BONE MINERAL DENSITY LOSS IN WOMEN WHO INITIATED ANTIHYPERTENSIVE MEDICATIONS DURING THE MENOPAUSAL TRANSITION IN A MULTICENTER, MULTIETHNIC, COMMUNITY-BASED COHORT STUDY: WOMEN'S HEALTH ACROSS THE NATION (SWAN)

by

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ABSTRACT

Objective: The objective of this study is to examine the association between initiating antihypertensive medications and bone mineral density (BMD) loss over time in women while transitioning through menopause.

Methods: Women who initiated antihypertensive use during menopausal transition were selected from the Study of Women across the Nation. Nonusers were matched to users with two methods: frequency matching and propensity score matching. Femoral neck, total hip and lumbar spine BMD were assessed annually and rate of loss was calculated and used as outcomes. Mixed-effects regression modeling strategy was used to examine the association between antihypertensive use and BMD loss.

Results: Among 2365 eligible women, we identified new users of angiotensin-converting enzyme inhibitors (ACE), beta blocker, and thiazide diuretics (N= 98, 107, and 99, respectively) and frequency-matched nonusers (N=1001). After propensity score matched sets were created, 69 ACE, 88 beta blocker and 76 thiazide users were matched with equal numbers of nonusers. After adjustment for potential confounders, both methods show that thiazide diuretics have a protective effect on femoral neck, total hip and lumbar annualized BMD loss compared to nonusers. Neither ACE nor beta blocker has an association with BMD loss at any anatomic site.

After matched using propensity scores, it shows that thiazide has a significantly protective effect on lumbar spine during late- and post- menopause, but not during pre- /peri-menopause.

Conclusion: In this cohort of women across the menopausal transition, use of thiazide diuretics is associated with a decreased rate of bone loss at the lumbar spine, total hip and femoral neck and use of ACE or beta blocker is not associated with bone loss at any of the sites.

Public Health Significance: The findings in this study provided reassurance for women who were using ACE or beta blocker to control blood pressure during the menopause transition, because neither of them has any negative effect on BMD loss during the transition. The results of this study also encourage clinicians to integrate the benefits of using thiazide diuretics, from the prospective of protecting the bone loss, into patients' education, especially for women during late- or post-menopause if there are no other contraindications.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

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1.0 INTRODUCTION

Approximately 6,000 women reach menopause every day (over 2 million per year) in the United States.[1] Women's health, especially bone health, is critical during this transition phase because research shows that bone mineral density (BMD) loss begins before the cessation of menses, accelerates 1 year before the final menstrual period (FMP) and slows 2 years after it.[2] Approximately one in two women over age 50 will break a bone because of osteoporosis.[3]

Osteoporosis is defined as a disease characterized by low bone mass and deterioration of bone structure, causing bone fragility and increased risk of fracture. According to the statistics provided by the National Osteoporosis Foundation, about 80% of the estimated 10 million Americans with osteoporosis, are women.[3] Moreover, osteoporosis and hypertension share a similar etiopathology and often coexist.[4] Since the 1960s, antihypertensive medications have been discovered and are increasingly and regularly used among patients. Assessing the relationship between exposure to antihypertensive medications and BMD loss is essential for people who are diagnosed as hypertensive and at risk for osteoporosis, especially for women during the menopausal transition.

The Study of Women's Health Across the Nation (SWAN) is a multiethnic, multicenter, longitudinal community-based cohort study of the psychosocial and biological changes that occur during the menopausal transition.[5] The bone data from the SWAN study was obtained annually from five study sites (Boston, Pittsburgh, Detroit, Oakland, and Los Angeles areas)

among the 7 sites in SWAN, allowing for research on women during the menopause transition period. The aim of this secondary analysis is, distinguished from previous research, to investigate the effect of initiating antihypertensive medications on BMD loss, with a focus on transmenopause. This analysis will quantify the effect of antihypertensive medications exposure during the transition on bone health and will help healthcare professionals incorporate knowledge of antihypertensive medications into treatment and patient education.

1.1 SWAN STUDY

SWAN, first funded since 1994, is sponsored by the National Institute on Aging and the National Institute of Nursing Research.[6] This study has 7 clinical field sites in the United States, including Detroit, MI (University of Michigan), Boston, MA (Massachusetts General Hospital), Chicago, IL (Rush Presbyterian-St. Luke's Medical Center), Oakland, CA (University of California Davis and Kaiser Permanente), Los Angeles, CA (University of California at Los Angeles), Newark, NJ (Mount Sinai Medical Center), and Pittsburgh, PA (University of Pittsburgh), with a central reproductive hormone laboratory, and a coordinating center located at the University of Pittsburgh Graduate School of Public Health (Pittsburgh, PA).

SWAN surveyed over 16,000 women and enrolled 3302 women in the study who were aged 42 to 52 at the study entry (1996-97); all had an intact uterus and at least one ovary and had had one or more menstrual periods during the previous 3 months.[5] They were not pregnant, breast-feeding, or taking reproductive hormones.[5] Among the women enrolled, 1550 were Caucasian, 935 African American, 286 Hispanic, 250 Chinese and 281 Japanese. They were followed annually and all follow-up visits were scheduled based on the individual's baseline

index date (the day on which the first appointment for the baseline visit occurred). At each SWAN visit, the participants were evaluated for a wide spectrum of physiological, physical, behavioral and psychological measures. Details of the study design and recruitment process have been published previously.[7]

1.2 STUDY BACKGROUND

1.2.1 Bone Metabolism and Menopause

In our body, bones are constantly remodeling. Approximately every ten years our skeleton is being completely renewed.[8] During the bone turnover cycles, bone-resorbing osteoclasts and bone-forming osteoblasts are coupled and controlled by a variety of hormones and cytokines, as well as by mechanical loading.[9,10] An increased activity of osteoclasts or decreased activity of osteoblasts leads to a decrease in bone mass or osteoporosis.

Osteoporosis is a disease characterized by low bone mass and deterioration of bone structure, causing bone fragility and increased risk of fracture. According to the World Health Organization (WHO), osteoporosis is defined as having a BMD value less than 2.5 standard deviations from the young-adult normal value (T-score < 2.5).[11] Millions of people worldwide suffer from osteoporosis, causing a big burden on the health system.[8] Currently osteoporosis is an epidemic in the United States with approximately 9.1 million women and 2.8 million men afflicted with the disease in 2010.[12]

Almost eighty percent of people who suffer from osteoporosis are women, with women over the age of 50 having the greatest risk of developing the disease.[12] Since the average age of a

woman having her final menstrual period (FMP) is around 50, postmenopausal women have a high risk of developing osteoporosis. However, SWAN investigators found that BMD loss even began before the FMP. Greendale et al[13] found that femoral neck BMD loss in White women started approximately 1 year before the FMP (year -1) and sharply declined about 1.76% annually until approximately 2 years after the FMP(year +2), thereafter, the BMD loss rate decelerated to 1.15% per year and did not cease. Similarly, the steep decline of femoral neck BMD occur in African American, Chinese and Japanese women as well, with a loss rate of 1.42%, 2.17% and 2.13% respectively from year -1 to +2, and a loss rate of 1.09%, 1.01% and 1.24% respectively after year +2. The cumulative 10 years lumbar spine BMD loss was 10.6% in White women and 7.38% was lost during the transmenopause.[2] Similarly, cumulative 10 year femoral neck loss was 9.1% in White women and 5.8% was lost during the transmenopause.

The accelerated BMD loss during transmenopause has clinical implications, most seriously bone fracture, which can lead to patient disability or even death. Various treatments are currently available to reduce the impact of bone fragility, but there is a lack of comprehensive treatment for the whole musculoskeletal system that leads to osteoporotic fracture.[14] New treatments or prevention methods are needed, especially for women across the menopause. The SWAN study provides the investigators a means to explore secondary prevention strategies for menopausal women once bone loss has started or bone fractures have occurred.

1.2.2 Blood Pressure and Menopause

Since the 1980s, a number of studies in both humans and animal models of hypertension have suggested an association between hypertension and osteoporosis.[15-18] Hypertension is one of the major risk factors for cardiovascular disease and also one component of the metabolic syndrome,

of which the incidence increases substantially during perimenopause and early menopause.[19] Martins et al, using data from NHANES III, conducted a cross-sectional study showing that gender or sex hormones have a prominent role in blood pressure regulation.[20] Cross-sectional studies also show that postmenopausal women are at a higher risk of hypertension compared to age-matched men[20] or their premenopausal counterparts[19]. Several cross-sectional studies concluded that either elevated blood pressure or incidence of hypertension is related to menopause independently of age by comparing two groups of women either in premenopausal status or in postmenopausal status. However, several limitations of these studies are noted including: not controlling for BMI and ethnicity in Weiss et al' study[21] and self-reported menopausal status not being verified retrospectively in Staessen et al's research[22].

Furthermore, longitudinal and cross-sectional studies have produced mixed results on the relationship between hypertension and osteoporosis. Janssen et al[23] investigated the metabolic syndromes occurred in the nature history of the menopausal transition among 949 participants, with 9 years' follow-up in SWAN. They found that systolic blood pressure increased with aging but not significantly (p=0.07) and there was no effect of menopause on blood pressure. The study of health in Pomerania[24] which was conducted on Mediterranean population, also failed to detect the significant change of blood pressure during the passage from premenopause to postmenopause independently of age with up to 6 years' follow-up, but the conclusion may not be applicable to other ethnic groups or populations with different geographical or cultural conditions. The finding in Pizarra study[24] was that the women who went from premenopause to postmenopause experienced no significant changes in blood pressure as compared with the women who did not yet have menopause. However, the sample size was insufficient to detect small effect sizes of interest.

Despite contradictory opinions on the effect of menopause on blood pressure, several mechanisms may explain the development of hypertension in postmenopausal women. These mechanisms involve cardiovascular risk factors, including weight and lipid levels; endothelial dysfunction, oxidative stress, inflammatory mediators and activation of the renin angiotensin and sympathetic systems.[19] It remains uncertain whether these physiological changes are caused by the menopausal transition itself or by aging.

Moreover, an age-related decrease in blood pressure control rates were more pronounced in women than in men, as shown by the Framinghan Heart Study.[25] It is still unknown whether the decline in blood pressure control in women is due to sexual hormone related treatment resistance or due to non-optimal pharmacologic management in the clinical setting. Thus, the SWAN study provides the investigators with longitudinal observational data from the clinical setting, to answer further research questions regarding the effect of drug choices, independent of biological changes occurring during menopause.

1.2.3 Factors associated with Bone Mineral Density

Factors that are associated with bone mineral density include demographic, socioeconomic, genetic, hormonal, and nutritional factors, as well as body weight, and lifestyle choices such as diet (Calcium/Vitamin D supplement, alcohol consumption), smoking and exercise. Lack of exercise, low body mass index (BMI), absence of menses, family history of osteoporosis, alcohol abuse, and smoking are all the risk factors that lead to low BMD. Low BMI[26] or low body weight affects premenopausal BMD and perimenopausal bone loss, shown by previously published SWAN data.[2] Calcium and Vitamin D supplements show a protective effect on BMD.

The hypothesis of estrogen deficiency causing postmenopausal osteoporosis was first published in 1941 by Albright et al[19], and this mechanism has been strongly supported by studies showing that estrogen administration prevented bone loss induced by oophorectomy in perimenopausal women.[27,28] Although the mechanisms by which estrogen regulates bone turnover are not well understood, studies in animals suggest that estrogen acts by altering the activities of factors that regulate osteoblast and osteoclast precursors.[29,30] Estrogen therapy is an effective option that increases bone mass; however, it produces a decrease in both bone formation and resorption associated with decreased remodeling. Moreover, because large randomized trials of hormone replacement therapy have called into question the long assumed protective effect of estrogen in heart disease risk, long term use of estrogen for increasing or maintaining BMD is not recommended.[31]

Comorbidities or medications taken for other indications can profoundly affect BMD.[10] Effects that directly or indirectly affect bone metabolism may be beneficial or harmful. Cancer treatment-induced bone loss is generally more rapid and severe than bone loss associated with menopause in women.[32] Other diseases, such as diabetes, arthritis or osteoarthritis, hyperthyroidism, and psychiatric comorbidities have demonstrable decreases in BMD.[33]

The advances in the osteoporosis therapeutic field have been very significant over the last two decades. Other pharmacotherapies available for the management of the patients with low BMD are classified into two groups: antiresorptive and anabolic agents. The antiresorptive or anticatabolic agents, such as bisphosphonates, selective estrogen receptor modulators, hormone therapy, suppress or attenuate the activity of the bone-resorbing cells, the osteoclasts, hence stopping bone loss and increasing bone strength. Anabolic agents, such as parathyroid hormone, induce bone formation, reversing in part the deterioration induced by the osteoporosis

progression. Although there are various treatments available for postmenopausal osteoporosis[10], it is still important to develop preventive measures.

Moreover, there are concerns regarding the side effects of the high dose or prolonged medications therapy. Following a thorough review of available safety data, the FDA has determined an osteoporosis and fracture warning on the over-the-counter proton pump inhibitor medication which has been used long-term to manage Gastroesophageal Reflux Disease.[34] It is important to observe any drug effects over time, especially for drugs that need to be taken regularly, such as antihypertensive agents, especially when there is a lack of randomized controlled trials.

1.2.4 Antihypertensive Medication Use and Bone Mineral Density

If not treated effectively, hypertension can lead to an increased risk of heart attack, stroke and renal failure. The earliest pharmacological remedies to treat hypertension included nitrites, thiocyanates, dehydrogenated alkaloids of ergot, pyrogens, and veratrum viride.[35] The most important breakthrough in the history of the drug treatment of hypertension came with the discovery of the orally effective diuretic, chlorothiazide in the late 1960s.[35]

Antihypertensive medication use among US adults with hypertension has significantly increased over the past 10 years.[36] The treatment of hypertension is based on the prescription of four major classes of antihypertensive drugs. According to the National Health and Nutrition Examination Survey from 2001 to 2010, the use of thiazide diuretics, β-blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers increased by 23%, 57%, 55%, and 26%, respectively.[36] Despite the wide range of drugs available to lower blood pressure, there has not been a novel antihypertensive mechanism entering the market in more than a decade,

resulting in very few new drug therapies for hypertension.[37] Therefore, it is crucial for physicians to optimize their antihypertensive therapies with the drugs available on the market.

It has been confirmed in several studies that thiazide diuretics have a positive effect on BMD. To the author's knowledge, there are three randomized controlled trials conducted to prove the protective effect. One randomized, double-masked, placebo-controlled trial examined the effect of chlorthalidone compared with placebo on three appendicular sites (calcaneus, distal radius and proximal radius) in 113 postmenopausal women in 1995, with an average of 2.6 years follow-up time[38], and another randomized, double-blind, placebo-controlled trial was conducted using hydrochlorothiazide compared with using placebo in 320 healthy normotensive adults whose ages ranged from 60 to 79 and were followed for 3 years.[39] The latter study shows that percentage increase of posterior-anterior spine BMD after treatment of low-dose hydrochlorothiazide (25mg per day) for 6 month was significantly greater (p =0.005) than the percentage increase in placebo group. However, only modest effects (0.82%; p = 0.12) were observed over 3 years. The study also concludes that the treatment effect on women is greater than men. Another clinical trial[40] was performed for 2 years (and then a 2-year extension) on 185 healthy postmenopausal women to examine hydrochlorothiazide's effect (verse placebo) on BMD change over time. They found significant between-groups (hydrochlorothiazide and placebo) differences over the course of 4 years regarding the change in bone density of the total body (0.9%, $p< 0.001$), legs (1.0%, $p = 0.002$), mid-forearm (1.1%, $p = 0.03$), and ultradistal forearm (1.4%, $p= 0.04$), whereas, the BMD changes measured cumulatively for 4 years were at the lumbar spine (0.9%, $p= 0.76$) and femoral neck (0.4%, $p = 0.53$) did not differ between groups.

Cross-sectional and longitudinal studies performed in humans have also demonstrated a slower rate of bone loss with thiazide diuretics therapy. Cauley et al[38], using a cross-sectional study, came to a conclusion that women at least 65 years old and using thiazide diuretics for more than 10 years had significantly higher bone mass (calcaneus, distal radius and proximal radius distal radius) than women who had never used thiazide diuretics. Wasnich et al[37] concluded that rates of bone loss at all three sites (calcaneus, distal radius and proximal radius) were significantly reduced among men who took thiazide diuretics for an average of 11.9 years when compared with men who took antihypertensive durgs other than thiazide. Sower et al[41] conducted a prospective study and suggested that current users of the thiazide class of medications had less 5-year cumulative radial bone loss (5.0% vs 7.4%, $p = 0.0035$) than women without current thiazide use. In conclusion, the small scale, short term randomized controlled trials cannot provide conclusive proof regarding thiazide' protective effect on BMD loss and the outcomes of the observational studies are rarely measured for lumbar spine, total hip and femoral neck. Thus, observational studies provide important complementary evidence.

For other antihypertensive drugs, there have been conflicting results. Of note, to the author's knowledge, there have been no randomized controlled trials conducted in regard to angiotensin-converting enzyme inhibitors (ACE) or beta blocker with BMD values as the outcomes and there are fewer observational data, as well, for ACE or beta blocker than for thiazide diuretics. A cross-sectional study[42] conducted in Chinese women whose age range from 65 to 92, shows that ACE use compared to nonuse was associated with higher femoral neck BMD (0.015 g/cm2, $p = 0.035$) in women, but not associated with total hip or lumbar spine. Based on a 1-year prospective cohort study of 50 postmenopause women with hypertension

using fosinopril, García-Testal et al[43] reported the loss of BMDs in lumbar spine and femoral neck was not significanly prevented.

Likewise, the effect of beta blocker on human bones has been considered in only a few studies and the data are not consistent. Shuman et al[44] used data from Dubbo Osteoporosis Epidemiology Study, which included 2203 women with a mean age of 68.7. In women, beta blocker users had higher femoral neck BMD ($p < 0.01$) and higher lumbar spine BMD ($p < 0.01$) than those not on beta blocker in cross-sectional analyses. Pasco et al[45] found that beta blocker use compared to nonuse was associated with a higher BMD at the total hip (2.5%, $p = 0.03$) and ultradistal forearm (3.6%, $p = 0.04$) in a population based, case-control study that used data for women, older than 50 years, and enrolled in the Geelong Osteoporosis Study. However, other studies have found beta blocker use and BMD loss to be unrelated. Rejnmark et al[46] failed to find any significant difference in BMD at the lumbar spine and femoral neck between beta blocker treated and untreated women using a cross-sectional design with data from Danish Osteoporosis Prevention Study. Reid et al[47] cannot identify any effect of beta blocker use on loss of hip or os calcis BMD over a mean follow-up of 4 years.

It is possible that other groups of antihypertensive drugs, such as loop diuretics, spironolactone, calcium channel blockers and nitrates have an effect on BMD loss, but this paper only investigated the effects of ACE, beta blocker and thiazide diuretics. Other groups of antihypertensive drugs were neither reviewed, nor included in the following analyses due to the small number of people taking them. The following table (Table 1) summarizes the mechanism of ACE, beta blocker and thiazide for hypertension treatment, as well as potential effects on bone health.

Table 1: Summary of Mechanism of Antihypertensive Drugs and Bone Health

	Mechanism to treat hypertension	Potential Mechanism affecting bone health
ACE	The renin-angiotensin-aldosterone system (RAAS) plays a central role in the control of blood pressure and has been an important target of antihypertensive drug. ACE inhibitors affects RAAS axis.[48]	Osteoblasts and osteoclasts express angiotensin-II type 1 receptor in cell cultures. Angiotensin-II induces the expression of receptor activator of NF-kappa B ligand (RANKL) in osteoblasts, leading to the activation of osteoclasts.[49] In animal studies, both ACE inhibitors and ARBs have been shown to preserve BMD.[50]
Beta Blockers	Beta-adrenergic receptors are the sympathetic components of the autonomic nervous system. Beta blockers inhibit beta-adrenergic receptors, thus used in the treatment of hypertension.[51]	In animal models, substantial evidence shows that sympathetic nerve fibers in bone tissue and functional adrenergic receptors in osteoblasts and osteoclasts.[52] Another study shows that fenoterol, a beta-2 agonist, nearly doubled RANKL mRNA in human osteoclasts.[53]
Thiazide	Thiazide inhibits Na^+/ Cl^- co-transporters (NCCs) by decreasing sodium reabsorption, which leads to decreased extracellular fluid and plasma volume. The volume loss results in decreased blood pressure.[54]	Thiazide inhibits the NCCs and the NCCs are also expressed in human osteoblast and osteoblast-like cells.[55] If osteoblast cells were blocked by thiazide, it will enhance bone calcium uptake, consequently has a positive effect on BMD.

2.0 METHODS

2.1 STUDY DESIGN

To determine whether antihypertensive medication (ACE, beta blocker and thiazide) use was associated with lower bone loss rates, this study adopted three key design features: new-user design, frequency matching and propensity score matching.

The new-user design[56] identifies users who start a course of treatment with the medication of interest. Unlike using current medication users for the comparative group, the new-user design mainly identifies short-term users of the medication, which optimizes the ability to control for disease risk factors that may be altered by long-term medication use. In observance of the new-user design, exclusion criteria were defined as follows: any use of antihypertensive medications at the first SWAN visit, any use of antihypertensive drugs other than the category of interest (ACE, beta blocker and thiazide) before or at the baseline visit, and any combination use of antihypertensive drugs across categories at the visit of drug initiation. In this analysis, the study baseline visit for users was defined as the visit prior to the one where medication use was initiated, as long as the baseline visit was no more than 2 years before drug initiation. People without a defined baseline (no prior visits within 2 years of drug initiation) were excluded from the study. Any visit before the defined baseline was also excluded.

The nonusers were defined as the participants who did not use any antihypertensive drugs throughout the SWAN study. Frequency matching was used to establish a comparable baseline for nonusers against the defined baseline for the antihypertensive drug users, with the aim of balancing the baseline characteristics, especially for menopause status. Frequency matching was designed by randomly assigning each nonuser a visit number (considered as baseline visit number) and the distribution of the randomly assigned visit numbers was patterned after the distribution of baseline visits for the users. The random number designating a SWAN visit was generated using SAS function (ranuni). Visits, if any, before the assigned baseline for the nonusers were excluded from this secondary analysis. The method was used in previous publication.[57]

Although frequency matching was used to balance the menopause status at baseline for each group, other characteristics at the baseline were still not balanced between the users and nonusers. When there are apparent baseline differences between groups, the possibility of bias arises: the outcome differences may not be due to the effect of the treatment per se, but rather on characteristics that initially determined whether or not a participant received a given treatment. Propensity score matching was used to eliminate biases and to improve balance of the measured baseline factors at the design phase of the observational study. Propensity scores were generated using the baseline characteristics, which were selected by stepwise regression, to estimate the probability of women being treated with antihypertensive medication versus not. The nonusers were matched to the users based on the propensity score.

2.2 STUDY POPULATION

The study population was identified from 2,365 women enrolled in the SWAN bone substudy. The SWAN bone substudy is being carried out in five of the seven clinical sites (see Chapter 1.1), located in Boston, Pittsburgh, the Detroit area, Oakland, and Los Angeles areas, who were self-defined as Caucasian, African American, Chinese, or Japanese (approximately half the women in each locale were self-defined Caucasians).[2]

Participants who have a baseline BMD measurement and at least one additional measurement were eligible for this analysis. Nonusers and new users that are exclusive to one category of antihypertensive medication were identified as shown in Figure 1. The study included all women who met these criteria, but did not include women who used antihypertensive other than ACE, beta blocker or thiazide.

Written informed consent was obtained from all participants, and each site's protocol was conducted with approval from an institutional review board. The secondary data analysis presented in this paper has been approved by the SWAN Publication and Presentation (P&P) administrator and the P&P Chair.

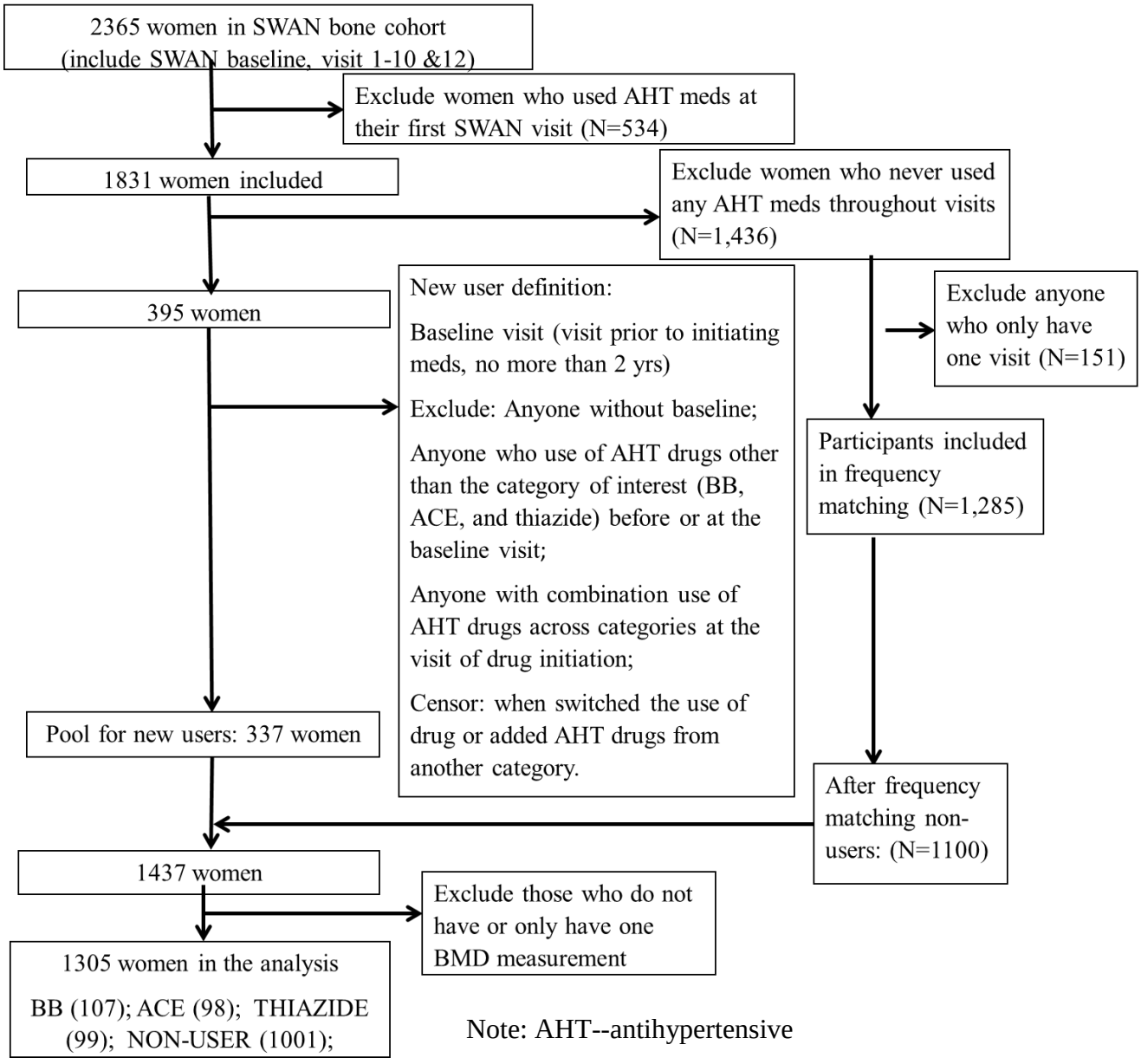


Figure 1: Flowchart of the Study Cohort of Antihypertensive Drug Use and BMD Loss Analysis

2.3 STUDY EXPOSURE

The primary exposure of interest was antihypertensive medication use which was categorized as ACE, beta blocker or thiazide; antihypertensive drug users were compared to nonusers who had never used any types of antihypertensive medications. Censoring occurred when any visit switched the use of drug to a different category or any visit started combination therapy (added antihypertensive drug(s) from a different category).

Medication data were coded using the Iowa Drug Information System (IDIS)[58] and collected annually from both the interview portion and the specimen collection form. Medication information collected included identifiable prescription medication, over the counter medication that had been determined to be important by the pharmacoepidemiologist assisting SWAN or a Coordinating Center data analyst, and medications that are currently classified as over the counter medication that were classified as prescription medication at the start of SWAN. Dosage information was not available.

2.4 STUDY OUTCOMES

Outcomes of interest in this study were BMD loss of lumbar spine, femoral neck, and total hip, which were normalized to the defined baseline. To define the BMD loss, two types of loss rate, annual rate of change (measured BMD value at visit X/ measured BMD value at defined baseline) and loss rate change from the referent group (need to be added to the annual rate of

change of the referent group to get the absolute value), were used in this secondary analyses. Women in the SWAN Bone Study have annual measurements of lumbar spine, total hip and femoral neck aBMD by Hologic dual-energy X-ray absorptiometry (DXA).[2] All SWAN measurements were made with QDR software. The scan machine model used at UCDavis and Pittsburgh is QDR 2000 prior to visit 8 and QDR 4500 was used from visit 8 forward; at the other 3 sites, QDR 4500 was used throughout all visits. To ensure comparable measurements between sites, Synarc machine drift correction factors specified to each site and scan date were applied on both lumbar spine and total hip BMDs. Moreover, the cross-calibration analyses between the QDR 2000 machine and QDR4500 machine were performed at the CC using 40 people at each site. Only certified DXA operators can analyze scans for the SWAN study. Densitometer's instruction manuals were provided to each site uniformly informing the scan specifications, positioning of the subject and defining the regions of interest. The quality control program for DXA measurements in SWAN has been published.[59]

A local Hologic anthropomorphic spine phantom was measured daily on each densitometer and a circulating Hologic anthropomorphic spine phantom was measured periodically on all densitometers. If retroactive analyses of these phantom measurements reveal significant longitudinal and/or cross-sectional deviation in the calibration of any densitometer, participant measurements from that densitometer during that interval are retroactively adjusted by the SWAN quality control center to eliminate this effect. These measures include low energy X rays of the lumbar spine, total hip, and whole body, which will provide an indication of bone strength, and predisposition to sustain fractures.

For the spine region, the scan includes the vertebrae and sometimes there are collapsed vertebrae, vertebrae with focal sclerosis, or a metal overlying the spine. So in the calculation of

the total BMD, vertebrae situations listed above are excluded from statistical analysis and spine BMD value is recalculated as sum (Bone Mineral Content) divided by the sum (Area) based on non-excluded vertebras if more than one region is useable. On the other hand, if a woman is determined to have only one usable region at any SWAN visit, the spine scan is excluded at that visit and subsequent visits. Unlike the spine scans, the hip scan or femoral neck scan variables are not recalculated based on useable regions and the values are set to be missing if the regions are not useable.

The BMD measurements selected in the analysis are among measurements from baseline through the SWAN follow-up visit 10 and visit 12. The visit 11 was not included in any of the analysis, because it is a non-funded visit, and quality of data collected cannot be guaranteed.

2.5 OTHER PARAMETERS

Several variables are considered as potential confounders and included in analyses. In SWAN, demographic variables (age, race, site, and education), annual income, marital status, life style factors (smoking history and alcohol consumption), self-assessed health status, social support, center for epidemiologic studies depression scale (CES-D), physical activity, and medication use (prescription hormone therapy (HT), bisphosphonates, proton pump inhibitors, thiazolidinediones, selective serotonin reuptake inhibitors, statins, beta-blockers, oral steroids, inhaled steroids, anti-convulstants, thiazide diuretics) were determined by either an interview-administered or a self-administered questionnaire. Comorbid conditions (rheumatoid arthritis,

chronic obstructive pulmonary, and diabetes) were self-reported or determined from metabolic syndrome, or medication use.

SWAN participants underwent physical measurements annually of weight and height. BMI was calculated using weight in kilograms divided by the square of height in meters. Physical activity was assessed using an adaptation of the Baecke questionnaire.[60] It is a self-reported instrument assessing sports, household, and daily routine, on the basis of the average responses to questions about various activities with scores ranging from 1(lowest) to 5(highest). A total physical activity score was calculated as the sum of the individual scores.

Final menstrual period (FMP) was defined as the initial day of the last menstrual period preceding 12 consecutive months of amenorrhea, identified retrospectively. The women in whom we have not been able to observe a natural transition either used hormone therapy or had a hysterectomy. Menopause status was determined using self-reported bleeding patterns and categorized as pre-menopausal (if they had monthly bleeding during each of the last 3 months and noted no change in their prior individual menstrual pattern) or early perimenopausal (if they had menstrual bleeding in one, two, or three of the last 3 months and also noted a change in bleeding pattern from their prior menstrual pattern), or late menopausal (no menstrual bleeding for at least 3 months but no more than 12 months), and post-menopausal period (no menstrual bleeding for at least 12 months). Women reporting hysterectomy or oophorectomy were classified as surgically menopausal. Menopause transition stage was updated at each study visit. However, menopause status included in the analysis was redefined using FMP date, because a study using SWAN data found poor agreement between annual interview and menstrual calendar data for early menopausal transition.[61] Therefore, for women who had a FMP date, pre-/ peri-menopausal status was defined as one year before the FMP, late menopausal status was defined

as from one year before the FMP to 2 years after menopause, and postmenopausal status was classified as 2 years after menopause. On the other hand, for women whose FMP date was not yet confirmed, the self-report menopause status was used with the pre- and peri-menopause status collapsed into one group.

3.0 DATA ANALYSES

3.1 LINEAR MIXED-EFFECT MODELING

Descriptive statistics of the baseline demographic variables, annual income, education, marital status, BMI, smoking history and alcohol consumption, self-assessed health status, social support, physical activity, medication use, self-reported comorbid conditions were compared across medication groups. Continuous variables were analyzed using ANOVA and Kruskal-Wallis tests, whereas categorical variables were analyzed using χ^2. Variables were transformed when necessary.

The rate of loss in femoral neck, total hip and lumbar spine BMD were normalized to the baseline BMD and an annual percentage change was obtained. The normalized bone mineral density measurements were used as the response variables. This approach provided clinically interpretable results and allowed comparison to other studies.

Linear mixed-effect regression modeling strategy was used, with random intercepts and slopes for each menopause stage. Because the rate of BMD loss varies greatly by menopause stage as discussed in Chapter 1.2.1, the fundamental modeling strategy was piecewise-defined, which accounted for the natural heterogeneity of BMD slopes in different menopause stages (see Appendix B). Because the interaction between drug effect and menopause stages was not statistically significant, the models were built under the assumption that the drug effect and

menopause stages act independently on BMD loss, in other words, their contributions are additive. Effect modification of the relationship between medication group and time and between menopause statuses on BMD loss (over time on the drug) was examined by the cross product term in the model. The coefficients estimated in the base model are intercepts for medications, pre-/peri-, late- and post- menopause status and slopes by medication group and slope by menopause status. Repeated measures were accounted by the correlation structure within subjects of random effect variance-covariance matrix (G matrix). For intercept and time variables (see Appendix B), the covariance between observations (G matrix) on the same subject were AR (1) with the assumption that correlation between repeated measures of BMD decreased toward zero with increasing time. For intercepts of late- and post-menopausal stages, the pattern of covariance between observations were not specified, which means that unstructured (UN) G matrix was used in the RANDOM statement. The same G matrix was also used for the slopes of all three menopausal stages. Default structure of error variance-covariance matrix (R) was used, which assumed that the errors for each subject were independently and identically distributed.

Restricted Estimation Maximum Likelihood (REML) was used by the procedure PROC MIXED from the SAS System Software (Version 9.3). The Satterthwaite approximation[62] for computing the denominator degrees of freedom for the tests of fixed effects was specified (ddfm=kr) in the procedure. It was used to adjust the estimated standard errors for fixed effects because the data were highly unbalanced. Akaike's information criterion (AIC) and Schwarz's Bayesian criterion (SBC) were used to select different covariance structures and to compare goodness of fit between models with the same fixed effects. Models with AIC or SBC values closest to zero were selected.

The covariates included in the adjusted model were selected using the likelihood ratio test (LRT), with statement (method=ml) specified in the PROC MIXED procedure. The models with forced in variables were considered as nested models, and the models that added other variables were considered as reference models. The LRT calculates the changes in deviance (-2LL) between the nested and reference models and statistically tests the change using a χ^2 distribution, with degrees of freedom equal to the change in number of parameters from the nested model to the referent model. The LRT was used to assess the model fit and test hypotheses about fixed-effect parameters among the variables that potential confound the relation. The selection process was based on comparing the values of likelihood functions for two models, with the same variance-covariance parameters. Variables that were forced in the models were pre-decided, including total number of comorbidities, physical activities, and other medication use (hormone therapy, diabetes related medication use, bisphosphonates and antidepressants). All analyses were performed in SAS (version 9.3).

3.2 PROPENSITY SCORE ANALYSES

The propensity score was defined as the probability of receiving the antihypertensive treatment (ACE, beta blocker or thiazide). Propensity score matching was used to improve the balance of the distributions of observed baseline factors between the treatment group and control group. Therefore, propensity score matching was used to maximize exchangeability across groups and estimate the average treatment effect. In this analysis, three sets of nonuser comparative groups

were generated separately to match ACE, beta blocker, and thiazide groups and three different models (one for each drug) were built for the same outcome.

Propensity scores were estimated using binary logistic regression models with the treatment choices as the outcomes. The models were built using stepwise regression which selected baseline variables that are associated with the outcome. Figure 2 shows the density plot of predicted probability (p-hat) which was generated from the model and shows the comparison between the drug group and nonusers before optimal matching. Optimal matching SAS macros were used to match the nonusers who have the closet logit transformed p-hat to the drug groups. The largest possible absolute differences between the logit (p-hat) for drug group and a valid compatible matched nonuser group were set as 1. Propensity score matching created 1:1 matched pairs of ACE vs. nonusers, beta blocker vs. nonusers, and Thiazide vs. nonusers. Figure 3 shows the density plot of p-hat for drug group and optimal matched nonusers. The macros were developed by Mayo Clinic staff in SAS System Software (Version 9.3) (Copyright 2005 Mayo Foundations for Medical Education and Research). A similar modeling strategy was used for the propensity matched sets of women with regard to linear mixed effect regression of BMD as described in Chapter 3.1.

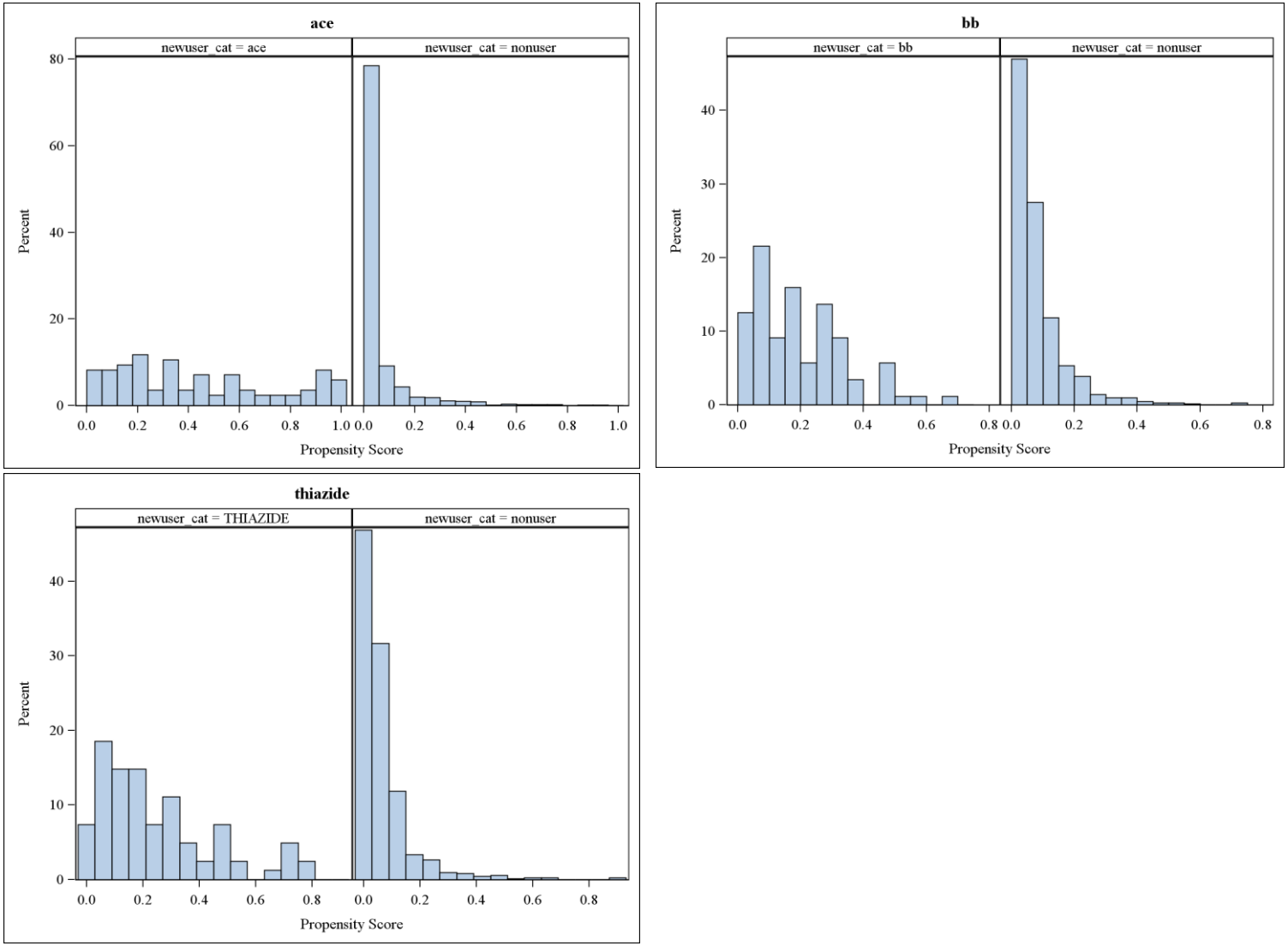


Figure 2: Propensity Score Distribution Comparison between Antihypertensive Users (ACE, beta blocker and Thiazide) and Nonuser before propensity score matching.

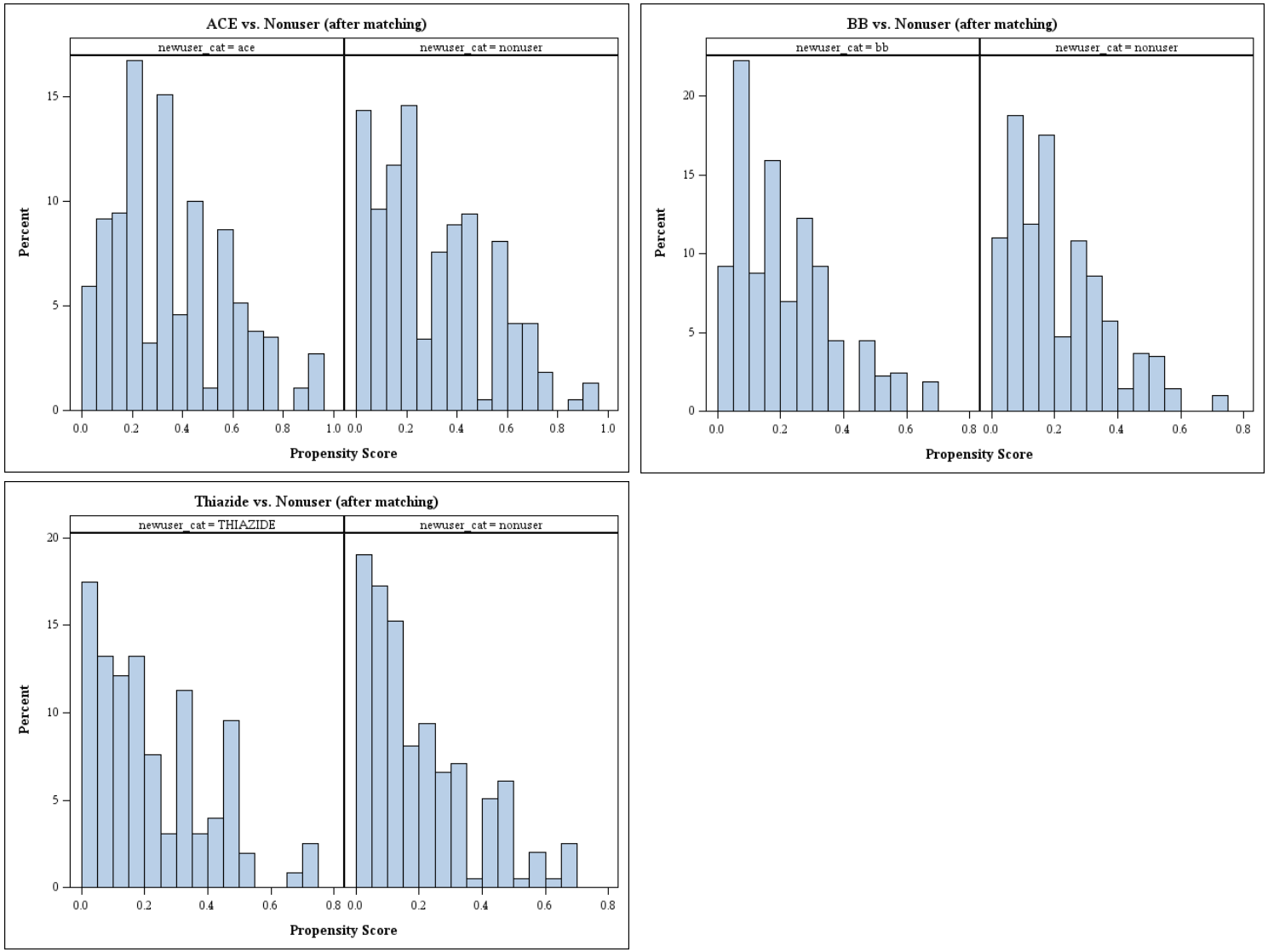


Figure 3: Propensity Score Distribution Comparison between Antihypertensive Users (ACE, beta blocker and Thiazide) and Nonuser after propensity score matching.

4.0 RESULTS AND CONCLUSIONS

4.1 BASELINE CHARACTERISTICS

Of the 2,365 women enrolled in the SWAN bone substudy, 534 were excluded because they reported use of a antihypertensive drug at the initial SWAN visit and 58 were excluded for lacking eligible baseline (no prior visits within 2 years of drug initiation) or because of using antihypertensive drugs across categories at baseline. Among the 1,436 women who never used any antihypertensive medications, 151 were excluded for only having one visit in total, and 185 women could not be matched to the users' baseline visit distribution and were excluded from the analysis. In the end, 132 participants were excluded due to lack of a follow-up BMD. The final analytic cohort has 1305 women in total, among which there are 107 (8.2%) women who initiated beta blocker use during the follow-up period, 98 (7.5%) who initiated ACE use, 99 (7.6%) who initiated thiazide use and also there are 1001 (76.7%) women who did not report any antihypertensive drug use at any visit included (Table 3). The top 3 prevalent drugs used within each category are displayed in Table 2, along with drug structure and time introduced into market.

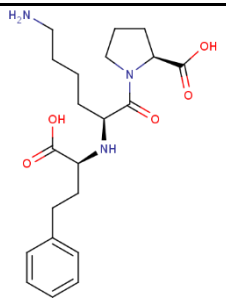
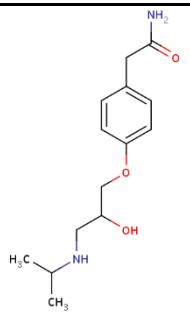
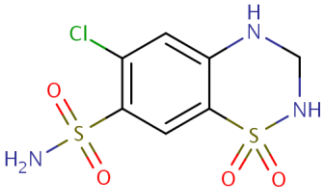
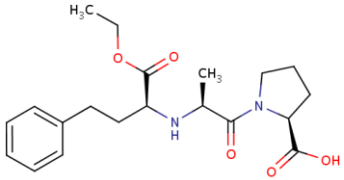
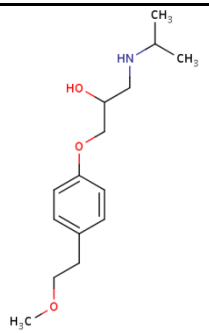
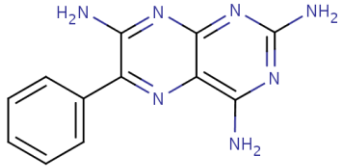
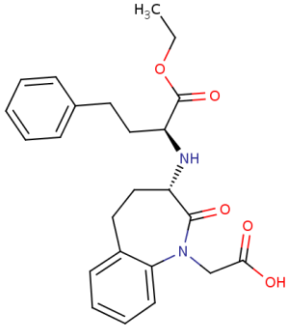
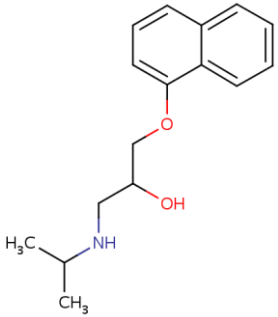
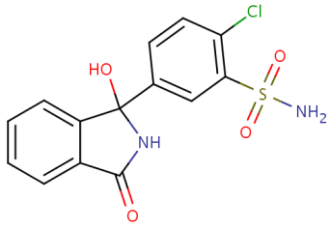
A total of 1305 participants were included in the analyses, with an average age of 51.5 (±4.2) years. The average follow-up time of subjects included in the analysis is 7.5 (±3.6) years. The baseline characteristics of the participants included in the study are detailed in Table 3. The

antihypertensive class initiated varied significantly (p-value <0.05) by demographic variables, including sites, education, income; self-reported comorbidities (heart problems, hyperlipidemia, and arthritis); medication use (diabetes related medication and proton pump inhibitor); and lifestyle factors (alcohol/tobacco use), and other risk factors including BMI, menopause status, self-rated health status, physical activity, and CES-D.

The nonuser group generally had a higher education level, higher income, lower BMI, less current or past tobacco use, higher alcohol consumption, higher physical activity and had a predominantly white population and relatively less black people compared to the all of the user groups. Almost 60% of the participants in the nonuser group self-classified as having excellent or very good health status, with less self-reported overactive or underactive thyroid conditions, hyperlipidemia, and arthritis; and in terms of medication use, nonuser had less use of diabetes related medications, antidepressants, and proton pump inhibitor.

Populations who were using regimens involving ACE and thiazide were more similar to one another in terms of menopause status, CES-D score, and BMI. Both groups had a higher percent of postmenopausal women at the baseline, higher depression score, and more obese people than populations using regimens involving beta blocker or nonusers. The baseline BMD values at lumbar spine, total hip and femoral neck were imbalanced, but the baseline values were not adjusted in the models, because the outcomes used were BMD values which were normalized to the baseline.

Table 2: Top 3 Drugs Which Initiated within Each Antihypertensive Category (Time introduced into therapy and structure)

ACE	Structure	Beta Blocker	Structure	Thiazide	Structure
Lisinopril (Early 1990s)		Atenolol (1980s)		Hydrochloro-thiazide (1970s)	
Enalapril (1980s)		Metoprolol (1970s)		Triamterene (1964)	
Benazepril (1991)		Propranolol (1970s)		Chlor-thalidone (1980s)	

*Note: Time introduced into therapy was listed under the drug names.

Table 3: Baseline Characteristics of Subjects included in Study Cohort

Characteristic	ACE (N=98)	BB (N=107)	Thiazide (N=99)	Nonuser (N=1001)	p-value
Demographics					
Age, mean, SD	52.1, 4.2	51.3, 4.3	52.0, 4.2	51.1, 4.2	0.0636
Race/Ethnicity, %					p>=.50
Caucasian	54.1	48.6	37.4	57.1	
Black	28.6	23.4	47.5	16	
Chinese	8.2	14	12.1	12.7	
Japanese	9.2	14	3	14.2	
Site, %					
Michigan	30.6	23.4	21.2	14.1	0.004
MGH	21.4	14	32.3	19.3	
UCDavis	12.2	19.6	19.2	23.9	
UCLA	17.3	23.4	6.1	25.4	
Pittsburgh	18.4	19.6	21.2	17.4	
Education, %					0.0016
High School or less	3.1	6.5	2.1	2.3	
High School/some college	66.3	42.1	58.8	44.1	
College degree	10.2	21.5	15.5	25.9	
Post College	20.4	29.9	23.7	27.8	
Marital status, %					p>=.50
Single/Never married	15.3	17.8	15.2	12.8	
Currently married	59.2	63.6	63.6	67.8	
Separated/Widowed/Divorced	25.5	18.7	21.2	19.3	
Income, %					0.0008
<20K	9	12.9	7.5	5.9	
20-<35K	13.5	13.9	16.1	9.6	
35-<50K	23.6	13.9	16.1	15.6	
50-<75K	18	16.8	25.8	24.3	
>75K	36	42.6	34.4	44.6	
BMI at visit 0, mean, SD	31.2, 6.8	27.7, 6.6	30.5, 6.4	26.4, 5.9	<.0001
BMI category, %					<.0001
Obese	52	29.5	53.5	22.7	
Overweight	28.6	31.4	26.3	28.3	
Normal	19.4	39	20.2	49	

Table 3 (cont.) Baseline Characteristics of Subjects included in Study Cohort

Characteristic	ACE (N=98)	BB (N=107)	Thiazide (N=99)	Nonuser (N=1001)	p-value
Self-reported Comorbidities					
Heart problem, %	1	3.7	0	0.7	0.0909
Thyroid, %	14.6	15.9	11.1	10	0.0403
Cancer, %	2	0	1	0.9	p>=.50
Hyperlipidemia, %	28.6	19.6	21.2	13.8	<.0001
Arthritis or osteoarthritis, %	20.6	19.6	22.2	13	0.0037
Osteoporosis, %	4.1	2.8	2	3.4	p>=.50
Other Medication Use					
Ever Reported HT Use, %	25.5	29	35.4	24	0.2169
Diabetes Related Medication, %	24.5	2.8	2	1.7	<.0001
Bisphosphonates, %	1	1.9	2	2.7	0.2572
Hydantoins, %	2	4.7	0	1.3	0.1045
Antidepressants, %	17.3	14	14.1	11.6	0.076
H2 Antagonists, %	0	7.5	7.1	3.4	p>=.50
Proton Pump Inhibitors, %	7.1	4.7	7.1	2.9	0.0131
Supplement Calcium (mg), %	31.9	23.7	36.8	36.7	0.082
Supplement Vitamin D (IU), %	8.3	3.9	9.2	7.8	p>=.50
Other Risk Factors of Interests					
Menopausal status, %					0.0863
Post menopause	28.6	15.8	26.8	18.7	
Late menopause	37.4	41.1	29.3	38.5	
Pre-/Peri-menopause	34.1	43.2	43.9	42.9	
Self-rated Health Category, %					<.0001
Fair or poor	12.4	17.5	11.2	10.5	
Good	46.4	40.8	51	30.1	
Excellent or very good	41.2	41.7	37.8	59.4	
Alcohol intake, %					0.0135
No (< once/month)	56.4	60.2	49.5	46.8	
Moderate use(> once/month)	20.2	21.4	34.3	27.6	
High use (>=2 times/week)	23.4	18.4	16.2	25.5	
Tobacco use, %					0.0008
Never	77.3	83	73.7	87.8	
Past	5.2	6.6	8.1	3.5	
Current	17.5	10.4	18.2	8.7	

Table 3 (cont.) Baseline Characteristics of Subjects included in Study Cohort

Characteristic	ACE	BB	Thiazide	Nonuser	p-value
	(N=98)	(N=107)	(N=99)	(N=1001)	
Physical Activity, mean, SD	7.3, 1.4	7.7, 1.5	7.6, 1.6	8.0, 1.5	<.0001
Binary CES-D Score, %	28.4	16	22.2	16.1	0.0097
CES-D Scale Score, mean, SD	11.6, 11.3	8.8, 8.5	9.9, 10.2	8.5, 8.4	0.1225
Social Support Scale Score, mean, SD	12.5, 2.9	12.5, 3.0	12.9, 2.8	13.0, 3.0	0.093
Ever fracture a bone, %	1	2.8	4	2.3	p>=.50
Reported high blood pressure, %	21.9	7.5	25.5	3.6	<.0001
BMD, g/cm2 (SD)					
Lumbar spine, mean, SD	1.08, 0.15	1.03, 0.13	1.06, 0.15	1.02, 0.15	0.0003
Total hip, mean, SD	0.98, 0.15	0.92, 0.14	0.97, 0.14	0.92, 0.14	<.0001
Femoral neck, mean, SD	0.83, 0.13	0.78, 0.12	0.85, 0.14	0.80, 0.13	0.0004

Abbreviations: BB, Beta Blocker; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HT, hormone therapy; Michigan, University of Michigan, Ann Arbor, Michigan; MGH, Massachusetts General Hospital, Boston, Massachusetts; UCDavis, University of California, California; UCLA, University of California, Los Angeles, California; Pittsburgh, University of Pittsburgh, Pittsburgh, Pennsylvania; CES-D, Center for Epidemiologic Studies Depression Scale.

4.2 BASELINE CHARACTERISTICS FOR PROPENSITY SCORE ANALYSES

Propensity score matching was used with a 1:1 matching ratio and the comparison baseline table is shown in Table 4. The 69 ACE users, 88 beta blocker users and 76 diuretics users, were matched with an equal number of nonusers, respectively. Although there is a general pattern of balanced baseline characteristics in the matched sets and a great improvement relative to the unmatched comparisons, a few recurring differences stand out. Use of H2 antagonists between ACE users and nonusers is imbalanced (p =0.0011) and there is no one in the ACE group who uses H2 antagonists, whereas 14.5% of nonusers reported the using it. The use of anticonvulsants is imbalanced between beta blocker users and nonusers (p =0.0437) with only 4.5% of ACE users and 0% nonusers. The mean of social support scale score for beta blocker users is 12.3 (±3.1) and 13.4 (±2.7) for nonusers. There are significantly (p =0.0063) more current and past smokers in the thiazide users compared to the nonusers.

Table 4: Baseline Characteristics of Subjects Initiated ACE, Beta Blocker, Thiazide and Propensity Score Matched Nonusers

Characteristic	ACE	Nonuser	p-value	BB	Nonuser	p-value	Thiazide	Nonuser	p-value
	(N=69)	(N=69)		(N=88)	(N=88)		(N=76)	(N=76)	
Demographics									
Age, mean, SD	52.0, 4.1	52.0, 4.3	p>=.50	51.3, 4.4	51.9, 4.5	0.3645	52.0, 4.4	52.7, 4.3	0.2945
Race/Ethnicity, %			p>=.50			0.3198			0.3713
Caucasian	56.5	59.4		51.1	58		35.5	47.4	
Black	23.2	24.6		20.5	18.2		47.4	35.5	
Chinese	10.1	7.2		14.8	14.8		13.2	13.2	
Japanese	10.1	8.7		13.6	9.1		3.9	3.9	
Site, %			p>=.50			0.2173			p>=.50
Michigan	29	27.5		22.7	27.3		21.1	23.7	
MGH	21.7	20.3		15.9	18.2		31.6	21.1	
UCDavis	15.9	13		19.3	23.9		21.1	25	
UCLA	17.4	17.4		25	18.2		6.6	9.2	
Pittsburgh	15.9	21.7		17	12.5		19.7	21.1	
Education, %			p>=.50			p>=.50			p>=.50
High School or less	4.3	4.3		6.8	3.4		2.7	2.6	
High School/some college	63.8	63.8		40.9	48.3		58.1	59.2	
College degree	14.5	11.6		22.7	21.8		14.9	15.8	
Post College	17.4	20.3		29.5	26.4		24.3	22.4	
Marital status, %			p>=.50			0.0847			p>=.50
Single/Never married	13	13		19.3	8		17.1	15.8	
Currently married	60.9	58		63.6	71.6		64.5	63.2	
Separated/Widowed/Divorced	26.1	29		17	20.5		18.4	21.1	

Table 4(cont.): Baseline Characteristics of Subjects Initiated ACE, beta blocker, Thiazide and Propensity Score Matched Nonusers

Characteristic	ACE	Nonuser	p-value	BB	Nonuser	p-value	Thiazide	Nonuser	p-value
	(N=69)	(N=69)		(N=88)	(N=88)		(N=76)	(N=76)	
Income, %			0.0453			p>=.50			p>=.50
<20K	6.2	11.9		13.1	6.8		7.1	9.3	
20-<35K	9.2	23.9		9.5	12.5		17.1	17.3	
35-<50K	24.6	16.4		13.1	15.9		15.7	20	
50-<75K	21.5	17.9		20.2	21.6		27.1	17.3	
>75K	38.5	29.9		44	43.2		32.9	36	
BMI at visit 0, mean, SD	30.4, 6.2	30.7, 6.6	p>=.50	27.6, 6.4	28.8, 7.0	0.2736	29.9, 6.6	30.3, 6.3	p>=.50
BMI category, %			p>=.50			0.4585			1
Obese	52.2	47.8		29.5	33		47.4	48.7	
Overweight	27.5	29		33	35.2		30.3	27.6	
Normal	20.3	23.2		37.5	31.8		22.4	23.7	
Self-reported Comorbidities									
Heart problem, %	0	1.4	0.3173	3.4	3.4	1	0	2.6	0.1559
Thyroid, %	11.6	14.5	p>=.50	13.6	12.5	p>=.50	10.5	12	p>=.50
Cancer, %	1.4	0	0.3173	0	3.4	0.0832	0	1.3	0.3173
Hyperlipidemia, %	24.6	27.5	p>=.50	21.6	19.3	p>=.50	17.1	26.3	0.1699
Arthritis or osteoarthritis, %	22.1	21.7	p>=.50	19.3	22.7	p>=.50	22.4	17.1	0.4165
Osteoporosis, %	4.3	2.9	p>=.50	3.4	6.9	0.3059	1.3	3.9	0.3125
Other Medication Use									
Ever Reported HT Use, %	23.2	23.2	1	21.6	20.5	p>=.50	25	22.4	p>=.50
Diabetes Related Medication,%	20.3	14.5	0.3707	3.4	4.5	p>=.50	1.3	5.3	0.1739
Bisphosphonates, %	1.4	1.4	1	2.3	3.4	p>=.50	2.6	2.6	1
Hydantoins, %	1.4	1.4	1	4.5	0	0.0437	0	1.3	0.3173

Table 4(cont.): Baseline Characteristics of Subjects Initiated ACE, beta blocker, Thiazide and Propensity Score Matched Nonusers

Characteristic	ACE	Nonuser	p-value	BB	Nonuser	p-value	Thiazide	Nonuser	p-value
	(N=69)	(N=69)		(N=88)	(N=88)		(N=76)	(N=76)	
Other Medication Use (cont.)									
Antidepressants, %	15.9	13	p>=.50	17	18.2	p>=.50	7.9	7.9	1
H2 Antagonists, %	0	14.5	0.0011	9.1	9.1	1	6.6	3.9	0.469
Proton Pump Inhibitors, %	4.3	2.9	p>=.50	3.4	6.8	0.306	7.9	7.9	1
Supplement Calcium (mg), %	39.2	42.9	p>=.50	23.8	31.8	0.3126	35.6	37.3	p>=.50
Supplement Vitamin D(IU),%	11.8	14.3	p>=.50	4.8	12.1	0.1362	11.9	15.3	p>=.50
Other Risk Factors of Interests									
Menopausal status, %						p>=.50			0.3098
Post menopause	26.1	24.6	p>=.50	15.9	20.5		27.6	26.3	
Late menopause	39.1	39.1		39.8	37.5		27.6	43.4	
Pre-/Peri-menopause	34.8	36.2		44.3	42		44.7	30.3	
Self-rated Health Category, %			p>=.50			p>=.50			p>=.50
Fair or poor	10.1	18.8		19	21.6		11.8	15.8	
Good	47.8	27.5		38.1	25		48.7	39.5	
Excellent or very good	42	53.6		42.9	53.4		39.5	44.7	
Alcohol intake, %			p>=.50			p>=.50			p>=.50
No (< once/month)	58	47.8		60.2	58		51.3	48.7	
Moderate use(> once/month)	18.8	31.9		21.6	19.3		32.9	30.3	
High use (>=2 times/week)	23.2	20.3		18.2	22.7		15.8	21.1	
Tobacco use, %			0.2072			0.0622			0.0063
Never	79.7	87		79.3	90.9		69.7	89.5	
Past	4.3	4.3		8	2.3		7.9	1.3	
Current	15.9	8.7		12.6	6.8		22.4	9.2	

Table 4(cont.): Baseline Characteristics of Subjects Initiated ACE, beta blocker, Thiazide and Propensity Score Matched Nonusers

Characteristic	ACE	Nonuser	p-value	BB	Nonuser	p-value	Thiazide	Nonuser	p-value
	(N=69)	(N=69)		(N=88)	(N=88)		(N=76)	(N=76)	
Physical Activity, mean, SD	7.3, 1.4	7.4, 1.3	p>=.50	7.8, 1.6	7.9, 1.4	0.4488	7.5, 1.7	7.7, 1.5	p>=.50
Binary CES-D Score, %	23.2	20.3	p>=.50	18.2	22.7	0.4561	21.1	17.1	p>=.50
CES-D Scale Score, mean, SD	10.1, 10.5	10.3, 9.0	0.4843	9.2, 8.7	10.3, 8.8	0.3942	9.5, 9.5	9.2, 9.2	p>=.50
Social Support Scale Score, mean, SD	12.8, 2.8	12.3, 3.3	0.4844	12.3, 3.1	13.4, 2.7	0.0099	12.9, 2.9	12.6, 3.2	p>=.50
Ever fracture a bone, %	1.4	4.3	0.312	3.4	2.3	p>=.50	5.3	3.9	p>=.50
Reported high BP, %	19.1	14.5	0.4706	8	11.4	0.46	21.1	23.7	p>=.50
BMD, g/cm2 (SD)									
Lumbar spine, mean, SD	1.07, 0.16	1.07, 0.17	p>=.50	1.02, 0.14	1.03, 0.17	1	1.05, 0.14	1.05, 0.17	p>=.50
Total hip, mean, SD	0.96, 0.14	0.99, 0.15	0.3078	0.92, 0.15	0.95, 0.16	0.2427	0.97, 0.14	0.98, 0.15	p>=.50
Femoral neck, mean, SD	0.81, 0.12	0.85, 0.16	0.1826	0.78, 0.12	0.84, 0.16	0.0203	0.84, 0.15	0.84, 0.16	p>=.50
Estimated Probability [a], mean, SD	.32, .23	.31, .22	p>=.50	.20, .14	.20, .15	p>=.50	.21, .17	.21, .16	p>=.50

Abbreviations: BB, Beta Blocker BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HT, hormone therapy; Michigan, University of Michigan, Ann Arbor, Michigan; MGH, Massachusetts General Hospital, Boston, Massachusetts; UCDavis, University of California, California; UCLA, University of California, Los Angeles, California; Pittsburgh, University of Pittsburgh, Pittsburgh, Pennsylvania; CES-D, Center for Epidemiologic Studies Depression Scale; BP, blood pressure.

[a] Propensity Score (ACE vs. Nonuser) was estimated based on baseline variables including average SBP, average DBP, ethnicity, site, menopause status, BMI, CES-D, education, alcohol consumption, ever used antidepressants, ever used HT, ever used H2 antagonists, ever used diabetes related medication, ever used bisphosphonates, total number of comorbidities, self-reported health condition, hyperlipidemia, self-reported thyroid and physical activity. Propensity Score (BB vs. Nonuser) was estimated based on baseline variables including average SBP, ethnicity, menopause status, BMI, alcohol consumption, ever used antidepressants, ever used H2 antagonists, total number of comorbidities, and self-reported heart problem. Propensity Score (Thiazide vs. Nonuser) was estimated based on baseline variables including average DBP, age, site, menopause status, BMI, ever used antidepressants, ever used proton pump inhibitor, and self-reported health condition.

4.3 MULTIVARIATE MIXED MODELS (FREQUENCY MATCHING)

Table 5 and 6 show the unadjusted model for predicting lumbar spine, total hip and femoral neck BMD over time. Table 7 and 8 present the models adjusted for age, ethnicity, site, BMI, number of comorbidities, physical activity, and other medication use (hormone therapy, diabetes related medication, bisphosphonates and antidepressants).

In the unadjusted model, the average rates of change among nonusers are shown in the Table 9 and the slope of lumbar spine BMD was -0.414% per year, -0.567% per year for total hip BMD and -0.676% per year for femoral neck BMD. Compared to the nonusers, thiazide users have a significantly lower annualized rate of BMD loss at lumbar spine (-0.215%, p =0.0005), total hip (-0.213%, p =0.02) and femoral neck (-0.310%, p <0.0001). ACE is not associated with lumbar spine (p =0.07), total hip (p =0.64) or femoral neck (p=0.91). Beta blocker use is not significantly associated with annualized loss of lumbar spine BMD (p =0.13) or total hip BMD (p =0.95), but beta blocker shows a nominal significance (p =0.05) on protecting femoral neck BMD loss but is not statistically significant after adjustment for multiple comparisons nor is it significant in the multivariable models (p =0.06) shown in Table 8.

In the adjusted model, Caucasian women with average BMI of 27.64kg/m^2 and average age of 53.77 years were treated as the reference sample. Age, BMI, and physical activity were used as time-varying variables and Pittsburgh was used as the reference level for site. Chinese and Japanese were grouped as the "Asian" category. The total number of self-reported

comorbidities was counted (maximum was 7) and other medication use was recoded depending on whether the drug was ever used at the visit of interest from self-reported medication data.

After adjustment for the above variables, thiazide's protective effect is consistent with the unadjusted models. Compared to nonusers, we estimate that the lumbar spine BMD loss decreased by 0.381% (p =0.0002) per year, total hip BMD loss decreased by 0.229% (p =0.0046) per year and femoral neck BMD loss decreased by 0.375% (p <.0001) per year. Neither ACE nor beta blocker had any effect on BMD on any of the sites. Although thiazide has a protective effect on BMD loss, the slope of the thiazide group still sharply declined in late menopause.

Table 5: Unadjusted Mixed Model for Lumbar Spine, Total Hip and Femoral Neck (Frequency Matching)

	Lumbar Spine		Total Hip		Femoral Neck	
Variable	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value
Intercept	97.810%	<.0001	98.710%	<.0001	97.860%	<.0001
Medication Category		0.0007		0.0002		<.0001
ACE (vs. Non)	0.393%	0.133	-0.032%	0.8808	0.009%	0.972
BB (vs. Non)	0.478%	0.0389	0.413%	0.0257	0.815%	0.0004
Thiazide (vs. Non)	0.873%	0.0004	0.782%	<.0001	1.496%	<.0001
Pre-/Peri-menopause	2.276%	<.0001	1.776%	<.0001	1.828%	<.0001
Late menopause	-1.248%	<.0001	-0.919%	<.0001	-0.714%	<.0001
Post menopause	-1.328%	<.0001	-1.122%	<.0001	-1.104%	<.0001
Slope						
Time	-0.567%	<.0001	-0.414%	<.0001	-0.676%	<.0001
Time*Medication		0.0012		0.1075		0.0003
Time*BB (vs. Non)	0.150%	0.1309	-0.005%	0.9453	0.169%	0.0542
Time*ACE (vs. Non)	0.185%	0.0672	0.038%	0.6426	-0.010%	0.9102
Time*Thiazide (vs. Non)	0.352%	0.0005	0.201%	0.015	0.366%	<.0001
Time*Pre/Peri menopause	0.564%	<.0001	0.529%	<.0001	0.556%	<.0001
Time*Late menopause	-0.602%	<.0001	-0.343%	<.0001	-0.278%	<.0001
Time*Post menopause	-0.171%	0.0034	-0.260%	<.0001	-0.183%	0.0017

Abbreviations: Non, Nonusers; BB, Beta Blocker; #, number; Time, Time been on the drug.

Table 6: Influence of Antihypertensive Medications on Annual BMD Slopes Summary for Lumbar Spine, Total Hip and Femoral Neck Bone Mineral Density (BMD) (Frequency Matching--Unadjusted Model)

	Annual BMD Slopes Summary for Lumbar Spine, Total Hip and Femoral Neck BMD (95% Confidence interval)		
	Lumbar Spine	Total Hip	Femoral Neck
Referent [a]	***-0.567% (-0.622% , -0.512%)***	***-0.414% (-0.459% , -0.369%)***	***-0.676% (-0.730% , -0.622%)***
Change from referent group [c]			
ACE	0.185% (0.084% , 0.285%)	0.038% (-0.044% , 0.121%)	-0.010% (-0.099% , 0.079%)
Beta Blocker	0.150% (0.051% , 0.250%)	-0.005% (-0.085% , 0.075%)	***0.169% (0.082% , 0.257%)***
Thiazide	***0.352% (0.251% , 0.453%)***	***0.201% (0.119% , 0.284%)***	***0.366% (0.274% , 0.457%)***

[a] Slope referent values are for Nonusers.
[b] Statistically significant associations are shown in ***bold italic*** typeface.
[c] To get slopes for medications, number needs to be added to the nonuser slope referent values.

Table 7: Adjusted Mixed Model for Lumbar Spine, Total Hip and Femoral Neck (Frequency Matching)

	Lumbar Spine		Total Hip		Femoral Neck	
Variable	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value
Intercept	97.530%	<.0001	98.160%	<.0001	98.210%	<.0001
Medication Category		<.0001		<.0001		<.0001
ACE (vs. Non)	0.478%	0.0847	0.189%	0.4022	-0.263%	0.3457
BB(vs. Non)	0.617%	0.0084	0.399%	0.0319	0.777%	0.0008
Thiazide (vs. Non)	1.145%	<.0001	0.955%	<.0001	1.431%	<.0001
Pre-/Peri-menopause	1.898%	<.0001	1.550%	<.0001	1.645%	<.0001
Late menopause	-0.955%	<.0001	-0.400%	0.0132	-0.244%	0.232
Post menopause	-0.886%	<.0001	-0.500%	0.0032	-0.542%	0.0074
Slope						
Time	-0.481%	<.0001	-0.320%	<.0001	-0.599%	<.0001
Time*Medication		0.0004		0.0384		0.0002
Time*BB (vs. Non)	0.162%	0.0986	-0.020%	0.8032	0.164%	0.0608
Time*ACE(vs. Non)	0.185%	0.0645	0.044%	0.5897	-0.017%	0.8503
Time*Thiazide(vs.Non)	0.381%	0.0002	0.229%	0.0046	0.375%	<.0001
Time*Pre/Peri menopause	0.555%	<.0001	0.512%	<.0001	0.543%	<.0001
Time*Late menopause	-0.586%	<.0001	-0.340%	<.0001	-0.271%	<.0001
Time*Post menopause	-0.136%	0.0205	-0.220%	<.0001	-0.160%	0.0058
Demographics						
Age (center at 53.77)	-0.155%	<.0001	-0.160%	<.0001	-0.139%	<.0001
Ethinicity		0.0003		<.0001		0.1236
Black (vs. Caucasion)	-0.662%	<.0001	-0.600%	<.0001	-0.347%	0.0409
Asian (vs. Caucasion)	0.118%	0.4637	0.063%	0.6271	0.007%	0.9659
SITE		0.0019		<.0001		<.0001
UCDavis(vs. Pittsburgh)	0.113%	0.5475	0.230%	0.1263	-0.612%	0.0016
UCLA (vs. Pittsburgh)	0.169%	0.3575	-0.700%	<.0001	-0.872%	<.0001
Michigan(vs. Pittsburgh)	0.577%	0.0017	-0.400%	0.0073	-1.184%	<.0001
MGH (vs. Pittsburgh)	0.603%	0.0006	-0.160%	0.2458	-0.906%	<.0001
BMI (center at 27.64)	0.067%	<.0001	0.052%	<.0001	0.079%	<.0001
Total # of Comorbidities	-0.102%	0.0521	0.026%	0.5348	0.025%	0.6436
Physical Activity	-0.012%	0.7125	0.034%	0.2066	-0.012%	0.7304
Other Medication Use						
Ever Reported HT Use	0.241%	0.1284	0.485%	0.0001	0.350%	0.0283
Diabetes Related Meds	-0.492%	0.0734	-1.050%	<.0001	-0.027%	0.9228
Bisphosphonates	2.006%	<.0001	1.455%	<.0001	1.543%	<.0001
Antidepressants	0.259%	0.0553	0.249%	0.0213	0.373%	0.0076

Abbreviations: Non, Nonusers; BB, Beta Blocker; #, number; Time, Time been on the drug.

Table 8: Influence of Antihypertensive Medications on Annual BMD Slopes Summary for Lumbar Spine, Total Hip and Femoral Neck Bone Mineral Density (BMD) Adjusted by Age, BMI, and Race (Frequency Matching) [a]

	Annual BMD Slopes Summary for Lumbar Spine, Total Hip and Femoral Neck BMD a (95% Confidence interval)		
	Lumbar Spine	Total Hip	Femoral Neck
Referent [b]	*-0.48% (-0.537%, -0.425%)*	*-0.321% (-0.366%, -0.276%)*	*-0.599% (-0.654%, -0.544%)*
Change from referent group [c]			
ACE	0.185% (+0.085%, +0.286%)	0.044% (-0.037%, +0.124%)	-0.017% (-0.106%, 0.072%)
Beta Blocker	0.162% (+0.064%, +0.261%)	-0.019% (-0.097%, +0.059%)	0.164% (0.076%, 0.251%)
Thiazide	***0.381% (+0.281%, +0.481%)***	***0.229% (+0.149%, +0.310%)***	***0.375% (0.284%, 0.466%)***
BMI (kg/m2) [c]	***0.067% (+0.057%, +0.077%)***	***0.052% (+0.044%, +0.060%)***	***0.079 (0.069%, 0.089%)***
Raced			
Black	***-0.662% (-0.828%,-0.496%)***	***-0.600% (-0.733%, -0.468%)***	***-0.347% (-0.517%, -0.177%)***
Other	0.118% (-0.043%, +0.279%)	0.063% (-0.066%, +0.192%)	0.007% (-0.159%, 0.173%)
Age (years) [c]	***-0.155% (-0.173%, -0.138%)***	***-0.161% (-0.175%,-0.147%)***	***-0.139% (-0.157%, -0.121%)***

[a] In addition to the variables listed, the model is also adjusted for site, physical activity, total number of comorbidities, ever use hormone therapy, ever used diabetes related medications, ever used bisphosphonates, ever used antidepressants. Slope referent values are for white women of average age (53.77 years), average BMI (27.64kg/m2).

[b] Slope referent values are for White nonusers.

[c] To get slopes for Medications, BMI, Race and Age, number needs to be added to the white and nonuser slope referent values.

[d] Statistically significant associations are shown in ***bold italic*** typeface.

4.4 MULTIVARIATE MIXED MODELS (PROPENSITY SCORE MATCHING)

Due to the design of propensity score matching, models were created to compare each drug of interest with nonusers separately. In the models for each drug class (Table 9-11), results were similar to results of frequency matching. Compared to no antihypertensive use, neither ACE nor beta blocker use was significantly related to BMD loss at any measured sites (Table 9 and 10). Table 11 shows that thiazide significantly slowed down the lumbar spine BMD loss by 3.157% (p =0.05) and decelerated the femoral neck BMD loss by 3.387% (p =0.0112). Unlike previous results shown in Table 7, for thiazide users, the rate of bone loss at the total hip was not significantly decelerated (1.643%, p =0.16).

The interaction between drug effect and menopause stage was tested for each pair comparison, and only the interaction between menopause status and thiazide on lumbar spine BMD loss was significant. This means that the effect of thiazide on lumbar spine BMD varied by the stage of menopause. The thiazide effects during menopausal transition are summarized in Table 12. Thiazide use during the late and post menopause decreases the lumbar spine BMD loss by 0.491% (95% CI: 0.026%-0.956%) and 0.452% (95% CI: 0.040%, 0.864%), respectively.

Table 9: Adjusted Mixed Model for Lumbar Spine, Total Hip and Femoral Neck among ACE Users and Matched Nonusers (Propensity Score Matching)

	Lumbar Spine		Total Hip		Femoral Neck	
Variable	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value
Intercept	98.860%	<.0001	99.240%	<.0001	97.710%	<.0001
Medication Category						
ACE (vs. Non)	-0.015%	0.9707	-0.542%	0.1667	0.176%	0.7004
Pre-/Peri-menopause	0.606%	0.366	0.141%	0.8193	2.189%	0.0034
Late menopause	-3.440%	<.0001	-2.285%	0.0008	-1.427%	0.0853
Post menopause	-2.993%	<.0001	-1.805%	0.0107	-1.661%	0.0447
Slope						
Time	0.340%	0.2473	0.120%	0.6719	-0.311%	0.3191
Slope						
Time*ACE (vs. Non)	0.042%	0.7765	-0.185%	0.214	-0.099%	0.4872
Time*Pre-/Peri-menopause	-0.042%	0.8861	0.229%	0.4126	0.707%	0.0302
Time*Late menopause	-1.317%	<.0001	-0.628%	0.025	-0.510%	0.1039
Time*Post menopause	-0.929%	0.0014	-0.539%	0.052	-0.441%	0.1517
Demographics						
Age (center at 53.77)	-0.046%	0.4431	-0.139%	0.0134	-0.078%	0.2535
Ethinicity		0.1495		0.8985		0.5339
Black (vs. Caucasion)	0.335%	0.4856	0.125%	0.7793	0.188%	0.7365
Asian (vs. Caucasion)	-1.225%	0.0635	-0.234%	0.7019	-0.813%	0.2778
SITE		0.0043		0.0456		0.1796
UCDavis (vs. Pittsburgh)	1.731%	0.0159	0.877%	0.1872	-0.140%	0.8645
UCLA (vs. Pittsburgh)	1.935%	0.0058	-0.416%	0.5242	-1.001%	0.2155
Michigan (vs. Pittsburgh)	2.091%	0.0003	0.489%	0.3615	0.222%	0.7395
MGH (vs. Pittsburgh)	1.796%	0.0022	1.027%	0.0586	0.801%	0.2365
BMI (center at 27.64)	-0.044%	0.1953	-0.031%	0.3231	-0.005%	0.8884
Total # of Comorbidities	-0.099%	0.5599	0.101%	0.5209	0.290%	0.1295
Physical Activity	0.048%	0.6739	0.065%	0.5416	0.041%	0.7502
Other Medication Use						
Ever Reported HT Use	0.152%	0.7618	0.190%	0.6816	-0.277%	0.6155
Diabetes Related Meds	0.328%	0.495	-0.534%	0.2303	-0.231%	0.6728
Bisphosphonates	0.978%	0.1975	1.746%	0.0132	0.016%	0.9849
Antidepressants	-0.359%	0.3683	0.297%	0.4176	-0.066%	0.884

Abbreviations: Non, Nonusers; #, number; Meds, Medications; Time, Time been on the drug.

Table 10: Adjusted Mixed Model for Lumbar Spine, Total Hip and Femoral Neck among Beta Blocker Users and Matched Nonusers (Propensity Score Matching)

	Lumbar Spine		Total Hip		Femoral Neck	
Variable	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value
Intercept	99.390%	<.0001	97.950%	<.0001	100.290%	<.0001
Medication Category						
Beta Blocker (vs. Non)	0.688%	0.0843	0.554%	0.0415	1.189%	0.0003
Pre-/Peri-menopause	2.429%	<.0001	1.471%	0.0004	0.857%	0.0968
Late menopause	-0.334%	0.6107	0.057%	0.9033	-0.660%	0.2437
Post menopause	-0.242%	0.6974	-0.992%	0.027	-1.194%	0.0311
Slope						
Time	-0.650%	0.0058	-0.462%	0.0036	-0.683%	0.0003
Slope						
Time*BB (vs. Non)	0.042%	0.8325	-0.039%	0.72	0.223%	0.0775
Time*Pre/Peri menopause	0.775%	0.0001	0.433%	0.0056	0.686%	0.0004
Time*Late menopause	-0.187%	0.3942	0.033%	0.8383	0.255%	0.1867
Time*Post menopause	0.027%	0.8997	-0.201%	0.1906	-0.200%	0.2833
Demographics						
Age (center at 53.77)	-0.011%	0.8234	-0.101%	0.0046	-0.177%	<.0001
Ethinicity		0.0006		0.0021		0.7632
Black (vs. Caucasion)	-1.833%	0.0007	-1.261%	0.0004	-0.317%	0.4624
Asian (vs. Caucasion)	1.114%	0.0538	0.095%	0.8078	0.006%	0.9898
SITE		0.0031		0.0009		0.001
UCDavis (vs. Pittsburgh)	-1.876%	0.0104	-0.880%	0.0711	-2.071%	0.0005
UCLA (vs. Pittsburgh)	-2.082%	0.002	-1.565%	0.0005	-1.383%	0.0123
Michigan (vs. Pittsburgh)	0.274%	0.6444	-0.979%	0.0147	-1.729%	0.0004
MGH (vs. Pittsburgh)	-0.760%	0.2301	-1.539%	0.0002	-0.789%	0.1206
BMI (center at 27.64)	0.044%	0.1437	0.028%	0.1873	0.035%	0.1684
Total # of Comorbidities	-0.264%	0.0957	0.184%	0.0873	0.008%	0.9502
Physical Activity	-0.064%	0.5081	0.100%	0.1486	-0.091%	0.2787
Other Medication Use						
Ever Reported HT Use	0.302%	0.5366	0.549%	0.1147	-0.313%	0.4613
Diabetes Related Meds	-0.207%	0.7844	-0.729%	0.1976	0.779%	0.2538
Bisphosphonates	1.278%	0.1806	2.705%	0.0001	1.620%	0.0595
Antidepressants	0.269%	0.434	0.336%	0.2069	0.461%	0.1609

Abbreviations: Non, Nonusers; BB, Beta Blocker; #, number; Meds, Medications; Time, Time been on the drug.

Table 11: Adjusted Mixed Model for Lumbar Spine, Total Hip and Femoral Neck among Thiazide Users and Matched Nonusers (Propensity Score Matching)

	Lumbar Spine		Total Hip		Femoral Neck	
Variable	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value
Intercept	98.470%	<.0001	99.300%	<.0001	98.080%	<.0001
Medication Category						
Thiazide (vs. Non)	1.240%	0.0051	0.721%	0.0288	1.355%	0.0012
Pre-/Peri-menopause	0.272%	0.7621	1.460%	0.034	1.076%	0.2245
Late menopause	-1.674%	0.0587	-0.458%	0.4757	0.073%	0.9293
Post menopause	-0.414%	0.6085	-0.415%	0.5008	0.384%	0.6208
Slope						
Time	-0.005%	0.9856	0.133%	0.5582	-0.552%	0.0588
Time*Medication						
Time*Thiazide(vs. Non)	0.316%	0.0466	0.164%	0.1638	0.339%	0.0112
Time*Pre/Peri menopause	-0.296%	0.3388	0.085%	0.7228	0.160%	0.6165
Time*Late menopause	-1.055%	0.0003	-0.604%	0.0071	-0.298%	0.3095
Time*Post menopause	-0.648%	0.019	-0.664%	0.0021	-0.167%	0.5523
Demographics						
Age (center at 53.77)	-0.016%	0.7934	-0.148%	0.001	-0.120%	0.0382
Ethinicity		0.0597		0.0067		0.0002
Black (vs. Caucasion)	-0.816%	0.1077	-0.679%	0.0679	-1.079%	0.0283
Asian (vs. Caucasion)	1.034%	0.0976	1.171%	0.0127	2.146%	0.0006
SITE		0.0095		0.106		<.0001
UCDavis (vs. Pittsburgh)	-2.260%	0.0007	-0.628%	0.2064	-2.801%	<.0001
UCLA (vs. Pittsburgh)	-1.978%	0.0045	-1.344%	0.0104	-3.706%	<.0001
Michigan (vs. Pittsburgh)	-0.651%	0.3023	-0.348%	0.4522	-1.124%	0.0676
MGH (vs. Pittsburgh)	-0.496%	0.3826	-0.059%	0.8881	-1.044%	0.0593
BMI (center at 27.64)	0.192%	<.0001	0.080%	0.0006	0.120%	0.0001
Total # of Comorbidities	-0.472%	0.0064	-0.218%	0.0939	-0.522%	0.0024
Physical Activity	0.231%	0.0463	0.009%	0.9206	0.141%	0.2111
Other Medication Use						
Ever Reported HT Use	-0.979%	0.1362	-0.027%	0.9561	0.744%	0.2137
Diabetes Related Meds	-0.134%	0.8722	-1.244%	0.0499	0.652%	0.4213
Bisphosphonates	-1.820%	0.1037	0.310%	0.7165	-0.492%	0.6485
Antidepressants	-0.416%	0.4108	-0.797%	0.0398	0.077%	0.8807

Abbreviations: Non, Nonusers; #, number; Meds, Medications; Time, Time been on the drug.

Table 12: Influence of Thiazide Use on Annual Rates of Change of Lumbar Spine Bone Mineral Density (BMD) by Menopause Status Adjusted by Age, BMI, and Race (Propensity Score Matching, 3-way interaction) [a]

Annual BMD Slopes Summary for Lumbar Spine by Menopause Status (95% Confidence interval)			
	Pre/Peri Menopause	Late Menopause	Post Menopause
Referent [b]	0.267% (-0.495%, 1.029%)	-0.704% (-1.415%, 0.007%)	-0.271% (-0.947%, 0.405%)
Change from referent group [c]			
Thiazide	-0.164%(-0.851%, 0.524%)	***0.491% (0.026%, 0.956%)***	***0.452% (0.040%, 0.864%)***
BMI (kg/m2) [c]	-0.104% (-0.446%, 0.237%)	-0.863% (-1.185%, -0.542%)	-0.456% (-0.763%, -0.150%)
Raced			
Black	-1.112% (-1.928%, -0.296%)	-1.871% (-2.667%, -1.075%)	-1.464% (-2.245%, -0.683%)
Other	0.738% (-0.194%, 1.670%)	-0.021% (-0.934%, 0.892%)	0.386% (-0.512%, 1.284%)
Age (years) [c]	-0.312% (-0.681%, 0.057%)	-1.071% (-1.421%, -0.721%)	-0.664% (-0.999%, -0.329%)

[a] In addition to the variables listed, the model is also adjusted for site, physical activity, total number of comorbidities, ever use hormone therapy, ever used diabetes related medications, ever used bisphosphonates, ever used antidepressants. Slope referent values are for white women of average age (53.77 years), average BMI (27.64kg/m2).

[b] Slope referent values are for White nonusers.

[c] To get slopes for Medications, BMI, Race and Age, number needs to be added to the white and nonuser slope referent values.

[d] Statistically significant associations are shown in ***bold italic*** typeface.

5.0 DISCUSSION

5.1 SUMMARY OF STUDY FINDINGS

This study used two methods to examine the longitudinal relationship between antihypertensive medication (ACE, beta blocker and thiazide) use and changes in BMD at lumbar spine, total hip and femoral neck. The results shown by different models were generally consistent with each other. Thiazide showed a protective effect on lumbar spine, and femoral neck, and a marginal positive effect on total hip. In particular, use of thiazide during late and postmenopause had a positive effect on the lumbar spine BMD. The results for ACE and beta blocker were consistent and showed no association with BMD loss at any of the sites.

Thiazide diuretics were found to have a protective effect on femoral neck, total hip and lumbar spine BMD loss among the women who were prescribed thiazide diuretics to treat hypertension. Although in the randomized controlled trials (presented in Chapter 1.2.4), the results generally tend to be similar with the results in this paper, the results obtained from the RCT are regarding specific drug within thiazide diuretics category in normotensive women, rather than hypertensive women. In the observational studies[37,38,40], bone sites, calcaneus, distal radius, and proximal radius, were prevalently under studied, but the BMD data collected for femoral neck, total hip and lumbar spine were rarely seen, to the author's knowledge.

ACE was not found to affect BMD loss compared to nonuse and the result is consistent with the 1-year prospective cohort study reported by García-Testal et al[43], with no significant relation between ACE and BMD loss. However, in a cross-sectional study invoving 1929 women (161 ACE users and 1768 nonuser), Lynn et al[42] found that higher femoral neck BMD associated with ACE use compared to nonuse. However, in Lynn et al's study, the combination use of drug (including ACE/thiazide and ACE/beta blocker) was not excluded and may contribute to the significant protective effect.

The nonsignificant findings regarding beta blocker in this study are consistent with the findings in the Geelong Osteoporosis Study[45] and Study of Osteoporotic Fracture[47]. In the Dubbo Osteoporosis Epidemiology Study[44], baseline characteristics indicate that, in women, beta blocker users had significantly higher femoral neck BMD and lumbar spine BMD. However, this study used cross-sectional BMD value and could not evaluate the BMD loss over time.

Two potential mechanisms may explain these results. First, thiazide has an overall positive effect on BMD by inhibiting the Na^+/Cl^- co-transporters in human osteoblast and osteoblast-like cells, thereafter enhancing bone calcium uptake. Second, one of the potential reasons that the protective effect for thiazide is not significant during the pre-/peri-menopause may be that serum follicle-stimulation hormone level is still low and the contribution of the thiazide towards BMD protection is not prominent compared to the hormone's contribution. However, this explanation needs to be validated by further studies. Another potential reason is that there is no significant BMD loss during pre-/peri menopause regardless of drug use.[13] On the basis of the fact that use of thiazide does not increase BMD value, we may form the hypotheses that thiazide either interact with the nature bone metabolism indirectly or inhibit the targets competing with another biological component.

5.2 CONTRIBUTION TO THE LITERATURE

The results from this study agreed with and expanded previous research. Previous studies mainly focused on BMD change over two cross-sectional time point or the cumulative change over time. In contrast to previous studies, this study used standardized annually collected data for women across the menopause and has validated menopause status retrospectively. Because of the study design of SWAN, this secondary analysis can differentiate drug effect in each menopause status, which has not been published elsewhere. Moreover, this study was rigidly designed and the results were cross validated by two methods (frequency matching and propensity score matching).

A large body of literature has reported association between fracture risk and antihypertensive drugs, but without any BMD data. Outcomes of this study can be used to support the role of BMD loss in terms of fracture. This analysis has many advantages over existing work, with a focus on an age group of women that was not often covered by other literature. This study also captured the extent to which BMD loss in the menopausal transition may be slowed down due to the use of thiazide diuretics.

This study also examined the long term BMD loss after initiating antihypertensive therapy. It enabled us to quantify the effect over approximately 7.5 (±3.6) years; double the length of follow-up time of the randomized controlled trials published so far.[39] It is difficult and expensive to conduct a randomized clinical trial with the long follow-up time required for a trial to have sufficient power to observe differences. Moreover, to accrue BMD related endpoint is often too expensive to make it feasible. Thus, the alternate option to estimate the causal effect is to use propensity score analyses. This study used propensity score matching to minimize the

differences of characteristics at the baseline between groups in order to ensure the exchangeability and positivity, which was rarely used in previous literature regarding this topic.

5.3 STUDY LIMITATIONS AND FUTURE RESEARCH

Study limitations are listed below, mainly including concern of overt bias, optimal matching, cofounding by indication. The imbalances of characteristics between groups prior to treatment were shown in Table 3, causing concern of overt bias that may have affected the conclusion draw from Chapter 4.3. Overt bias is defined as the treated and control groups differ prior to treatment in ways that matter for the outcomes under studied. Although overt bias is common in the observational studies and it may not lead to substantially different conclusions, overt bias still creates problems in distinguishing whether the outcome associates with the drug use or the conditions that lead to the drug use. In this study, we used the new user design to limit this bias by excluding the prevalent users at the SWAN study baseline, who may have more severe hypertension compared to the new users. We also used propensity score matching to verify the conclusion.

The tradeoff of using propensity score matching (optimal matching) is that some women in the treatment groups may be excluded due to incomplete matches, which reduces statistical power. Incomplete matches may result due to two reasons: missing data of the covariates when multivariate analysis is used to calculate the propensity score or not enough overlap in propensity scores between treatment group and control group.[63] After the propensity score matching, there are still 69 women in the ACE group, 88 in the beta blocker group and 76 in the thiazide group,

as compared to the original identified 98, 107, and 99 respectively. Similar conclusions, drawn from the propensity score analysis and the frequency matching analysis, provided strong supportive evidence.

Confounding by indication remains as a potential bias in this study, as it does in any other pharmacoepidemiology study.[64] Blood pressure, in this study, is a time-varying confounder, which may be affected by the exposure and may also affect the BMD, and we did not count for this. Conditioning on blood pressure not only creates selection bias but also prevents identification of the total effect of the exposure to antihypertensive medication. However, blood pressure at baseline is used to estimate the propensity score, because it is associated with treatment selection.

This study has a sub-cohort of women who were prescribed antihypertensive medications to treat hypertension symptoms and only included women across menopause, so the results cannot be generalized to men, to normotensive women, or to younger women who have not started the menopause transition. The population selected for this analysis does not stand for overall SWAN population; therefore, the annualized rate of the BMD loss presented here is not comparable with the annualized BMD loss rate for the overall SWAN population. Drug dosage information for exploring dose-event relationships is not available.

Future research can be done to answer the following questions. Firstly, future research is needed to understand the mediators of thiazide's protective effect on BMD loss. Thiazide leads to the varied effect on BMD loss during each menopausal period whether or not thiazide interacts with other biological changes (e.g. the level of serum follicle-stimulation hormone). Secondly, the safety of thiazide use among normotensive women needs to be further evaluated for osteoporosis prevention in healthy women during the menopause transition. Thirdly, whether

the reduced BMD loss was due to controlled blood pressure or the drug itself remains as questions that need to be answered. In the end, future research should be done to validate thiazide's protective effect on the BMD loss when used as an add-on therapy to other antihypertensive medications, because two or more agents from different pharmacologic classes are often needed to achieve adequate blood pressure control.

5.4 PUBLIC HEALTH SIGNIFICANCE

The findings in this study provided reassurance for women who were using ACE or BB to control blood pressure during the menopause transition, because neither of them has any negative effect on BMD loss during the transition. The results of this study also encourage clinicians to integrate the benefits of using thiazide diuretics, from the prospective of protecting the bone loss, into patients' education, especially for women during late- or post-menopause if there are no other contraindications.

APPENDIX A

GLOSSARY OF ABBREVIATIONS

The following terms are listed alphabetically:

ACE--angiotensin-converting enzyme inhibitors

BB--beta blocker

BMD--bone mineral density

BMI--body mass index

CES-D--center for epidemiologic studies depression scale

FMP--final menstrual period

GERD--Gastroesophageal Reflux Disease

HT-- hormone therapy

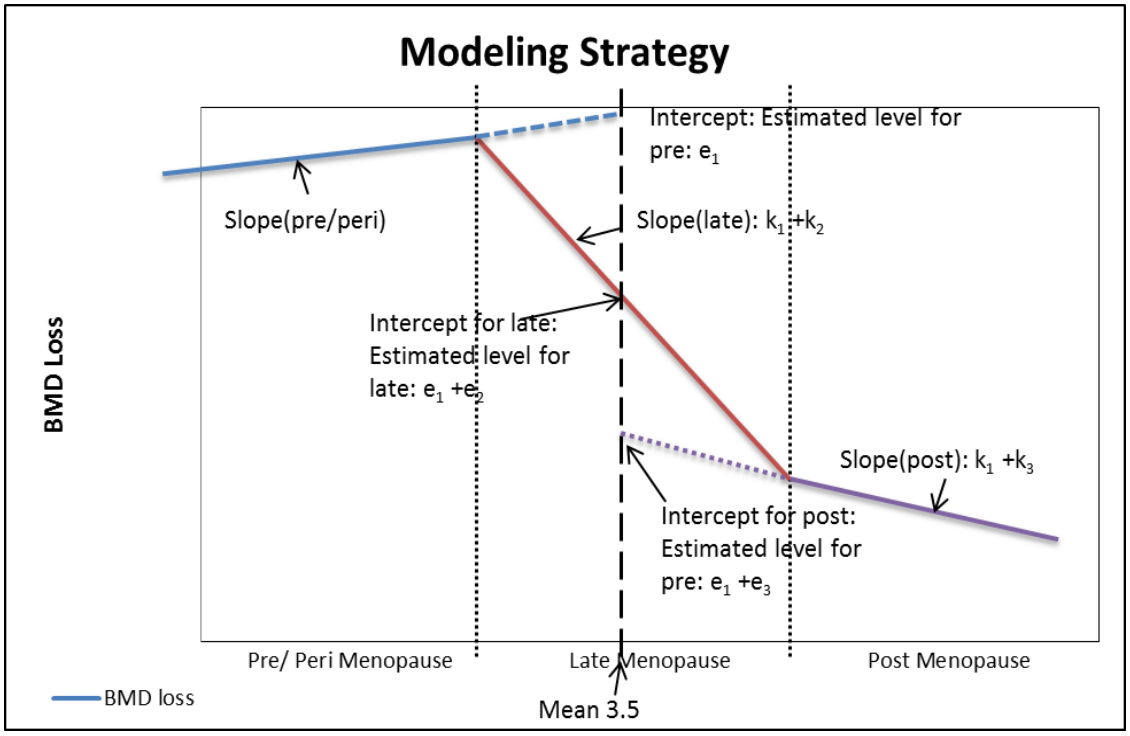
IDIS--Iowa Drug Information System

RAAS--renin-angiotensin-aldosterone system

SWAN--Study of Women's Health Across the Nation

APPENDIX B

MODELING STRATEGY



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