Title Page

**The Effects of Thyroid Medication on Bone Mineral Density: A Study of Women’s Health Across the Nation (SWAN) Pharmacoepidemiology Study**

by

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Abstract

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**The Effects of Thyroid Medication on Bone Mineral Density: A Study of Women’s Health Across the Nation (SWAN) Pharmacoepidemiology Study**

Eric E. Edwards, MPH

University of Pittsburgh, 2019

**Abstract**

**Background:** As women progress through menopause, bone mineral density (BMD) slowly decreases due to estrogen loss. Several studies suggest that medications taken for hypothyroidism do not have a significant effect on the rate of BMD loss, while others suggest that thyroid medication could decrease BMD and increase the risk of fractures, osteoarthritis, and osteoporosis.

**Objective:** To determine if women who initiate thyroid medication for hypothyroidism experience an increase loss in BMD at the femoral neck (FN), hip or spine compared to non -initiators.

**Methods:** We investigated changes in BMD associated with new use of thyroid hormone therapy in a prospective longitudinal cohort of mid-life women. BMD and medication use were measured annually over a period of 16 years. Propensity score matching (PS) was applied to balance baseline characteristics of women who did and did not initiate thyroid medications. Mixed model regression was used to examine annualized change in BMD. Covariates with a known impact on bone health (age, race, body mass index (BMI), menopausal status, thiazide diuretic and hormone use) were included in all models.

**Results:** Our cohort included 356 women (n=178 in each group) with a mean age of 52.9 (SD=5.6) years and BMI of 29.4 (SD=6.8). 64.3 % of the women were Caucasian, 19.9% African American, 9.6% Chinese and 6.2% Japanese. Median follow-up time was 9.5 years. After adjusting for the variables mentioned above, the annual rate of bone loss at the FN, hip and spine for the treatment and control groups were FN (- 0.71% vs. – 0.85% p= 0.22), hip (-0.57% vs -0.66% p=0.47), and spine (-0.59 vs -0.71% p=0.41).

**Conclusion:** After employing a pharmacoepidemiology design and PS matching there were no significant differences in BMD between those who used thyroid medications and those who did not at either the FN, total hip, or spine.

**Public Health Significance:** The findings in this study will help women who are using thyroid hormone therapy during the menopausal transition know that it does not affect their risk of developing osteoporosis or bone loss. The results of this study can help inform clinicians to better focus their time in known variables that affect BMD loss such as thiazide diuretics, body mass index, smoking status, and diabetes. This information will help doctors better educate patients on preventative measures that can help decrease the rate of bone loss through the menopausal transition.

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Preface

I would like to thank my family and friends for supporting me through my graduate education. This program has taught me so much about the public health practice both in research and in application. Dr. Nancy Glynn has served as an amazing mentor, advisor, and influencer. She has taught me so much through our professional and academic relationship and with her guidance, I can take graduate level knowledge and apply it in my professional career. I would also like to thank my research advisor and essay reader, Dr. Kristine Ruppert, for her patience and guidance throughout this process. Finally, I would like to thank the University of Pittsburgh for providing me with an extraordinary graduate educational experience and I am grateful for the tools I have been provided with in public health so that I can implement them in my future career as a clinical pharmacist. It is recommended that acknowledgments, nomenclature used, and similar items should be included in the Preface.

# Introduction

One of the leading public health concerns in the United States is osteoporosis since it affects 20 million Americans and causes osteoporotic fractures in approximately 1.7 million Americans per year 1. Osteoporosis is defined as the deterioration of bone mass and is responsible for a risk in fracture incidence. Menopause is a contributing factor that increases the risk of bone loss in women, resulting in a decrease in 50% of their trabecular bone and 30% of their cortical bone over a lifetime 1,2.

A study by Greendale showed that the rate of bone mineral density (BMD) loss in women was greatest from one year prior through two years after the FMP, termed the transmenopause. Postmenopausal loss rates, those occurring between two and five years after the FMP, were less than those observed during transmenopause. Cumulative, 10-year LS BMD loss was 10.6%; 7.38% was lost during the transmenopause. Cumulative FN loss was 9.1%; 5.8% was lost during the transmenopause 3.

Hypothyroidism is a condition when your thyroid gland does not produce enough hormone 4. By age 50, one in every twelve women has a significant degree of hypothyroidism, and by age 60, it is one woman out of every six 5. Hypothyroidism is known to impact bone mineral homeostasis because of the decrease in three crucial hormones that are meant regulate bone mineral homeostasis: parathyroid hormone, 1,25 (OH)2D, and calcitonin 4.The prevalence of hypothyroidism in the general population ranges from 3.8-4.6% 6,7. The Whickham survey gives an annual incidence of hypothyroidism of 4.1 per 1000 in women 8.

 The Study of Women’s Health Across the Nation (SWAN) is a seven-center, longitudinal cohort study of the menopause transition in a community-based sample of women from multiple ethnic groups 4. BMD of the lumbar spine and proximal femur has been measured annually in women at five SWAN sites. SWAN is the first large-scale, multiethnic, longitudinal cohort study to assess BMD across the entire menopause transition 4. Thus, SWAN provides a unique opportunity to examine the effects on thyroid hormone (TH) medication use and changes in BMD across the menopause transition.

#  Literature Review

## Bone Physiology

Osteoblasts are specialized cell that forms bone and have an essential function in remodeling bone 9. Osteoblasts are also vital in the production of bone matrix proteins and bone mineralization 9. They are developed from pluripotent mesenchymal stems cells signaled under the direction of a characterized suite of regulatory transcription factors, specifically osterix and runt-related factor 2 10. Mature osteoblasts signal the production for matrix mineralization and immature osteoblasts regulate the formation of osteoclasts 11,12,13.

 Osteoclasts originate from colony-forming unit- granulocyte macrophage hematopoietic cell in the bone marrow 14. Differentiation requires contact with stromal cells of the osteoblastic lineage in the bone marrow and stimulation by receptor activator of nuclear factor 𝛋-𝛃 ligand (RANKL), which is released by immature osteoblasts that binds to RANK on the osteoclasts 15. The effects of RANKL on osteoclasts are strictly regulated by osteoprotegerin, which is a decoy receptor of RANKL that is also secreted by osteoblast precursors 16. Orchiectomy causes the proliferation of osteoblast precursors that secretes RANKL that stimulates osteoclast propagation and activation, which results in bone loss 17.

 Osteocyte formation occurs during bone formation when a portion of osteoblasts undergoes terminal separation and entombment by mineralized osteoid 18. Osteocytes are held in liquid-filled lacunae inside the mineralized bone, which represents 90-95% of the total bone cells 19. Osteocytes have long dendrite-like prominences that interact with other osteocytes inside the mineralized, and with osteoblasts located on the bone surface 20. Osteocytes signal bone remodeling by responding to mechanical stimuli to prevent the buildup of bone microdamage 20.

### Bone mineral density testing

 A bone mineral density test can provide a patient with their overall bone health. A commonly used test to assess BMD is the central dual-energy x-ray absorptiometry, or DXA test. This test measures BMD at the hip and spine. These results are then compared to the peak bone mineral density of a 30-year-old adult, and the results are expressed with a T-score 21. A T-score of 0 means that the BMD is equal to the BMD of a young healthy adult 21. The differences between the BMD and the healthy young adult normal measure are referred to as the standard deviation (SDs). Negative numbers represent a lower BMD and higher risk for fracture incidence 21 A T-score that is between +1 and -1 is considered the normal range for a healthy adult 21. A T-score that is between -1 and -2.5 is considered to be an individual with a low bone mass 21. T-scores that are at -2.5 or lower are considered to be criteria for a case of osteoporosis in a young healthy adult 21. The lower the T-score value below 0, the more severe the osteoporosis progression is.

### Thyroid Dysfunction and Menopause

 The incidence of thyroid diseases such as hypothyroidism, nodular goitre, and cancer is highest in elderly and postmenopausal women 22. It is difficult to distinguish the symptoms because they can appear to be nonspecific or common with the menopausal and ageing process 22. If thyroid dysfunction goes unrecognized, it can lead increase the risk of fractures. These fractures are caused by a decrease in the BMD, making them more fragile and susceptible to breaking.

One of the most important factors in bone development is the ability for the thyroid to function properly. Thyroid hormones are controlled by the thyroid hormone receptors (TR) 23. The subtypes of TR are TRa1, TRa2, TRɃ1, TRɃ2 receptors. When these receptors are deficient or dysfunctional TRa, they will cause growth retardation, delays in bone maturity, perturbations in the bone mineralization, and a decrease in BMD 24,25. T3 regulates the bone growth and bone mineralization; stimulates IL-6 and IL-8 while it intensifies the effects of IL-1 and IL-6, controls the production of osteocalcin, collagen type 1, and increases proliferation, differentiation and apoptosis of osteoblast 24.

 The thyroid hormone has a vital role in endochondral ossification, linear growth, maintenance of bone mass, skeletal development, and fracture healing 26. These are common issues seen in the elderly population, specifically women who experience a higher number of fractures due to osteoporosis. During the early stages of hypothyroidism, bone growth starts to cease in growth, mineralization, and formation. Normal TH levels are maintained through the classic negative feedback loop which involves the hypothalamus-pituitary-thyroid (HPT) 27. The major TH is the pro-hormone T4, which is chemically converted to a more potent TH, T3. Hypothyroidism is usually treated with medications that directly impact the level of T3 and T4 in the body. When these two hormones are regulated, they can stimulate optimal skeletal development.

 When the thyroid does not produce enough hormones to function properly, a patient is considered to have hypothyroidism, one of the more prevalent thyroid diseases. If untreated during childhood it can lead to growth retardation, disturbances of endochondral ossification, delayed bone age and persistent short stature 24,25,28. Other morphological studies suggest that thyroid epithelium undergoes a degenerative process, which will lead to its flattening, and with the size of the thyroid follicles diminishing, fibrous connective tissue and lymphoid tissue proliferates 27. As the thyroid shrinks, the hormones secreted lower in both strength and frequency. This has a major impact on the skeletal development and bone mineral density of the individual.

 Hyperthyroidism occurs when the thyroid gland is overactive, thus increasing the rate of metabolism. With the lack of research on the effects of hyperthyroidism and its effect on BMD loss, a study published in 1993 looked to investigate the effects of endogenous subclinical hyperthyroidism due to solitary autonomously functioning thyroid nodule and its effects on bone metabolism and association of risk for the development of osteoporosis. A cross-sectional study was used to measure BMD in premenopausal women and post-menopausal women. These participants were placed into three categories: non-toxic nodular goiter (n=32), subclinical hyperthyroid (n=37), and toxic solitary autonomous thyroid nodule (n=22). These were matched by age and menopausal phase with a control group (n=68). The BMD sites that were measured were femoral neck, midshaft radius, and spine.

 In the non-toxic nodular goiter group, BMD for lumbar spine and neck did not differ from menopausal status and age-matched reference population. The L2-L4 site of the lumbar spine showed a statistically significant decrease in BMD, but only in the toxic nodular goiter group, and this decrease was more efficient in post-menopausal females (p<0.001) than in premenopausal females (p<0.05) 29. At the femoral neck and midshaft radius, the average BMD measurements were slightly but still significantly lower only in the post-menopausal hyperthyroid group compared to the control (p<0.01) 29. In the toxic solitary nodule, the BMD of femoral neck and midshaft radius was significantly decreased in both pre- and post-menopausal cohorts 29. This study concluded that BMD in lumbar spine, femoral neck, and midshaft of the radius are not significantly decreased in pre-menopausal patients with endogenous subclinical hyperthyroidism, resulting from a solitary autonomously functioning thyroid nodule 29.

 A prospective study, published in 1980, looked to investigate 22 patients diagnosed with hyperthyroidism and showed a 12.5% lower BMD at the lumbar spine when compared to healthy control group 30. It showed that lumbar bone mineral content increased by 3.7% after one year 30. Their findings show that an excess of thyroid hormones could lead to a negative spinal mineral balance and the results of BMD loss was clinically insignificant but still partially reversible through anti-thyroid treatment 30.

### Thyroid Hormone Medication Use and Bone Mineral Density

 Studies regarding T3 have shown that it does not stimulate, inhibit, or proliferate osteoblast cells, but impacts osteoblast cell activity 31. Specifically, it increases the expression of osteoblast differentiation markers collagen, osteocalcin, osteopontin, alkaline phosphatase, MMP9, and MMp13 in osteoblast 32,33-36. T3 also influences key pathways that form osteoblast and osteoblast differentiation 26. This supports the theory that T3 stimulates osteoblast activity both directly and indirectly. On the other hand, T3 stimulates osteoclastic bone resorption in the presence of osteoblasts, but this does not occur if the osteoblasts are absent 37,38. This implies that the TH indirectly stimulates osteoclasts through the expression of RANKL and other cytokines involved in osteoclastogenesis 39. This study supports that the functioning of TH does have an impact on the two important cells involved in bone resorption.

 One study looked at a total of 991 Caucasian women between the ages 50-98. Of the 991 study participants, 196 were identified as TH users for an average length of 20.4 years 40. These participants were compared to the remaining 795. Results showed that women who had been taking the daily (D) thyroxine-equivalent dose of 200 𝞵g or greater had a significantly lower BMD at the midshaft radius and hip compared to women who were taking less than 200𝞵g 40. The daily dose of 1.6𝞵g/kg and higher was associated with decreased bone mass at the hip, ultradistal radius, midshaft radius, and lumbar spine when compared to the non-TH users. On the other hand, daily dose lower than 1.6𝞵g/kg was not associated with lower BMD levels 40. These results were independent of age, BMI, smoking status, thiazide use, corticosteroids, and estrogen use. Their results indicate that long-term TH use at thyroxine-equivalent doses of 1.6 𝞵g/kg or larger was statistically significant with osteopenia at all four sites. These results suggest that increase in TH could decrease BMD in postmenopausal women.

Another study conducted in 1988 investigated the long-term effects of Levothyroxine (T4) in premenopausal women on their BMD 41. The BMD of 31 premenopausal women was age and weight matched to 31 women who did not experience bone abnormalities or take thyroid medication 41. The premenopausal women who received the T4 treatment had a 12.8% lower bone density at the femoral neck and 10.1% lower bone density at the femoral trochanter compared to their controls 41. On the other hand, both groups had similar measurements in the lumbar spine recorded between both groups. These results suggest that premature exposure to T4 might not provide protective effects to BMD loss, but potentially enhance the progression of BMD loss in the hip and increase the total risk of age-related BMD loss.

 The treatment of hypothyroidism became a controversial topic, which led to the publication of a study in 1994 42. This study looked at the effects of T4 in the treatment of hypothyroidism, while considering confounding variables in the potential relationship. Ducan et al. collected measurements of 202 Caucasian women who were currently taking thyroid hormone to determine its effect on BMD measured by a number of clinical characteristics and parameters associated with thyroid hormone therapy 42. BMD measurements of the spine (L2-L4) were taken from 195 participants and BMD ate three sites of the hip were taken from 157 participants. The BMD of the proximal radius was measured in 124 subjects. Their findings showed that an increase in age and any history of previous thyrotoxicosis were related to the deteriorating effect on spine BMD 42. BMI was also found to have a positive correlation with spine BMD 42. The dose or duration of the thyroid hormone, type of underlying thyroid disease, history of thyroidectomy, or serum-free thyroxine index had no effect on either the initial BMD or any change in spine BMD over time 42. In hip BMD, age showed an inverse relationship in that as age increased hip BMD decreased 42. History of thyrotoxicosis was associated with a decrease in hip BMD at all three sites (0.05<p<0.10) 42. Calcium use, estrogen use, nodular thyroid disease, thyroidectomy, duration of hormone Rx, and average dose showed no clinical statistically significant effect on spine BMD. The same variables excluding average dose had no statistically significant effect on proximal radius BMD 42. Their study concluded that thyroid hormone therapy was not associated with any significant effect on BMD of the spine or hip, but there was an observed decrease in proximal radius BMD that was related to previous thyrotoxicosis and to dose of thyroid hormone.

 Another study had somewhat similar results in that there could be a protective effect from using thyroid medication for slowing down the deterioration of BMD in postmenopausal women. Ribot’s research was a prospective study of the femoral and vertebral BMD in 49 hypothyroid participants who were given replacement doses of levothyroxine (135+/- 32 𝞵g/day); they were compared them to an untreated group. These women were separated into two groups: one received detectable serum of thyroid and the other received an undetectable serum. Among the primary hypothyroid patients, there was an average decrease of 5.4% (p<0.001) in the vertebral BMD, 7.3% (P<0.001) for trochanter, and 7% (P<0.001) in the femoral neck was recorded after one full year of treatment 43. An ANOVA analysis was conducted to ensure that the observed decrease in BMD was not due to age or menopause status 43. Within the cross-sectional component of this study, there was no significant difference observed in vertebral BMD from the age-matched normal values measured in patients who are either receiving either a substituted or suppressive dose. There was an effect on BMD loss around the first year of treatment, but no significant loss during year two of the study 43.

A more recent study in 2014 looked to investigate previous research on the effects of thyroid hormone therapy on BMD 41. The study had 150 women over the age of 50, with 100 diagnosed with hypothyroidism and 50 healthy women were all divided into three groups: Group A consisted of subjects with hypothyroidism, group B consisted of subjects with primary hypothyroidism for at least 2 years and were taking levothyroxine as treatment, group C consisted of healthy individuals (control group) 41. A blood sample was taken to determine concentration of thyroid stimulating hormone (TSH) and the bone densitometry was used to determine the BMD, which was reported as a T-score to determine the stage of osteoporosis. The T-score of the lumbar vertebrae and femoral neck were taken with a DXA and were compared between the three groups. The study showed that there was not a significant difference in femoral neck T-score between the three groups, but the T-score at lumbar spine L2-L4 regions were significantly different (P=0.01) 41. In group B, the prevalence of osteoporosis was 56% higher than the other treatment and control group 41. The ANOVA test that was conducted indicated that there was a T-score at lumbar spine L2-L4 region between groups 2 and 3 (P=0.027) and between group 1 and 2 (P=0.034) was significantly different 41. The regression analysis conducted did not show any correlation between serum TSH levels and T-score at femoral neck and lumbar spine (P>0.05), but after they removed the effect of the baseline TSH level in group 2, there was a significant difference in prevalence of osteoporosis at the lumbar spine between all groups (P=0.01) 41. Their findings suggest that the treatment of hypothyroidism with thyroid hormones can reduce bone mineral density.

### Other Factors Associated with Bone Mineral Density

*Negative Factors*

As a woman transitions through menopause, decreasing levels of testosterone and estrogen have a negative effect on BMD 14,44,45,46,47. Other factors that negatively affect BMD include smoking, chronic alcohol use, diabetes, glucocorticoid use, and inactivity 48,49,50,51.

*Positive Factors*

 Certain medications have been found to have a positive effect on BMD; these include

Thiazide diuretics, hormone replacement therapy, insulin use calcium and vitamin D 3,53,54,55. African American women were also found to have a higher BMD compared to Caucasian, Japanese and Hispanic women 3.

#  Methods and Analysis

## Study Design

SWAN began in between1996–1997, with the purpose of studying health changes during mid-life in a multi-ethnic community-based cohort of 3,302 women. The overall aim of SWAN is to examine a wide variety of health-related issues as women transition through menopause. The full study design and procedures, including recruitment and medication collection protocols have been described in detail elsewhere 50. Briefly, seven sites across the USA enrolled women between the ages of 42 and 52 if they were pre or early perimenopausal, had an intact uterus, had at least one intact ovary, were not currently taking hormone therapy or oral contraceptives, were not pregnant or lactating, and had ≥1 menstrual period in the previous three months.  After enrollment, women were seen annually. Five of the seven sites conducted a bone health study, with BMD as one of its main outcomes; this involved 2365 women 51.

The current analysis examined whether the initiation of thyroid hormone use in participants was associated with an increased loss of BMD at the femoral neck (FN), total hip and spine. To examine this, a new-user design was employed. A new-user design is a pharmacoepidemiology method that identifies a group of participants who initiate a drug, that is, who have never used thyroid medication before the start of the follow-up.

For those who had not reported thyroid hormone use, we randomly selected a frequency-matched non-user group a comparable baseline. This was to ensure a similar distribution of the baseline visit between the two groups. Then using propensity score matching we created a matched cohort of TH users and non-users. PS matching usually involves the formation of pairs of treated and untreated subjects with similar propensity scores. Greedy matching is commonly used for the formation of these pairs. This means that for a given treated subject, the closest untreated subject within the specified caliper distance is selected for matching to the treated subject. Logistic regression models are mostly used and those baseline variables that influence treatment assignment are included in the model. In this analysis, the following variables were used to calculate the PS: age, race/ethnicity osteoporosis, diabetes, and BMI

### Assessment of Medication Use

Medication use was assessed at each SWAN study visit. Participants were asked about medications taken in the last three months, and responses were verified by visual inspection of medication bottles. If the participant forgot to bring medication containers to the study visit, a review of medication lists was performed. Each medication was then classified into its generic name and assigned a code according to a computerized medication dictionary (Iowa Drug Information Service (IDIS) Drug Vocabulary, College of Pharmacy, University of Iowa, Iowa City, IA).  Dosage information was not consistently listed and thus was not used for these analyses.

### Bone mineral density measurements

The BMD (g/cm2) of the lumbar spine and femoral neck were measured annually using Hologic instruments (Hologic Inc, Waltham, Massachusetts). Three sites used Hologic 4500A models at baseline; two of these sites later upgraded to Discovery models, one at follow-up visit 12 and one at follow-up visit 13. Two sites started with 2000 models at baseline and both of upgraded to 4500 models at follow-up visit 8. Each site that upgraded its hardware scanned 40 volunteers on both old and new machines to develop cross-calibration regression equations, which were applied by the SWAN Coordinating Center. A standard quality control (QC) program was conducted in collaboration with QC centers at Synarc Inc (San Francisco, CA) from baseline to follow-up visit 10 and with the USCF DXA Quality Assurance Center (San Francisco, CA) thereafter. QC included daily phantom measurements, quarterly review of the daily QC plots by the QC centers with correction factors applied for drift if needed, local site review of all scans, and review of problem scans by a member of the SWAN Bone Committee. Short-term in vivo measurement variability was 0.014 g/cm2 (1.4%) for the LS and 0.016 g/cm2 (2.2%) for the FN.

### Statistical Analysis

Descriptive statistics (mean, median, and range) of the baseline demographic variable, were calculated. Variables were transformed where necessary. Standardized mean differences (SMD) were then calculated to examine improvement in balance between the two groups. To compare the annualized rate of change in BMD among the TH users and non-users, a piecewise mixed-effects regression modeling strategy that included a random intercept and slope was used. BMD loss three years prior to medication use and up to seven years after medication use was examined. Factors selected a priori for inclusion in the base models included Age, Race, Body Mass Index (BMI), Menopausal Status, Thiazide Diuretic, and HRT. For consistency, if a covariate was found to be significant at one anatomical site (e.d., femoral neck), that covariate was forced into the other sites. Therefore, all final models for each comparison group contain the same covariates and included only the a priori variables and other covariates with P values <0.05. SAS version 9.3 (SAS Institute, Inc., Cary, North Carolina) was used for the analyses. A greedy matching caliper was set at 0.2 of the standard deviation of the logit of the PS 56.

### Covariates

Initial BMD measurements were taken of femoral neck, spine, and total hip. Other comorbidities were measured. Level of education was collected and separated into two groups; less than or equal to high school or greater than high school. Current tobacco use was determined and either classified or not currently using. The number of participants at each of the four sites (Michigan, Mass General, UC Davis, UCLA, and Pittsburgh) was determined. The menopausal status identified where each woman was in the menopausal transition. Women were categorized into either the pre-menopausal, early or late perimenopause, post-menopause, unknown due to hormone therapy or hysterectomy. Current medication use was also collected on hormone (estrogen/progesterone), bisphosphonates, thiazide diuretics, selective estrogen receptor modulators (SERMs), and gonadotropin-releasing hormone (GnRH) use. The comorbid conditions that were collected were hyperlipidemia, hypertension, osteoarthritis, osteoporosis, cancer, cardiovascular disease, and diabetes. Current fractures measured along with how participants would categorizes their overall health.

# Results

*Study Sample*

A total of 3302 women were enrolled in the SWAN study and among them, 2365 women were enrolled in the bone cohort study. Women were categorized into TH users (n=313) and non-users (n=2052) based on their self-reported use of thyroid hormone use. After removing the prevalent users (n=133) and PS matching, 178 users and non-users remained in our study cohort (Figure 1).

Women not enrolled in SWAN bone cohort

n=937

Women who never initiated thyroid medication

n=2052

SWAN Bone Cohort

n=2365

Women enrolled in SWAN study

N=3302

Women who initiated thyroid medication

n=313

Incident thyroid medication users

n=180

Prevalent users n=133

Final Sample after propensity score matching

n= 178 (1279 observations) user group

n=178 (1282 observations) non-users group

Figure 1 Flow Chart: Study Cohort

## Data Analysis

### Baseline Characteristics

Table 1 shows that the non-TH user group on average was older than the TH user group with an average age of 53.02 and standard deviation (SD) of 5.71. The BMI, and BMD for femoral neck, spine, hip, and total is similar between both cohorts with the only difference being the total comorbidities experienced by both groups. Table1 also shows that the non-thyroid users experienced an average of 1.35 total comorbidities and 1.52 in the thyroid user cohort. The larger percentage of total cohort had more than a high school education degree (n=266) and comprised of 75.6% of the cohort. Tobacco use was reported in 41.9% of the total population (n=149), but no significant difference in terms of reported use between the non-user (n=78) 44.1% and user group (n=71) 40.3%.

The sample was primarily Caucasian women (n=229) 64.3%, with a relatively equal distribution between non-users and users among the four ethnicities reported seen below in the Table 1. Most women were in the early/late perimenopausal or postmenopausal transition period (n=154) 44.3% and (n=125) 35.1% respectively. The most prevalent comorbid conditions reported by the total cohort were hyperlipidemia (n=147) 41.3%, hypertension (n=151) 43.4%, and osteoarthritis (n=143) 40.3%. A total of 292 women self-reported as being in excellent, very good, or good health.

Table 1 Baseline Demographics

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Total** | **Non-Thyroid Medication User** | **Thyroid Medication User** | **SMD** |
|   | **N=356** | **n=178** | **n=178** |  |
| **Variable**  | n  | % | n | % | n | % |  |
| Age, mean (SD) | 52.90 | 5.60 | 53.00 | 5.70 | 52.80 | 5.50 | **0.036** |
| BMI, mean (SD) | 29.40 | 6.80 | 29.40 | 7.20 | 29.50 | 6.80 | **0.009** |
| Femoral Neck BMD, g/cm2 mean (SD) | 0.81 | 0.12 | 0.81 | 0.12 | 0.81 | 0.12 | **0.072** |
| Spine BMD, g/cm2 mean (SD) | 1.05 | 0.14 | 1.10 | 0.15 | 1.00 | 0.14 | **0.076** |
| Hip BMD, g/cm2 mean (SD) | 0.94 | 0.14 | 0.94 | 0.13 | 0.95 | 0.14 | **0.047** |
| **Tobacco use** |  |
| current | 149 | 41.9% | 78 | 43.8% | 71 | 39.9% | **0.076** |
| **Race/Ethnicity** |  |
| White | 229 | 64.3% | 117 | 65.7% | 112 | 62.9% | **0.059** |
| Black | 71 | 19.9% | 37 | 20.8% | 34 | 19.1% | **0.042** |
| Chinese | 34 | 9.6% | 13 | 7.3% | 21 | 11.8% | **0.150** |
| Japanese | 22 | 6.2% | 11 | 6.2% | 11 | 6.2% | **0.000** |
| **Menopausal Status** |  |
| Pre | 19 | 5.3% | 9 | 5.1% | 10 | 5.6% | **0.025** |
| Early Peri | 131 | 36.8% | 66 | 37.1% | 65 | 36.5% | **0.012** |
| Late Peri | 23 | 6.5% | 12 | 6.7% | 11 | 6.2% | **0.023** |
| Natural Post | 125 | 35.1% | 61 | 34.3% | 64 | 36.0% | **0.035** |
| Surgical Post with BSO | 15 | 4.2% | 11 | 6.2% | 4 | 2.2% | **0.197** |
| Unknown dt HT or Hysterectomy | 43 | 12.1% | 19 | 10.7% | 24 | 13.5% | **0.086** |
| **Medication Use** |  |  |  |  |  |  |
| Hormone Replacement Therapy  | 142 | 39.9% | 74 | 41.6% | 68 | 38.2% | **0.069** |
| Bisphosphonates | 37 | 10.4% | 15 | 8.4% | 22 | 12.4% | **0.129** |
| Thiazide | 54 | 15.2% | 28 | 15.7% | 26 | 14.6% | **0.106** |
| **Comorbid conditions** |  |  |  |  |  |  |
| Hyperlipidemia | 147 | 41.3% | 70 | 39.3% | 77 | 43.3% | **0.080** |
| Hypertension | 151 | 42.4% | 75 | 42.1% | 76 | 42.7% | **0.011** |
| Osteoarthritis | 143 | 40.2% | 63 | 35.4% | 80 | 44.9% | **0.201** |
| Osteoporosis | 33 | 9.3% | 17 | 9.6% | 16 | 9.0% | **0.019** |
| Cancer | 24 | 6.7% | 11 | 6.2% | 13 | 7.3% | **0.045** |
| CVD | 12 | 3.4% | 4 | 2.2% | 8 | 4.5% | **0.125** |
| Diabetes  | 36 | 10.1% | 21 | 11.8% | 15 | 8.4% | **0.112** |
| **Overall Health** |  |
| Excellent/VG/Good | 292 | 82.0% | 154 | 86.5% | 138 | 77.5% | **0.220** |
| Fair/Poor | 61 | 17.1% | 23 | 12.9% | 38 | 21.3% |  |

\*SMD=Standardized Mean Difference, other variables examined included education, study site, selective estrogen receptor modulators (SERMS) and gonadotropin-releasing hormone (GnRH).

### Annual rate of change in BMD in femoral neck, lumbar spine, and total hip with covariate adjustments

Table 2 shows the type 3 test to determine which covariates have a significant effect on BMD in the neck. BMD in the femoral neck was found to be statistically significantly associated with BMI (p<0.0001). It was also found to be statistically significantly associated with menopausal status and thiazide diuretic, (p<0.0001) and (p<0.0041), respectively. Table 3 shows covariates that were statistically significant with the BMD in the hip were BMI (p<0.0001), race (p<0.0098), menopausal status (p<0.0001), and thiazide diuretic use (p<0.0013). Table 4 shows that BMD in lumbar spine was statistically significantly associated with age (p<0.0001), race (p<0.0273), menopause status (p<0.0001), and thiazide diuretic (p<0.0069).

Table 2 Covariate efforts on BMD in femoral nect



Table 3 Covariate efforts on BMD in hip

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Table 4 Covariate efforts on BMD in lumbar spine

****

###  Annual rate of change in BMD in femoral neck, lumbar spine, total hip

Figure 2 shows the annual rate of change in the femoral neck, total hip, and lumbar spine through the course of the study. At the FN a decrease of -0.71% was found in the TH users compared to non-users -0.85% decrease (p<0.22). At the total hip, TH users group experienced a -0.57% loss and -0.66% in the non- users group (p<0.47). The TH users group experienced a -0.59% BMD loss in lumbar spine and -0.71% BMD loss in the non- users group with a (p<0.41). These results were adjusted for age, race, BMI, menopausal status, thiazide diuretics and hormone use.

\*Adjusted for age, race, body mass index (BMI), menopausal status, thiazide diuretic and hormone use

Figure 2 Annual rate of bone loss in lumbar spine, femoral neck, and toal hip after medication initiation

### Piecewise Rate of change in BMD

 Table 2 shows the average estimated BMD decrease in each site for 3 years prior to thyroid medication initiation and seven years after baseline (thyroid initiation). Figure 3 shows the annual change in BMD loss in each site of the users over time. The estimated BMD loss in the neck prior to the initiation of thyroid medication was -0.74% each year for 3 years until baseline visit. After thyroid medication was initiated, there was an observed -0.71% decrease in BMD in the neck each year for seven years. The decrease in BMD loss in the neck was found to be significant (p<0.0001) within the user group. The estimated BMD loss in the hip prior to the initiation of thyroid medication was -0.27% for 3 years until baseline visit. After thyroid medication was initiated, there was an observed -0.57% decrease in BMD in the hip each year for seven years. The decrease in BMD loss in the hip was found to be significant (p<0.0001) within the user group. The estimated BMD loss in the spine prior to the initiation of thyroid medication was -0.69% for 3 years until baseline visit. After thyroid medication was initiated, there was an observed -0.59% decrease in BMD in the spine each year for seven years. The decrease in BMD loss in the spine was found to be significant (p<0.0001) within the user group.

Table 5 Estimates for within group differences in BMD loss

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Site** | **Label** | **Estimate** | **Error** | **DF** | **t-value** | **Pr > |t|** | **Alpha** | **Lower** | **Upper** |
| Femoral Neck | Early users | -0.00735 | 0.001414 | 292 | -5.20 | <.0001 | 0.05 | -0.01013 | -0.00457 |
| Late users | -0.00709 | 0.000911 | 287 | -7.78 | <.0001 | 0.05 | -0.00888 | -0.00529 |
| Hip | Early users | -0.00269 | 0.001191 | 318 | -2.26 | 0.0247 | 0.05 | -0.00503 | -0.00034 |
| Late users | -0.00574 | 0.000868 | 277 | -6.61 | <.0001 | 0.05 | -0.00745 | -0.00403 |
| Lumbar Spine | Early users | -0.00699 | 0.001458 | 309 | -4.79 | <.0001 | 0.05 | -0.00986 | -0.00412 |
| Late users | -0.00589 | 0.001063 | 258 | -5.54 | <.0001 | 0.05 | -0.00798 | -0.00379 |

Figure 3 Percent BMD loss in femoral neck, hip, lumbar spine

# Discussion

We studied a longitudinal cohort of women who were transitioning through menopause to determine if thyroid medication had an effect on bone mineral density loss in the femoral neck, lumbar spine, or hip. In this study, we found no significant changes between the user and non-user groups. A previously mentioned study that looked at the effects of long-term L-Thyroxine and its effect on BMD was greater than our results indicated 41. Their users experienced a 12.8% decrease in femoral neck and a 10.1% decrease in femoral trochanter compared to the non-users they were matched to 41. Their results also showed a similar decrease in the lumbar spine bone density in both groups 41 This study showed a greater decrease in BMD compared to our results and differs from our findings in that thyroid medications does not appear to prevent or provide a significant protective effect against BMD loss in lumbar spine, femoral neck, or hip.

 Another study looked to investigate the effects of L-thyroxine on 202 Caucasian women and its effect on lumbar spine, three sites of the hip, and proximal radius 42. Their literature review is consistent with ours in finding previous studies that an increase in age (p<0.001), higher BMI (p<0.0001), history of thyrotoxicosis (p<0.05) are associated with a decrease in lumbar spine BMD. Their results indicated that thyroid medication therapy was not statistically significantly associated with BMD loss in the spine or total hip. There was a significant association between a decrease in BMD loss in the proximal radius and previous thyrotoxicosis and the average dose of thyroid hormone 42. Our study looked only at lumbar spine, hip, and femoral neck and adjusting for the same a priori variables, we found the same results, no significance.

 The effects of levothyroxine were measured in a cross-sectional descriptive study on 150 women over the age of 50 41. Their results showed that there was no statistically significant differences of femoral neck T-score between the group recently diagnosed with hypothyroidism, patients with hypothyroidism who have been treated with two years with levothyroxine, and a non-hypothyroidism group (control). This differs from our study in that they compared three groups in which two did not receive thyroid medication (hypothyroid diagnosed and control), and a user group who was diagnosed. Their results also reflect a two year treatment and with the same thyroid medication. The T-score at the lumbar spine was statistically significantly different (p=0.01) in BMD loss. These results indicate that there may be a potential relationship between thyroid medication use and lumbar spine BMD loss, but there are many potential biases when conducting a cross-sectional study. Since a cross-sectional study is a snapshot of the effect of an independent variable on a dependent, there is always potential for a misrepresentation of the true relationship. Cross-sectional studies also do not capture those who experience an observable outcome after the measure was taken.

# Study Limitations and Future Research

 Strengths of this analysis include the ability to compare bone loss density among four ethnic groups over time and obtain longitudinal measurements, SWAN total sample size, propensity score matching, and a new users design, the population on the racial/ethnic groups is very representative for the US population. The use of the new user’s study design to decrease the potential overt bias by excluding those who have been on thyroid medication prior to the SWAN baseline assessment.

Some limitations could have affected our final results. Prior SWAN research does show the rate of BMD throughout the menopausal transition, so this provided us information on how BMD loss increases and decreases over time. Another major concern in observational studies is overt bias, which can potentially create problems in determining whether the outcome associates with the drug use or the conditions that lead to the drug use. Another limitation of this study is the difference in thyroid hormone in the blood between each participant. Thyroid levels vary between participants; thus, the effect of thyroid medication will differ based on this factor, thus some participants might experience a more protective effect from the thyroid medication.

 In conclusion, this analysis does not confirm that thyroid medication will either accelerate or decelerate the rate of bone mineral density loss in femoral neck, total hip, and lumbar spine in TH users. There is a trend that shows there may be some relationship in that the treatment group experiences a potentially slower decrease in BMD, but this trend is not significant in either group. Future research should include a larger sample size to investigate the relationship. Future research should also investigate other medications to see if they could have an effect on BMD loss in women.

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