gynoid fat mass ratio were assessed with use of ENCORE software, version 12.2. Weight, and waist and hip circumferences were measured in duplicate. Weight was measured using a clinical scale (Toledo Weight Plate, Model I5S, Bay State, and Systems Inc. Burlington, MA). Knee height was measured with a Harpenden pocket stadiometer (also used for standing height). Knee height was used to estimate standing height for participants who could not stand, or who had a spinal curvature. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared [84, 85].
Laboratory Assays

Serum high sensitivity C-reactive protein (hsCRP) was measured with a solid-phase, enzyme-labeled chemiluminescent immunometric assay (Immulite 1000, Diagnostic Products Corporation (DCP) Los Angeles, CA 90045-5597), as specified by the manufacturer (PILKCR-7, 2003-11-25). The intra- and inter-assay CV% were 6.0% and 7.3% respectively.

Serum TNF-α & IL-6 were each measured by non-cross-reacting enzyme-linked immunoassays (ELISA), with use of monoclonal and polyclonal antibodies for the analysis of specific cytokine antigens (Quantikine ELISA, R&D Systems, Minneapolis, MN, USA). At the NEL, for IL-6, the intra-assay CV% is (1.6-4.2%) and the inter-assay CV% is (3.3-6.4%). For TNF-α, the intra-assay CV% is 5.3-8.8% and the inter-assay CV% is (10.8-16.7%). Plasma cholesterol was measured with EDTA plasma using an enzymatic endpoint reaction in a Cobas Mira Analyzer with Roche Diagnostics Item #44307. Ciba Corning Normal and Abnormal assayed control serum (Items #9702 and #9705) and Sigma Diagnostics elevated lipid control (Item #L1008) were used as controls [86]. HDL-C (mg/dL) was analyzed using EDTA plasma with the enzymatic endpoint reaction in a Cobas Mira Analyzer with Roche Diagnostics (Item #44120), and was calibrated with a Roche Diagnostics Cobas Calibrator with Roche Diagnostic Cholesterol Reagent (Item #44307) Dextran Sulfate-Magnesium Precipitating Reagent. The standard was Roche Diagnostics Cobas Calibrator Serum, (Item #44157), and the controls were Sigma Assayed Bi-Level HDL-C Cholesterol Controls, (Items #H4009 and #H5009) (Allan, Poon et al. 1974; Warnick, Benderson et al. 1982). LDL-C was calculated with use of the Friedewald formula, except in the cases when triglyceride (TG) concentration
exceed 400 mg/dL [87]. TG was analyzed with an enzymatic endpoint reaction on the Olympus AU400e with Olympus Triglyceride Reagents (OSR6033) (Olympus America Inc., Melville, NY).

**Potential Covariates:**

Potential covariates include age (continuous), sex (categorical), socio-demographic parameters such as education (categorical), income (continuous), smoking status (categorical), alcohol intake (categorical), total energy intake (continuous), physical activity (continuous/categorical). Information was obtained from all participants on the following variables: age, education, and household income. This questionnaire was designed based on questions from NHANES III [88], HHANES [89], the National Health Interview Survey Supplement on Aging [90], and refined during our previous field experience. Health-related behaviors: Information about current and past use of alcohol and smoking and about physical activity were obtained. Seasonality was defined 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.

Diabetes was considered present if fasting blood glucose exceeded 125 mg/dL or if the participant was being treated with insulin or oral hypoglycemic agents. Serum glucose was measured at baseline and 2 years, using an enzymatic, kinetic reaction on the Olympus AU400e with Olympus Glucose Reagents (Olympus America Inc., Melville, NY) at the NEL.
Data Entry and Quality Control

Participants were coded with a subject identification number. All data were entered and managed at the BPRHS center at Northeastern University on password-protected computers. Data entry reports were generated periodically to detect and minimize missing data and data entry errors. When missing data were identified in questionnaires, participants were contacted by phone within two weeks to retrieve the information. Data were backed up each day and stored on our central server using unique subject identification numbers.

Power and sample size:

The BPRHS study is ongoing and currently has baseline and 2 year-data through home visits on 1502 and 1276 participants, respectively. In addition, we have bone and body composition measurements on 970 subjects. The proposed sample size estimates were selected to assure that we have at least 90% power to detect expected associations that are reliable but consistent with published studies. We derived our sample size estimates and the estimated power from the standard normal approximation to the Fisher logarithmic transformation for linear correlations, which are likely conservative given that multiple linear regression models generally provide greater precision, smaller standard errors, and greater power.
STATISTICAL ANALYTICAL PLAN

Analyses were conducted with SAS© version 9.3 (SAS Institute Inc., Cary, NC). Formal hypothesis testing was two-sided, with a nominal type I error of 0.05. We assessed the modeling assumptions of non-linearity, and potential outliers and influential data were considered. Plots and graphs were used to summarize the distributions of primary analytic variables and other descriptive data. For all aims, results were summarized using regression estimates, p-values, and 95% CI. Geometric means are reported for log-transformed variables. Analyses were adjusted for covariates, including sex, age, seasonality, etc. Potential confounding and effect modifiers were checked for by including interaction term into the regression model. For continuous data, independent sample t-tests were employed; for categorical data, chi-square test for differences in proportions was employed. Only participants who met the eligibility criteria for the intervention and with complete data for the primary analytical variables were included in this analysis. Table 7 lists variables included in our statistical models.
### TABLE 7 Summary of calculated outcome measures and covariates

<table>
<thead>
<tr>
<th>Variables</th>
<th>Measurement</th>
<th>Factor</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Predictor variable</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure</td>
<td>Serum 25(OH)D</td>
<td>Continuous/categorical</td>
<td>ELISA</td>
</tr>
<tr>
<td><strong>Outcome variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro-inflammatory cytokines</td>
<td>IL-6 (mg/L)</td>
<td>1&lt;sup&gt;0&lt;/sup&gt;</td>
<td>ELISA</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td>2&lt;sup&gt;0&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>hsCRP</td>
<td>2&lt;sup&gt;0&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Adiposity</td>
<td>BMI</td>
<td>1&lt;sup&gt;0&lt;/sup&gt;</td>
<td>Measurement tools</td>
</tr>
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<td></td>
<td>WC</td>
<td>2&lt;sup&gt;0&lt;/sup&gt;</td>
<td>DXA</td>
</tr>
<tr>
<td></td>
<td>TBF</td>
<td>2&lt;sup&gt;0&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Lipid profile</td>
<td>HDL-C (mg/dL)</td>
<td>1&lt;sup&gt;0&lt;/sup&gt;</td>
<td>Lipid concentration</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>2&lt;sup&gt;0&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LDL-C</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>2&lt;sup&gt;0&lt;/sup&gt;</td>
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<tr>
<td><strong>Covariates</strong></td>
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</tr>
<tr>
<td>Other blood measure</td>
<td>Albumin (g/L)</td>
<td>Continuous/categorical</td>
<td>Kinetics</td>
</tr>
<tr>
<td></td>
<td>Creatinine (µmol/L)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Parathyroid hormone</td>
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<tr>
<td></td>
<td>White blood cell counts</td>
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<td></td>
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<tr>
<td></td>
<td>Seasonality (blood drawn)</td>
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<tr>
<td>Demographics</td>
<td>Age</td>
<td>categorical/continuous</td>
<td>Designated questionnaire &amp; measurement tools</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>education</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>poverty</td>
<td></td>
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<td></td>
<td>Smoking (y/n)</td>
<td>categorical/continuous</td>
<td></td>
</tr>
<tr>
<td>Health and Life Style Factors</td>
<td>Alcohol (gm/day)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>physical activity level</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td># Blocks walking/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVD</td>
<td>Systolic BP</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Diastolic BP</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Heart disease</td>
<td>categorical</td>
<td></td>
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<td>CVD medications</td>
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<td></td>
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<td>Vitamin D intake</td>
<td>Vitamin D dietary</td>
<td></td>
<td>FFQ</td>
</tr>
<tr>
<td></td>
<td>Vitamin D supplement</td>
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</tr>
</tbody>
</table>
The Specific Aims

**Specific Aim One:** To evaluate the association between baseline serum 25(OH)D concentration (nmol/L) and lipid profile constituent concentration (mg/dL), namely, HDL-C, LDL-C, TG, and TC over time.

*Primary Hypothesis One:* lower serum 25(OH)D concentration is associated with 2.5 year change in HDL-C concentration.

**Specific Aim Two:** To evaluate the association between serum 25(OH)D concentration with IL-6, TNF-α, and hsCRP.

*Primary Hypothesis Two:* lower serum 25(OH)D concentration is associated with higher circulating levels of inflammatory markers.

**Specific Aim Three:** to evaluate the association between serum 25(OH)D concentration and adiposity.

*Primary Hypothesis Three:* lower serum 25(OH)D concentration is associated with higher adiposity as measured by BMI, and as a secondary aim, by waist circumference (WC).

**Specific Aim Four:** to evaluate the cross-sectional association between serum 25(OH)D concentration with total and regional adiposity

*Primary Hypothesis Four:* lower serum 25(OH)D concentration is associated with higher total and regional adiposity measures as measured by dual-energy x-ray absorptiometry (DXA), including total body fat (TBF).
FIGURE 8 Diagram illustrates the relationship between serum 25(OH)D and the various CVD risk factors.
BIBLIOGRAPHY


CHAPTER TWO

Longitudinal Association of serum 25(OH)D concentration and Dyslipidemia among Older Puerto Ricans:
The Boston Puerto Rican Health Study & Boston Puerto Rican Osteoporosis Study

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Boston, MA, USA 02115

Presented at Experimental Biology 2013 (Oral Presentation)
ABSTRACT

Vitamin D deficiency is highly prevalent. Several reports suggest an association between plasma 25-hydroxyvitamin D concentration (25(OH)D) and cardiovascular disease (CVD). We examined, cross-sectionally and longitudinally, the relation between serum 25(OH)D concentration and lipid profile of 970 Puerto Rican adults, 270 men and 700 women, aged 45-75 years. Cross sectional analyses at the two year time point showed that high-density lipoprotein cholesterol (HDL-C) was significantly associated with plasma 25(OH)D ($P<0.01$); but not low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), or total cholesterol (TC).

In longitudinal analyses, baseline-25(OH)D was significantly associated with subsequent 2.5-year change in plasma HDL-C ($\beta = -0.045 \pm 0.02$, $P =0.007$) controlling for age, sex, baseline-HDL-C concentration, BMI, follow-up time, physical activity, total energy, alcohol, smoking, seasonality, poverty, and education. This relationship remained significant after adjustment for CVD medications and heart disease status. After controlling for potential confounders, baseline-25(OH)D was a significantly positive predictor of the change in plasma TG concentration over time ($\beta = 0.40 \pm 0.02$, $P =0.03$).

In conclusion, even though 25(OH)D serum concentration was positively associated with HDL-C in our cross-sectional analyses, the longitudinal analyses showed that higher 25(OH)D serum concentration did not translate into an increase of the atheroprotective HDL-C, nor a decrease in the not-atheroprotective plasma TG in this population of older Puerto Rican adults living in the Boston area.
Abbreviations:

25-hydroxyvitamin D (25(OH)D); cardiovascular disease (CVD); confidence interval (CI); United States (USA); food-frequency questionnaire (FFQ); high-density lipoprotein (HDL-C); low-density lipoprotein (LDL-C); triglycerides (TG); total cholesterol (TC); vitamin D receptor (VDR); cytochrome-P450 enzymes (CYP-450s).
INTRODUCTION

Vitamin D receptor (VDR) is found not only in bone metabolism tissues, but in a variety of tissues, making it likely that vitamin D actions extend beyond maintaining calcium homeostasis and skeletal integrity [93, 94]. Achieving optimum vitamin D status with only dietary sources is not generally feasible [26, 29-31]; rather, endogenous synthesis is the most efficient way. This occurs via the ultraviolet-β activation of 7-dehydrocholesterol producing the D₃ form, cholecalciferol [32]. This later binds to the vitamin D-binding protein (VDBP) and is transported to the liver, where it gets its first hydroxylation at the 25 position by 25-hydroxylase, producing 25-hydroxyvitamin D (25(OH)D). The second hydroxylation occurs at the 1-α position by the actions of one of the cytochrome-P450 enzymes (CYP-450s), namely, CYP27B1, a 1α-hydroxylase, converting 25(OH)D to 1α,25(OH)₂D, calcitriol. 25(OH)D is the blood biomarker that determines the status of vitamin D; it is characterized by a relatively long half-life of about 3 weeks. 1α,25(OH)₂D is the active metabolite that binds to VDR, yet it has very short half-life, up to only 12-15 hours [32, 34-36]. Vitamin D pathways are exquisitely regulated by parathyroid hormone (iPTH), serum calcium (Ca⁺) and phosphate (P_i) concentration, as well as by the negative feedback inhibition by 1α,25(OH)₂D concentration [37, 95]. iPTH under the influence of low serum calcium increases the second hydroxylation, thus, increasing 1α,25(OH)₂D [95].

Notwithstanding increased risk of osteoporosis and lower bone mineral density [40], vitamin D deficiency has been associated with increased risk of mortality [96] as well as several chronic illnesses, including: CVD, hypertension [20, 23, 62], cancer [45, 63], obesity, type-2-diabetes [48, 64] and inflammatory disorders [67, 97, 98]. In the last
decade, a wide range of scientific evidence has linked vitamin D deficiency to an increased risk of cardiovascular disease (CVD) [6, 25, 99, 100]. Dyslipidemia, which is characterized by either low plasma HDL-C, high LDL-C and/or high TG concentration, is a major risk factor for CVD concentration [101, 102]. Several epidemiological and ecological studies have shown that lower 25(OH)D concentration is associated with an unfavorable lipid profile [103-107]. However, divergent results were observed when intervention studies investigated the effect of vitamin D supplementation on a number of CVD risk factors that included the lipid profile [108].

Ethnic minorities, largely, and Puerto Ricans, explicitly, are reported to have a greater burden of health disparities compared to non-Hispanic whites, thus increasing the risk of developing chronic illness [109-111]. In their systematic review, Renzaho and colleagues showed that ethnic minorities are substantially predisposed to a greater risk of vitamin D deficiency among all age groups. They further conclude that a number of indications link vitamin D deficiency to obesity-related chronic diseases [112].

The aim of this study is to determine the longitudinal association between serum 25(OH)D concentration and lipid profile components, namely, HDL-C, LDL-C, TC, and TG (mg/dL), among participants of the Boston Puerto Rican Health Study (BPRHS) and the Boston Puerto Rican Osteoporosis Study (BPROS), living in the Greater Boston Area.
MATERIALS AND METHODS

Study Population and Data Collection

Description of the recruitment and methods of the BPRHS [83] and its ancillary study, the BPROS [113], are described in detail elsewhere. Concisely, at both time points, a bilingual interviewer conducted home visits and administrated detailed questionnaires on general background, socioeconomic status, education, household income, health and health behaviors that include medical diagnosis, detailed use of medication(s), depression and a psychological acculturation scale. They also administered questionnaires on dietary intake, food security, activities of daily living, migration status, stress, depressive symptomology, and cognitive function. Moreover, the interviewer collected information about anthropometrics, physical performance measures and blood pressure. The following day, the study phlebotomist collected 24-hr urine, 12-hr fasting blood and saliva. By December 2010, 1504 participants completed baseline visits. Once the 2-yr follow up was completed, participants were re-consented for the BPROS. By May 2013, 1257 participants completed 2-year follow-up visits and 970 of these completed the BPROS study. All study protocols were approved by the Institutional Review Boards at Tufts Medical Center and Northeastern University, and each participant provided written informed consent.

There were no significant differences in socio-demographic characteristics between those with complete or incomplete information. The present study includes complete longitudinal data for 700 Puerto Rican women and 270 Puerto Rican men.
Covariates Assessments and Definition

At each time point, in addition to the designed questionnaires, physical function tests were completed and anthropometric measures were taken. Blood pressure measures were obtained at three times during the interview. Weight, and waist and hip circumferences were measured in duplicate. Weight was measured using a clinical scale (Toledo Weight Plate, Model I5S, Bay State, and Systems Inc. Burlington, MA). Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared [27, 28]; BMI was categorized into: recommended (BMI<25), overweight (25-30), and obese (≥30) [114]. For the purpose of this analysis, vitamin D deficiency was defined as serum 25(OH)D concentration <50 nmol/L, insufficiency between 50-75 nmol/L, and sufficiency as >75 nmol/L [54, 115]. Seasonality was defined as summer, July to September; fall, October to December; winter, January to March; and spring, April to June. Smoking status was categorized into yes or no for the current behavior. Vitamin D from supplements was defined as: minimal 0-10 µg/day (200-400 IU/day); and high ≥10 µg/day (≥400 IU/day). Participant’s education was measured at baseline and defined by the cutoff point of 8th grade, while poverty was measured at each time point and defined by the U.S. Department of Health and Human Services federal poverty levels. Dietary Vitamin D intake was estimated from a semi-quantitative food-frequency questionnaire (FFQ) specifically designed for this population [91]. This FFQ has been validated against serum carotenoids [116], and vitamin B12 [117] in a Hispanic population aged ≥60 years. Those with energy intakes <600 or >4800 kilocalories and/or >10 questions blank on the FFQ were excluded from the analyses. Nutrient intakes were calculated with the Nutrition Data System for Research (NDS-R) software version 2007 (Nutrition
Coordinating Center, University of Minnesota, Minneapolis, MN, Food and Nutrient Database 2007, released 2007). Alcohol intake (gm) was captured by the FFQ. Heart disease status was self-reported. Medication classifications were based primarily on AHFS Drug Information® (AHFS DI®) pharmacologic and therapeutic classification 2005. We only used cardiovascular pharmacologic medications based on the prefix 24. Cardiovascular medication classification included: cardiac drugs, including glycoside such as digoxin; antilipemic agents-HMG CoA reductase- such as Lipitor; omega-3 and -6 supplements; hypotensive agents such as Thiazide; vasodilating-agents- including nitrates, Viagra and Dypyridamole; alpha, beta and calcium channel blockers; ACE inhibitors; angiotensin II inhibitors; and any hypertension medication [118].

**Laboratory assays**

Fasting blood samples (12-h) were drawn by a certified phlebotomist at the participant’s home on the morning after the home interview, or as soon thereafter as possible and kept at 4°C. Plasma was separated within 4 hours and was used for the analysis of plasma lipid concentration. Aliquots of the isolated serum were saved and stored at -80°C until processed. Serum 25(OH)D concentration was measured by extraction followed by 25I radioimmunoassay Packard COBRA II Gamma Counter (DiaSorin Inc., Stillwater, MN 55082 catalog # 68100E); intra- and inter-assay CV% 10.8% and 9.4%, respectively. Serum creatinine was assessed with a colorimetric, kinetic reaction using the Olympus AU400e with Olympus Creatinine Reagents (OSR6178) (Olympus America Inc., Melville, NY); intra-and inter-assay CV% were 2.0%, and 4.0%, respectively. Plasma TC, HDL-C and TG were measured enzymatically on the Olympus
AU400. For TC, Olympus reagents (OSR6116) were used; intra- and inter-assay CV% were 1.8%, and 2.2%, respectively. For HDL-C, Olympus reagents (OSR6156) were used. For TG, Olympus reagents (OSR6033) were used; intra- and inter-assay CV% were 2.8%, and 2.7%, respectively. LDL-C and VLDL-C were calculated based on the Friedewald equation from TC, HDL-C and TG i.e. VLDL=TG/5 and LDL-C =TC – (VLDL + HDL-C) [119].

**Statistical analyses:**

All statistical analyses were conducted with SAS 9.1.3 (SAS Institute Inc., Cary, NC); and all tests were 2-sided with \( P < 0.05 \) to be considered statistically significant. Dependent skewed variables were natural log-transformed to normalize their distributions before the analysis. Demographic and clinical characteristics were compared using Student’s \( t \)-test for continuous variables and the chi square tests for categorical variables. Characteristics were compared by sex using two-tailed \( t \)-tests for two independent samples, and \( \chi^2 \) test for differences in proportion. Paired \( t \)-tests were used to compare characteristics across time points. Pearson partial linear correlation analysis was used to examine relationships between serum 25(OH)D concentration with lipid profile at each time point, adjusting for age and sex. Additionally, we constructed multivariate regression analysis using general linear models to examine change in the plasma concentration of the lipid profile components, with baseline serum 25(OH)D concentration. A Risk factor model (RF-model) included: adjustment for age (y), baseline-25(OH)D (nmol/L), BMI, baseline-lipid component, follow-up time (mos), and sex in the full model. The Life-Style model (LS-model) included: physical activity, total
energy (kcal/day), current smoking status (y/n), alcohol (g/day), seasonality, poverty level, and education in addition to the RF-model covariates. To control for confounding by indications, the CVD model was used to adjust for the use of any cardiovascular medication (y/n) as well as the prevalence of heart disease (y/n) [120]. For all multivariate regression models, we checked assumptions of linearity and homogeneity by examining outcome residuals against the exposure. For all final models, we performed influence diagnostics tests to check for outliers and influential points. We also tested all of our models for any potential effect modification by sex, with covariates in our models, such as smoking, by including an interaction term with the exposure variable. Because the lipid profile might be sex-specific, we constructed sex-stratified analyses in addition to the full model. The baseline concentration of each lipid profile component was included as a covariate to all of our models to control for regression to the mean [121]. To reduce bias and augment precision, we conducted multiple imputation analysis to consider the uncertainty that missing values might convey within our dataset. We created a total of five imputed data sets with (seed= 32, 37854) using procedure (PROC MI); we included all covariates that are considered risk factors and being used in any of our models. We used (PROC MIANALYZE) to combine all the five imputed data sets, thus, producing valid statistical inferences that accounts for within and between imputation variation [122]. We found that these missing values produced no significant impact on our analysis.
RESULTS

Complete serum 25(OH)D concentration was available for 650 women and 255 men at both baseline and 2-yr. The mean ages of men and women were 59.5 ± 8.0 and 60.5 ± 8.0, respectively (Table 1). The mean follow-up time was 33.3± 12 months for women and 30.5 ± 49 months for men. At baseline, the mean serum 25(OH)D of women and men were, 44.0 ± 17.5 and 43.0 ± 16.1 nmol/L, respectively, while the 2-year values were 50.1 ± 19.0 and 47.0 ± 17.5 nmol/L, respectively. The mean 25(OH)D at 2-yr was 5.5 nmol/L higher compared to baseline (P<0.0001) (Figure 1.1). Compared to men, women had significantly higher plasma HDL-C, LDL-C and TC (mg/dL) at both time points; while men had consistently higher plasma TG (mg/dL) and serum creatinine (µmol/L). All lipid profile components increased significantly over time (P<0.0001). The prevalence of reported heart disease status went up by 9 and 12% among men and women, respectively. In addition, use of cardiovascular medication increased by 12% and 10.5% and of lipid lowering medication by 14% and 17%, for men and women, respectively. While the mean BMI values did not differ across, the two time points, women consistently had significantly higher mean BMI than men, (P<0.0001). In contrast, waist circumference did not differ by sex, but was significantly higher at 2-yr compared to baseline (P<0.0001).

There was no significant change over time in dietary vitamin D intake. However, women had higher dietary vitamin D intake (IU/day) and higher vitamin D-containing supplement use than men; while men had higher energy intake (kcal/day) than women at both time points. There was a significant reduction in total energy consumed (P < 0.001) over the 2.5 years for both men and women. The men walked significantly more
miles/day, i.e. 2 miles/day, compared to 1.4 miles/day \((P<0.0001)\) for the women; men also had higher physical activity scores and reported more current smoking and alcohol consumption than women.

Among the lipid profile components, only HDL-C at 2-yr was significantly correlated with serum 25(OH)D \((r = 0.1, P =0.01)\) (Table 1.2). In longitudinal analysis (Tables 1.3 and 1.4) baseline-25(OH)D was not associated with change in plasma HDL-C concentration controlling for age, sex, BMI and follow-up time. However, in the CVD model adjusting for physical activity, total energy (kcal/day), alcohol (gm/day), smoking (y/n), seasonality, poverty level, education, heart disease (y/n) and taking CVD medications (y/n), a significant association between baseline-25(OH)D and change in plasma HDL-C concentration emerged \((\beta = -0.04 \pm 0.02, P =0.01)\), but in an unexpected negative direction. Consistently, all three models explained about 62.5% of the variation in the 2.5-year change in plasma HDL-C. Similarly, after differences in confounders were controlled in LS-model, baseline-25(OH)D was significantly associated with change in plasma TG, but in the unexpected positive direction \((\beta = 0.40 \pm 0.02, P =0.03)\). This model explained only 30% of the variation in 2.5-year change in plasma TG. However, when controlling for the CVD related covariates, the significant association was attenuated \((\beta = 0.34, P =0.05)\). Changes in plasma LDL-C and TC also tended to be positively associated with baseline 25(OH)D, but these did not reach significance level \((P >0.05)\).

Smoking status was found to be an effect modifier of the association between baseline-25(OH)D and HDL-C. The interaction between baseline-25(OH)D and smoking status \((P =0.04)\) was significant, thus, we stratified the analysis by smoking status for all
of the lipid profile components. Interestingly, baseline-25(OH)D was inversely yet significantly associated with the change in plasma HDL-C concentration over time ($\beta = -0.06, P =0.002$) only among non-smokers, when controlling for age, sex, BMI and follow-up time. This association was strengthened after controlling for all life style covariates ($\beta = -0.06, P =0.0002$) and CVD covariates ($\beta = -0.07, P =0.0003$). The stratified models explained approximately 63 to 65% of the variation in 2.5-year change in plasma HDL-C among non-smokers. On the other hand, null associations were found among current smokers (Figure 1.2). Additionally, even though the interaction term between baseline-25(OH)D and smoking status within the LDL-C models were not significant, the stratified analysis showed that among currently smoking participants, baseline-25(OH)D was inversely associated with the change in plasma LDL-C concentration over time ($\beta = -0.25, P =0.02$), explaining roughly 42.3% of the model variation. The addition of lifestyle covariates did not attenuate the coefficient, but the significant association disappeared ($\beta = -0.23, P =0.05$) (Figure 1.3). Smoking status was not an effect modifier of the association with baseline-25(OH)D and either plasma TG or TC.

**DISCUSSION**

Although serum 25(OH)D concentration was positively associated with plasma HDL-C cross-sectionally, longitudinal analyses showed inverse associations. Baseline-25(OH)D demonstrated significant, negative association with 2.5-year change in plasma HDL-C concentration ($\beta = -0.04, P =0.01$), independent of important confounders.
including age, sex, BMI, total energy intake, physical activity score, smoking, alcohol, seasonality, poverty and education. This negative association remained after adjustment for CVD-related variables. Similar relationships were seen with plasma TG, but not with LDL-C and TC.

Our report is in contrast to several reports that indicate serum 25(OH)D concentration is positively associated with plasma HDL-C, and negatively associated with plasma LDL-C and TG [106, 107, 123-127]. In the prospective Health Professionals Follow-up Study (HPFS), Giovannucci and colleagues demonstrated a positive linear trend of LDL-C, HDL-C and TC, as well as a negative trend with plasma TG across serum 25(OH)D quartiles among 900 US men, ages 64 and older [125]. Another study showed that increasing serum 25(OH)D tertiles were independently associated with higher HDL-C and lower TG; but not TC [107]. Interestingly, some the earlier studies reported that both HDL-C and LDL-C were positively associated with serum 25(OH)D concentration, thus, both increasing plasma concentration. It is well known that increasing HDL-C and lowering LDL-C is desirable to improve CVD risk [2, 128]. A cross-sectional analysis of the Third National Health and Nutrition Examination Survey (NHANES III), found that serum 25(OH)D concentration was significantly inversely associated with TC, in 13,331 US men and women ages 20 years and older [96]. The study did not find an association between plasma HDL-C and serum 25(OH)D [96]. One longitudinal study, with a 14 year follow-up in 1762 participants, found that serum 25(OH)D and plasma TG were inversely associated [104]. Most prior studies were cross-sectional and no proof of a causal relationship can be inferred. In our longitudinal results, with control for numerous confounders, we saw different results.
Our results are in accordance with a few reports that found that serum 25(OH)D concentration may not be associated with an atheroprotective lipid profile [96, 104, 105, 123, 127]. One study found that TC was significantly associated with higher serum 25(OH)D concentration among 8,018 Norwegian men and women, aged 43-70 years [104]. Two other studies also found that plasma TG were positively associated with serum 25(OH)D; however, these latter studies were in children [123, 127].

Intervention studies in the form of placebo-controlled randomized trials (RCT) are most informative for causal relationships. Very few RCT have been conducted to assess the effect of vitamin D on lipid concentration, and most reported a null effect of vitamin D supplementation on lipid profile [108, 129-131]. One study showed that daily vitamin D supplementation (3320 IU/day) for one year significantly improved plasma TG: the supplemented group lowered plasma TG compared to placebo, -13.5% and +3% (P =0.001) correspondingly. However, the supplemented group also exhibited a significant increase in plasma LDL-C [129]. Conversely, another study did not show that a year of vitamin D supplementation, at 40,000 IU/week, changed plasma lipids in 438 overweight/obese participants [108]. Yet another study showed that vitamin D supplementation may not be atheroprotective in postmenopausal women [130]. Most of these RCT can be criticized for low sample size, which may have contributed to the null results.

Through stratification, we observed two distinct phenomena in the relationship between plasma HDL-C, in particular, and serum 25(OH)D concentration. First, only women retained the significant inverse association between serum 25(OH)D and the change in plasma HDL-C concentration during follow up, thus, mirroring the association
of the combined data. The other explanation is that due to lack of power, we could not
detect a significant association of serum 25(OH)D and the change in plasma HDL-C
concentration among men. The significant association of serum 25(OH)D on HDL-C
among non-smokers was surprising, although similar results were reported previously
[132]. Among participants of the Tromsø study, smokers had 15-20% higher serum
25(OH)D concentration than non-smokers. The pathophysiological mechanism by which
smoking may impact serum 25(OH)D concentration or the metabolism of 1,25(OH)₂D
has not been investigated. However, it has been suggested that smoking might influence
25-hydroxylase (CYP2R1) thus, lowering serum 25(OH)D concentration [133]. Another
point that might explain this paradox is that, smoking contains nicotine, which exerts
anti-inflammatory properties [134, 135].

Our results disagree with several epidemiologic and ecological studies regarding
the role of serum 25(OH)D on lipid profile as a major risk factor for CVD. One
explanation for our unexpected finding is that vitamin D induces the intestinal absorption
of dietary calcium, thus lowering calcium fecal excretion [37, 95]. This might affect the
amount of fatty acids in the feces, due to a reduction in the formation of insoluble fatty
soaps [136]. This could lead to an increased rate of the absorption of fatty acids, thus
higher plasma LDL-C and TG concentration, in particular, and TC in general [137]. In
addition, higher vitamin D could decrease cholesterol excretion because of the lower
availability of calcium within the intestine, and hence lower binding to bile acids, with
lower conversion of cholesterol to bile acids [138]. One study suggested that higher
calcium intake may lower LDL-C by increasing vitamin D A 15 week weight loss trial
showed that, among 63 healthy, overweight/obese women, the daily supplementation of
600 mg of calcium and 200 IU of vitamin D significantly improved LDL-C as well as LDL: HDL-C ratio, while improvements in HDL-C and TG approached significance [139].

Our findings should be interpreted within the context of a few limitations. First, our population is Puerto Rican, therefore our findings could be very specific to this particular admixture population, with genetic variation interacting not only with the environment, but social factors as well [140-143]. This may limit generalizability. Secondly, although we have controlled for many known potential covariates, residual confounding is still be a possibility.

In conclusion, our longitudinal analysis showed that higher 25(OH)D serum concentration did not translate into an increase of the atheroprotective plasma HDL-C, nor a decrease of plasma TG in this population of older Puerto Rican adults living in the Boston area. Moreover, our results showed that smoking may be an effect modifier of the longitudinal association of serum 25(OH)D and HDL-C, as well as LDL-C. The conflicting information in the literature suggests that more studies are needed to clarify the role of vitamin D on lipid profiles.
BIBLIOGRAPHY


48. Kwan, L.L., O.I. Bermudez, and K.L. Tucker, *Low vitamin B-12 intake and status are more prevalent in Hispanic older adults of Caribbean origin than in


<table>
<thead>
<tr>
<th></th>
<th><strong>Baseline (n=1504)</strong></th>
<th><strong>Two Year (n=970 )</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Women (n=1060)</strong></td>
<td><strong>Men (n=444)</strong></td>
</tr>
<tr>
<td>Age (y)</td>
<td>57.0 ± 7.3</td>
<td>56.0 ± 8.0</td>
</tr>
<tr>
<td>Education (&gt;8th grade %)</td>
<td>51.5</td>
<td>55.5</td>
</tr>
<tr>
<td>Poverty (%)</td>
<td>60.0</td>
<td>49.0**</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>44.0± 17.4</td>
<td>43.0 ± 16.5</td>
</tr>
<tr>
<td>Follow-up time (months)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Serum Lipids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>47.1 ± 12.2</td>
<td>40.5 ± 12.5***</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>110 ± 34.4</td>
<td>101 ± 35.2***</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>157 ± 99.0</td>
<td>175 ± 140**</td>
</tr>
<tr>
<td>Total cholestrol (mg/dL)</td>
<td>188 ± 40.5</td>
<td>174 ± 43.0***</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>68.0 ± 27.5</td>
<td>88.0 ± 45.0***</td>
</tr>
<tr>
<td>Heart disease (%)</td>
<td>20.5</td>
<td>22.0</td>
</tr>
<tr>
<td>Cardiovascular meds (%)</td>
<td>61.5</td>
<td>57.3</td>
</tr>
<tr>
<td>Lipid-lowering meds (%)</td>
<td>41.0</td>
<td>38.5</td>
</tr>
<tr>
<td><strong>Adiposity Measures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.2 ± 7.0</td>
<td>30.0 ± 5.3***</td>
</tr>
<tr>
<td>Waist Circumference(cm)</td>
<td>102.2 ± 15.4</td>
<td>103.0 ± 13.5</td>
</tr>
<tr>
<td><strong>Other Covariates</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variable</td>
<td>Male Mean ± SD</td>
<td>Female Mean ± SD</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>----------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Miles walking/day</td>
<td>1.0 ± 1.5</td>
<td>1.4 ± 2.0</td>
</tr>
<tr>
<td>Physical Activity Score</td>
<td>31.0 ±1.13</td>
<td>32.1±1.20</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>20.1</td>
<td>32.5</td>
</tr>
<tr>
<td>Alcohol drinking (%)</td>
<td>36.0</td>
<td>52.0</td>
</tr>
<tr>
<td>Alcohol intake (gm/day)</td>
<td>3.0 ± 13.5</td>
<td>14.0 ± 45.0</td>
</tr>
<tr>
<td>Vitamin D supp (&gt;200IU/day) (%)</td>
<td>30.0</td>
<td>24.1</td>
</tr>
<tr>
<td>Total vitamin D (IU/day)</td>
<td>290.5±172.0</td>
<td>280.0± 178.0</td>
</tr>
<tr>
<td>Total Energy (kcal/day)</td>
<td>2100 ± 1100</td>
<td>2750 ± 1430</td>
</tr>
</tbody>
</table>

BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D.

*Geometric mean ± SD

1 Data are presented as mean ± SD for continuous variables or as % for categorical variables

Means were compared using t-test for between sex (within each time points): *P-value <0.05, ** P-value <0.001, ***P-value <0.0001

Means were compared using paired t-test for between time points: §P-value <0.05, §§P-value <0.001, §§§P-value <0.0001

Percentages were compared using Chi-square test for between sex (within each time points): $P-value <0.05, $$$P-value <0.001, $$$$ P-value <0.0001
**TABLE 1.2:** Pearson partial linear correlation of serum 25(OH)D concentration and adiposity phenotype at baseline and 2-year follow-up

<table>
<thead>
<tr>
<th>Lipid Profile Components (mg/dL)</th>
<th>Baseline</th>
<th>Two-Year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25(OH)D</td>
<td>p-value</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.01</td>
<td>0.7</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.01</td>
<td>0.7</td>
</tr>
<tr>
<td>TC</td>
<td>-0.03</td>
<td>0.3</td>
</tr>
<tr>
<td>TG</td>
<td>0.005</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Pearson partial linear correlation coefficient (r) is age, sex and seasonality adjusted

Abbreviations: high-density lipoprotein (HDL-C); low-density lipoprotein (LDL-C); triglycerides (TG); total cholesterol (TC); 25-hydroxy vitamin D concentration (25(OH)D)
TABLE 1.3 Regression coefficients (± SE) from longitudinal linear regression analysis of lipid profile components and baseline serum 25(OH)D concentration

<table>
<thead>
<tr>
<th>Baseline 25(OH)D (nmol/L)</th>
<th>Lipid Profile Components</th>
<th>Change in HDL-C</th>
<th>Change in LDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ALL (n=866)</td>
<td>Men (n=246)</td>
</tr>
<tr>
<td></td>
<td>β ± SE</td>
<td></td>
<td>β ± SE</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>β ± SE</td>
<td>β ± SE</td>
</tr>
<tr>
<td>Risk Factor model</td>
<td>-0.025 ± 0.01</td>
<td>0.1</td>
<td>-0.01 ± 0.02</td>
</tr>
<tr>
<td>Life-Style model</td>
<td>-0.045 ± 0.01</td>
<td>0.007</td>
<td>-0.04 ± 0.03</td>
</tr>
<tr>
<td>CVD Model</td>
<td>-0.04 ± 0.02</td>
<td>0.01</td>
<td>-0.035 ± 0.03</td>
</tr>
</tbody>
</table>

Risk factor model: adjusted for age, BMI, baseline-lipid-component (mg/dL), follow-up time (mos), and sex, in the case of the combined data.
Life-Style model: adjusted for model 1 variables physical activity, total energy (kcal/day), alcohol, smoking, seasonality, poverty level, education and sex in the case of the combined data

CVD model: adjusted for model 1+ 2 variables plus heart-disease (y/n), taking cardiovascular agents (y/n) and sex in the case of the combined data
TABLE 1.4 Regression coefficients (± SE) from longitudinal linear regression analysis of lipid profile components and baseline serum 25(OH)D concentration

<table>
<thead>
<tr>
<th>Baseline 25(OH)D (nmol/L)</th>
<th>Lipid Profile Components</th>
<th>Change in TG</th>
<th>Change in TC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALL (n=844)</td>
<td>Men (n=228)</td>
<td>Women (n=615)</td>
</tr>
<tr>
<td>Risk Factor model</td>
<td>β ± SE</td>
<td>p</td>
<td>β ± SE</td>
</tr>
<tr>
<td>0.22</td>
<td>0.2</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>± 0.2</td>
<td>± 0.5</td>
<td>± 0.15</td>
<td>± 0.15</td>
</tr>
<tr>
<td>Life-Style model</td>
<td>0.4</td>
<td>0.03</td>
<td>1.0</td>
</tr>
<tr>
<td>± 0.2</td>
<td>± 0.5</td>
<td>± 0.17</td>
<td>± 0.17</td>
</tr>
<tr>
<td>CVD Model</td>
<td>0.34</td>
<td>0.05</td>
<td>1.0</td>
</tr>
<tr>
<td>± 0.2</td>
<td>± 0.5</td>
<td>± 0.15</td>
<td>± 0.15</td>
</tr>
</tbody>
</table>

Risk factor model: adjusted for age, BMI, baseline-lipid-component, time f/up (mos), and sex in the case of the combined data. Life-Style model: adjusted for model 1 variables physical activity, total energy (kcal/day), alcohol, smoking, seasonality, poverty level, education and sex in the case of the combined data.
CVD model: adjusted for model 1 variables plus heart-disease (y/n), taking cardiovascular agents (y/n) and sex in the case of the combined data.¹
FIGURE 1.1 Prevalence of low serum 25-hydroxyvitamin D concentration among Puerto Rican men (n=254) and women (n=667). Serum 25(OH)D concentration was categorized as: severe deficiency $\leq 25$ nmol/l; deficiency $25-50$ nmol/l, insufficiency $50-75$ nmol/L, and sufficiency $\geq 75$ nmol/L. Values are percentage of individuals.
FIGURE 1.2 The association between serum (25)OH at baseline and the change in HDL a (mg/dL) over time modified by smoking.
M1: adjusted for age, BMI, baseline-lipid-component (mg/dL), time f/up (mos), and sex.
M2: adjusted for model 1 variables physical activity, total energy (kcal/day), alcohol, seasonality, poverty level, education.
M3: adjusted for model 1 + 2 variables plus heart-disease (y/n), taking cardiovascular agents (y/n)
FIGURE 1.3 The association between serum (25)OH at baseline and the change in LDL among others over time modified by smoking.
M1: adjusted for age, BMI, baseline-lipid-component (mg/dL), time f/up (mos), and sex.
M2: adjusted for model 1 variables plus physical activity score, total energy (kcal/day), alcohol, seasonality, poverty level, education
M3: adjusted for model 1+ 2 variables plus heart-disease (y/n), taking cardiovascular agents (y/n)
CHAPTER THREE

An Inverse Association of Vitamin D Serum Concentration and Inflammatory Markers

The Boston Puerto Rican Osteoporosis Study

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ABSTRACT

Vitamin D deficiency has been linked to obesity, metabolic syndrome and autoimmune diseases. Several reports suggest an association between serum 25-hydroxy vitamin D concentration (25(OH)D) and inflammatory markers. We cross-sectionally examined the relation between serum 25(OH)D concentration and serum inflammatory markers in 963 Puerto Rican adults: 268 men and 695 women, aged 45-75 years. After adjusting for age, sex, and seasonality, serum 25(OH)D was significantly negatively associated with tumor necrosis factor-alpha (TNF-α) and Interleukin 6 (IL-6) (P<0.01 and P<0.04, respectively), but not with high-sensitivity C-reactive protein (hsCRP). These associations remained significant adjusting for waist circumference (WC), total body fat (TBF), and abdominal fat mass; and for smoking status, alcohol intake, vitamin D supplementation and physical activity. In conclusion, lower serum 25(OH)D concentration was significantly associated with higher concentration of inflammatory markers in this population of older Puerto Rican adults living in the Boston area. Longitudinal studies are needed to clarify causal pathways.
**Abbreviations:**

25-hydroxyvitamin D concentration (25(OH)D); android fat-mass (AFM); cardiovascular disease (CVD); confidence interval [CI]; dual-energy x-ray absorptiometry (DXA); gynoid fat-mass (GFM); high-sensitivity C-reactive protein (hsCRP); Human tumor necrosis factor-alpha (TNF-α); Interleukin 6 (IL-6); Life-style model (LS-model); Non-steroidal anti-inflammatory drugs (NSAIDs); Obesity model (O-model); Intact parathyroid hormone (iPTH); percent body fat (%TBF); Risk factor model (RF-model); total body fat (TBF); white blood cell (WBC); food-frequency questionnaire (FFQ).
INTRODUCTION

It is well recognized that vitamin D actions extend beyond calcium metabolism and bone health [93, 94]. As a steroid hormone, vitamin D functions as a gene modulator for several hundred genes. It regulates both-directly and indirectly the proliferation and differentiation of immune cells including macrophages, monocytes and lymphocytes; initiating a phosphorylation cascade of mitogen-activated protein kinases (MAPK) and extracellular signal-regulated kinase (ERK); potentially participating in a cross-talk with the genomic pathway to modulate gene expression [37-39, 144]. The expression of VDR in various types of cells, including immune cells, supports the assumption that vitamin D may exert some immune modulator actions [4, 145, 146]. To date, a wide range of immune responses have been reported for vitamin D, including the down regulation of a number of inflammatory responses, such as Interleukin 6 (IL-6), Human tumor necrosis factor-alpha (TNF-α); and interferon-γ (IFN-γ) [4, 72, 145, 147] as well as high-sensitivity C-reactive protein (hsCRP), a non-specific inflammatory marker [148, 149]. However, most of these reports are from animal studies, along with some in vitro work [147, 150-152]. Few have investigated the association of vitamin D and inflammation in humans [98, 153-155]. One observational study showed that serum 25(OH)D concentration was inversely associated with TNF-α in women, after controlling for potential covariates [154]. Another study showed that not only TNF-α, but also IL-6 and hsCRP, were inversely associated with 25(OH)D [98]. In a double-blind, randomized, placebo-controlled trial (RCT), nine months of daily supplementation with 2000 IU of vitamin D and 500 mg of calcium resulted in an improved cytokine profile; in particular, lower TNF-α concentration were seen compared to baseline [153]. In a large randomized intervention trial, Beilfuss and colleagues demonstrated one year of supplementation with vitamin D in the range between 40,000 to 20,000 IU/week, led to
marginally significant lower serum IL-6 concentration in the intervention arm compared to the placebo arm. Interestingly, however, they observed significantly elevated hsCRP concentration within the intervention arm [155].

Ethnic minorities generally, and Puerto Ricans specifically, carry a higher burden of health disparities compared to non-Hispanic whites; which translates to a greater risk of chronic disease [109-111]. Several studies have reported that ethnic minorities tend to have higher concentration of inflammatory marker [156]. One systematic review showed that ethnic minorities were considerably more likely to have vitamin D deficiency, across all age groups. They concluded a number of potential mechanisms link vitamin D deficiency to obesity-related chronic disease, including inflammation [112].

The aim of this study was to determine the cross-sectional association between serum 25(OH)D concentration and inflammatory markers, namely, IL-6, TNF-α, and hsCRP, among participants of the Boston Puerto Rican Osteoporosis Study (BPROS).

**MATERIALS AND METHODS**

**Study Population and Data Collection**

Recruitment and data collection for the BPROS are described in detail elsewhere [113]. In brief, a bilingual interviewer conducted home visits and administrated a questionnaire that collects information on socioeconomic status, education, household income, health and health behaviors, medical diagnoses, current medications, depression, and psychological acculturation. The interviewer measured anthropometrics, physical performance and blood pressure. The following day, the study phlebotomist collected 24-hr urine, 12-hr fasting blood and saliva.
Participants were provided with written instructions for collecting urine, taking PABA tablets, and fasting for the blood draw. Two days before the scheduled visit, participants were given a reminder phone call and reinforced instruction for urine collection and overnight fasting. Participants were asked to provide the 24-hour urine collection in two containers, one for every 12 hours. Once the 2-yr follow up visit was complete, participants were re-consented for the BPROS. They visited the Human Nutrition Research Center on Aging (HNRCA) at Tufts University for bone density and body composition measurements, and an additional blood draw. Participants then completed additional questionnaires on sun exposure, family history of osteoporosis, and history of fracture. All laboratory measurements for this study were performed by the Nutrition Evaluation Laboratory at Tufts University.

By May 2013, 1257 participants completed 2-year follow-up visits and 970 completed the BPROS study. All study protocols were approved by the Institutional Review Boards at Tufts Medical Center and Northeastern University, and each participant provided written informed consent. There were no significant differences in socio-demographic characteristics between those with complete or incomplete information. The present study includes complete data for 700 Puerto Rican women and 270 Puerto Rican men.

**Covariates and Exposure Assessments**

At each time point, in addition to the designed questionnaires, physical function tests were completed and anthropometric measures were taken. Weight, and waist and hip circumferences were measured in duplicate. Weight was measured using a clinical scale (Toledo Weight Plate, Model 15S, Bay State, and Systems Inc. Burlington, MA). Body mass index (BMI)
was calculated as weight (kg) divided by height (m) squared [84, 85]. Diastolic and systolic blood pressure were measured with an electronic sphygmomanometer (Dinamap TM Model 8260, Critikon, Tampa, FL), at three time points during home visits by trained interviewers: once near the beginning, the middle, and then near the end of the interview, after a short rest during which the subject was seated quietly. The average of the second and third readings was used in the analysis.

Education was measured at baseline and defined using the 8th grade as a cutoff point; poverty was measured at each time point and defined by the U.S. Department of Health and Human Services federal poverty levels. Dietary vitamin D intake was estimated from a semi-quantitative food-frequency questionnaire (FFQ) that was specifically designed for this population [91]. This FFQ has been validated against serum carotenoids [116], and vitamin B12 [117] in a Hispanic population aged ≥ 60 years. Those with energy intakes < 600 or > 4800 kilocalories and/or > 10 questions blank on the FFQ were excluded from the analyses. Nutrient intakes were calculated with the Nutrition Data System for Research (NDS-R) software version 2007 (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, Food and Nutrient Database 2007, released 2007). Alcohol intake (gm) was captured by the FFQ. For the purpose of this paper, vitamin D deficiency was defined as serum 25(OH)D concentration below 50 nmol/L, insufficiency between 50-75 nmol/L, and sufficiency above 75 nmol/L [26, 157].

Seasonality was defined as summer, July to September; fall, October to December; winter, January to March; and spring, April to June. Smoking status was categorized as yes or no for the current behavior. Vitamin D from supplements was defined as: minimal 0-10 µg/day (200-400 IU/day); and high ≥ 10 µg/day (≥ 400 IU/day).
Laboratory assays

Fasting blood samples (12-h) were drawn by a certified phlebotomist at the participant’s home on the morning after the home interview, or as soon thereafter as possible. Aliquots were saved and stored at -80°C until processed. Serum 25(OH)D concentration was measured by extraction, followed by $^{25}$I radioimmunoassay Packard COBRA II Gamma Counter (DiaSorin Inc., Stillwater, MN 55082 catalog # 68100E); intra- and inter-assay CV% were 10.8% and 9.4%, respectively. Intact serum parathyroid hormone (IPTH) was detected by two-site chemiluminescent enzyme-labeled immunometric assay (Diagnostic Products Corporation (DCP) Los Angeles, CA ) [158]; intra- and inter-assay CV% were 5.5%, and 7.9%, respectively. Serum creatinine was assessed with a colorimetric, kinetic reaction using the Olympus AU400e with Olympus Creatinine Reagents (OSR6178) (Olympus America Inc., Melville, NY); intra- and inter-assay CV% were 2.0%, and 4.0%, respectively. The hsCRP was measured by solid-phase chemiluminescent immunometric assay; intra- and inter-assay CV% were 6.0%, and 7.3%, respectively. Both IL-6 was and TNF-α were measured using commercial radioimmunoassay kit (R&D Systems Inc, Minneapolis, MN). For IL-6, the intra- and inter-assay CV% was 1.6-4.2 % and 3.3-6.4 %, respectively. For TNF-α, the intra- and inter-assay CV% was 5.3-8.8 % and 10.8-16.7 % respectively. WBC (mm$^3$) was measured by electronic impedance using ABX Pentra 60 C+ (Baker Instruments Corp., Allentown, PA)

Statistical analyses:

All statistical analyses were conducted with SAS 9.1.3 (SAS Institute Inc, Cary, NC); and all tests were 2-sided, with $P <0.05$ considered statistically significant. Dependent skewed variables were natural log-transformed to normalize their distributions before the analysis.
Demographic and clinical characteristics were compared using Student’s $t$-tests for continuous variables, and chi square tests for categorical variables. Characteristics were compared across sex using two-tailed $t$-tests for two independent samples, and $\chi^2$ test for differences in proportion. We applied Pearson Linear correlations to examine relationships between serum 25(OH)D concentration and the different inflammatory markers, adjusting for age and sex. Further, we applied the general linear models procedures (PROC GLM) to model the cross-sectional associations between 25(OH)D (continuous) and inflammatory markers (continuous), and adjusted for potential confounding variables. Linear trends of the inflammatory variables across tertiles of 25(OH)D were adjusted for sex, seasonality, and age as well as other potential covariates. The multivariate regression models for both IL-6 and TNF-$\alpha$ are: **Risk-Factor model (RF-model)**: adjusted for age (y), sex, seasonality, WBC count (mm$^3$), and use of non-steroidal anti-inflammatory (Y/N); the **Obesity model (O-model)**: serum iPTH (pg/ml), BMI, total-fat mass (g), and abdominal fat-mass (AFM), in addition to the prior model’s covariates; the **Life-Style model (LS-model)**: adjusted for the prior two models, in addition to physical activity, current smoking status (y/n), alcohol intake (g/d), poverty level, and education. Some models were adjusted for further covariates. The multivariate regression models for hsCRP are: **Risk-Factor model (RF-model)**: Sex, age seasonality, the use of non-steroidal anti-inflammatory medication (Y/N), energy intake (kcal/day), smoking (Y/N), and WBC count (mm$^3$) (to control for current infection). **Obesity model (O-model)**: RF-model+ BMI, total fat-mass (g), diastolic blood pressure and diabetes (Y/N). **Comprehensive Model (C-model)**: RF-model+ O-model, poverty, and education. Linear trend tests were performed by assigning each participant the median 25(OH)D for each tertile category, and using it as a continuous variable in PROC GLM. For all linear models, we checked the assumptions of linearity and homogeneity through
examining the outcome residuals against the exposure. For all final models, we performed influence diagnostics tests to check for outliers and influential points.

**RESULTS**

The mean ages of women and men were 60.3 ± 7.5 and 59.5 ± 8.0, respectively (Table 2.1). The mean serum 25(OH)D concentration for women was 50.1 ± 19.0, and was significantly higher ($P = 0.01$) than for men, 47.0 ± 17.5 nmol/L. There was no significant difference between men and women for most inflammatory markers, with the exception of hsCRP; where women had significantly higher concentration than men, 3.5± 3.3 and 2.3±3.2 mg/L, respectively. Women also had significantly higher adiposity measures than men, with the exception of WC, where no significant difference was found (although this implies higher WC for women relative to recommended WC). Puerto Rican women had significantly higher dietary vitamin D and supplement intakes, and lower total energy intake (kcal/day) than men. Puerto Rican men walked significantly more miles/day and had higher physical activity score compared to women. More men reporting current smoking and current alcohol consumption compared to women, while more women used non-steroidal anti-inflammatory drugs (NSAID) than did men 34.0% vs. 18.0%, respectively.

Both IL-6 and TNF-α concentrations showed significant inverse associations with 25(OH)D, ($r = -0.1, P =0.0005$) and ($r = -0.11, P =0.001$), respectively (Table 2.3). Per unit increase in 25(OH)D, there was a corresponding significant reduction in IL-6 ($\beta = -0.03 \pm 0.001, P =0.01$) and TNF-α ($\beta= -0.002 \pm 0.001, P =0.02$). Further adjustment for covariates of both the
obesity and the life-style models did not attenuate these significant inverse associations. Conversely, hsCRP showed no significant association with 25(OH)D in any model (Table 2.4).

We further evaluated the adjusted means of each inflammatory marker across the tertiles of serum 25(OH)D concentration. Participants in the second and third tertile of 25(OH)D had significantly lower IL-6 concentration compared to those in the lowest tertile, < 40 nmol/L, \( P = 0.04 \) and \( P = 0.003 \), respectively (Figure 2.1). A similar trend was observed for TNF-\( \alpha \); those in the highest tertile of 25(OH)D had significantly lower TNF-\( \alpha \) concentration compared to those in the lowest tertile of 25(OH)D, < 40 nmol/L (\( P = 0.007 \)) (Figure 2.2). There were significant decreasing trends in IL-6 and TNF-\( \alpha \) (\( P\text{-trend} = 0.002 \) and \( P\text{-trend} = 0.001 \)) across 25(OH)D tertiles. No significant trend was observed for hsCRP concentration (Figure 2.3)

**DISCUSSION**

The present study provides evidence of an inverse association between serum 25(OH)D and inflammatory markers, namely, IL-6 and TNF-\( \alpha \), among participants of the BPROS; irrespective of age, sex, seasonality, adiposity phenotype and lifestyle factors that include smoking, physical activity and alcohol consumption. This population, as reported before, exhibits a high prevalence of vitamin D deficiency [159]. After adjustment for potential covariates, serum 25(OH)D concentration remained significantly associated with IL-6 and TNF-\( \alpha \), but was not associated with hsCRP. These results are in accordance with several other reports on the association between lower serum 25(OH)D concentration and inflammatory markers [98, 154, 155]. These results are also consistent with *in vitro* and animal studies that showed TNF-\( \alpha \) suppression by vitamin D [97, 152, 160, 161] and IL-6 [97, 147, 162]. One RCT, in heart failure
patients, demonstrated that 9 months of daily supplementation with 2000 IU of vitamin D and 500 mg of calcium significantly lowered serum TNF-α concentration [153]. Another RCT also demonstrated a suppression of TNF-α concentration with a daily dose of a preparation that contained 3332 IU of vitamin D [129].

The lack of association between serum 25(OH)D and hsCRP is not in agreement with a number of cross-sectional reports [98, 163]. Possible explanations include that, first, hsCRP concentration can be affected by acute phase reactions due to viral infection, which may increase hsCRP by 50,000 fold. However, this is unlikely to be the case with the presence of constant yet potent stimuli [149]. Another point to emphasize is that this specific population has a high prevalence of health disparities that include metabolic syndrome, depression, and diabetes, which might mask the association [109, 164]. Additionally, in this population the mean of 25(OH)D concentration is low, with widespread vitamin D deficiency and thus, limited distribution and a very low number of sufficient levels to show significant differences. In our analysis, the adjustment of white blood cell counts, which is an indicator of current infection, did not improve the null association between hsCRP and serum 25(OH)D concentration.

Higher serum concentration of inflammatory markers such TNF-α, IL-6 and hsCRP have emerged as independent risk factors for CVD, diabetes and several autoimmune diseases, and are often correlated with clinical impairment [165, 166]. Therefore, attenuating inflammatory marker concentration may reduce risk of these conditions. When bound to its receptor, 1,25(OH)2D, the active metabolite, has the ability to attenuate the high levels of inflammatory markers by down-regulating a number of related genes [37-39, 144]. Our data suggest that serum 25(OH)D concentration explains about 6-20% of the variation in the IL-6 and TNF-α concentration, respectively, among our participants.
The findings of this report need to be understood in the context of some limitations. As a cross-sectional study, it cannot provide further insight about causation or long-term status. Longitudinal studies with two or more time points are needed to investigate the role of serum 25(OH)D concentration on inflammatory response. Although we were able to adjust for a number of known cofounders, residual confounding might still a possibility.

In conclusion, the present study supports the hypothesis that inflammatory markers such as IL-6 and TNF-α are inversely associated with serum 25(OH)D concentration in Puerto Rican adults. These findings support the need to re-examine the determination of optimal vitamin D status. However, more studies, especially longitudinal prospective studies, are needed to fully characterize the relationship between serum 25(OH)D concentration and inflammatory markers.
BIBLIOGRAPHY

6. *2010 ICD-9-CM.*

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### TABLE 2.1 Descriptive characteristic of the men and women of The Boston Puerto Rican Osteoporosis Study

<table>
<thead>
<tr>
<th>Variables</th>
<th>Women (n=699)</th>
<th>Men (n=273)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>60.3 ± 7.5</td>
<td>59.5 ± 8.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Education (above 8th grade %)</td>
<td>49.0</td>
<td>44.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Poverty (%)</td>
<td>59.0</td>
<td>49.2</td>
<td>0.009</td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>50.1 ± 19.0</td>
<td>47.0 ± 17.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Adiposity Variables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.0 ± 7.0</td>
<td>30.2 ± 5.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>104 ± 15</td>
<td>104 ± 14</td>
<td>0.4</td>
</tr>
<tr>
<td>Total Fat Mass (kg)</td>
<td>36.0 ± 1.5</td>
<td>26.5 ± 1.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Android Fat mass (kg)</td>
<td>3.4 ± 1.3</td>
<td>3.0 ± 1.4</td>
<td>0.0007</td>
</tr>
<tr>
<td>Inflammatory Markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum hsCRP (mg/L) $^\S$</td>
<td>3.5± 3.3</td>
<td>2.3 ± 3.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum TNF-α (pg/ml) $^\S$</td>
<td>3.0 ± 1.5</td>
<td>3.1 ± 1.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Serum IL-6 (pg/ml) $^\S$</td>
<td>3.2 ± 2.3</td>
<td>3.1 ± 2.1</td>
<td>0.9</td>
</tr>
<tr>
<td>WBC x10³/mm³</td>
<td>6.7 ± 2.1</td>
<td>6.8 ± 2.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Other Covariates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of NSAIDs (%)</td>
<td>34.0</td>
<td>18.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Current- smoking (%)</td>
<td>18.0</td>
<td>30.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Current- alcohol drinking (%)</td>
<td>28.2</td>
<td>42.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------</td>
<td>------</td>
<td>---------</td>
</tr>
<tr>
<td>Physical Activity Score$^\S$</td>
<td>31.3 ± 4.0</td>
<td>33.0 ± 6.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Miles walking/day$^\S$</td>
<td>1.4 ± 2.0</td>
<td>2.0 ± 2.4</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

**Dietary Measures**

<table>
<thead>
<tr>
<th>Vitamin D Supplement (&gt;200IU/day) $^#$ (%)</th>
<th>30.2</th>
<th>18.5</th>
<th>0.0002</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total vitamin D intake (IU/day)$^|$</td>
<td>288 ± 152</td>
<td>257 ± 123</td>
<td>0.002</td>
</tr>
<tr>
<td>Total energy intake (kcal/day)</td>
<td>1770 ± 825</td>
<td>2250 ± 1200</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D.

$^1$Data are presented as mean ± SD for continuous variables or as % for categorical variables

$^2$Geometric mean ± SD

Means were compared using t-tests. Percentages were compared using the Chi-square test ($\chi^2$).

$^\S$The range for the physical activity score = 0-35.5 for Puerto Rican men and 26.4 for Puerto Rican women. Range for Miles/day =0-22 for men and 0-20 miles/day for Puerto Rican women.

$^\#$The recommended dietary allowance (RDA) for vitamin D for men and women aged 19-70 years =15 ug/day [82]

$^\|$Adjusted for energy intake using ANOVA (PROC GLM; SAS Institute, Cary, NC).
**TABLE 2.2** Relationships between serum 25(OH)D concentration and inflammatory markers

<table>
<thead>
<tr>
<th>Inflammatory Markers</th>
<th>Serum 25(OH)D concentration</th>
<th>$r$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum hsCRP (mg/L)</td>
<td></td>
<td>- 0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>Serum TNF-α (pg/ml)</td>
<td></td>
<td>- 0.1</td>
<td>0.0005</td>
</tr>
<tr>
<td>Serum IL-6 (pg/ml)</td>
<td></td>
<td>- 0.11</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Pearson partial linear correlation coefficient ($r$) is age and sex adjusted

25(OH)D, 25-hydroxyvitamin D; hsCRP, high-sensitivity C-reactive protein; TNF-α, Tumor necrosis factor-alpha; IL-6, Interleukin 6.
TABLE 2.3: Adjusted associations between inflammatory markers and 25(OH)D concentration.

<table>
<thead>
<tr>
<th>Serum 25(OH)D (nmol/L)</th>
<th>IL-6</th>
<th></th>
<th>TNF-α</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
<td>p</td>
<td>β</td>
</tr>
<tr>
<td><strong>Risk Factor</strong></td>
<td>-0.003</td>
<td>0.001</td>
<td>0.01</td>
<td>-0.002</td>
</tr>
<tr>
<td><strong>Obesity</strong></td>
<td>-0.003</td>
<td>0.001</td>
<td>0.01</td>
<td>-0.0015</td>
</tr>
<tr>
<td><strong>Life Style</strong></td>
<td>-0.0024</td>
<td>0.001</td>
<td>0.03</td>
<td>-0.002</td>
</tr>
</tbody>
</table>

1Regression coefficients (± SE)

**Risk Factor Model:** Sex, age seasonality, WBC count (mm$^3$), and use of Aspirin (Y/N)

**Obesity Model:** Risk factor model + BMI, total fat-mass and android fat-mass

**Life Style Model:** Risk-factor-model+ Obesity-model, smoking and Physical Activity Score
TABLE 2.4: Regression coefficients (± SE) from multivariate regression analysis of hsCRP (mg/dL) and serum 25(OH)D concentration.

<table>
<thead>
<tr>
<th>Serum 25(OH)D (nmol/L)</th>
<th>hsCRP (mg/dL)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
<td>p</td>
</tr>
<tr>
<td>Risk Factor Model</td>
<td>0.002</td>
<td>0.002</td>
<td>0.3</td>
</tr>
<tr>
<td>Obesity Model</td>
<td>0.001</td>
<td>0.001</td>
<td>0.3</td>
</tr>
<tr>
<td>Comprehensive Model</td>
<td>0.0005</td>
<td>0.001</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Risk Factor Model**: Sex, age seasonality, WBC count (mm$^3$), and use of non-steroidal anti-inflammatory (Y/N), energy intake (kcal/day) smoking (Y/N).

**Obesity Model**: Risk factor model + BMI, total fat-mass (g) and android fat-mass (g), diastolic blood pressure and diabetes (Y/N)

**Comprehensive Model**: Risk-factor-model+ Obesity-model, poverty, and education.
FIGURE 2.1 Adjusted means (±SEM) of IL-6 concentration (pg/ml) by tertiles of adjusted serum 25(OH)D concentration among 893 Puerto Rican men and women. The LS-Means adjusted for sex, age, BMI, total fat-mass, android fat-mass, physical activity, and smoking.

* Different from referent group (25(OH)D < 40 nmol/L) (Dunnett adjustment for multiple comparisons; $P < 0.05$)
FIGURE 2.2 Adjusted means (±SEM) of TNF-α concentration (pg/ml) by tertiles of adjusted serum 25(OH)D concentration among 893 Puerto Rican men and women. The LS-Means adjusted for sex, age, BMI, total fat-mass, android fat-mass, physical activity, and smoking.

* Different from referent group (25(OH)D < 40 nmol/L) (Dunnett adjustment for multiple comparisons; P < 0.05)
FIGURE 2.3 Adjusted means (±SEM) of hsCRP concentration (mg/dL) by tertiles of adjusted serum 25(OH)D concentration among 893 Puerto Rican men and women. The LS-Means adjusted for sex, age, BMI, total fat-mass, WC count, use of NSAID’s, physical activity, and smoking. Dunnett adjustment for multiple comparisons; \( P > 0.05 \)
Poster Presentation:

PDF of the presentation of Chapter Two data at Experimental Biology 2013
CHAPTER FOUR

Longitudinal Association of Vitamin D Serum Concentration & Adiposity Phenotypes

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ABSTRACT

Vitamin D deficiency is prevalent, especially among ethnic minorities. There are several cross-sectional studies on the association between serum 25-hydroxy vitamin D concentration (25(OH)D) concentration and adiposity measures such as body mass index (BMI), and waist circumference (WC). We examined the longitudinal association between BMI and WC on serum 25(OH)D concentration in 970 Puerto Rican adults: 270 men and 700 women, aged 45-75 years. Our analyses showed negative linear correlations at two time points between BMI (\(r = -0.1, P = 0.02\)) and WC (\(r = -0.1, P = 0.009\)) and serum 25(OH)D concentration. Multivariate analysis showed that baseline-BMI was significantly inversely associated with change in serum 25(OH)D concentration (\(P < 0.02\)), controlling for age and sex. This association remained significant after adjusting for vitamin D supplement use, smoking, physical activity and alcohol intake. Baseline-WC was not significantly associated with change in serum 25(OH)D concentration. The major finding of the present study is that obesity appears to have a negative consequence on serum 25(OH)D concentration in this population of older Puerto Rican adults living in the Boston area, which might accelerate vitamin D deficiency status.
ABBREVIATIONS:

25-hydroxyvitamin D (25(OH)D); cardiovascular disease (CVD); confidence interval (CI);
United States (USA); food-frequency questionnaire (FFQ); body mass index (BMI)
INTRODUCTION

The prevalence of overweight and obesity has increased among all socioeconomic classes, leading to an increased risk of associated mortality and morbidity [167]. A recent report suggested that vitamin D deficiency is another re-emerging global health problem that might further contribute to an increase in the prevalence of related-chronic diseases [168]. Several factors are known to influence 25-hydroxy vitamin D [25(OH)D] concentration, including age, sex, sun exposure, skin pigmentation, ethnicity, adiposity, and physical activity [55, 56].

Ethnic minorities, generally, and Puerto Ricans, specifically, shoulder the greatest burden of health disparities, including a greater risk of chronic disease [109-111]. In the United States obesity continues to rise, specifically among minorities such as Hispanics, i.e. 39.1% [169, 170]. Additionally, a systematic review concluded that ethnic minorities have a significantly higher risk of vitamin D deficiency among all age groups. They further concluded that there is a number of indications that link vitamin D deficiency to obesity-related chronic disease [112].

The association between vitamin D deficiency and obesity has been consistently documented, indicating that obesity is inversely associated with serum 25(OH)D [132, 171-173]. One of the postulated theories to explain this phenomenon is the sequestering of vitamin D into adipose tissue, thus affecting its bioavailability for further metabolism [174, 175]. Another is that obese individuals tend to have lower outdoor physical activity and greater time spent indoors [173]. In the cross-sectional Tromsø study, BMI was significantly inversely associated with serum 25(OH)D [132]. The Insulin Resistance Atherosclerosis (IRAS) Family study reported that among Hispanics, 25(OH)D was inversely associated with adiposity phenotype; however, it was not associated with 5-yr change in adiposity [176]. Another study demonstrated that serum
25(OH)D concentration was inversely associated with BMI ($r = -0.18$, $P = 0.005$) and WC ($r = -0.14$, $P = 0.03$) [177].

The aim of this study was to determine the longitudinal association between serum 25(OH)D concentration in relation to BMI and WC among participants of the Boston Puerto Rican Health Study (BPRHS) and the Boston Puerto Rican Osteoporosis Study (BPROS) in the Greater Boston Area.

**MATERIALS AND METHODS**

**Study Population and Data Collection**

Recruitment and methods of the BPRHS [83] and its ancillary study, the BPROS [113], are described in detail elsewhere. Concisely, at both time points, a bilingual interviewer conducted home visits and administrated detailed questionnaires on general background, socioeconomic status, education, household income, health and health behaviors that include medical diagnosis, detailed use of medication(s), depression and a psychological acculturation scale. They also answered questionnaires on dietary intake, food security, activities of daily living, migration status, stress, depressive symptomology, and cognitive function. Moreover, the interviewer collected information about anthropometrics, physical performance measures and blood pressure. The following day, the study phlebotomist collected 12-hr urine, 12-hr fasting blood and saliva. By December 2010, 1504 participants completed baseline visits. Once the 2-yr follow up was completed, participants were re-consented for the BPROS. By May 2013, 1257 participants completed 2-year follow-up visits and 970 of which completed the BPROS study. These participants contributed 24-hr urine measures as well. All study protocols were approved
by the Institutional Review Boards at Tufts Medical Center and Northeastern University, and each participant provided written informed consent.

There were no significant differences in socio-demographic characteristics between those with complete or incomplete information. The present study includes complete longitudinal data for 700 Puerto Rican women and 270 Puerto Rican men.

**Covariates Assessments and Definition**

Enrolled participants completed questionnaires that captured information on general background and socioeconomic status, dietary intake, food security, health history, health behaviors, health insurance, medical conditions and detail use of medication(s), activities of daily living instrument, migration status, stress, depressive symptomology, and cognitive function. Moreover, anthropometric and blood pressure measures, and physical function tests, were completed. Weight, and waist and hip circumferences were measured in duplicate. Weight was measured using a clinical scale (Toledo Weight Plate, Model I5S, Bay State, and Systems Inc. Burlington, MA). Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared [27, 28]; BMI was categorized into: recommended (BMI <25), overweight (25-30), and obese (≥ 30) [43]. For the purpose of this analysis, vitamin D deficiency was defined as serum 25(OH)D concentration below 50 nmol/L, insufficiency between 50-75 nmol/L, and sufficiency above 75 nmol/L [44, 45]. Seasonality was defined as summer, July to September; fall, October to December; winter, January to March; and spring, April to June. Smoking status was categorized into yes and no for the current behavior. Vitamin D from supplements was defined as: minimal 0-10 µg/day (200-400 IU/day); and high ≥ 10 µg/day (≥ 400 IU/day).
Education level was measured at baseline and defined using the cutoff point of 8th grade while poverty was measured at each time point and defined following the U.S. Department of Health and Human Services. Dietary vitamin D intake was estimated from a semi-quantitative food-frequency questionnaire (FFQ) that was specifically designed for this population [46]. This FFQ has been validated against serum carotenoids [47], and vitamin B12 [48] in a Hispanic population aged ≥ 60 years. Those with energy intakes < 600 or > 4800 kilocalories and/or > 10 questions blank on the FFQ were excluded from the analyses. Nutrient intakes were calculated with the Nutrition Data System for Research (NDS-R) software version 2007 (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, Food and Nutrient Database 2007, released 2007). Alcohol intake (gm) was captured by the FFQ. Heart disease status was self-reported by the participants.

**Laboratory assays**

Fasting blood samples (12-h) were drawn by a certified phlebotomist at the participant’s home on the morning after the home interview, or as soon thereafter as possible. Aliquots were saved and stored at -80°C until processed. Serum 25(OH)D concentration was measured by extraction followed by $^{25}$I radioimmunoassay Packard COBRA II Gamma Counter (DiaSorin Inc., Stillwater, MN 55082) catalog # 68100E with intra-assay and inter-assay CV% of 10.8% and 9.4%, respectively. Serum creatinine was assessed with a colorimetric, kinetic reaction using the Olympus AU400e with Olympus Creatinine Reagents (OSR6178) (Olympus America Inc., Melville, NY) with intra-assay and inter-assay CV% of 2.0%, and 4.0%, respectively.
Statistical analyses:

All statistical analyses were conducted with SAS 9.1.3 (SAS Institute Inc., Cary, NC); and all tests were 2-sided with \( P < 0.05 \) considered statistically significant. Dependent skewed variables were natural log-transformed to normalize their distributions before the analysis. Demographic and clinical characteristics were compared using student \( t \)-test for continuous variables and chi square for categorical variables. Characteristics were compared by sex using two-tailed \( t \)-test for two independent samples, and with \( \chi^2 \) test for differences in proportion. Paired \( t \)-test was used to compare characteristics across time points. Pearson partial linear correlation analysis was used to examine the relationship between serum 25(OH)D concentration with adiposity phenotype at each time point, adjusting for age and sex. We constructed general linear models (PROC GLM) to model longitudinal associations between the change in 25(OH)D concentration (continuous) and adiposity phenotype (continuous). Because adiposity phenotype might be sex-specific, we constructed sex-stratified analyses in addition to the full model, even if the by-sex interaction term was not significant. Model-1 was adjusted for age (y), baseline-25(OH)D (nmol/L), serum creatinine (µmol/L), follow-up time (months), and sex in the full model. In addition to model-1 covariates, model-2 included seasonality, dietary vitamin D & supplements intake. Model-3 was adjusted for model-1 and 2 covariates plus physical activity, alcohol intake (g), current smoking status (y/n), poverty level and education. Least-square means and linear trends of 25(OH)D across the BMI categories were assessed using PROC GLM and adjusted for multiple comparisons using Dunnett’s adjustment, with the lowest group as the reference group. The model was repeated for each time point, independent of the other. The model was adjusted for age (y), sex, seasonality, current smoking status (y/n), total energy intake (kcal/d), physical activity, serum creatinine, dietary vitamin D & vitamin D supplement intake.
For all multivariate regression models, we checked the assumptions of linearity and homogeneity through examining the outcome residuals against the exposure. For all final models, we performed influence diagnostics tests to check for outliers and influential points. We also tested all of our models for potential effect modifications by sex, with covariates such as smoking, by including interaction terms in the model. The baseline concentration of each lipid profile component was included as a covariate to all of our models to control for regression to the mean [121]. To reduce bias and augment precision, we conducted multiple imputation analysis to consider the uncertainty that missing values might convey within our dataset. We created a total of five imputed data sets with (seed= 32, 37854) using procedure (PROC MI); we included all covariates that are considered risk factors and being used in any of our models. We used (PROC MIANALYZE) to combine all the five imputed data sets, thus, producing valid statistical inferences that accounts for within and between imputation variation [122]. We found that these missing values produced no significant impact on our analysis.

**RESULTS**

Serum 25(OH)D concentration was available for 650 women and 255 men at baseline and 2-yr. The mean ages of men and women were 59.5 ± 8.0 and 60.5 ± 8.0, respectively. The mean follow-up time for women was 33.3± 12 months while men had a mean follow-up time equal to 30.5 ± 49 months. At baseline, the mean serum 25(OH)D of the women and men was, 44.0 ± 17.5 and 43.0 ± 16.1 nmol/L, respectively, while the 2-year 50.1 ± 19.0 and 47.0 ± 17.5 nmol/L, respectively. Among all participants, the mean of 25(OH)D at 2-yr was 5.5 nmol/L higher compared to baseline \( (P<0.0001) \). Compared to men, women at the 2-yr time point had
significantly higher serum 25(OH)D ($p=0.02$). Figure 3.1 shows the monthly distribution of serum 25(OH)D for the two time points.

While BMI values did not differ between the two time points within each sex, Puerto Rican women did exhibit significantly higher BMI compared to Puerto Rican men, i.e. 33.0 and 30 ($P < 0.0001$), respectively. On the contrary, WC did not differ between both sex, but it was significantly higher at 2-yr compared to baseline ($P < 0.0001$). No significant change in the participants’ dietary vitamin D intake. However, Puerto Rican women had higher dietary vitamin D intake (IU/day) and higher use-rate of VD-containing supplements. Overall men had higher energy intake (kcal/day) compared to women at both time points. On the other hand, there was a clear significant reduction in total calories consumed, i.e. $\mu = 365$ kcal/day ($P < 0.001$) within the 2.5 years of follow-up.

Table 2 shows the age and sex Pearson linear correlation coefficients of serum 25(OH)D concentration and adiposity phenotype. BMI and WC showed significant negative linear correlation with serum 25(OH)D at both time points. Baseline-BMI ($r = -0.1, P = 0.006$) and Baseline-WC ($r = -0.1, P = 0.02$), 2yr-BMI ($r = -0.1, P = 0.01$), 2yr-WC ($r = -0.1, P = 0.01$).

The longitudinal linear regression model (Table 3) showed that baseline-BMI was significantly inversely associated with change in serum 25(OH)D concentration ($\beta = -0.17, P = 0.04$) controlling for age, sex, creatinine, and follow-up time. In model-2, the inclusion of seasonality, dietary vitamin D & supplements did not alter the regression coefficient or its significance. Nevertheless, the additional adjustment of the association for miles/day, alcohol, smoking, poverty index, and education status (model-3), strengthened the inverse association ($\beta = -0.21, P = 0.01$). The adjusted-mean serum 25(OH)D was significantly lower with increased
obesity among the participants at both time points, \( P \)-trend <0.01 and <0.002, respectively (Fig. 1). At baseline, participants with BMI \( \geq 40 \) had significantly lower 25(OH)D (38.5 \( \pm \) 1.5 nmol/L) compared to BMI \( \leq 25 \) (44.5 \( \pm \) 1.2 nmol/L), while at the 2-yr time point, participants with BMI who were classified as obese had significantly lower 25(OH)D (45.5 \( \pm \) 1.2 nmol/L) compared to BMI \( \leq 25 \) (50.1 \( \pm \) 1.5 nmol/L).

**DISCUSSION**

The results of our longitudinal analysis confirm the inverse association between low serum 25(OH)D concentration and BMI. Our observation of lower adjusted mean serum 25(OH)D concentration in obese, severely obese and morbidly obese participants is in agreement with many studies [132, 172, 176, 177]. Our longitudinal multivariate linear regression showed that baseline-BMI was negatively associated with the change in serum 25(OH)D concentration (\( P =0.01 \)), independent of covariates including age, sex, serum creatinine, physical activity, smoking, alcohol, seasonality, poverty and education. Conversely, we did not observe similar longitudinal associations with WC, although it was significantly correlated with serum 25(OH)D at both time points.

The mechanism that can explain the phenomena of low serum 25(OH)D and increased adiposity risk is not clear. A review by Earthman and colleague discusses a number of proposed theories that link Vitamin D deficiency to adiposity [178]. One theory suggests that because Vitamin D is fat-soluble, it might be sequestered within adipose tissue. They further suggest that the adipose tissue of obese individuals might have higher 25(OH)D uptake and storage relative to lean individuals [48, 178]. Another theory suggests that due to the fact that adiposity, especially
visceral adiposity, contributes to a high inflammatory state, this in turn, contributes to lower serum 25(OH)D concentration [178, 179]. Another hypothesis suggests that because of increased adiposity, there may possibly be an increase of the action of 24-hydroxylase, leading to amplified catabolism of vitamin D [178, 180]. Consequently, further research is needed to elucidate our understanding of how vitamin D may be affected by adiposity or how vitamin D affects obesity.

More than 60% of the participants of BPROS had serum 25(OH)D concentration below 50 nmol/L, which is considered deficient, according to the Endocrine Society [115]. A recent report by the National Health and Nutrition Examination Survey (NHANES) III, showed that more than 42% of their 2001-2006 participants had a vitamin D concentration below 50 nmol/L; when stratified by ethnicity, Hispanics were second highest in vitamin D deficiency with 70% [181]. Our prior report indicated that among 1502 participants of the BPRHS, more than 70% had serum 25(OH)D concentration below 50 nmol/L at baseline, concurring with the NHANES III data [159]. This greater risk of vitamin D deficiency may be related to greater risk of health disparities [182]. A report by Palacios and colleagues showed that Puerto Ricans who are residing in Puerto Rico, had higher serum 25(OH)D concentration, approximately 75 nmol/L, compared to our population, at baseline 43.4 ± 17.2 nmol/L [159] and 2yr 50.0 ± 19.0 nmol/L. This difference in serum 25(OH)D concentration is likely due to sun exposure, generally, and latitude, specifically, i.e. Puerto Rico is at 18.26° while Boston is at 42.35° [26].

The present study has limitations: we did not have direct measures of sun exposure at baseline. However, our study has substantial strengths: a total large sample size with low attrition and the use of a detailed questionnaire with an FFQ that allowed detailed information.
In summary, serum 25(OH)D concentration was inversely associated with 2.5-yr change in BMI. The adjusted-mean serum 25(OH)D concentration showed a declining trend, at both time points, with greater BMI, especially when reaching class III obesity.
BIBLIOGRAPHY


**TABLE 3.1**: Descriptive characteristic of men and women in the Boston Puerto Rican Health Study (BPRHS) & Boston Puerto Rican Osteoporosis Study (BPROS)

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n=1504)</th>
<th></th>
<th>Two Year (n=970)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women (n=1050)</td>
<td>Men (n=445)</td>
<td>Women (n=700)</td>
<td>Men (n=270)</td>
</tr>
<tr>
<td><strong>Age (y)</strong></td>
<td>57.0 ± 7.3</td>
<td>56.0 ± 8.0</td>
<td>60.3 ± 7.5</td>
<td>59.5 ± 8.0</td>
</tr>
<tr>
<td><strong>Education (&gt;8th grade %)</strong></td>
<td>51.5</td>
<td>55.5</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Poverty (%)</strong></td>
<td>60.0</td>
<td>49.0$$^s$$</td>
<td>59.0</td>
<td>48.5$$^s$$</td>
</tr>
<tr>
<td><strong>Serum 25(OH)D (nmol/L)</strong> $$^s$$</td>
<td>44.0± 17.4</td>
<td>43.0 ± 16.5</td>
<td>50.1 ± 19.0</td>
<td>47.0 ± 17.5*</td>
</tr>
<tr>
<td><strong>Follow-up time (months)</strong></td>
<td>--</td>
<td>--</td>
<td>33.3± 12</td>
<td>30.5± 49</td>
</tr>
<tr>
<td><strong>BMI (kg/m^2)</strong></td>
<td>33.2 ± 7.0</td>
<td>30.0 ± 5.3$$^s$$</td>
<td>33.0 ± 7.0</td>
<td>30.2 ± 5.5$$^s$$</td>
</tr>
<tr>
<td><strong>Waist Circumference (cm) $$^s$$</strong></td>
<td>102.2 ± 15.4</td>
<td>103 ± 14</td>
<td>104 ± 15</td>
<td>104 ± 14</td>
</tr>
<tr>
<td><strong>Miles walking/day $$^s$$</strong></td>
<td>1.0 ± 1.5</td>
<td>1.4 ± 2.0$$^s$$</td>
<td>1.4 ± 2.0</td>
<td>2.0 ± 2.4 $$^s$$</td>
</tr>
<tr>
<td><strong>Current- smoking (%)</strong></td>
<td>20.1</td>
<td>32.5$$^s$$</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Current- alcohol drinking (%)</strong></td>
<td>36.0</td>
<td>51.0$$^s$$</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Vitamin D Supplement (&gt;200IU/day) (%)</strong></td>
<td>30.0</td>
<td>24.1</td>
<td>30.2</td>
<td>18.5$$^s$$</td>
</tr>
<tr>
<td><strong>Total vitamin D intake (IU/day)</strong></td>
<td>291 ± 172</td>
<td>280 ± 178</td>
<td>288 ± 152</td>
<td>257 ± 123.$^*$$</td>
</tr>
</tbody>
</table>

BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D.

1. Data are presented as mean ± SD for continuous variables or as % for categorical variables.

Means were compared using t-test for between sex (within each time points): *P-value <0.05, ** P-value <0.001, ***P-value <0.0001.

Means were compared using paired t-test for between time points: $^sP$-value <0.05, $^{ss}P$-value <0.0001.

Percentages were compared using Chi-square test for between sex (within each time points): $^sP$-value <0.05, $^{ss}P$-value <0.001, $^{sss}P$-value <0.0001.
**TABLE 3.2** Pearson partial linear correlation of serum 25(OH)D concentration and adiposity phenotype at baseline and 2-year

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th></th>
<th>Two-Year</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25(OH)D</td>
<td>p-value</td>
<td>25(OH)D</td>
<td>p-value</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.1</td>
<td>0.006</td>
<td>-0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>WC</td>
<td>-0.1</td>
<td>0.02</td>
<td>-0.1</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Pearson partial linear correlation coefficient (r) is age and sex adjusted

Abbreviations: BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D; WC, waist circumference; AFM, abdominal fat mass; TBF, total body fat; %TBF, total body fat percentage
TABLE 3.3 Regression coefficients (± SE) of adiposity phenotypes on plasma 25(OH)D concentration

<table>
<thead>
<tr>
<th>Change in 25(OH)D (nmol/L)</th>
<th>Baseline-BMI</th>
<th></th>
<th></th>
<th>Baseline-WC</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALL</td>
<td>Men</td>
<td>Women</td>
<td>ALL</td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td></td>
<td>(n=866)</td>
<td>(n=246)</td>
<td>(n=620)</td>
<td>(n=864)</td>
<td>(n=245)</td>
<td>(n=619)</td>
</tr>
<tr>
<td>Model 1</td>
<td>-0.17 (± 0.1)</td>
<td>0.04</td>
<td>-0.25 (± 0.2)</td>
<td>0.1</td>
<td>-0.13 (± 0.1)</td>
<td>0.1</td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.22 (± 0.1)</td>
<td>0.01</td>
<td>-0.26 (± 0.2)</td>
<td>0.1</td>
<td>-0.15 (± 0.1)</td>
<td>0.1</td>
</tr>
<tr>
<td>Model 3</td>
<td>-0.22 (± 0.1)</td>
<td>0.01</td>
<td>-0.3 (± 0.2)</td>
<td>0.1</td>
<td>-0.20 (± 0.1)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Model 1: adjusted for age, baseline 25(OH)D, serum creatinine, time f/up (mos), and sex in the case of the combined data
Model 2: adjusted for model 1 variables plus seasonality, dietary vitamin D & supplements and sex in the case of the combined data
Model 3: adjusted for model 1 variables plus physical activity, alcohol, smoking, poverty level, education and sex in the case of the combined data
FIGURE 3.1 The monthly distribution of serum 25(OH)D for the participants of BPRHS at both time points
FIGURE 3.2 The adjusted means of serum 25(OH)D concentration within the categories of class III obesity LS-Means adjusted for Sex, age seasonality, miles/day, vitamin D supplements, dietary VD, smoking, and total calories. * Different from referent group (BMI $\geq 25$) (Dunnett adjustment for multiple comparisons; $P < 0.05$)
PDF of the presentation of Chapter Three data at Vitamin D Workshop 2013
CHAPTER FIVE

The Association of Total and Regional Adiposity with Serum 25-hydroxyvitamin D Concentration

The Boston Puerto Rican Osteoporosis Study (BPROS)

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ABSTRACT

Vitamin D deficiency is prevalent, especially among ethnic minorities. There are several cross-sectional studies on the association between serum 25-hydroxyvitamin D concentration (25(OH)D) and adiposity phenotype; specifically: total body fat (TBF), percent body fat (%TBF), android fat-mass (AFM) and gynoid fat-mass (GFM), using dual-energy x-ray absorptiometry (DXA). We examined this association among 970 Puerto Rican adults, 270 men and 700 women; aged 45-75, years from the Boston Puerto Rican Osteoporosis study. Our analyses showed that TBF-in kilograms-or %TBF was not significantly associated with serum 25(OH)D. Moreover, AFM showed inverse significant association with serum 25(OH)D in both Puerto Rican men and women, whereas GFM showed a positive significant association with serum 25(OH)D among Puerto Rican women only. This suggests that obesity is negatively associated with serum 25(OH)D concentration in this population of older Puerto Rican adults living in the Boston area.
ABBREVIATIONS:

25-hydroxyvitamin D concentration (25(OH)D); android fat-mass (AFM); cardiovascular disease (CVD); 95% confidence interval (95% CI); dual-energy x-ray absorptiometry (DXA); gynoid fat-mass (GFM); Life-Style model (LS-model); Obesity model (O-model); Parathyroid Hormone, Intact (iPTH); percent body fat (%TBF); Risk Factor model (RF-model); total body fat (TBF); United States (USA).
INTRODUCTION

Ethnic minorities, generally, and Puerto Ricans, specifically, are shown to carry a greater burden of health disparities than non-Hispanic whites, which results in a greater risk of chronic disease [109-111]. Obesity in the United States (USA) is on the rise, especially among minorities such as Hispanics, i.e. 38.7% (95% confidence interval [CI], 33.5%- 43.9%) [169, 170, 183]. A 2010 report by Flegal indicated that Hispanic men and women exhibit different prevalence of obesity after the age 40; 37.4% (95% CI, 29.0-45.8) and 46.6% (95% CI, 37.3-55.9), respectively [183]. Casa and colleagues found that ethnicity might be associated with moderately higher prevalence of obesity compared to non-Hispanic whites; with about a 5% higher BMI. They also reported that percentage body-fat was about 4% greater among Hispanic, relative to non-Hispanic white, women-especially in the arms and trunks areas [184].

Android obesity, or central obesity, is when the predominant fat deposition is in the abdomen, while gynoid obesity is when the predominant fat deposition occurs in the hip area [185]. Android obesity is thought to be related to increased risk of cardiovascular disease (CVD), while gynoid obesity is less related to CVD [186]. Whereas most of the research on the association between serum 25(OH)D concentration and adiposity measures have focused on BMI, it is not clear how AFM and GFM are associated with serum 25(OH)D concentration.

Vitamin D deficiency is emerging as a global health problem that may lead to an increase of chronic disease, including CVD, osteoporosis and other immune-related diseases [56, 168]. Several factors are known to influence serum 25(OH)D concentration,
including age, sex, sun exposure, skin pigmentation, ethnicity, adiposity, and physical activity [49, 55, 56]. It is suggested that some ethnic minorities are more susceptible to an increased risk of vitamin D deficiency than others [176, 187]. In a systematic review, Renzaho and colleagues discuss that ethnic minorities have a significantly higher risk of vitamin D deficiency among all age groups. They conclude that there is a number of indications linking vitamin D deficiency to obesity-related chronic disease among minorities [112]. Our prior report showed that the mean serum 25(OH)D concentration in Puerto Rican adults in the Greater Boston Area was 43.5 ± 16.5 nmol/L [159]. One postulated theory to explain this phenomenon is the sequestering of vitamin D into adipose tissue, thus affecting its bioavailability for further metabolism [49, 174, 175]. Another is that people who are obese tend to have lower outdoor physical activity, and increased time spent indoors [173]. Numerous studies have investigated the association between adiposity and serum 25(OH)D concentration, applying different indexes of adiposity [48, 60, 64, 132, 172, 173, 176, 184, 188-190]. The Longitudinal Aging Study Amsterdam showed that TBF was inversely associated with 25(OH)D [172]. A study in south-east Texas showed that among Hispanics, the mean serum 25(OH)D was 48 nmol/L, and was inversely associated with %TBF \( (r = -0.28, \ P < 0.001) \) and TBF \( (r = -0.33, \ P < 0.001) \) [188]. A study of Puerto Ricans in Puerto Rico showed that serum 25(OH)D was significantly inversely correlated with %TBF \( (r = -0.24, \ P = 0.02) \) [60].

The aim of this cross-sectional study was to determine the association between serum 25(OH)D concentration in relation to total body fat-mass (TBF) and percent-body-fat (%TBF), and its various components, in the participants of the Boston Puerto Rican
Osteoporosis Study (BPROS). We included android fat-mass (AFM) and gynoid fat-mass (GFM), using dual-energy x-ray absorptiometry (DXA).

MATERIALS AND METHODS

Study Population and Data Collection

Description of the recruitment and methods of the BPROS are described in detail elsewhere [113]. Concisely, a bilingual interviewer conducted home visits and administrated a detailed questionnaire on socioeconomic status, education, household income, health and health behaviors that includes: medical diagnosis and current medication use, depression, and psychological acculturation. Additionally, the interviewer collected anthropometrics, physical performance measures, and measured blood pressure. The day after this interview, the study phlebotomist collected 12-hr urine and 12-hr fasting blood and saliva. Once the 2-yr follow up was complete, participants were re-consented for the BPROS. By May 2013, 1257 participants completed 2-year follow-up visits and 970 had completed the BPROS study, which included a 24-hr urine collection. All study protocols were approved by the Institutional Review Boards at Tufts Medical Center and Northeastern University, and each participant provided written informed consent.

There were no significant differences in socio-demographic characteristics between those with complete or incomplete information. The present study includes complete data for 700 Puerto Rican women and 270 Puerto Rican men.
Covariates Assessments and Definition

For the purpose of this analysis, vitamin D deficiency was defined as serum 25(OH)D concentration below 50 nmol/L, insufficiency between 50-75 nmol/L, and sufficiency above 75 nmol/L [26, 157]. Seasonality was defined as summer, July to September; fall, October to December; winter, January to March; and spring, April to June. Smoking status was categorized into yes or no for the current behavior. Vitamin D from supplements was defined as: minimal 0-10 µg/day (200-400 IU/day); and high ≥ 10 µg/day (≥ 400 IU/day). Participant’s education was measured at baseline and defined using the cutoff point of 8th grade while poverty was measured at each time point and defined by the U.S. Department of Health and Human Services federal poverty levels. Dietary vitamin D intake was estimated from a semi-quantitative food-frequency questionnaire (FFQ) specifically designed for this population [91]. This FFQ has been validated against serum carotenoids [116], and vitamin B12 [117] in a Hispanic population aged ≥ 60 years. Those with energy intakes < 600 or > 4800 kilocalories and/or > 10 questions blank on the FFQ were excluded from the analyses. Nutrient intakes were calculated with the Nutrition Data System for Research (NDS-R) software version 2007 (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, Food and Nutrient Database 2007, released 2007). Alcohol intake (g) was captured by the FFQ.
Exposure Assessment:

All fat-mass measures were obtained using DXA whole-body scans (Lunar Prodigy, GE Healthcare, Madison, WI) using software Encore version 6.1. Total body scans were carried out on consenting participants, and all scans followed a standardized protocol to ensure consistency. Daily quality assurance scans were performed, as well as weekly scans of various thickness phantoms to examine the stability of the instrument. The precision of the total body fat and lean tissue were, 0.94% and 0.77%, respectively [191]. Android and gynoid measurements were based on the percentage of fat within each body region. AFM (kg) was defined as the area between the ribs and the pelvis. The upper demarcation is 20% of the distance between the iliac crest or the pelvis and the femoral neck. The GFM (kg) was defined as the region including the hips and upper thighs overlapping leg and trunk regions. Its upper demarcation, below the pelvis cut, is defined as 1.5 times the android height, with total height as twice the height of the AFM region (Figure 4.1). The lateral boundaries of both AFM and GFM were defined as arms and legs [192]. Percentage total body fat, %TBF, is determined as a ratio of total-fat-mass to total body mass, both in (kg).

Laboratory assays

Fasting blood samples (12-h) were drawn by a certified phlebotomist at the participant’s home on the morning after the home interview, or as soon thereafter as possible. Aliquots were saved and stored at -80°C until processed. Serum 25(OH)D concentration was measured by extraction followed by $^{25}$I radioimmunoassay Packard COBRA II Gamma Counter (DiaSorin Inc., Stillwater, MN 55082) catalog # 68100E
with intra-assay and inter-assay CV% of 10.8% and 9.4%, respectively. Serum intact parathyroid hormone (iPTH) was detected by solid-phase, two-site chemiluminescent enzyme-labeled immunometric assay using the Immulate 1000 Diagnostic Products Corporation (Los Angeles, CA 90045-5597) with intra-assay and inter-assay CV% of 5.5%, and 7.9%, respectively. Serum creatinine was assessed with a colorimetric, kinetic reaction using the Olympus AU400e with Olympus Creatinine Reagents (OSR6178) (Olympus America Inc., Melville, NY) with intra-assay and inter-assay CV% of 2.0%, and 4.0%, respectively.

**Statistical analyses:**

All statistical analyses were conducted with SAS 9.1.3 (SAS Institute Inc, Cary, NC); and all tests were 2-sided with \( P < 0.05 \) considered statistically significant. Dependent skewed variables were natural log-transformed to normalize their distributions before the analysis. Demographic and clinical characteristics were compared using Student’s \( t \)-test for continuous variables and chi square tests for categorical variables. Characteristics were compared by sex using two-tailed \( t \)-test for two independent samples, and with \( \chi^2 \) test for differences in proportion. Pearson partial linear correlation analysis was used to examine the relationship between serum 25(OH)D concentration with adiposity, adjusting for age and sex. We constructed general linear models (PROC GLM) to model the cross-sectional associations between 25(OH)D (continuous) and fat-mass (continuous). The Risk Factor model (RF-model) was adjusted for age (y), serum creatinine (nmol/L), serum iPTH (pg/ml), and sex in the full model. The Dietary model (D-model) included serum seasonality, dietary vitamin D and supplements, and total
energy (kcal/day) in addition to the prior model covariates. The Life-Style model (LS-model) was adjusted for prior models in addition to physical activity, current smoking status (y/n), alcohol intakes (g/d), poverty level, and education. For all linear models, we checked the assumptions of linearity and homogeneity through examining the outcome residuals against the exposure. For all final models, we performed influence diagnostics tests to check for outliers and influential points.

RESULTS

The mean ages of women and men were 60.3 ± 7.5 and 59.5 ± 8.0, respectively (Table 1). The mean serum 25(OH)D for women was 50.1 ± 19.0, significantly higher ($P = 0.01$) compared to men, 47.0 ± 17.5 nmol/L. Women had significantly higher adiposity than men for most measures. Women also had significantly higher dietary vitamin D and supplement intakes. Men walked significantly more miles/day and had higher physical activity scores compared to women. Men also reported higher levels of smoking and alcohol consumption compared to the women.

Only AFM was significantly negatively correlated with serum 25(OH)D ($r = -0.1, P =0.02$), while TBF approached significance ($r = -0.06, P =0.07$) (Table 4.3). The multivariate regression analysis of the associations of DXA TBF-in kilogram-or %TBF-were not significantly associated with serum 25(OH)D, adjusting for potential covariates (Table 4.3).

In the RF-model, AFM was significantly negatively associated with serum 25(OH)D ($\beta =-0.003, P =0.009$). This inverse association remained significant after further adjustment for the covariates ($\beta =-0.003, P =0.01$). Even though the interaction
term of AFM and sex was not significant, but regional adiposity might be sex-specific, thus, we stratified by sex. Among Puerto Rican men, the additional adjustment for the covariates of the LS-model did not attenuate the association between AFM and serum 25(OH)D (β = -0.002, $P = 0.008$) explaining approximately 22% of the variability in serum 25(OH)D. Among Puerto Rican women, only the additional adjustments of LS-model covariates appreciably emphasized the significantly inverse association between AFM and 25(OH)D (β = -0.003, $P = 0.03$).

GFM was not a significant predictor of serum 25(OH)D when controlling for the covariates of the RF-model. However, with additional adjustment for the covariates of D-model and LS-model, GFM was positively significantly associated with 25(OH)D (β = 0.002, $P = 0.02$). Interestingly, when the analyses were stratified by sex, the positive associations remained significant among Puerto Rican women only (β = 0.002, $P = 0.04$) (Figure 4.2).

**DISCUSSION**

These results provide support to the current dialogue on adiposity, *in general*, and fat-mass in terms of amount and region-*in particular*, with vitamin D status. By initially focusing on TBF-as measured by DXA-and serum 25(OH)D concentration, we did not discern any significant association. Similarly, none of our models were significant for %TBF. On the other hand, when we further investigated the role of regional adiposity, *precisely*, android and gynoid fat-mass, our results showed a distinct picture. AFM was negatively associated with serum 25(OH)D after adjusting for several potential covariates
including, age, serum creatinine, serum iPTH, seasonality, supplements and dietary vitamin D, smoking, alcohol, and total energy, thus, demonstrating the independent negative association of android adiposity on serum 25(OH)D concentration. In contrast, GFM was significantly positively associated with serum 25(OH)D ($P =0.02$) independent of age, serum creatinine, serum iPTH, seasonality, supplements and dietary vitamin D, smoking, alcohol, and total energy.

We reported earlier that vitamin D deficiency remains prevalent in this Hispanic subgroup, indicating that vitamin D deficiency is not a transient health concern, thus, predisposing this population to higher levels of mortality and morbidity [182]. Recently, we reported that the change in BMI over time was negatively associated with the change in serum 25(OH)D concentration ($P =0.01$) independent of various known factors including age, sex, serum creatinine, physical activity, smoking alcohol, seasonality, poverty and education [193]. Taken together, our results are in agreement with a number of epidemiological studies demonstrating that measures of adiposity including TBF, BMI, and regional adiposity are inversely associated with serum 25(OH)D concentration [48, 49, 132, 171-173].

The association between vitamin D and adiposity, in general, and regional adiposity, specifically, was explained by Lumb and colleagues in 1971. They were the first to introduce the hypothesis of vitamin D sequestration in the adipose tissue and muscle [76]. Later on, they substantiated that hypothesis and confirmed the link between adiposity and vitamin D [77]. Subsequently, Wortsman and colleagues confirmed the aforementioned premise, stating that deposition within the adipose tissue lowers the bioavailability of serum 25(OH)D and 1,25(OH)2D leading to vitamin D
insufficiency/deficiency. They showed lower serum 25(OH)D and higher iPTH among obese people [48]. AFM has been associated with higher mortality independent of BMI [194] and is a known risk factor for many serious health disparities including CVD, hypertension, dyslipidemia and insulin resistance [194-196], as well as inflammation [179, 197].

The results of our study should be interpreted within the context of some limitations. First, due to the cross-sectional nature, causality cannot be inferred, but the results are in agreement with a number of cross-sectional studies [172, 188, 190]. Another potential limitation is that with the use of DXA, the differentiation between visceral and subcutaneous fat is not feasible. However, it was shown by Savgan-Gurol and colleagues that the percentage fat shown by DXA was significantly associated with visceral fat ($r = -0.83$, $P < 0.0001$), while the association with subcutaneous was lower ($r = -0.77$, $P < 0.001$) [198]. This only suggests that the importance of android fat-mass as an indicator of central obesity might be greater compared to the relative distribution of visceral and subcutaneous fat.

In conclusion, our findings of the negative, significant association of AFM on serum 25(OH)D concentration; while GFM was positively associated with serum 25(OH)D concentration, highlights the importance of differences in body composition on vitamin D status. Although it is clear that some of the association between regional adiposity and serum 25(OH)D concentration may be attributed to the sequestration of vitamin D in adipose tissues, it is still considered a complex relationship and more work is needed to elucidate the relationship between adipose tissue and vitamin D and the effect both to overall health.
BIBLIOGRAPHY


FIGURE 4.1 The illustrations of android region (AFM) and gynoid region (GFM)
FIGURE 4.2 The β coefficients of the association between serum 25(OH)D concentration with gynoid and android fat among Puerto Rican men and women adjusted for age and serum creatinine (mg/dL), serum iPTH (pg/ml), total fat-mass (g), seasonality, dietary vitamin D and supplements, total energy (kcal/day), physical activity, alcohol, smoking, poverty level, education and sex in the All data.
**TABLE 4.1** Descriptive characteristics of men and women in Boston Puerto Rican Osteoporosis Study (BPROS)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Women (n=699)</th>
<th>Men (n=273)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>60.3 ± 7.5</td>
<td>59.5 ± 8.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Education (above 8th grade %)</td>
<td>49.0</td>
<td>44.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Poverty (%)</td>
<td>59.0</td>
<td>49.2</td>
<td>0.009</td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>50.1 ± 19.0</td>
<td>47.0 ± 17.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Adiposity Variables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.0 ± 7.0</td>
<td>30.2 ± 5.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>104.0 ± 15.0</td>
<td>104.3 ± 14.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Total Fat-mass (kg)</td>
<td>36.0 ± 1.5</td>
<td>26.5 ± 1.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Android Fat-mass (kg)</td>
<td>3.4 ± 1.3</td>
<td>3.0 ± 1.4</td>
<td>0.0007</td>
</tr>
<tr>
<td>Gynoid Fat-mass (kg)</td>
<td>6.0 ± 2.1</td>
<td>4.1 ± 2.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Android:Gynoid</td>
<td>1.05 ± 0.13</td>
<td>1.3 ± 0.22</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Average fat percentage</td>
<td>44.4 ± 6.5</td>
<td>30.0 ± 7.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Other Covariates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current- smoking (%)</td>
<td>18.0</td>
<td>30.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Current- alcohol drinking (%)</td>
<td>36.0</td>
<td>51.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Physical Activity Score</td>
<td>31.3 ± 4.0</td>
<td>33.0 ± 6.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Miles walking/day</td>
<td>1.4 ± 2.0</td>
<td>2.0 ± 2.4</td>
<td>0.0002</td>
</tr>
<tr>
<td>Dietary Measures</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>p-value</td>
</tr>
<tr>
<td>-------------------------------------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Vitamin D supplement (&gt;200IU/day) # (%)</td>
<td>30.2</td>
<td>18.5</td>
<td>0.0002</td>
</tr>
<tr>
<td>Total vitamin D intake (IU/day) §</td>
<td>287.5 ± 152.0</td>
<td>257.0 ± 123.0</td>
<td>0.002</td>
</tr>
<tr>
<td>Total energy intake (kcal/day)</td>
<td>1770.0 ± 825</td>
<td>2250 ± 1200</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D.

1 Data presented as mean ± SD for continuous variables, or as % for categorical variables

2 Geometric mean ± SD

For between sex’s comparisons, t-test was used for means and Chi-square test χ²-test was used for percentages.

‡ Range for Physical Activity Score = 35.5 for men and 26.4 for women. Range for Miles/day = 22 for men and 20 miles/day for women.

# The recommended dietary allowance (RDA) for vitamin D for men and women aged 19-70 years = 15 ug /day [82]

‖ Adjusted for energy intake using ANOVA
**TABLE 4.2** Pearson partial linear correlation of serum 25(OH)D concentration and adiposity phenotype at 2-year

<table>
<thead>
<tr>
<th></th>
<th>2-year</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>25(OH)D</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBF</td>
<td>-0.06</td>
<td>0.07</td>
</tr>
<tr>
<td>%TBF</td>
<td>-0.03</td>
<td>0.3</td>
</tr>
<tr>
<td>AFM</td>
<td>-0.1</td>
<td>0.02</td>
</tr>
<tr>
<td>GFM</td>
<td>-0.04</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Pearson partial linear correlation coefficient (r) is age and sex adjusted

Abbreviations: BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D; WC, waist circumference; AFM, abdominal fat-mass; TBF, total body fat; %TBF, total body fat percentage
TABLE 4.3 Regression coefficients (± SE) from cross-sectional regression analysis of TBF and %TBF on plasma 25(OH)D concentration.

<table>
<thead>
<tr>
<th>Serum 25(OH)D (nmol/L)</th>
<th>Adiposity Phenotype</th>
<th>TBF</th>
<th>%TBF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
<td>P-value</td>
</tr>
<tr>
<td>Risk Factor Model</td>
<td>-0.0001</td>
<td>0.00005</td>
<td>0.2</td>
</tr>
<tr>
<td>Dietary Model</td>
<td>-0.00005</td>
<td>0.00005</td>
<td>0.2</td>
</tr>
<tr>
<td>Life-Style Model</td>
<td>-0.0001</td>
<td>0.00005</td>
<td>0.1</td>
</tr>
</tbody>
</table>

TBF, total body fat; %TBF, total body fat percentage
Risk Factor Model: adjusted for age and creatinine (mg/dL), serum iPTH (pg/ml) and Dietary Model: adjusted for model 1 variables plus seasonality, dietary vitamin D and supplements, total energy (kcal/day).
Life-Style Model: adjusted for model 1+2 variables plus physical activity, alcohol, smoking, poverty level and education.
TABLE 4.4 Regression coefficients (± SE) from cross-sectional regression analysis of AFM and GFM on plasma 25(OH)D concentration.

<table>
<thead>
<tr>
<th>Serum 25(OH)D (nmol/L)</th>
<th>Adiposity Phenotype</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AFM β ± (SE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk Factor Model</td>
<td>-0.003 ± 0.001</td>
<td>0.009</td>
<td>-0.004 ± 0.003</td>
<td>0.1</td>
<td>-0.002 ± 0.001</td>
<td>0.1</td>
<td>0.001 ± 0.001</td>
</tr>
<tr>
<td>Dietary Model:</td>
<td>-0.003 ± 0.001</td>
<td>0.01</td>
<td>-0.002 ± 0.001</td>
<td>0.008</td>
<td>-0.001 ± 0.0005</td>
<td>0.2</td>
<td>0.002 ± 0.001</td>
</tr>
<tr>
<td>Life-Style Model</td>
<td>-0.003 ± 0.001</td>
<td>0.01</td>
<td>-0.002 ± 0.001</td>
<td>0.01</td>
<td>-0.003 ± 0.001</td>
<td>0.03</td>
<td>0.002 ± 0.001</td>
</tr>
</tbody>
</table>

AFM, android-fat-mass; GFM, gynoid-fat-mass.

**Risk Factor Model**: adjusted for age and serum creatinine (mg/dL), serum iPTH (pg/ml), total fat-mass (g) and sex in the case of the combined data.

**Dietary Model**: adjusted for model 1 variables plus seasonality, dietary vitamin D and supplements, total energy (kcal/day).

**Life-Style Model**: adjusted for model 1+2 variables plus physical activity, alcohol, smoking, poverty level, education.
CHAPTER SIX

DISCUSSION AND SUMMARY

Understanding the association between plasma 25-hydroxyvitamin D concentration (25(OH)D) concentration and cardiovascular disease (CVD) is important. This dissertation was designed to examine the multiple pathways through which serum 25(OH)D as the biomarker of vitamin D status could affect longitudinally as well as cross-sectionally a number of CVD associated risk factors, namely, lipid profile, adiposity, and inflammation among the participants of the Boston Puerto Rican Health Study (BPRHS) and the Boston Puerto Rican Osteoporosis Study (BPROS).

Foremost, it is well documented in the literature that dyslipidemia, i.e. high plasma LDL-C, TG and low plasma HDL-C, is highly associated with increased risk of CVD along with other factors such as high blood pressure [2, 128, 199]. In the first report of this doctoral work, our results are in apparent contrast to scores of reports that indicate serum 25(OH)D concentration is positively associated with plasma high-density lipoprotein (HDL-C), and negatively associated with plasma low-density lipoprotein (LDL-C), total cholesterol (TC), and triglycerides (TG) [4-10]. We showed that even though serum 25(OH)D concentration was positively associated with plasma HDL-C cross-sectionally, the longitudinal analyses illustrated different yet interesting associations; it showed that baseline-25(OH)D demonstrated a negative, yet significant, association with 2.5-year change in plasma HDL-C concentration ($\beta = -0.04, P = 0.01$); an association independent of important putative confounders including age, sex, body mass index (BMI), total energy, physical activity, smoking, alcohol, seasonality, poverty and education. We
demonstrated this association not only within the life-style model, but throughout the confounding by indication adjustment in our CVD-model. Similar relationships were found with plasma TG, but not with LDL-C and TC. In the prospective Health Professionals Follow-up Study (HPFS), Giovannucci and colleagues demonstrated a positive linear trend of LDL-C, HDL-C and TC, as well as a negative trend with plasma TG across serum 25(OH)D quartiles among 900 US men, ages 64 and older [125]. Another study showed that serum 25(OH)D was independently associated with higher HDL-C and lower TG; but not TC [107]. Interestingly, two studies reported that both HDL-C and LDL-C were positively associated with serum 25(OH)D concentration, thus, both are being affected in the same direction, i.e. increasing their plasma concentration. It is well known that one favorable approach to lower CVD risk is to increase HDL-C and lower LDL-C [2, 128]. A report by Melamed and colleagues, found that when analyzing Third National Health and Nutrition Examination Survey (NHANES III), serum 25(OH)D concentration were significantly inversely associated with TC, among 13,331 US men and women ages 20 years and older. However, they found no association between plasma HDL-C and serum 25(OH)D among their participants [96]. Another longitudinal study with a 14 year follow up found that among 1762 participants, serum 25(OH)D and plasma TG were inversely associated [104]. Few key differences can be outlined and prior studies are mostly cross-sectional; no proof of a causal relationship can be inferred. One difference to illustrate is that our results are longitudinal, and we have controlled for numerous confounders, which were not necessarily available among previous cross-sectional studies.

On the contrary our results are in accordance with a number of reports that found serum 25(OH)D concentration might not be associated with atheroprotective lipid profile [96, 104, 105, 123, 127]. A study by Jorde and colleagues found that TC was significantly associated with
increased serum 25(OH)D concentration among 8,018 Norwegian men and women, ages 43-70 years [104]. Two other studies found that plasma TG were positively associated with serum 25(OH)D; however, both studies investigated this effect among children [123, 127]. Intervention studies in the form of placebo-controlled randomized trials (RCT’s) are designed to confirm or dispute any causal relationship. Exceptionally few RCT have been conducted to investigate the question in discussion, and their results have largely been inconsistent on the effect of vitamin D supplementations on improving lipid profile [108, 129-131]. A study by Zittermann and colleagues showed that daily vitamin D supplementation (3320 IU/day) for one year had a significant effect on plasma lipids. They found that the supplemented group had lower plasma TG compared to the placebo, -13.5% and +3% (P =0.001) correspondingly; nonetheless, the supplemented group exhibited a significant increase in plasma LDL-C [129]. Conversely, this was not the case for the study by Jorde and colleagues; they were not able to demonstrate that one year of vitamin D supplementation, 40,000 IU/week, improved the plasma lipids of the 438 overweight/obese participants [108]. Another study showed that vitamin D supplementation might not be atheroprotective among postmenopausal women [130]. Most of these RCT’s can be criticized for a rather low sample size, which might have contributed to the null results.

The relationship between plasma HDL-C and serum 25(OH)D concentration is particularly noteworthy. The significance of the interaction term of smoking status with serum 25(OH)D was interesting. The significant impact of serum 25(OH)D on HDL-C among non-smokers was unexpected, however similar results were reported by Jorde and colleagues [132]. They found that among the participants of the Tromsø study, smokers had higher levels of serum 25(OH)D concentration; about 15-20%, compared to non-smokers. They thought it might be due to a difference in methodology, i.e. immunological vs. LC-MSMS, and excluded them from the
analysis. The pathophysiological mechanism by which smoking may impact serum 25(OH)D concentration or the metabolism of 1,25(OH)₂D has not been investigated. However, it has been discussed that smoking might influence 25-hydroxylase (CYP2R1) thus, lowering serum 25(OH)D concentration [133]. Another point that might explain this paradox is that, smoking contains nicotine, which exerts anti-inflammatory properties [134, 135].

Despite the disagreement of our results with many epidemiologic and ecological studies for the protective effective role of serum 25(OH)D on lipid profile as a major risk factor for CVD, the contradictory yet significant evidence we show cannot be disregarded. One explanation for these phenomena is that vitamin D induces the intestinal absorption of dietary calcium, thus lowering calcium fecal excretion [37, 95]. This might affect the amount of fatty acids in the fecal due to the reduction in the formation of insoluble fatty soaps [136]. Thus, leading to an increased rate of the absorption of fat, thus higher plasma LDL-C and TG concentration, in particular, and TC in general [137]. In addition, higher vitamin D might decrease cholesterol excretion because of the lower availability of calcium within the intestine, and hence less binding to bile acids [138]; this would translate into less cholesterol converting into bile acids. One study suggested that higher calcium intake might augment the prior phenomena and lower LDL-C by increasing not only vitamin D but it has been accompanied by calcium supplements. A 15-week weight loss trial study by Major and colleagues showed that among 63 healthy, overweight/obese women, the daily supplementation of 600 mg of calcium and 200 IU of vitamin D significantly improved LDL-C as well as LDL: HDL-C ratio, while HDL-C and TG approached significance level [139].

Secondly, there is considerable evidence that inflammation is one element of the development of CVD. Higher serum concentration of inflammatory markers such TNF-α, IL-6
and hsCRP have emerged as independent risk factors for CVD [165, 166]. Therefore, attenuating these inflammatory markers concentration may influence risk of such illnesses. Vitamin D may have the ability to attenuate the high levels of inflammatory markers by down-regulating a number of genes [37-39, 144].

A second aim of this doctoral work was to study the *cross-sectional* association between serum 25(OH)D and inflammatory markers, namely, interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α) as well as high-sensitivity C-reactive protein (hsCRP) among the participants of the BPROS. Our data suggest that serum 25(OH)D concentration explained about 6-20% of the variation in the IL-6 and TNF-α concentration, respectively. Our data provides evidence of an inverse association between serum 25(OH)D and inflammatory markers, namely, IL-6 and TNF-α, among the participants of the BPROS; irrespective of age, sex, seasonality, adiposity phenotype and lifestyle factors that include smoking, physical activity and alcohol consumption. This population, as reported before, exhibits a high prevalence of vitamin D deficiency [159]. Our findings from the multivariate regression analysis, especially for IL-6 and TNF-α, showed that irrespective of the various constructed models, serum 25(OH)D concentration remained a significant predictor of IL-6 and TNF-α, but not of hsCRP, after controlling for potential covariates. These results are in accordance with other reports on the association between serum 25(OH)D concentration and inflammatory markers [98, 154, 155]. In further support, our results are consistent with *in vitro* and animal studies that showed TNF-α suppression by vitamin D [97, 152, 160, 161] and IL-6 [97, 147, 162]. A RCT by Schleithoff and Colleagues, demonstrated that 9 months of daily supplementation of 2000 IU of vitamin D and 500 mg of calcium significantly lowered serum TNF-α concentration compared to baseline
among congestive heart failure participants [153]. In addition, another RCT demonstrated an inhibition of TNF-α concentration with 3332 IU of vitamin D daily [129].

The lack of association between serum 25(OH)D and hsCRP is not in concurrence with a number of cross-sectional reports [98, 163]; there are a number of plausible explanations. First, hsCRP concentration can be affected by acute phase reactant due to viral infection. However, that might not be the case with the presence of constant yet potent stimuli [149]. Another point to emphasize is that this specific population has a high prevalence of health disparities that includes metabolic syndrome, depression, and diabetes, which might mask the association [109, 164]. Additionally, in this population the mean of 25(OH)D concentration reaches only to 50 nmol/L; indicating widespread vitamin D deficiency and thus, very limited distribution and a very low number of sufficient levels to show significant differences. In our analysis, the adjustment of white blood cell counts, which is an indicator of current infection, did not improve the null association between hsCRP and serum 25(OH)D concentration.

Thirdly, adiposity increases the risk of numerous chronic diseases such as CVD and type-2-diabet among others [200, 201]. We intended for our third report of this doctoral work to investigate the magnitude of serum 25(OH)D concentration being affect by adiposity phenotype over time. Our results confirm an inverse association between low serum 25(OH)D concentration and adiposity phenotype i.e. body mass index (BMI) and waist circumference (WC). Our observation of lower adjusted mean serum 25(OH)D concentration in obese, severely obese and morbidly obese participants is in agreement with many studies [132, 172, 176, 177]. Baseline-BMI was negatively associated with the change in serum 25(OH)D concentration ($P =0.01$), independent of known factors including age, sex, serum creatinine, physical activity, smoking, alcohol, seasonality, poverty and education. We adjusted for covariates in three different models,
all of which showed significant longitudinal negative association. Conversely, we did not observe similar associations with WC, even though it was significantly correlated with serum 25(OH)D at both time points.

A mechanism that can explain the phenomena of low serum 25(OH)D and increased adiposity risk is not clear. The review by Earthman and colleague discusses number of proposed theories that link Vitamin D deficiency to adiposity [178]. One of the theories suggests that since Vitamin D is fat-soluble it might be sequestered within adipose tissue. They further suggest that the adipose tissue of obese individuals may have higher 25(OH)D uptake and storage relative to lean individuals [48, 178]. An additional theory suggests that due to the fact that adiposity, especially visceral adiposity, is considered a high inflammatory state, this contributes to lower serum 25(OH)D concentration [178, 179]. Finally, with increased adiposity, there may be an increase of the action of 24-hydroxylase, thus, leading to amplified catabolism of Vitamin D [178, 180]. Consequently, further research is needed to further elucidate our understanding of how vitamin D might be affected by adiposity or even how vitamin D affects obesity itself.

The majority of the participants of BPROS- more than 60%- had serum 25(OH)D concentration below 50 nmol/L, which is considered a deficiency according to the Endocrine Society [115]. The most recent report by the National Health and Nutrition Examination Survey (NHASES) III, showed that more than 42% of their 2001-2006 participants had vitamin D below 50 nmol/L; when stratified by ethnicity, Hispanics were second highest in vitamin D deficiency with 70% [181]. Our prior report indicated that among 1502 participants of the BPRHS, more than 70% had serum 25(OH)D concentration below 50 nmol/L at baseline, concurring with the NHANES III data [159]. This greater risk of vitamin D deficiency may be related to greater risk of health disparities [182]. A report by Palacios and colleagues showed that Puerto Ricans who
are residing in Puerto Rico, had higher serum 25(OH)D concentration, approximately 75 nmol/L, compared to our population, at baseline 43.4 ± 17.2 nmol/L [159] and 2yr 50.0 ± 19.0 nmol/L. One theory to illustrate here is that this difference in serum 25(OH)D concentration might be due to sun exposure, generally, and latitude, specifically, i.e. PR’s latitude is 18.26° while Boston’s latitude is 42.35° [26].

Lastly, we aspired in our fourth report of this doctoral work to not only explore the association between serum 25(OH)D concentration and adiposity phenotype, in general, but rather to investigate in more depth that association total as well as regional fat mass. Our report initially focused on total body fat (TBF)-as measured by dual-energy x-ray absorptiometry (DXA) and serum 25(OH)D concentration. Similarly, percent body fat (%TBF) was not significantly associated with 25(OH)D. On the other hand, when we further investigated the role of regional adiposity, android (AFM) and gynoid fat-mass (GFM), results differed. AFM, was negatively associated with serum 25(OH)D after adjusting for several potential covariates including, age, serum creatinine, serum iPTH, seasonality, supplements and dietary vitamin D, smoking, alcohol, and total energy. On the other hand, GFM was significantly positively associated with serum 25(OH)D ($P=0.02$) independent of age, serum creatinine, serum PTH, seasonality, supplements and dietary vitamin D, smoking, alcohol, and total energy.

We reported earlier that vitamin D deficiency remains prevalent in this Hispanic subgroup, indicating that vitamin D deficiency is not a transient health concern, thus, predisposing this population to higher levels of mortality and morbidity [182]. Recently, we reported that the change in BMI over time was negatively associated with the change in serum 25(OH)D concentration ($P=0.01$) independent of various known factors including age, sex, serum creatinine, physical activity, smoking alcohol, seasonality, poverty and education [193].
Taken together, our results are in agreement with a number of epidemiological studies demonstrating that measures of adiposity including TBF, BMI, and regional adiposity are inversely associated with serum 25(OH)D concentration [48, 49, 132, 171-173].

Our findings should be interpreted within the context of a few limitations. First, our population is primarily Puerto Ricans, therefore our findings could be very specific to this particular admixture population, with genetic variation interacting not only with the environment, but social factors as well [140-143]; thus, generalizability may not be an option. Additionally, even though we have controlled for all the known potential covariates, residual confounding is still a possibility.

Some of our findings came from our cross-sectional study; hence, it provides neither further insights about causation nor long-term status. Longitudinal studies with two or more time points are ideal alternative to investigate the role of serum 25(OH)D concentration on CVD risk factors; because serum 25(OH)D concentration can be assessed before the outcome, thus, allowing us to avoid any potential bias. Nevertheless, our findings show very unique results to the Puerto Rican population. Another limitation is that we have no direct measure of sun exposure at baseline. Another potential limitation is that with the use of DXA, the differentiation between visceral and subcutaneous fat is not feasible. However, it was shown by Savgan-Gurol and colleagues that the percentage fat shown by DXA was significantly associated with visceral fat \( r = -0.83, P < 0.0001 \), while the association with subcutaneous was lower \( r = -0.77, P < 0.001 \) [198]. This only suggests that the importance of android fat-mass as an indicator of central obesity might be greater compared to the relative distribution of visceral and subcutaneous fat. Another potential bias that might affect our results is selection bias that might have occurred in our study. Even though selection bias may not be possible to appropriately
evaluate how it may affected our results, it is possible that those who declined to participate in our study may have a higher socioeconomic status, as well as highly educated, thus, may higher probability of having sufficient serum 25(OH)D concentration [187]

In conclusion, our longitudinal analysis showed that higher 25(OH)D serum concentration did not translate into an increase of the atheroprotective plasma HDL-C, nor a decrease of the un-atheroprotective plasma TG in this population of older Puerto Rican adults living in the Boston area. Moreover, our results showed that smoking might be an effect modifier of the longitudinal association of serum 25(OH)D and HDL-C, as well as LDL-C. Granted our work should be replicated in other populations, our findings provide opposing yet significant evidence of the effect of vitamin D on plasma lipid profile.

Secondly, our study supports the hypothesis that inflammatory markers such as IL-6 and TNF-α are inversely associated with serum 25(OH)D concentration among the participants of the BPROS. These findings support the need to re-examine the determination of optimal vitamin D status. However, more studies, especially longitudinal prospective studies, are needed to fully characterize the relationship between serum 25(OH)D concentration and inflammatory markers.

Thirdly, serum 25(OH)D concentration was inversely associated with 2.5-yr change in BMI. The adjusted-means of serum 25(OH)D concentration showed declining trend, at both time points, with the increase in BMI especially when reaching class III obesity. Our findings of the negative significant association of AFM on serum 25(OH)D concentration provides compelling evidence that AFM may affect serum 25(OH)D concentration; while GFM was positively associated with serum 25(OH)D concentration. Even though it is clear that some segments of the regional adiposity and serum 25(OH)D concentration relationships may be attributed to the sequestration of vitamin D in adipose tissues, it is still considered a complex relationship and
more work is needed to elucidate the relationship between adipose tissue and vitamin D and the effect both to overall health.

Taken together, it appears that serum 25(OH)D concentration is strongly influenced by adiposity, which is considered a chronic low-grade inflammatory condition, thus, by sequestering 25(OH)D within the adipose tissue, neither the high inflammatory condition might be ameliorated nor dyslipidemia could be improved by vitamin D. Figure 5.1 summarize the findings of this dissertation work.

Vitamin D deficiency/insufficiency has emerged as an autonomous risk factor of cardiovascular mortality. Thus, the need for an enhanced understanding of the epidemiological data as well as the pathophysiological pathways by which this pleiotropic hormone may or may not affect cardiovascular health is imperative. This understanding will result in better informing of future clinical trials and ultimately results in improved evidence-based therapeutic recommendations. Research findings by Bidchoff-Ferrari and Colleagues estimated that 1000 IU/day of vitamin D supplements 50% of the younger and older adults to sufficient levels of serum 25(OH)D concentration, i.e. 75 nmol/L. However, it will take twice as much of that, meaning 2000 IU/day to bring 85-90% of the population to those sufficient concentration [202]. Thus, for ethnic minorities who are facing higher health disparities may require 2000 IU/day of vitamin D supplement. Despite the fact that it is premature to make such recommendations for the rationale of precluding CVD risk, achieving sufficient concentration of 25(OH)D is essential. Even though there is plethora of scientific information of vitamin D and its receptor, there is an urgent necessity to further understand and elucidate the biochemical and transcriptional effect of vitamin D and its various analogs on CVD, as well as to search for new targets to treat CVD and ultimately reduce health disparities.
Notwithstanding the above limitations, our study has substantial strengths: a large sample size with low attrition; we have complete data on 1504 Puerto Rican men and women at baseline, 1257 at 2-year and 970 within the ancillary bone study. This large sample size is ample to detect differences with minimally 90% power. In addition, we have used detailed questionnaires along with an FFQ that allowed detailed information.

In summary, our findings suggest that risk factors of CVD are highly associated with lower 25(OH)D concentration among Puerto Rican men and women. Results from this dissertation work may provide guidance for future interventional studies for an understudied yet fast growing population that appears to have high health disparities. Improving vitamin D status may not solve all of their health disparities; however, it might lower their risk of developing chronic disease.
FIGURE 5.1 Diagram illustrates the relationship between serum 25(OH)D and the various CVD risk factors among the participants of the BPRHS
BIBLIOGRAPHY


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APPENDIX
Serum Vitamin D Concentration, Dietary Sources, and its relation to BMI among Puerto Ricans Living in the Greater Boston Area\textsuperscript{1,2}

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Abbreviations used: Boston Puerto Rican Health Study (BPRHS), 25-hydroxyvitamin D (25(OH)D), body mass index (BMI), National Health and Nutrition Examination Survey (NHANES), Recommended Dietary Allowance (RDA).

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ABSTRACT

Puerto Ricans have greater health disparities compared to the general population. Vitamin D deficiency is highly prevalent among ethnic minorities. Cross-sectional analyses included data from 1504 Puerto Ricans, 445 men and 1050 women, aged 45-75 years in Boston, Massachusetts. Our aim was to determine serum 25-hydroxy vitamin D concentration (25(OH)D) and associated contributing factors as well as test the hypothesis that lower serum 25(OH)D is inversely associated with body mass index (BMI). Associations between 25(OH)D and likely determinants were tested with multivariable linear regression. Low 25(OH)D concentration was defined as <50 nmol/L. Dietary data from food frequency questionnaire were used to rank intake sources of vitamin D using PROC RANK. Mean serum 25(OH)D concentration was 43.5 ± 16.5 nmol/L for men and 43.1 ± 16.0 nmol/L for women. 70.0% of participants had 25(OH)D <50 nmol/L. Milk products contributed 34% of total vitamin D intake, followed by supplements, 21.5%. Among women, but not men, higher intake of supplements was associated with significantly higher serum 25(OH)D concentration. Never-smokers had significantly higher serum 25(OH)D compared to current-smokers (P=0.01), but alcohol intake was not associated with vitamin D status. The multivariate model showed that season (summer vs. others), supplement use, dietary vitamin D intake, number of miles walked/day and serum albumin were each positively associated (all P<0.05), while BMI was inversely associated (P=0.0007), with serum 25(OH)D. Women with BMI ≥40 had significantly lower serum 25(OH)D concentration compared to lower BMI. In conclusion, vitamin D deficiency is prevalent among Puerto Rican adults living in Boston, MA, and this is associated with BMI and several behavioral measures, including intake, smoking and outdoor walking.
INTRODUCTION

Vitamin D is naturally present in a limited number of food sources; but is available through synthesis from its subcutaneous precursors with sun exposure[82]. Although 25-hydroxy vitamin D [25(OH)D] is the measureable form and best clinical measure of status, there is no consensus on a cutoff point for adequate vitamin D concentration. Most experts define deficiency as 25(OH)D <50 nmol/L[115, 157]. Worldwide, vitamin D insufficiency is a major problem, affecting more than 1 billion children and adults [26]. In the US, data from the Third National Health and Nutrition Evaluation Survey (NHANES III) showed that approximately 41% of men and 53% of women were considered vitamin D deficient [203]. A more recent NHANES report indicated that vitamin D deficiency was more prevalent among blacks and Hispanics than non-Hispanic whites, at 82.1% and 69.2% respectively [182]. A systematic review reported that the mean of serum 25(OH)D for immigrants and/or ethnic minorities ranged between 24 to 60 nmol/L [171]. Vitamin D deficiency results in calcium and phosphorous abnormalities and may lead to secondary hyperparathyroidism [115]. Due to its fat-soluble nature, adults with BMI >30 are at higher risk of vitamin D deficiency, which may be due, in part, to body fat sequestration [115]. Several studies have reported associations between obesity and lower serum 25(OH)D concentration [48, 49, 64, 173].

In the US, Puerto Ricans are the second largest Hispanic subgroup [204]. Recent publications have confirmed the presence of important health disparities as well as low socioeconomic status and education in this population [205]. Several reports have discussed the high prevalence of obesity, type 2 diabetes, hypertension, depression and cognitive decline among Puerto Ricans, relative to other Hispanic groups [164, 205-207]. However, we are aware of no reports of vitamin D status in this population. Therefore, our aim was to describe the
distribution of serum 25(OH)D concentration among Puerto Rican men and women living in the greater Boston area, and to explore its relationship with traditional determinants of 25(OH)D, including BMI.

MATERIALS AND METHODS

Study Population
Self-identified Puerto Rican adults, aged between 45-75 years, were recruited in the greater Boston area as participants in the Boston Puerto Rican Health Study (BPRHS). The BPRHS is an ongoing longitudinal study, started in 2005, to examine the role of social and psychological stress on physiological dysfunction and related health outcomes among 1504 participants. Study design, sampling, and recruitment methods have been described in detail [83]. Briefly, after selecting census tracks that contained at least 25 Puerto Rican adults, aged 45 years and older, blocks that contained ≥ 10 Hispanic adults, aged 45 and older, were enumerated. Other recruitment methods were through random approach at community events (9.4%) or as respondents to flyers or community members’ referral (6.9%). Participants were excluded if they had serious health conditions or advanced dementia, had no permanent address, or planned to move away within two years. The study protocol was approved by the Institutional Review Boards at Tufts Medical Center and Northeastern University, and each participant provided written informed consent.

For the current analysis, participants with missing data on variables needed for the analysis were excluded (n=93) as well as those with serum 25(OH)D concentration greater than 250 nmol/L (n=1) [55]. There were no significant differences in baseline sociodemographic characteristics.
between those with complete or incomplete information. The present study includes 1400 participants with complete baseline data available at the time of analysis.

**Field data collection**

During home interviews, data on age, sex, education, household income, smoking status, alcohol use, medical history and current medication use were collected by interviewer administered questionnaire. Detailed description of the field data collection is available in previous publications [83, 109]. Serum 25(OH)D concentration was categorized as deficient when <50 nmol/L, insufficient between 50-75 nmol/L, and sufficient when > 75 nmol/L[26, 157]. BMI was calculated as weight (kg) divided by height (m)² and defined by World Health Organization criteria: recommended (BMI<25), overweight (25-30), obese (30-35), and very obese (≥40) [208]. For subset analyses, we further categorized BMI using the class III obesity definition [114]. Seasonality was defined as summer, July to September; fall, October to December; winter, January to March; and spring, April to June. Age was categorized into two groups: 45 to 59 years and 60-75 years. Both smoking status and alcohol were categorized into yes and no for the current behavior.

Dietary vitamin D intake was estimated from a semi-quantitative food-frequency questionnaire (FFQ) that was specifically designed for this population [91]. This FFQ has been validated against serum carotenoids [116], and vitamin B12 [117] in a Hispanic population aged ≥ 60 years. Those with energy intakes < 600 or > 4800 kilocalories and/or > 10 questions blank on the FFQ were excluded from the analyses. Nutrient intakes were calculated with the Nutrition Data System for Research (NDS-R) software version 2007 (Nutrition Coordinating Center,
University of Minnesota, Minneapolis, MN, Food and Nutrient Database 2007, released 2007). Vitamin D from supplements was defined as: minimal or no supplement < 5 µg/day (≤ 200IU/day); moderate 5-10 µg/day (200-400 IU/day); and high ≥ 10 µg/day (≥ 400 IU/day). Total vitamin D (food and supplements) was categorized according to the current RDA (15 µg/day or 600 IU/day) for vitamin D as: ≤½ the RDA (7.5 µg/day or 300 IU/day); >½ RDA- the RDA (7.5-15 µg/day or 300-600 IU/day); and ≥ the RDA (10 µg/day or 600 IU/day) [209]. Dietary only vitamin D (µg/day) was categorized into quintiles. Serum creatinine concentration (nmol/L) was categorized into quartiles. Serum albumin (g/L) was categorized as normal 34 – 54 g/L, or high, ≥ 54 g/L. Miles/day walked was calculated from blocks reported and categorized as: 0 miles/day, up to 0.5 mile/day, and more than 0.5 mile/day.

Laboratory assays

Fasting blood samples (12-h) were drawn by a certified phlebotomist at the participant’s home on the morning after the home interview, or as soon thereafter as possible. Aliquots were saved and stored at -80°C until processed. Serum 25(OH)D concentration were measured by extraction followed by 25I radioimmunoassay Packard COBRA II Gamma Counter (DiaSorin Inc., Stillwater, MN 55082) catalog # 68100E with intra-assay and inter-assay coefficients of variation of 10.8% and 9.4%, respectively. Serum albumin was detected with a dye binding, endpoint reaction using the Olympus AU400e with Olympus Albumin Reagents (OSR6102) (Olympus America Inc., Melville, NY) Serum creatinine was assessed with a colorimetric, kinetic reaction using the Olympus AU400e with Olympus Creatinine Reagents (OSR6178) (Olympus America Inc., Melville, NY).
Statistical analyses

All statistical analyses were conducted with SAS 9.1.3 (SAS Institute Inc, Cary, NC); and all tests were 2-sided with P < 0.05 considered statistically significant. Skewed variables were natural log-transformed to normalize their distributions before analysis. We calculated age-adjusted mean serum 25(OH)D concentration across categories for known and potential determinants of vitamin D status, and tested for linear trend with the general linear models procedure (PROC GLM). Dunnett’s and Tukey’s tests were used for multiple comparison adjustment. Linear regression analysis was used to quantify the association between age, sex, education, poverty status, smoking status (yes/no), alcohol use (yes/no), number of miles walked/day, BMI, serum albumin, and serum creatinine as independent variables, and serum 25(OH)D concentration as a dependent variable. Age-adjusted mean serum 25(OH)D was calculated and plotted against medians for deciles of vitamin D intake. For comparison with general U.S. adult dietary intakes, food groups were constructed according to the USDA classification. Food sources of vitamin D intake were identified and ranked by the total intake from foods. All dietary variables were energy adjusted.

RESULTS

Complete serum 25(OH)D concentration and dietary intake information was available for 1316 participants, 935 women and 381 men. The mean of serum 25(OH)D among the BPRHS participants was about 43.5 ± 16.1 nmol/L. Approximately 70% had low vitamin D status, <50 nmol/L, whereas only 4.2% of men and 3.5% of women had serum 25(OH)D concentration >75 nmol/L (Figure 6.1). The mean ages of men and women were 56.6 ± 8 and 57.3 ± 7.5,
respectively (Table 6.1). Women had significantly lower serum albumin, serum creatinine, and walked fewer miles/day compared to men. In addition, women had significantly higher BMI and poverty status than did men. More women than men were taking supplements with vitamin D, and women tended to have higher total vitamin D intake. Serum 25(OH)D concentration was associated with total vitamin D intake as defined by the RDA. The consumption of less than $\frac{1}{2}$ RDA (7.5 µg/day) of total vitamin D intake was significantly lower than the $\frac{1}{2}$ RDA – RDA and ≥ RDA increased serum 25(OH)D concentration significantly compared to lower amounts, 48 and 50 nmol/L compared to 40.3 nmol/L (Figure 6.2). The median intake of dietary vitamin D (5.3 µg/day) and higher contributed to significantly higher serum 25(OH)D concentration (nmol/L) compared to the referent group intake of 5.22 µg/day ($P = 0.02$, $P = 0.005$ and $P = 0.01$, respectively (Figure 6.3).

Men and women consuming daily supplements with at least 5µg/day of vitamin D had significantly higher serum 25(OH)D concentration than those consuming supplements with less than 5µg/day of vitamin D ($P < 0.0001$) (Table 6.2). The season of examination was associated with serum 25(OH)D concentration ($P < 0.0001$), with lower concentration in the winter and spring, relative to the summer. Higher serum creatinine concentration was associated with significantly higher serum 25(OH)D concentration ($P = 0.009$ and $P = 0.02$ for men and women, respectively). Among women only, older women, aged 60-75 years, had significantly higher serum 25(OH)D than did women aged 45-59 y ($P = 0.007$). BMI had an inverse relationship with age-adjusted serum 25(OH)D concentration ($P$-trend 0.001). Compared to never-smokers, women who were current smokers had significantly lower 25(OH)D concentration ($P = 0.02$). Women, but not men, had significantly higher serum 25(OH)D concentration when walking more miles/day ($P$-trend =0.01). Among men, lower serum albumin concentration was associated
with lower serum 25(OH)D ($P = 0.05$). Alcohol intake was not significantly associated with 25(OH)D.

In a full model with adjustment for other variables, serum 25(OH)D remained significantly associated with dietary vitamin D intake ($P < 0.0001$), use of vitamin D-containing supplements ($P < 0.0001$), seasonality ($P < 0.0001$), and number of miles walked/day ($P < 0.05$) (Table 6.3). There was a significant interaction between sex and vitamin D supplement use ($P = 0.008$) (Figure 6.4). Among women, supplements with vitamin D in the range of 4.5-10 µg/day and those $\geq 10$ µg/day, were associated with significantly higher mean serum 25(OH)D concentration, 45 nmol and 51.3 nmol/L, respectively, compared with non-supplement use ($P < 0.0001$), 40 nmol/L. However, among men, only supplements providing 5-10 µg/day of vitamin D were associated with significantly higher mean serum 25(OH)D concentration compared with non-supplement use ($P < 0.0001$).

Another interaction was between seasonality and age ($P = 0.0008$) (Figure 6.5). Younger participants, ages 45-59 years, had significantly higher 25(OH)D in both summer and fall, 50.5 nmol/L and 49.0 nmol/L, respectively, relative to winter and spring, (41.5 nmol/L and 39.0 nmol/L, respectively, $P < 0.0001$). However, among older participants, ages 56 - 75 years, serum 25(OH)D did not differ significantly across the four seasons.

Subset analyses of the women showed that serum 25(OH)D was significantly lower with increased obesity ($P$-trend = 0.001) when BMI was further categorized into obesity categories. Women with class III obesity, BMI $\geq 40$, had significantly lower serum 25(OH)D concentration (41.6 nmol/L) compared to lower BMI (Figure 6.4) adjusting for age, seasonality and vitamin D supplements, and number of miles walked/day. In addition to supplements, a range of foods contributed to vitamin D intake (Table 6.4). Fortified milk was the major contributor, at 34% of
total vitamin D intake from food. Fish ranked second, at 16.2%, as one of the food sources that is naturally rich of vitamin D. Eggs and ready-to-eat cereal each contributed about 4.5% to total dietary vitamin D intake.

DISCUSSION

These results highlight the high prevalence of vitamin D deficiency among Puerto Rican adults living in the greater Boston area, and show an inverse relationship between BMI and serum 25(OH)D concentration. The majority (70%) of the men and women in our study had low vitamin D status, with a mean circulating of 25(OH)D of 44.0 nmol/L—which is lower than that reported in the literature for several other populations [182, 210-212]. Our data concur with a recent NHANES report showing that 69.2% of U.S. Hispanics had low vitamin D. This greater risk of vitamin D deficiency may translate into greater risk of health disparities [182]. Although debated, most current recommendations suggest that serum concentration should be above 75 nmol/L [26, 29, 51, 82, 182]. We found that only 3% of Puerto Rican men and 4% of women achieved this [213]. This disparity may have various explanations, including skin pigmentation [31, 182]. Darker skin is associated with lower 7-dehydrocholesterol conversion to its active metabolite, which requires longer sunlight exposure [214]. In addition, these participants reside in Boston, which lies on the 43.3°N, with relatively low UV-B exposure [31]. Moreover, 50% of the BPRHS participants reported incomes below the poverty line, and 60% reported education of less than 8th grade [83] indicating low socioeconomic status. This may translate into fewer healthy food choices and limited access to supplements.
Unlike many reports, aging was not negatively associated with serum 25(OH)D concentration in this population [212, 215]. With aging, the presence of vitamin D precursors within the skin declines, which puts the elderly at higher risk of vitamin D deficiency [26, 216]. A recent report using NHANES III data reported significant declines with aging [215, 217] while others have reported no significant difference across age groups [182]. In our sample, the difference appears to be due to greater use of supplements, particularly among older women, relative to younger individuals. Vitamin D supplements as well as dietary vitamin D have previously been shown to be associated with serum 25(OH)D concentration [218, 219]. This study confirms the importance of vitamin D from supplementation and dietary sources, especially among women, on improving vitamin D status. In our population, 30% of women were consuming supplements with higher vitamin D concentration (≥ 5 µg/day or 200 IU/day) compared to 24% of men. In general, women tend to be more likely than men to be prescribed vitamin D-containing supplements, often for osteoporosis prevention. When compared to the RDA, levels, only 5% of this population is meeting the current RDA of vitamin D, which is 15 µg/day or 600 IU/day [209]. This low percentage meeting the RDA is consistent with poor health status, which has been observed among this population [109, 113, 220].

The relationship between BMI and serum 25(OH)D concentration among Puerto Rican women is noteworthy. Our results showed that with increased obesity, especially when reaching class III obesity, serum 25(OH)D concentration declined significantly. Our results indicate that serum 25(OH)D concentration reached a plateau at BMI 35 or less, compared to severely obese categories. Most studies have found an inverse relationship between obesity and serum 25(OH)D concentration [48, 64, 173]. One of the proposed theories that links obesity to lower 25(OH)D is that the latter leads to an increase in iPTH concentration, resulting in interference with lipolysis
and greater expression of fatty acid synthase [221]. Another theory suggests that lower 25(OH)D may be one of the causes of increased fat mass during the winter season [222]. It has also been shown that there is increased sequestration of 7-dehydrocholesterol in adipose tissue, thus fewer precursor molecules are available [48, 49]. This population has a high (57%) prevalence of obesity [83, 113, 223], combined with very low physical activity [83], both of which translate into low outdoor activity, thus reduced sun exposure, all of which are strong predictors of lower serum 25(OH)D concentration. This complex of factors may explain why this Puerto Rican population has very low serum 25(OH)D concentration.

Several limitations of our study should be noted. First, due to the cross-sectional design, associations cannot be translated into cause and effect relationships. In addition, residual confounding remains a possibility, even though we adjusted for several essential covariates. Third, we did not have a measure of sun exposure, which would have added predictive ability to the models, although we did include a measure of outdoor physical activity which is a likely proxy. Nevertheless, our study design was strengthened by the use of our validated FFQ that has been designed for this particular ethnicity group [116, 117]. This report of vitamin D status shows high prevalence of vitamin D deficiency in a large Puerto Rican adult population. Because vitamin D status has been associated with numerous health outcomes, it is likely that improving vitamin D status in this population at high risk of health disparities, could contribute to reducing health disparities.


<table>
<thead>
<tr>
<th>No.</th>
<th>Author(s)</th>
<th>Title</th>
<th>Journal</th>
<th>Volume</th>
<th>Issue</th>
<th>Pages</th>
</tr>
</thead>
</table>

144. 2010 ICD-9-CM.


<table>
<thead>
<tr>
<th></th>
<th>Women (n=1050)</th>
<th>Men (n=445)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td>57.3 ± 7.5</td>
<td>56.6 ± 8.0</td>
<td>0.1*</td>
</tr>
<tr>
<td>Education (&lt; 8th grade %)</td>
<td>48.0</td>
<td>44.0</td>
<td>0.1†</td>
</tr>
<tr>
<td>Poverty (%)</td>
<td>62.0</td>
<td>51.0</td>
<td>0.0003†</td>
</tr>
<tr>
<td><strong>BMI (kg/m^2)</strong></td>
<td>33.0 ± 7.0</td>
<td>30.0 ± 5.2</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>Serum Albumin (g/L)</td>
<td>42.5 ± 3.1</td>
<td>43.2 ± 3.6</td>
<td>0.001*</td>
</tr>
<tr>
<td>Serum Creatinine (nmol/L)</td>
<td>68.0 ± 27.5</td>
<td>88.0 45.1</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>Number of Miles Walked/day‡</td>
<td>1.0 ± 1.4</td>
<td>1.3  ± 1.6</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>43.1 ± 17.0</td>
<td>43.5 ± 16.5</td>
<td>0.6*</td>
</tr>
<tr>
<td>Vitamin D Supplement use (&gt; 5µg/day) (%)</td>
<td>30.0</td>
<td>24.0</td>
<td>0.02†</td>
</tr>
<tr>
<td>Dietary vitamin D intake*</td>
<td></td>
<td>(µg/day)</td>
<td>5.3± 2.7</td>
</tr>
<tr>
<td>Total vitamin D intake*</td>
<td></td>
<td>(µg/day)</td>
<td>7.3 ± 3.3</td>
</tr>
</tbody>
</table>

BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D.

1 Data are presented as mean ± SD for continuous variables or as % for categorical variables.

* Values were compared using t-test. † Values were compared using χ²-test.

‡ Range = 0-10 miles/day

* The recommended dietary allowance (RDA) for vitamin D for men and women aged 19-70 years =15 µg /day [82]

‖ Adjusted for energy intake using ANOVA (PROC GLM; SAS Institute, Cary, NC).
TABLE 6.2 Age-adjusted means of serum 25-hydroxyvitamin D (25(OH)D) concentration stratified by sex for different characteristics among BPRHS participants, ages 45-75 years

<table>
<thead>
<tr>
<th>Variables</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%) *</td>
<td>LSM</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45-59</td>
<td>651 (61.5%)</td>
<td>42.0</td>
</tr>
<tr>
<td>60-75</td>
<td>407 (38.5%)</td>
<td>45.0</td>
</tr>
<tr>
<td>Vitamin D Supplement (IU/day) † ‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 200</td>
<td>738 (70.0%)</td>
<td>41.1</td>
</tr>
<tr>
<td>200-400</td>
<td>224 (21.5%)</td>
<td>46.5</td>
</tr>
<tr>
<td>≥ 400</td>
<td>77 (8.5%)</td>
<td>53.1</td>
</tr>
<tr>
<td>Season of measurement †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer (July-September)</td>
<td>356 (34.5%)</td>
<td>44.5</td>
</tr>
<tr>
<td>Fall (October- December)</td>
<td>264 (26.0%)</td>
<td>46.0</td>
</tr>
<tr>
<td>Winter (January-March)</td>
<td>184 (18.0%)</td>
<td>41.1</td>
</tr>
<tr>
<td>Spring (April-June)</td>
<td>224 (21.5%)</td>
<td>38.2</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²) †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 25</td>
<td>114 (11.0%)</td>
<td>44.0</td>
</tr>
<tr>
<td>25-30</td>
<td>282 (27.3%)</td>
<td>45.5</td>
</tr>
<tr>
<td>30-35</td>
<td>307 (29.3%)</td>
<td>44.5</td>
</tr>
<tr>
<td>≥ 35</td>
<td>307 (29.3%)</td>
<td>44.5</td>
</tr>
</tbody>
</table>

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### Smoking Status

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>44.0</th>
<th>1.1</th>
<th>832 (79.5%)</th>
<th>291 (66.0%)</th>
<th>44.0</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>41.0</td>
<td>0.5</td>
<td>217 (20.5%)</td>
<td>149 (34.0%)</td>
<td>43.0</td>
<td>1.4</td>
</tr>
</tbody>
</table>

### Alcohol Intake

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>44.0</th>
<th>0.5</th>
<th>675 (64.5%)</th>
<th>211 (49.0%)</th>
<th>44.5</th>
<th>1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>44.0</td>
<td>0.8</td>
<td>376 (35.5%)</td>
<td>228 (51.0%)</td>
<td>43.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

### Serum Albumin (g/L)

<table>
<thead>
<tr>
<th></th>
<th>&lt; 38</th>
<th>42.0</th>
<th>2.2</th>
<th>49 (5%)</th>
<th>16 (4.0%)</th>
<th>35.0</th>
<th>4.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥ 38</td>
<td>43.1</td>
<td>0.5</td>
<td>964 (95%)</td>
<td>410 (96.0%)</td>
<td>44.0</td>
<td>0.8</td>
</tr>
</tbody>
</table>

### Serum Creatinine (nmol/L)

<table>
<thead>
<tr>
<th></th>
<th>35.5 - 61.0</th>
<th>41.5</th>
<th>1.0</th>
<th>280 (27.5%)</th>
<th>19 (4.5%)</th>
<th>34.5</th>
<th>4.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>62.0 - 70.0</td>
<td>42.5</td>
<td>1.0</td>
<td>298 (29.0%)</td>
<td>46 (11.0%)</td>
<td>40.0</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>71.0 - 79.5</td>
<td>45.3</td>
<td>1.1</td>
<td>223 (22.0%)</td>
<td>92 (21.5%)</td>
<td>42.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>80.0 - 698</td>
<td>44.1</td>
<td>1.1</td>
<td>221 (22.0%)</td>
<td>270 (63.0%)</td>
<td>45.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

### Miles Walked/day

<table>
<thead>
<tr>
<th></th>
<th>0 mile/day</th>
<th>41.3</th>
<th>1.1</th>
<th>263 (25.0%)</th>
<th>52 (12.0%)</th>
<th>40.5</th>
<th>2.4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0- 0.5 mile/day</td>
<td>42.5</td>
<td>0.8</td>
<td>395 (38.0%)</td>
<td>140 (32.0%)</td>
<td>44.0</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>&gt; 0.5 mile/day</td>
<td>45.0</td>
<td>1.0</td>
<td>367 (37.0%)</td>
<td>247 (56.3%)</td>
<td>44.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

---

1. Age-adjusted means were calculated with LSMEANS ANOVA (PROC GLM; SAS Institute, Cary, NC). Values presented are means
2. P-values for trend are calculated based on Dunnett adjustment for multiple comparisons (P > 0.05)
TABLE 6.3 Correlates\(^1\) of serum 25(OH)D concentration among Puerto Rican adults stratified by sex.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women (n=935)</th>
<th>Men (n=381)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\beta^*)</td>
<td>SEM</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.15</td>
<td>0.01</td>
</tr>
<tr>
<td>Seasonality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fall vs. Summer</td>
<td>2.0</td>
<td>0.15</td>
</tr>
<tr>
<td>Winter vs. Summer</td>
<td>-3.0</td>
<td>0.04</td>
</tr>
<tr>
<td>Spring vs. Summer</td>
<td>-6.1</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>-0.24</td>
<td>0.002</td>
</tr>
<tr>
<td>Miles walked/day</td>
<td>0.6</td>
<td>0.05</td>
</tr>
<tr>
<td>Vitamin D supplement (µg/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5 vs. 10 µg/day</td>
<td>-11.0</td>
<td>0.001</td>
</tr>
<tr>
<td>5-10 vs. 10 µg/day</td>
<td>-6.5</td>
<td>0.002</td>
</tr>
<tr>
<td>Dietary vitamin D (µg/day)(^†)</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Serum Albumin (g/L)</td>
<td>0.5</td>
<td>0.006</td>
</tr>
<tr>
<td>Smoking (No vs. Yes)</td>
<td>3.5</td>
<td>0.008</td>
</tr>
</tbody>
</table>

\(^1\)\(\beta\)-coefficients, SEM and \(P\)-values were calculated using PROC GLM; (SAS Institute, Cary, NC). All covariates were included in the model simultaneously, including adjustment for serum creatinine, alcohol intake education (< 8th grade %) and poverty.

\(^†\)Dietary vitamin D adjusted for energy intake.
TABLE 6.4 The least-square means of serum 25(OH)D concentration for the effect of vitamin D supplements categories by sex

<table>
<thead>
<tr>
<th></th>
<th>Women (n=935)</th>
<th>Men (n=381)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SEM</td>
</tr>
<tr>
<td>≤ 200 IU/day</td>
<td>40.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.70</td>
</tr>
<tr>
<td>200-400</td>
<td>45.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.14</td>
</tr>
<tr>
<td>≥ 400 IU/day</td>
<td>51.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.85</td>
</tr>
</tbody>
</table>

β coefficients, SEM and P-values were calculated using PROC GLM; (SAS Institute, Cary, NC). The model was adjusted for age, BMI, season, dietary vitamin D, miles walking/day, serum albumin, serum creatinine, smoking, alcohol intake education (< 8th grade %) and poverty.
TABLE 6.5 The least-square means of serum 25(OH)D concentration and seasons categories by age groups

<table>
<thead>
<tr>
<th></th>
<th>40-59 Years</th>
<th></th>
<th>60-75 Years</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SEM</td>
<td>β</td>
<td>SEM</td>
</tr>
<tr>
<td>Summer</td>
<td>50</td>
<td>1.50</td>
<td>46</td>
<td>1.5</td>
</tr>
<tr>
<td>Fall</td>
<td>50.3</td>
<td>1.15</td>
<td>48.5</td>
<td>1.32</td>
</tr>
<tr>
<td>Winter</td>
<td>41.3</td>
<td>1.35</td>
<td>45.2</td>
<td>1.75</td>
</tr>
<tr>
<td>Spring</td>
<td>38.4</td>
<td>1.32</td>
<td>43.4</td>
<td>1.65</td>
</tr>
</tbody>
</table>

β coefficients, SEM and P-values were calculated using PROC GLM; (SAS Institute, Cary, NC). The model was adjusted for sex, BMI, dietary vitamin D, miles walking/day, serum albumin, serum creatinine, smoking, alcohol intake education (< 8th grade %) and poverty.
**TABLE 6.6 Major Dietary Sources of Vitamin D intake among Puerto Rican adults**

<table>
<thead>
<tr>
<th>Rank</th>
<th>Food Groups</th>
<th>% contribution of dietary vitamin D intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Milk (whole, 2%, 1% &amp; milk products)</td>
<td>32.4</td>
</tr>
<tr>
<td>2</td>
<td>Supplements</td>
<td>28.7</td>
</tr>
<tr>
<td>3</td>
<td>Fish</td>
<td>16.2</td>
</tr>
<tr>
<td>4</td>
<td>Eggs</td>
<td>4.5</td>
</tr>
<tr>
<td>5</td>
<td>Cereal, Ready-to-eat, Cold</td>
<td>4.3</td>
</tr>
<tr>
<td>6</td>
<td>Beef</td>
<td>2.3</td>
</tr>
<tr>
<td>7</td>
<td>Processed Meat, Sausage, Frank</td>
<td>2.1</td>
</tr>
<tr>
<td>8</td>
<td>Chicken/Turkey</td>
<td>2.0</td>
</tr>
<tr>
<td>9</td>
<td>Pork</td>
<td>1.3</td>
</tr>
<tr>
<td>10</td>
<td>Ice cream, Sherbet, Frozen Yogurt</td>
<td>1.2</td>
</tr>
<tr>
<td>11</td>
<td>Yogurt</td>
<td>1.1</td>
</tr>
<tr>
<td>12</td>
<td>Cheese</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Food groups listed were the ones that contributed more than 0.8% of dietary vitamin D intakes.
FIGURE 6.1 Prevalence of low serum 25-hydroxyvitamin D concentration among Puerto Rican men (n=426) and women (n=1015). Serum 25(OH)D concentration was categorized: deficient ≤ 50 nmol/L, insufficient 50-75 nmol/L, and sufficient ≥ 75 nmol/L. Values are percentage of individuals.
FIGURE 5.2 Association between the median intake of total vitamin D as defined by the RDA and serum 25(OH)D concentration (nmol/L) for BPRHS participants (n=1433). The vitamin D RDA is 15 µg/day = 600 IU/day. Data points are least square means for serum 25(OH)D, adjusted for age, sex, and season, plotted against category of the RDA of vitamin D intake ($P$-trend <0.0001). RDA categories are significantly different from referent group ≤½ RDA (Dunnett adjustment for multiple comparisons; $P < 0.05$).
FIGURE 6.3 Association between vitamin D intake from food only sources (µg/day) and serum 25(OH)D concentration (nmol/L) among non-supplement users of the BPRHS participants (n=1036). Data points are least square means, adjusted for age, sex, and season, plotted at the median for each quintile category of energy-adjusted vitamin D intake from food ($P$-trend = 0.0007). Dietary intake of vitamin D equal to 5.28 µg/day and higher contributed to a significantly higher serum 25(OH)D concentration (nmol/L) compared to the referent group 5.23µg/day (Dunnett adjustment for multiple comparisons; $P < 0.05$).
FIGURE 6.4 Mean serum 25(OH)D concentration by BMI categories of Puerto Rican women (n=990), adjusted for age, season, dietary vitamin D and vitamin D supplement use (P-trend = 0.001). BMI ≥40 is significantly different from the referent group BMI ≥ 25; (Dunnett adjustment for multiple comparisons; P < 0.05).