IMPACT OF AGE AT NATURAL MENOPAUSE ON INCIDENCE OF IMMUNE-MEDIATED DISEASES: ASTHMA, HYPOTHYROIDISM, AND RHEUAMTOID ARTHRITIS IN POSTMENOPAUSAL CANADIAN WOMEN

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Abstract

The average life expectancy is increasing, and women are now expected to spend more than a third of their lives after menopause. Menopause is marked by major hormonal changes, particularly a significant decline in estrogen levels. Estrogen influences nearly all systems in the body, and an earlier age at menopause (ANM) results in an earlier cessation of estrogen production. Early ANM has been associated with an increased risk of various diseases, while later ANM has been linked to a higher risk of reproductive cancers. However, the relationship between ANM and immune-mediated diseases has not been well studied. Immune-mediated diseases such as asthma, hypothyroidism, and rheumatoid arthritis are more common in women and may be influenced by the timing of menopause. This study retrospectively examined postmenopausal women from the Canadian Longitudinal Study on Aging over a 10-year period. Multivariable Cox regression models were used to investigate the associations between ANM and the incidence of these diseases. The analysis showed that earlier ANM was linked to a reduced risk of asthma, while later ANM was associated with a lower risk of rheumatoid arthritis. No significant association was found between ANM and hypothyroidism. These findings suggest a potentially complex and differential role of estrogen in the immune system. They may inform healthcare professionals in monitoring ANM and evaluating risk for specific immune-mediated diseases. Further research is needed to understand the role of estrogen in the development of these diseases.

Keywords: age at natural menopause (ANM), asthma, hypothyroidism, rheumatoid arthritis, women's health, Canadian Longitudinal Study on Aging (CLSA), Canada

Dedication

Dedicated to women's health, and the pursuit of knowledge that supports it.

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Preface

Chapter 2 is published in *Menopause*, the Journal of the North American Menopause Society.

Chapter 3 is published in *PLOS one* Public Library of Science ONE

Chapter 4 is published in *Seminars in Arthritis and Rheumatism*

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List of Abbreviations

ANM: Age at natural menopause

BMI: Body mass index

CI: Confidence intervals

CLSA: Canadian Longitudinal Study on Aging

CVD: Cardiovascular diseases

ER-α: Estrogen receptor alpha

ER-β: Estrogen receptor beta

HRT: Hormone replacement therapy

HR: Hazard ratio

HT: Hormone therapy

IL-1: Interleukin-1

IL-1β: Interleukin-1 beta

IL-6: Interleukin-6

MICE: Multiple imputation by chained equations

NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells

ORE: Office of Research Ethics

OR: Odds ratio

POF: Premature ovarian failure

RA: Rheumatoid arthritis

T2: Type 2

T3: Triiodothyronine

T4: Thyroxine

TBG: Thyroid binding globulin

Th1: T-helper 1

Th2: T-helper 2

TNF- α: tumor necrosis factor alpha

TSH: Thyroid stimulating hormone

Chapter 1

General Introduction

Biology of Menopause

Menopause occurs due to depletion of ovarian follicular reserve.[1] The ovarian reserve, compromises follicles that house oocytes which are surrounded by granulosa cells, underpins fertility and menstrual cycling.[2] These follicles also represent the primary source of estrogen production.[1] As the follicular reserve diminishes, typically in midlife, systemic estrogen levels decline markedly, triggering the onset of menopause.[3]

In premenopausal women, estradiol is the dominant and most biologically potent form of estrogen, produced primarily by the ovaries. After menopause, the ovaries cease estradiol production, and the primary source of estrogen shifts to estrone, which is produced in adipose tissue via aromatization of androgens.[4] Estrone is a weaker form estrogen compared to estradiol.[5] A third form, estriol, is produced mainly during pregnancy and plays a limited role outside of that context.[4] The postmenopausal shift from estradiol to estrone has important implications for systemic inflammation and immune regulation, as discussed below.

Menopause and Estrogen Function

Estrogen plays a regulatory role across nearly all tissues, including the brain, vasculature, musculoskeletal system, and immune system.[3,6] The postmenopausal decline in estrogen has been implicated in the pathogenesis of multiple conditions including metabolic and cardiovascular disorders, autoimmune diseases and neurodegeneration.[6]

Age at Natural Menopause

Natural menopause is typically defined as 12 consecutive months of amenorrhea without an obvious cause such as an oophorectomy, radiotherapy or chemotherapy.[7] Across 21 studies in 10 countries, the average age at natural menopause has been found to range between 47 and 53 years.[7] In many studies the average age at menopause has been found to be 51 years.[8-11] Menopause occurring before the age of 40 years is considered to be primary ovarian insufficiency, which affects approximately 1% of the population, though its exact cause remains unknown.[7,12] Early menopause, defined as menopause occurring between 40 and 45 years, affects about 5% of the population.[12]

Health Implications of Early vs. Late Menopause

The timing of menopause has significant implications for long-term health. Early or Premature menopause has been associated with increased risks of cardiovascular disease, stroke, type 2 diabetes, osteoporosis, depression, cognitive decline, and lupus erythematosus.[1-3,6-19] In contrast, later menopause has been linked to elevated risk of reproductive organ cancers.[22,23] Women undergoing menopause earlier than average also face increased risk of premature morbidity and mortality, and reduced quality of life.[24,25] The hormonal shift associated with menopause, particularly the decline in estradiol levels, not only affects cardiovascular and metabolic health but also has profound implications for immune system function.[26] As a result, menopause may contribute to the onset or exacerbation of immune-mediated diseases.

Immune-Mediated Diseases: Epidemiology and Immunopathogenesis

Immune-mediated diseases encompass a broad range of conditions in which the immune system attacks the body's own cells (autoimmune diseases), becomes overactive (allergies), or fails to function properly (immunodeficiencies).[27] These diseases are characterized by chronic or

acute inflammation and tissue damage, often involving the overproduction of proinflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α) or an imbalance in T-helper 1(Th1)/ T-helper 2 (Th2) cytokine levels.[27] The global incidence of immune-mediated disease has risen rapidly, particularly in industrialized countries, posing growing medical and socioeconomic burdens.[28] These often lifelong diseases, substantially reduce quality of life and life expectancy,[28] and have been found to affect up to 10% of the world's population.[29] Sex hormones regulate the development and function of immune response and dysregulation of these hormones can contribute to immune-mediated diseases.[30] Immune-mediated diseases are more common in women than men and one proposed explanation involves hormonal differences, particularly the role of estrogen and progesterone in modulating immune responses.[31] In postmenopausal women, the loss of estrogen exacerbates the levels of proinflammatory cytokines,[26] increases sensitivity to these cytokines, and reduces production of anti-inflammatory cytokines.[21]

Rational and Objectives

Given the role of estrogen on immune function, the timing of menopause particularly early or late onset, may play a critical role in influencing immune-related disease risk. Although cardiovascular and metabolic consequences of early menopause are well documented,[15,32] its relationship with immune-mediated conditions remains poorly understood. This study aims to address this knowledge gap by focusing on three common immune-mediated diseases: asthma, hypothyroidism, and rheumatoid arthritis.[27,28,33,34] These diseases disproportionately affect women,[35-37] and are influenced by hormonal fluctuations during key reproductive transitions.[38-40] Asthma is associated with airway inflammation and has been shown to increase in onset after menopause.[41,42] Hypothyroidism, most often caused by autoimmune thyroiditis,[42] is sensitive to

hormonal changes and increasingly prevalent in aging women.[44,45] Rheumatoid arthritis (RA), a systemic autoimmune disease, also has strong links to hormonal status.[46] Despite their clinical relevance, the effect of age at natural menopause (ANM) on the risk of developing these conditions has not been well explored. Understanding this relationship between age at natural menopause and risk of these diseases could provide valuable insights into the long-term health consequences of reproductive aging and inform prevention and management strategies for to postmenopausal women. This dissertation aims to address the following three objectives, each presented in a self-contained, recently published manuscript:

Objective 1: To investigate the association between ANM and risk of asthma in postmenopausal women from the Canadian Longitudinal Study on Aging (CLSA)

Objective 2: To investigate the association between ANM and risk of hypothyroidism in postmenopausal women from the CLSA

Objective 3: To investigate the association between ANM and risk of rheumatoid arthritis in postmenopausal women from the CLSA.

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Chapter 2

The association between age at natural menopause and risk of asthma among postmenopausal women from the Canadian Longitudinal Study on Aging

Durmalouk Kesibi, MA, Michael Rotondi, PhD, Heather Edgell, PhD, and Hala Tamim, PhD

Abstract

Objective: This study aimed to investigate the association between age at natural menopause and incidence of asthma among postmenopausal Canadian women.

Methods: Women between the ages of 45-85 yr were followed for a 10-yr period. Analysis was restricted to naturally postmenopausal women who are nonsmokers and did not have asthma prior to menopause. Age at natural menopause was examined using the following categories: 40-44, 45-49, 50-54 (reference), and ≥55. Survival analysis was utilized to determine time to onset of asthma. Multivariable Cox regression analysis was performed to assess the relationship between age at natural menopause and asthma after adjusting for covariates.

Results: The multivariable Cox regression analysis showed a 30% decreased risk of asthma in women with age at natural menopause of 40-44 yr compared with age at natural menopause of 50-54 yr with a hazard ratio of 0.7 (95% confidence interval: 0.49-0.95).

Conclusions: Women with later ages at natural menopause may be at increased risk for asthma. Key Words: Age at menopause – Age at natural menopause – Asthma – CLSA – Incidence asthma.

Introduction

Menopause is a critical period in all women's lives as it is associated with major physiological changes. It is defined as the cessation of menstruation for 12 consecutive months.[1] It is also characterized by a loss of ovarian function leading to decreased levels of sex hormones: estrogen and progesterone.[2] The average age at natural menopause (ANM) is around 51 yr, whereas early-onset menopause is between 40 and 44 yr.[3] Women with an earlier age at menopause have been shown to be at an increased risk for many long-term health conditions, including cardiovascular diseases, diabetes, osteoporosis, and depression.[1,3-6]

Asthma is a common chronic disease affecting more than 300 million people worldwide.[7] The prevalence of asthma has been increasing over recent years,[8-10] and it has substantial economic costs.[8] Asthma ranks as one of the highest diseases for healthcare utilization.[11] In 2007, the economic burden of asthma was $56 billion in the United States.[11] Asthma is characterized by phases of reversible airflow obstruction caused by hyperactive contractions of the smooth muscles and by inflammation of the airway.[12] Its main symptoms include chest tightness, wheezing, dyspnea, and cough.[7]

Adult-onset asthma is asthma onset after childhood,[12] and it is typically more severe,[9,13,14] and more difficult to treat than childhood asthma.[11] Several studies have investigated the incidence of adult-onset asthma, with findings showing a higher incidence in females compared with males. Winer et al and Eagan et al reported a female-to-male asthma incidence of 4.9 versus 2.8 and 5.9 versus 4.4 per 1,000 person-years, respectively.

Hormonal links to asthma have been demonstrated by the reverse in gender prevalence of asthma. Asthma is more prevalent in boys in childhood; however, after puberty,[12] asthma becomes more prevalent in girls. Furthermore, females tend to have more severe asthma[15] and are less likely to have remission of the disease.[16] Studies have found a peak incidence of asthma around 40 yr, which is the age of menopause transition,[17] whereas other studies found a peak at average age at menopause.[13]

Some studies found that natural or synthetic increase of estrogen levels in the body lead to increased risk of asthma. It is evident from several studies that higher body mass index (BMI) levels in women lead to increased risk of asthma compared with women with lower BMI levels.[18-21] This may be due to the increased circulating estrogen in the body associated with higher BMI levels.[18,22] This relationship is supported by studies that found an increased asthma risk in women with higher BMI and not in men,[19-21] emphasizing the role of female sex hormones in the relationship. Furthermore, synthetic estrogen exposure seems to also promote asthma development. Rates of asthma diagnosis increased with use of hormone therapy (HT) and declined after termination of use.[23] Similarly, women using HT had a 63% increased risk of asthma, whereas women who stopped HT were two times more likely to quit asthma treatment.[24] Women with lower BMI levels on HT had a similar prevalence to asthma as obese women not on HT[18]; this may suggest the similar effect of synthetic estrogen to natural estrogen on asthma risk. A study found an association between use of HT and increased asthma risk in postmenopausal women in a dose-response relationship.[25] Another study on postmenopausal women found higher estrogen levels in women with severe asthma compared with women with mild asthma and controls,[26] whereas another study on premenopausal women showed an association between estrogen administration

and asthma risk.[14] These studies provide evidence for the effect of natural and synthetic estrogen on the risk of asthma in women.

Research on the association between menopause and asthma incidence is limited and has yielded conflicting results. Among these studies, Troisi et al reported a decreased risk of asthma in postmenopausal women compared with premenopausal women.[25] Conversely, a meta-analysis mostly based on cross-sectional studies found no significant difference in asthma incidence between premenopausal and postmenopausal women.[27] More recent prospective studies have indicated an increased risk in postmenopausal women. Specifically, Triebner et al found an increased risk for women with early and late menopause as compared with premenopausal women.[28] Another study by Matulonga-Diakiese et al found an increased risk of asthma in women with surgical menopause compared with premenopausal women, but no significant increased risk was found for women with natural menopause.[29] In the literature, only one study investigated the association of ANM and asthma risk and found that both earlier menopause, at or before 46 yr, and later menopause, at and after 55 yr, were associated with increased risk of asthma.[14] Given the lack of literature on the topic, this study aimed to investigate the association between ANM and incidence of asthma among nonsmoking postmenopausal Canadian women from the Canadian Longitudinal Study on Aging (CLSA).

Methods

Study design and sample

This research involved a secondary data analysis of the CLSA. CLSA is a Canada-wide prospective study of 51,338 men and women, between the ages of 45 and 85 yr over a 20-yr period. The study was launched in 2010, for the purpose of providing a better understanding of the physical, biological, and social changes associated with aging. The CLSA sample consists of two cohorts: tracking and comprehensive. At baseline, the tracking cohort was compromised of n = 21,241 (males: 10,406; females: 10,835) participants, randomly selected from across the 10 Canadian provinces with data collection happening over telephone interviews. However, the comprehensive cohort consisted of n = 30,097 (males: 14,777; females: 15,320) participants who were randomly selected from 7 of 10 Canadian provinces based on living within 25-50 km from 1 of the 11 CLSA data collection sites across Canada. Data collection from the comprehensive cohort consisted of an at-home interview and a physical examination at a CLSA data collection site. The CLSA data collection happens every 3 years, and three cycles of data collection have already been completed. Approximately 21.42% of the sample was lost to follow-up or died by follow-up 2. More details on CLSA method have been described elsewhere.[30,31]

This study was a retrospective analysis of the pooled tracking and comprehensive CLSA samples over three cycles of data collection (baseline, follow-up 1, and follow-up 2). CLSA sampling design excluded individuals who were unable to respond in English or French, or unable to understand the purpose of the study, or provide reliable data. Furthermore, individuals with cognitive impairment, living on First Nations reserves and settlements, living in long-term care institutions, and full-time members of the Canadian Armed Forces were also excluded.

Study participants

For this study, all male participants were initially excluded leaving a sample of 26,155 females. Additional exclusions were applied to restrict the sample to nonsmoking naturally

postmenopausal women with clear information on menopause status. Specifically, women with missing information on menopause, women who did not reach menopause, women with surgical menopause or with medically induced menopause, women with missing ANM, and women with age at menopause under 40 or over 67 yr (based on thresholds similar to Verschoor and Tamim)[32] were excluded. Women with missing information on asthma status, age at asthma, or had asthma prior to menopause were also excluded. Current smokers were excluded from the sample to limit residual confounding since smoking is a strong risk factor for asthma[33-36] and earlier age at menopause.[37] After applying all these exclusions, the study sample consisted of 14,406 participants. Additional details on excluded participants are provided in Figure 1.

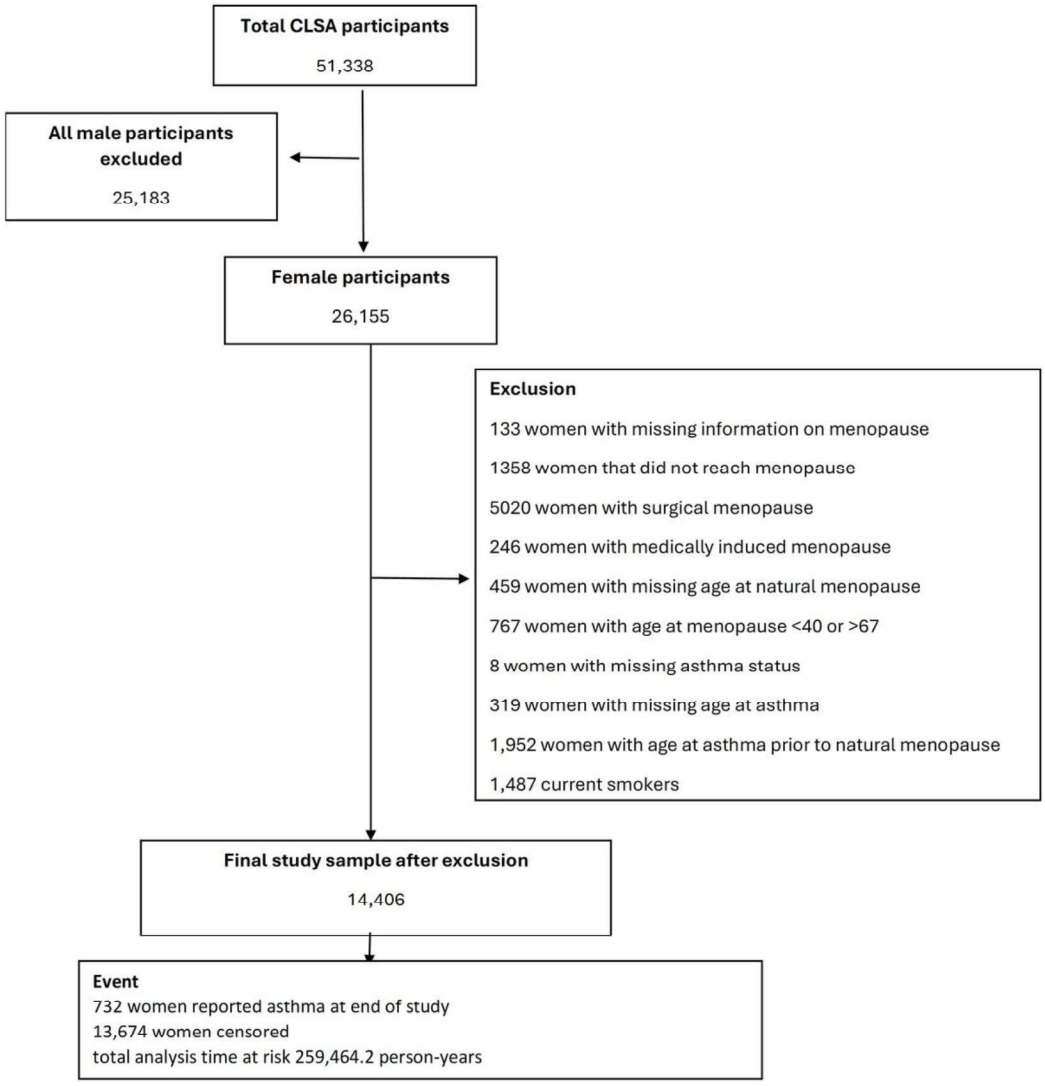

FIG. 1: Canadian Longitudinal Study on Aging (CLSA) participant chart.

Exposure assessment (ANM)

Self-reported ANM was ascertained from all three cycles by the following question, "*Have you gone through menopause, meaning that your menstrual periods stopped for at least 1 year and did not restart?*" answered by, "yes" or "no." Women who answered by "yes" were then asked, "*How old were you when your menstrual periods stopped for at least 1 year and did not restart?*" Answers were recorded in years of age. Age at menopause was examined in the following categories (40-44, 45-49, 50-54 [reference], and ≥55), similar to the categories presented by Mondul et al.[38]

Outcome assessment (incidence of asthma)

All postmenopausal women were asked about their asthma status with the following question, "*Has a doctor ever told you that you have asthma*?" answered by, "yes" or "no." Women who answered by "yes" were then asked about their age at asthma: "*At what age or in what year were you first told that you had asthma?*" reported in years. Women who answered by "no" were asked again in the next cycle. Information on asthma incidence was collected from all three cycles for the comprehensive cohort, and from follow-ups 1 and 2 of the tracking cohort.

TABLE 1 - Characteristics of study population according to age at natural menopause

	Age at Natural Menopause (years)								
	40-44		45-49		50-54		≥ 55		P-
Variables	**Unweighted**	**Weighted[a]**	**Unweighted**	**Weighted[a]**	**Unweighted**	**Weighted[a]**	**Unweighted**	**Weighted[a]**	**value[a]**
***N* (%)**	1,065(7.4%)	274,200 (7.8)	3,247(22.5%)	820,052 (23.2%)	7,386 (51.3%)	1,806,969 (51.1%)	2,708 (18.8%)	634,858 (18.0%)	
Sociodemographic									
Age at Baseline (years) mean (SD)	65.5 (10.2)	62.3 (10.2)	63.5 (10.5)	60.3 (10.2)	62.6 (9.9)	60.2 (9.9)	65.1 (9.0)	63.7 (9.1)	<0.001
Ethnicity									
White	1,023 (96.1%)	265596.1 (96.9%)	3,049 (94.0%)	769615.5 (93.8%)	6,994 (94.7)	1691857.6 (93.6%)	2,586 (95.5%)	606929.2 (95.6%)	0.006
Other[b]	33 (3.1%)	7122.7 (2.6%)	152 (4.7%)	34804.3 (4.2%)	317 (4.3%)	97845.7 (5.4%)	93 (3.4%)	16457.3 (2.6%)	
Marital status									
No partner	479 (45.0%)	98963.1 (36.1%)	1,294 (39.9%)	227835.4 (28.0%)	2,658 (36.0%)	469463.1 (26.0%)	1,013 (37.4%)	181974.3 (28.7%)	0.002
With Partner	586 (55.0%)	175236.4 (64.0%)	1,953 (60.2%)	592216.3 (72.2%)	4,728 (64.0%)	1337505.8 (74.0%)	1,695 (62.6)	452883.4 (71.3%)	
Employment									
Not currently working	735 (69.0%)	185926.3 (68.0%)	2,047 (63.0%)	477968.1 (58.3%)	4,476 (60.6%)	993875.6 (55.0%)	1,901 (70.2%)	428694.3 (67.5%)	<0.001
Currently working	321 (30.1%)	82464.4 (30.0%)	1,179 (36.3%)	336668.5 (41.1%)	2,869 (38.4%)	795342.2 (44.0%)	794 (29.3%)	201266.8 (31.7%)	
Education Level									
Less than high school	96 (9.0%)	62037.6 (22.6%)	194 (6.0%)	144834.9 (17.7%)	389 (5.3%)	279118.1 (15.4%)	165 (6.1%)	121165.4 (19.1%)	0.004
High school – some college	645 (60.1%)	177217.3 (64.6%)	1,806 (55.6%)	511039.9 (62.3%)	3,874 (52.5%)	1141806.1 (63.2%)	1,463 (54.0%)	386777.5 (61.0%)	
Bachelor's or higher	316 (29.7%)	32099.2 (11.7%)	1,237 (38.1%)	157824.7 (19.2%)	3,109 (42.1%)	383470.3 (21.2%)	1,075 (39.7%)	124865.3 (19.7%)	
Household income (CAD)									
<$20,000	73 (6.9%)	32984.2 (12.0%)	213 (6.6%)	52876.5 (6.5%)	344 (4.7%)	82844.2 (4.6%)	136 (5.0%)	40790.9 (6.4%)	0.000
20,000-50,000	329 (31.0%)	79699.2 (30.0%)	888 (27.4%)	218295.3 (26.6%)	1,800 (24.4%)	447758.4 (24.8%)	738 (27.3%)	178279.6 (28.1%)	
50,000-100,000	331 (31.1%)	90580.4 (33.0%)	1,049 (32.3%)	277226.4 (33.8%)	2,430 (32.9%)	597809.7 (33.1%)	910 (33.6%)	213655.3 (33.7%)	
>$100,000	222 (20.9%)	46170.0 (16.8%)	820 (25.3%)	214759.7 (26.2%)	2,257 (30.6%)	553861.3 (30.7%)	671 (24.8%)	143067.1 (22.5%)	
Health-related Factors									
Smoking									
Never	407 (38.2%)	98231.0(35.8%)	1,159 (35.7%)	276157.1 (33.7%)	2,908 (39.4%)	705649.3 (39.1%)	1,059 (39.1%)	254928.2 (40.2%)	0.022
Former	658 (61.8%)	175968.6 (64.2%)	2,088 (64.3%)	543894.7 (66.3%)	4,478 (60.6%)	1101319.6 (60.9%)	1,649 (60.9%)	379929.5 (59.8%)	
Alcohol consumption									
Never	141 (13.2%)	32305.4 (11.8%)	376 (11.6%)	99029.0 (12.1%)	755 (10.2%)	196085.1 (10.9%)	278 (10.3%)	69584.7 (11.0%)	0.471
Less than once weekly	413 (38.8%)	109183.6 (39.8%)	1,167 (35.9%)	309199.9 (37.7%)	2,485 (33.6%)	627103.0 (34.7%)	970 (35.8%)	229720.2 (36.2%)	
More than once weekly	466 (43.8%)	120136.6 (43.8%)	1,567 (48.3%)	374652.6 (45.7%)	3,886 (52.6%)	897857.5 (49.7%)	1,357 (50.1%)	313502.3 (49.4%)	
Leisure time physical activity									
Non-regular	502 (47.1%)	136038.1 (50.0%)	1,381 (42.5%)	381799.8 (46.6%)	3,013 (40.8%)	789024.2 (43.7%)	1,076 (39.7%)	273577.4 (43.1%)	0.060
Regular	562 (52.8%)	138079.6 (50.4%)	1,857 (57.8%)	436034.4 (53.2%)	4,365 (59.1%)	1016938.8 (56.3%)	1,627 (60.1%)	360650.2 (56.8%)	
Depression									
No (CES-D10<10)	863 (81.0%)	223239.2(81.4%)	2,629 (81.0%)	663944.5 (80.1%)	6,204 (84.0%)	1493026.6 (82.6%)	2,282 (84.3%)	528561.8 (83.3%)	0.515
Yes (CES-D10>=10)	202 (19.0%)	50960.3 (18.6%)	608 (18.7%)	155261.5 (18.9%)	1,158 (15.7%)	309046 (17.1%)	417 (15.4%)	105272.2 (16.6%)	
BMI (Kg/m^2) mean (SD)	28.0 (6.4)	28.0 (6.5)	27.1 (5.5)	27.3 (5.8)	27.0 (5.6)	26.9 (5.4)	27.5 (5.5)	27.(5.6)	0.006
Allergies									

No	652 (61.2%)	172320.5 (62.8%)	1,980 (61.0%)	516012.9 (62.9%)	4,566 (61.8%)	1109825.2 (61.4%)	1,658 (61.2%)	414679.9 (65.3%)	0.2952
Yes	409 (38.4%)	101289.3 (37.0%)	1,245 (38.3%)	300367.2 (36.6%)	2,777 (37.6%)	691495.3 (38.3%)	1,037 (38.3%)	219475.1(34.5%)	
Residence Type									
Urban	860 (80.8%)	202022.6 (73.7%)	2,582 (79.5%)	555548.7 (67.7%)	5,928 (80.3%)	1273987.6 (70.5%)	2,149 (79.4%)	453497.6 (71.4%)	0.3028
Rural	141 (13.2%)	56529.8 (20.6%)	452 (13.9%)	194396.3 (23.7%)	971 (13.2)	378041.9 (20.9%)	366 (13.5%)	124976.4 (19.7%)	
Other	64 (6.0%)	15647.2(5.7%)	213 (6.9%)	70106.8 (8.5%)	487 (6.6%)	154939.4 (8.6%)	193 (7.1%)	56383.7 (8.9%)	
***Reproductive Factors* mean (SD)**									
Duration of Oral Contraceptive (years)	6.7 (7.5)	6.6 (7.2)	7.7 (8.2)	8.2 (8.4)	7.6 (8.2)	8.1 (8.7)	7.2 (8.1)	7.4 (8.1)	0.006
Number of births	2.3 (1.4)	2.3 (1.4)	2.3 (1.2)	2.4 (1.4)	2.3 (1.4)	2.4 (1.3)	2.5 (1.3)	2.6 (1.3)	0.011
Age at Menarche (years)	12.8 (1.6)	12.7 (1.7)	12.8 (1.5)	12.8 (1.6)	12.8 (1.5)	12.9 (1.5)	13 (1.5)	13.0 (1.6)	0.092
Duration of use of any HT (years)	4.2 (7.2)	3.3 (6.4)	2.7 (5.6)	2.4 (5.1)	1.8 (4.2)	1.4 (3.8)	1.8 (4.2)	1.5 (3.6)	<0.001

All variables were obtained from baseline expect for number of births that was obtained from follow-up 1, age at menarche that was obtained from follow-up 2, and duration of oral contraceptives that was obtained from follow-ups 1 and 2.
Table is showing column percentages. Totals may not sum to 100% due to missing data.
95% CI, 95% confidence intervals; BMI, body mass index; CAD, Canadian dollars; CES-D, the Center for Epidemiological Studies Depression Scale; HT, hormone therapy; SD, standard deviation.
[a]Estimated using inflation weights.
[b]Other included South Asian, Chinese, Filipino, Latin American, Japanese, Southeast Asian, Korean, Arab, West Asian, Black, and other North American origins.

TABLE 2 - Associations between age at natural menopause and the risk of asthma onset

Variables	Adjusted HR (95% CI)[ab]
Age at Natural Menopause	
40-44	**0.7 (0.49-0.95)**
45-49	1.0 (0.7-1.2)
50-54 (ref)	1
≥55	1.0 (0.8-1.3)
Sociodemographic	
Age at Baseline (years)	**1.0 (1.0-1.0)**
Ethnicity	
White	1
Other[c]	1.2 (0.8-2.0)
Marital status	
No partner	1
With Partner	1.1 (0.9-1.3)
Employment	
Not currently working	1
Currently working	1.0 (0.7-1.3)
Education Level	
Less than high school	1.0 (0.7-1.4)
High school – some college	1.0 (0.8-1.3)
Bachelor's or higher	1
Household income (CAD)	
<$20,000	0.8 (0.5-1.3)
20,000-50,000	0.9 (0.6-1.2)
50,000-100,000	0.9 (0.6-1.2)
>$100,000	1
Health-related Factors	
Smoking	
Never	1
Former	**1.1 (1.0-1.2)**
Alcohol consumption	
Never	1
Less than once weekly	1.0 (0.7-1.4)
More than once weekly	0.9 (0.6- 1.2)
Leisure time physical activity	
Non-regular	1
Regular	1.0 (0.8-1.2)

Depression	
No (CES-D10<10)	1
Yes (CES-D10>=10)	**1.4 (1.1-1.8)**
BMI (Kg/m^2)	**1.1 (1.0-1.1)**
Allergies	
No	1
Yes	**1.8 (1.5-2.3)**
Residence Type	
Urban	0.9 (0.7-1.3)
Rural	1
Other	1.1 (0.7-1.8)
Reproductive Factors	
Duration of Oral Contraceptive	**1.0 (1.0-1.0)**
Number of births	1.0 (0.9- 1.1)
Age at Menarche (years)	0.9 (0.9-1.0)
Duration of use of any HT	**1.0 (1.0-1.0)**

Regression analysis was performed after implementing multiple imputation chained equation.
Bold numbers indicate the significant results with *P* value <0.05.
95% CI, 95% confidence intervals; BMI, body mass index; CAD, Canadian dollars; CES-D, the Center for Epidemiological Studies Depression Scale; HR, hazard ratio; HT, hormone therapy.
[a]Calculated HR and 95% CI using survey analytical weights and robust standard errors.
[b]Hazard ratio was adjusted for all variables including BMI as time-varying covariate.
[c]Other included South Asian, Chinese, Filipino, Latin American, Japanese, Southeast Asian, Korean, Arab, West Asian, Black, and other North American origins.

Covariates

Sociodemographic covariates included continuous measure of age at baseline; ethnicity (White, other), where "other" included South Asian, Chinese, Filipino, Latin American, Japanese, Southeast Asian, Korean, Arab, West Asian, Black, and other North American origins; marital status (with partner vs no partner); level of education (less than high school, high school to some college, bachelor's degree or higher); total annual household income in Canadian dollars (<20,000, 20,000-50,000, 50,000-100,000, >100,000); and employment (not currently working vs currently working). Health-related factors included smoking status (never vs former), alcohol consumption (never, less than once weekly, at least once weekly), and frequency of leisure time physical activity in the past year (nonregular vs regular), with regular defined as participation in physical activity at least once a week and self-reported allergies and depression categorized as yes or no. Depression was determined using "The Center for Epidemiological Studies short Depression scale," with a score of 10 on the scale suggesting presence of depressive symptomology.39 Height and weight were continuously measured and used to calculate BMI in kg/m2. In the tracking cohort, information to determine BMI was self-reported, whereas in the comprehensive cohort, it was measured. BMI was added to the model as a time-varying covariate. Reproductive factors were adjusted for and included as continuous variables: age at menarche in years, duration of oral contraceptives in years, number of births, and duration of HT in years. All covariates were measured at baseline except for number of births that was obtained from follow-up 1, age at menarche that was obtained from follow-up 2, and duration of oral contraceptives that was obtained from follow-ups 1 and 2.

Endpoints

Incidence of asthma after ANM was the outcome of interest in the study where women were followed for 10 yr. Women who did not develop asthma during the study were considered censored observations and contributed person-years to the study. Endpoints were defined as (a)

age at asthma onset and (b) age at last follow-up, which could be due to death, loss to follow-up, or end of the study's follow-up time.

Statistical analysis

Survival analysis was utilized to determine time to onset of asthma. Descriptive statistics were used to summarize the characteristics of the study population across different groups of ANM. The data are presented as means and standard deviations for continuous variables and compared using analysis of variance. However, data are presented as frequencies and percentages for categorical variables and compared using chi-square test. Kaplan-Meier survival curves were used for the bivariate analysis to determine time to onset of asthma after menopause, and a log-rank test was used to compare Kaplan-Meier survival curves. Missing data were addressed using multiple imputation by chained equations (MICE) using the “mi impute chained” function in Stata version 18.40 Moderate amounts of missingness were found for the three variables: age at menarche, oral contraceptives, and number of births with missingness being 23.1%, 18.4%, 13.9%, respectively. The majority of the missingness was due to loss to follow-up because these variables were collected at later follow-up cycles, not baseline, and some participants had dropped out of the study. This led us to adopt a missing-not-at-random assumption. All variables in the analysis were imputed for missing values using MICE. Specifically, we generated 20 imputed data sets and applied logistic regression to predict binary variables, ordered logistic regression for ordinal variables, and linear regression for continuous variables. Regression coefficients from each imputed dataset were combined using Rubin's approach.40 Adjusted Cox proportional hazard regression models were used to estimate the hazard ratios (HR) and their 95% confidence intervals (CI) for the association between ANM (in years: 40-44, 45-49, 50-54 [reference], or ≥55) and incidence of asthma. BMI, smoking, and HT were added to the model as interaction terms with ANM; however, no statistical significance for the interactions was found. The Cox proportional hazard assumption was examined using log-log plots. Inverse probability weights provided by the CLSA were used to make results generalizable to the Canadian population. Inflation weights were used for descriptive statistics, and analytics weights were used for regression analysis. A P value <0.05 was considered statistically significant. All statistical analyses were performed using Stata statistical software (version 18; StataCorp LLC, College Station, TX, USA).

Results

This study included 14,406 postmenopausal women (weighted N = 3,536,078). Incident asthma was reported by 732 women (5.08%). The incidence of asthma in the study considering the follow-up time of 259,464.2 person-years was 2.82 per 1,000 person-years. The mean age at baseline was 61.4 yr (SD = 10.0). Most of the sample was White (95.5%), and 62.2% were living with a partner. Over 50% of the sample had high school to some college education, was not currently working, and had a household income of over $50,000 a year. Former smokers comprised 62.2% of the sample, whereas 52.5% of women reported drinking more than once weekly, and 58.5% reported being physically active at least once a week. Most of the sample reported no current depression symptoms (83.5%). The mean BMI was reported as 27.2 kg/m2 (SD = 5.6). Allergies were reported by 38.2% of sample, and 80.0% reported living in an urban area. The average age at menarche was 12.8 (SD = 1.5), whereas the number of live births averaged 2.4 (SD = 1.4). The average number of years of use of oral contraceptives and HT was

7.5 (SD = 8.1) and 2.2 (SD = 4.8), respectively. The mean and median ANM were 50.7 and 51.0 yr, respectively. Although the median age at asthma was 62.0 yr. Table 1 compares descriptive statistics by ANM groups. ANM differed across several sociodemographic, health-related, and reproductive factors. Table 2 presents the results of adjusted HR and 95% CI. Women with ANM of 40-44 yr showed a 30% decreased risk for incidence of asthma as compared with women with ANM of 50-54 yr with an HR of 0.7 (95% CI: 0.49-0.95).

Figure 2 provides bivariate analysis using Kaplan-Meier curves of time to onset of asthma for each ANM category. The estimated P value is 0.7, indicating no significant difference between ANM categories on incidence of asthma. Figure 3 shows the Cox proportional hazard assumption using the log-log plots. Lines on the plot are approximately parallel but show some crossing, which indicates that the Cox proportional hazard assumption was likely violated. To ensure that the model remained robust against potential violations of the proportional hazard's assumption, BMI was included as a time-varying covariate and robust standard errors were used.

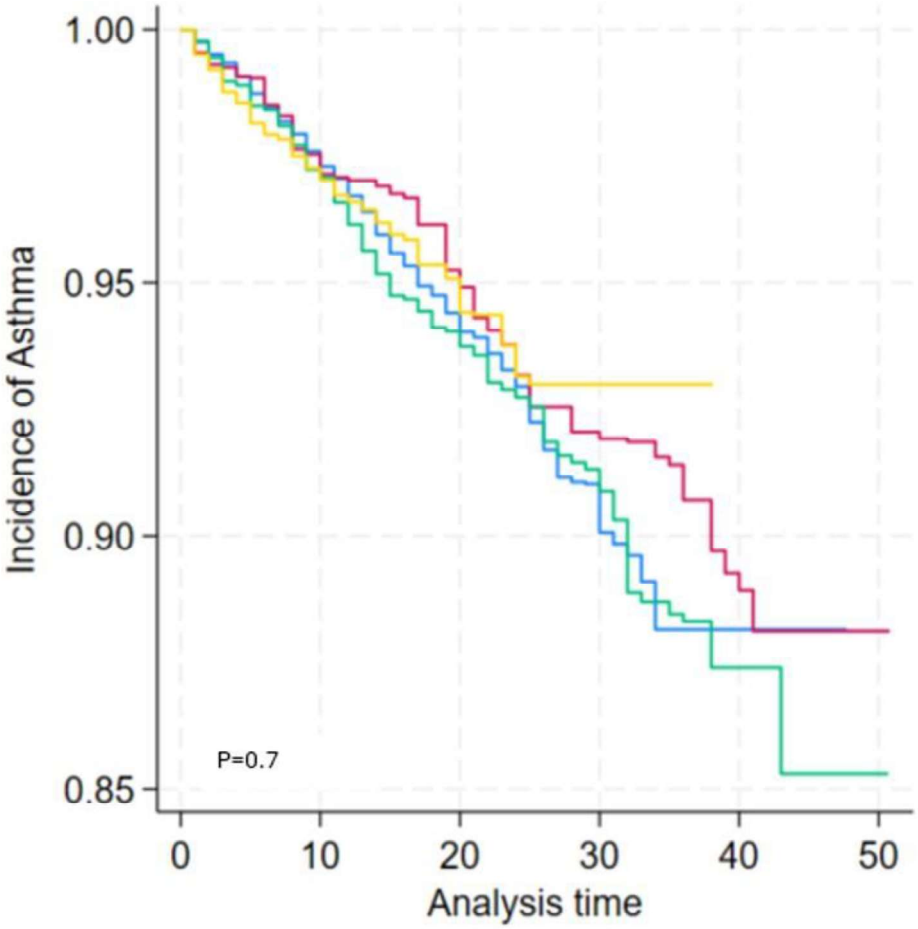

FIG. 2: Kaplan-Meier survival curves by age at natural menopause (ANM) groups.

Red represents ANM of 40–44 yr; green represents ANM of 45–49 yr; blue represents ANM of 50–54 yr; yellow represents ANM of ≥55 yr.

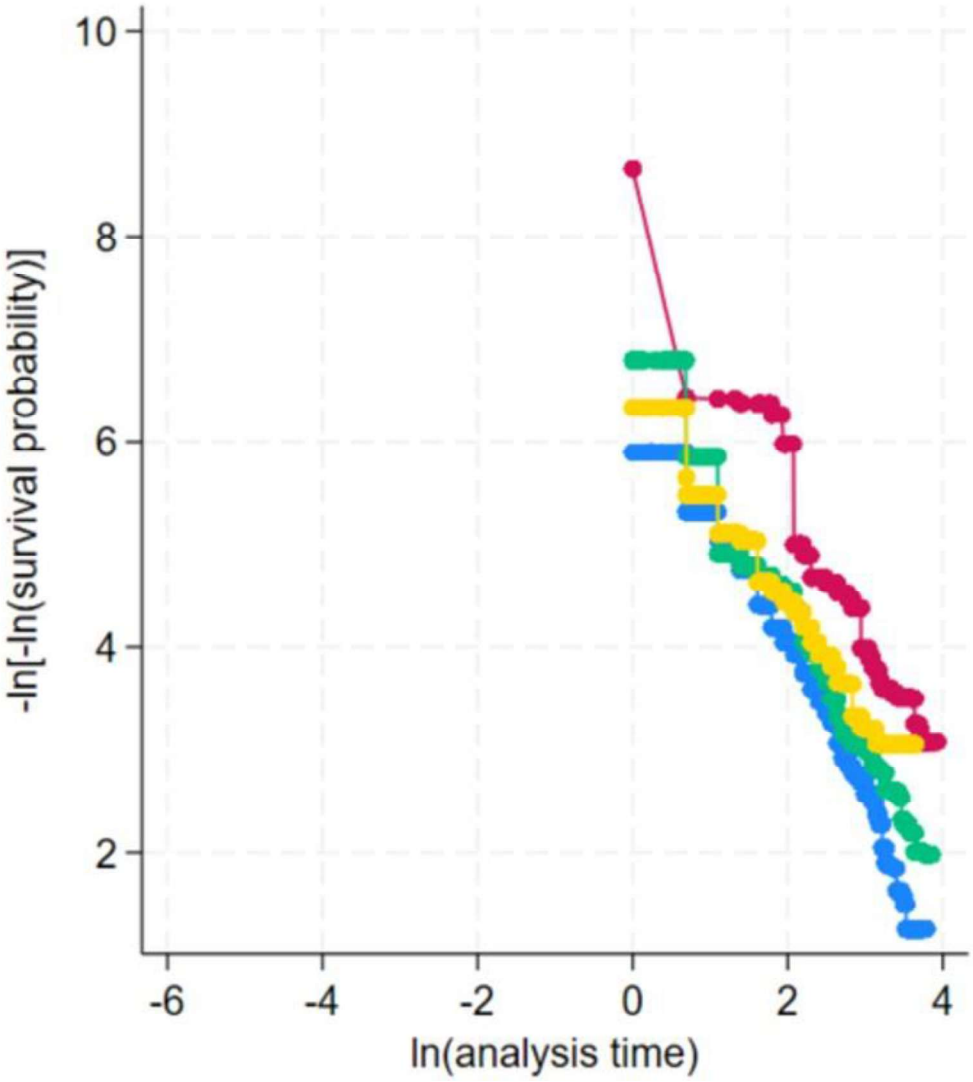

FIG. 3: Cox proportional hazard assumption using adjusted log-log plots.

Red represents age at natural menopause (ANM) of 40–44 yr; green represents ANM of 45–49 yr; blue represents ANM of 50–54 yr; yellow represents ANM of ≥55 yr.

Discussion

This study investigated the association between ANM and asthma incidence in nonsmoking postmenopausal Canadian women. Results from the study showed that early ANM of 40-44 yr is associated with a significantly reduced risk of asthma compared with ANM of 50-54 yr. Research on the association between menopause and asthma incidence has yielded conflicting results. Most studies have looked at menopause status and not age at menopause; however, the change in estrogen levels post menopause can provide some guidance on our

results. Consistent with our findings, a prospective study by Troisi et al reported a decreased risk of asthma in postmenopausal women compared with premenopausal women with an HR of 0.65 (95% CI: 0.46-0.92).[25] A meta-analysis found no significant difference in asthma incidence between premenopausal and postmenopausal women[27]; however, it was based primarily on cross-sectional studies. Conversely, two prospective studies have shown an increased asthma risk in postmenopausal women compared with premenopausal women. Specifically, Triebner et al (2015) observed an increased risk for early and late menopausal women compared with premenopausal women with HR of 2.11 (95% CI: 1.06-4.20) and 3.44 (95% CI: 1.31-9.05), respectively.[28] Another study by Matulonga-Diakiese et al found an increased risk of asthma in women with surgical menopause with an HR of 1.33 (95% CI: 1.01-1.75) compared with premenopausal women, whereas no significant increased risk was found for women with natural menopause.[29] However, the increased risk of asthma in these two studies could be confounded by BMI rather than menopause status. This was evident after the stratified analysis by BMI, which showed that the increased risk of asthma remained significant for women with higher BMI levels only. Fat tissue is the main source of estrogen in postmenopausal women.[29] Higher estrogen levels in women with higher BMI could be causing the risk of asthma in women in these studies.

In the literature, only one study investigated the association of ANM and asthma risk, and contradicting to our results, it found an increased risk of asthma in women with age at menopause onset at or before 46 yr (HR: 1.37; 95% CI: 1.24-1.51) and at or after 55 yr (HR: 1.15; 95% CI: 1.03-1.29).[14] However, this study controlled for smoking status but did not exclude current smokers like our study. The increased risk of asthma with early age at menopause could be related to a higher prevalence of smokers among the early age at menopause group.[37]

Several studies have demonstrated physiological mechanisms by which estrogen negatively affects asthma. Estrogen binding to ER-α in the lungs seems to promote airway hyperresponsiveness as evidenced in ER-α knockout mice, which shows reduced airway hyperresponsiveness.[41] Estrogen is an immunoenhancer and can control inflammatory and autoimmune diseases like asthma by promoting lung inflammation[42]; estrogen can increase Th2 cytokine production and alter eosinophil behavior; this contributes to T2 inflammation that underlies some asthma phenotypes.[42,43] A study on ovariectomized rats demonstrated reduced airway inflammation compared with nonovariectomized rats and the addition of estrogen reestablished airway inflammation.[44] These studies highlight the complex role of estrogen as a risk factor for asthma, through different pathways.

This study had several strengths, including its large population-based sample from across Canada, which provided a high degree of generalizability. We were able to include detailed information on sociodemographic, health-related, and reproductive factors, which increased precision and enhance generalizability. This is the second study to investigate the relationship between ANM and asthma incidence, and the first to exclude current smokers, thereby minimizing residual confounding by smoking and providing a more valid association between ANM and asthma. However, this study does have some limitations. The reliance on self-reported data could lead to recall and misclassification biases; however, previous studies have found a high reported accuracy for self-reported asthma status[27,45] and age at menopause.[28,32] The generalizability of the findings is affected by the CLSA exclusion criteria and the fact that almost

95% of the sample identified as White. Additionally, we are at risk for some violations of the proportional hazard assumption, but this was minimized by using robust standard errors.

Conclusions

This study showed that women with early menopause are at reduced risk of asthma and suggests the role of estrogen in increasing risk of asthma. Healthcare providers need to be aware of the potential role of this relationship and carefully monitor asthma symptoms in women with later ANM. More studies need to investigate physiological mechanisms of estrogen on asthma development and progression.

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The opinions expressed in this manuscript are the author's own and do not reflect the views of the CLSA.

Data are available from the CLSA (www.clsa-elcv.ca) for researchers who meet the criteria for access to deidentified CLSA data.

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Chapter 3

Associations between age at natural menopause and risk of hypothyroidism among postmenopausal women from the Canadian Longitudinal Study on Aging (CLSA)

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Abstract

Menopause is a key period in women's lives associated with major physiological changes. Early menopausal age has been linked to a range of adverse outcomes. Estrogen has been found to increase levels of thyroid binding proteins in the blood; however, its effect on hypothyroidism is not well investigated. To date limited studies were conducted to investigate the association between age at natural menopause and incidence of hypothyroidism, thus the objective of this study is to investigate the association between age at natural menopause and incidence of hypothyroidism among postmenopausal Canadian women. The study included women from the Canadian longitudinal study on aging that were followed for a 10-year period. Analysis was restricted to naturally postmenopausal women without hypothyroidism prior to menopause. Age at natural menopause was examined using the following categories 40-44, 45-49,50-54 (reference), and ≥55. Survival analysis was utilized to determine time to onset of hypothyroidism. Unadjusted and adjusted multivariable Cox regression models were used to assess the relationship between age at natural menopause and incidence of hypothyroidism. The multivariable Cox regression analysis showed no significant association between age at natural menopause and risk of hypothyroidism.

Introduction

Hypothyroidism is a condition where the thyroid gland does not make adequate amounts of thyroid hormones: Thyroxine (T4) and triiodothyronine (T3).[1] Thyroid hormones are carried to all tissues in the body where they help metabolism, maintain thermoregulation, and sustain the function of the brain, heart, muscles, and other organs.[2] Some signs and symptoms of hypothyroidism include fatigue, weight gain, constipation, cold intolerance, depression, hair loss, bradycardia, and goiter. [1] The main cause of hypothyroidism in the developed world is autoimmune dysregulation.[1] Hypothyroidism has been found to affect 4.6% of the US population.[3] The prevalence of hypothyroidism is three to seven times higher in women than men and the incidence increases with age.[4] A large study in the UK with twenty years of follow-up found an incidence of hypothyroidism of 0.6 per 1,000 person-years in males and 3.5 per 1,000 in females person-years.[5]

Thyroid function is controlled by the hypothalamic-pituitary-thyroid-axis.[6] The anterior pituitary gland releases thyroid stimulating hormone (TSH), which stimulates the thyroid gland to secret thyroid hormones (T3 &T4) into the blood where they have a negative feedback effect on TSH.[6] Thyroid hormones can exist in a free or bound form. Only the free form can enter target tissues, while the other form is bound to thyroid binding globulin (TBG) - a protein which carries thyroid hormones in the blood.[7] Hypothyroidism is usually indicated by high TSH and low free T4 levels.[8] While subclinical hypothyroidism (a milder form of the disease) is indicated by an elevated TSH and normal free T4.[9]

Menopause is an integral period in women's lives marked by major physiological changes, including a significant drop of estrogen.[10] The average age at menopause is 51 years,[10]

and both menopause and hypothyroidism affect reproductive hormones.[11] It has been found that 70% of hypothyroidism cases are in patients over the age of 50 years at the time of diagnosis,[12] with an increase of incidence in the postmenopausal period.[13] Further, subclinical hypothyroidism frequently exists or develops during menopausal transition and is mainly due to autoimmune dysfunction.[6] A recent study has found that 20-35% of women with premature ovarian failure (POF) have thyroid autoimmune diseases,[14] and guidelines suggest that women with POF must be measured for thyroid antibodies[15]. This could suggest a role of early menopause in thyroid disease.

Few studies have investigated the incidence of hypothyroidism due to changes in reproductive factors. A study found that the incidence of hypothyroidism decreased during pregnancy but increased sharply in the postpartum period.[16] One study looked at age at menarche and found that early menarche was associated with an increased risk of subclinical hypothyroidism; however, it was a cross-sectional study.[17] There is contradicting evidence about the effect of oral contraceptives use and risk of hypothyroidism. A study evaluating the long-term use of oral contraceptives found increased risk of hypothyroidism with an odds ratio (OR) of 4.71 (95% CI 1.7-12.9) after adjusting for sociodemographic, health-related and reproductive factors,[4] and additional studies have found that current use is associated with an increased incidence.[18] However, other studies have found no significant association between ever and current use of oral contraceptives,[19,20] yet these studies do not specify the type of oral contraceptives used.

Earlier age at menopause has been found to increase risk of several autoimmune and endocrinological diseases.[21-23] Only two studies have examined the association of age at menopause and the subclinical form of hypothyroidism, and they have shown contradicting

results.[6,17] Overall, no study has examined the effect of age at natural menopause (ANM) on risk of hypothyroidism in a longitudinal design. Some studies have shown that the presence of estrogen could increase thyroid disorders by increasing thyroid cell proliferation and leading to conditions like goiter.[24] This study will examine the association between ANM and incidence of hypothyroidism among postmenopausal women from the Canadian Longitudinal Study on Aging (CLSA).

Methods

Study design and sample

This study involved a secondary data analysis from CLSA. CLSA is a Canada wide study of 51,338 males and females between the ages of 45-85 years at recruitment over a 20-year period with the aim of understanding factors associated with the well-being of the aging population. CLSA consists of two cohorts: Tracking and Comprehensive. At baseline, the Tracking cohort included 21,241 participants (male: 10,406; female: 10,835), selected randomly from across the 10 Canadian provinces and interviewed by telephone. The Comprehensive cohort included 30,097 participants (male:14,777 & female: 15,320) who were randomly selected from 7 of the 10 Canadian provinces and had to be within 25-50 km from one of the 11 data collection sites. Data collection for this cohort involved an in-person home interview and more in-depth data collection at one of the data collection sites. CLSA data collection occurs every three years, with three cycles of data currently available (baseline, follow-up 1, and follow-up 2). Approximately 21.42% of the sample was lost to follow-up or had died by follow-up 2. More details on CLSA method can be found elsewhere.[25,26]

This study was a retrospective analysis of de-identified data accessed from CLSA on November 10, 2022. It included combined Tracking and Comprehensive cohorts, including three cycles of data collection (baseline, follow-up 1, and follow-up 2). The CLSA study design excluded residents of the Canadian territories, remote regions, Federal First Nations reserves, and other provincial First Nations settlements. It also excluded full-time members of the Canadian Armed Forces, individuals living in institutions, those unable to respond in English or French, and those who are cognitively impaired at recruitment. The CLSA study has been approved by McMaster University Health Integrated Research Ethics Board and by research ethics boards at all collaborating Canadian institutions. This study is a secondary data analysis of fully de-identified CLSA data approved by the York University, Office of Research Ethics (ORE) [STU 2022-114]. Further consent from participants was not required as all CLSA participants provided informed consent during primary data collection to have their de-identified data used in future research.

Study participants

All males were excluded, leaving 26,155 females in the study. Other exclusions included: women with missing information on menopause, women that did not reach menopause, women with surgical or medically induced menopause, women with missing age at natural menopause, women with age at menopause under 40 years or over 67 years, similar to cut-offs proposed by Verschoor & Tamim.[27] The study also excluded women with missing information on hypothyroidism incidence, and women with hypothyroidism prior to age at natural menopause. A participant flow diagram and common exclusions are shown in figure 4.

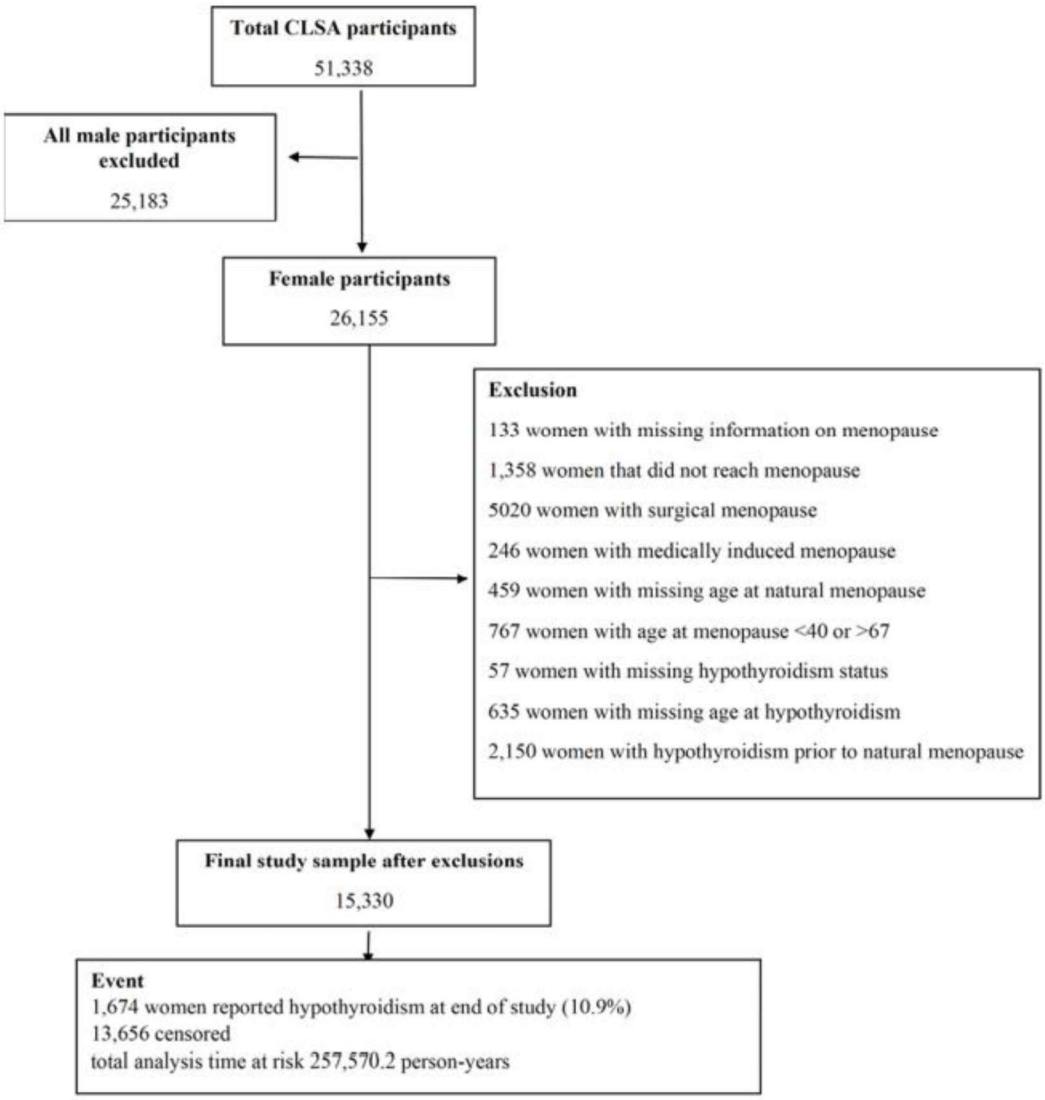

FIG. 4: Canadian Longitudinal Study on Aging (CLSA) participant chart.

Exposure assessment (age at natural menopause (ANM))

Self reported age at natural menopause was ascertained from all three cycles (baseline, follow-up1 & follow-up2) with the question: "*Have you gone through menopause, meaning that your menstrual periods stopped for at least one year and did not restart?*" answers were recorded as, "Yes" or "No". Women who answered "Yes" were then asked about their age at menopause: "*How old were you when your menstrual periods stopped for at least one year and*

did not re-start?" answers were reported in years of age. ANM was categorized into the following groups: 40–44, 45–49,50-54 (reference), and ≥55, similar to categories presented in Mondul et al.[28] and Brand et al.[21] Having ANM as categorical variable allows for better detection of non-linear relationships.

Outcome assessment (incidence of hypothyroidism)

Information on incidence of hypothyroidism following menopause was collected using the following questions. First, women were asked, "*Has a doctor ever told you that you have an UNDER-active thyroid gland (sometimes called hypothyroidism or myxedema)?answered by "*Yes" or "No". Women who answered by "Yes" were asked about their age of diagnosis: "*At what age, or in what year, were you first told you had hypothyroidism?*" answers were reported in years of age. Women who answered by "No" were asked in the next cycle. The Incidence of hypothyroidism was collected from all three cycles in the Comprehensive cohort and from follow-ups 1 & 2 cycles only of the Tracking cohort because information on age at hypothyroidism is not available from baseline Tracking.

Covariates

Sociodemographic factors included: Ethnicity (White, other), where "other" included, South Asian, Chinese, Filipino, Latin American, Japanese, Southeast Asian, Korean, Arab, West Asian, Black, and other North American origins, and education level (less than high school, high school to some college, bachelor's degree or higher). Health-related factors included: Smoking status (never, former, current), alcohol consumption (never, less than once weekly, at least once weekly), frequency of leisure time physical activity in the past year (nonregular, regular), with regular defined as participation in physical activity at least once a week. Height and weight were

used to calculate body mass index (BMI) in kg/m^2. In the Tracking cohort information to determine BMI was self-reported while in the Comprehensive cohort it was measured. The BMI cut-off for under weight was increased from the standard 18.5 kg/m^2 because very few participants had that low of a BMI.[29] BMI level was categorized as, underweight < 20.0 kg/m^2, normal weight = 20.0-24.99 kg/m^2, overweight = 25.0-29.99 kg/m^2, and obese >30 kg/m^2. Reproductive factors were also adjusted for and included age at menarche in years (≤11, 12-14, ≥15), duration of oral contraceptives in years (0-3,4-7,8-11, ≥12), number of births (0,1,2,3, ≥4), duration of hormone replacement therapy (HRT) in years (never, <1,1-2,3-4, ≥5), and HRT type (none, combined estrogen and progesterone, estrogen, and progestrone). All covariates were measured at baseline, except for number of births which was obtained from follow-up 1, age at menarche which was obtained from follow-up 2, and duration of oral contraceptives which was obtained from follow-ups 1 & 2.

Endpoints

Women were followed for 10 years and the incidence of hypothyroidism after menopause was the primary outcome measure. Women who did not develop hypothyroidism during the study period were considered censored observations but contributed person-years. The end of follow-up was defined at the earliest occurrence of one of the following events: a) incidence of hypothyroidism b) loss to follow-up or death or c) end of the study period.

Statistical analysis

Survival analysis was utilized to determine time to onset of hypothyroidism. Descriptive statistics by ANM categories were used to describe the sample. The data are presented in frequencies and percentages and compared using a chi-square test. Missing data was addressed

using multiple imputation by chained equations (MICE). Three variables, which were not collected at baseline but at later cycles (Oral contraceptive duration, number of births, and age at menarches), were found to have a moderate amount of missingness (17.6%, 13.5%, 22.8%, respectively), leading us to adopt a missing-not-at-random and multiple imputation strategy. We applied the MICE procedure using the 'mi impute chained' command in Stata version 18 to all variables.[31] Specifically, we generated 20 imputed data sets, using logistic regression for binary variable and ordered logistic regression for categorical variable. Regression coefficients were combined using Rubin's rule.[30] Kaplan-Meier survival curves were used for the bivariate analysis to determine time-onset of hypothyroidism, and a log-rank test was used to compare the survival curves. Unadjusted and adjusted cox proportional hazard regression models were used to estimate the hazard ratios (HR) and their 95% confidence intervals (CIs) for the association between ANM (in years:40–44, 45–49,50-54 (reference), and ≥55) and incidence of hypothyroidism. BMI and HRT were considered in the model as interaction terms with ANM and no significance was found. The Cox proportional hazard assumption was evaluated using log-log plots. Inverse probability weights provided by the CLSA were used to make results generalizable to the Canadian population.[31] Inverse probability weights provided by the CLSA were used to make results generalizable to the Canadian population. Inflation weights were used for descriptive statistics and analytics weights were used for regression analysis. A p-value of 0.05 was considered statistically significant. All statistics were calculated using Stata statistical software (version 18, StataCorp LLC, College Station, TX, USA).

Results

This study included 15,330 postmenopausal women (weighted $N = 4{,}009{,}706$). Exclusions are presented in figure 4. During the study's follow-up period 1,674 (10.9%) reported

hypothyroidism incidence with a follow-up time of 257,589.7 person-years, resulting in a hypothyroidism incidence of 6.5 per 1000 person-years. The mean age at baseline was 60.6 years. The mean and median ANM were 50.3(SD: 4.3) and 50 (IQR: 48-53) years, respectively, while the median age at hypothyroidism diagnosis was 60 years. Over half of the sample was between 45-64 years (68.5%) at baseline. Most of the sample was of White origin (94.4%). Over half of the sample had high school to some college education (62.5%). Only 12.6% of the sample were current smokers, and 12.5% reported ever having cancer. Around half the sample consumed alcohol more than once weekly (48.1%) and engaged in regular physical activity (52.8%). Normal BMI range was found in 36.8% of the sample. Oral contraceptive use of 12 years or more was found in 22.7% of the sample. the most common number of births was two children per family (34.4%). A large portion of the sample never used HRT (71.1%), and half of the sample had average age at menarche of 13 years (52.9%). Table 3 shows characteristics of the study population categorized by ANM. ANM differed on several sociodemographic, health-related, and reproductive factors.

Figure 5 shows the bivariate analysis using Kaplan-Meier survival curves depicting time to onset of hypothyroidism for each ANM category. The estimated p-value was 0.7, suggesting no evidence of a significant difference between ANM categories and incidence of hypothyroidism. Table 4 shows the unadjusted and adjusted multivariable cox regression model with HRs and their 95% CIs. No evidence of any significant associations between ANM and risk of hypothyroidism was found. Women with BMI levels between 25-29 and equal to or more than 30 showed an increased risk of hypothyroidism with HR of 1.2 (95% CI: 1.1-1.4) and HR of 1.4 (95% CI: 1.2-1.6), respectively. Figure 6 shows the Cox proportional hazard assumption using log-log plots. Lines on the plot are overlapping indicating a violation of the proportional

hazard assumption. Robust standard errors were used to ensure that the model remained robust against potential violations of the proportional hazard's assumption.

TABLE 3: Characteristics of study population according to age at natural menopause

Variables	Age at Natural Menopause								P-value[a]
	40-44		45-49		50-54		≥ 55		
	Unweighted	Weighted[a]	Unweighted	Weighted[a]	Unweighted	Weighted[a]	Unweighted	Weighted[a]	
N (%)	1,263 (8.2)	372021.3 (9.3%)	3,568 (23.3)	971274.8 (24.2%)	7,722 (50.4)	1980849.4 (49.4%)	2,777 (18.1)	685560.6 (17.1%)	
Ethnicity									
White	1,199 (94.9%)	358973.0 (96.5%)	3,344 (83.7%)	914957.8 (94.2%)	7,303 (94.6%)	1853987.9 (93.6%)	2,653 (95.5%)	656548.6 (95.8%)	0.0585
Other[a]	52 (4.1%)	10665.7 (2.9%)	168 (4.7%)	37955.6 (3.9%)	331 (4.3%)	98935.9 (5.0%)	98 (3.5%)	18347.2 (2.7%)	
Education Level									
Less than high school	120 (9.5%)	91559.1 (24.6%)	231 (6.5%)	198500.1 (20.4%)	428 (5.5%)	325897.5 (16.5%)	160 (5.8%)	131102.6 (19.1%)	<0.001
High school – some college	780 (61.8%)	240379.6 (64.6%)	2,0340 (57.0%)	598812.94 (61.7%)	4,101 (53.1%)	1247791.2 (63.0%)	1,499 (54.0%)	420812.7 (61.4%)	
Bachelor's or higher	355 (28.1%)	37237.1 (10.0%)	1,294 (36.3%)	169293.6 (17.4%)	3,180 (41.2%)	404729.1 (20.4%)	1,113 (40.1%)	131595.8 (19.2%)	
Health-related Factors									
Smoking									
Never	394 (31.2%)	98894.8 (26.6%)	1,104 (30.9%)	251546.3 (25.9%)	2,804 (36.3%)	665611.5 (33.6%)	1,019 (36.7%)	253527.0 (37.0%)	<0.001
Current	198 (15.7%)	75910.8 (20.4%)	461 (12.9%)	184735.3 (19.0%)	614 (8.0%)	201397.8 (10.2%)	614 (8.0%)	43236.3 (6.3%)	
Former	666 (52.7%)	194252.1 (52.2%)	1,982 (55.6%)	528763.9 (54.4%)	4,265 (55.2%)	1105441.9 (55.8%)	4,265 (55.2%)	384050.7 (56.0%)	
Alcohol consumption									
Never	177 (14.0%)	46184.4 (12.4%)	435 (12.2%)	122198 (12.6%)	791 (10.2%)	234717.9 (11.4%)	307 (11.1%)	84836.6 (12.4%)	0.5064
Less than once weekly	476 (37.7%)	148328.0 (39.9%)	1,309 (36.7%)	370393.0 (38.1%)	2,619 (33.9%)	685143.4 (34.6%)	978 (35.2%)	236342.1 (34.5%)	
More than once weekly	566 (44.8%)	163299.8 (43.9%)	1,680 (47.1%)	442139.8 (45.5%)	4,053 (52.5%)	983352.5 (49.6%)	1,399 (50.4%)	340672.2 (49.7%)	
Leisure time physical activity									
Non-regular	620 (49.1%)	184645.0 (49.6%)	1,1612 (45.2%)	485788.5 (50.1%)	3,275 (42.2%)	909015.0 (45.9%)	1,131 (40.7%)	306902.3 (44.8%)	0.0027
Regular	638 (50.5%)	184209.4 (49.5%)	1,946 (54.5%)	483231.0 (49.7%)	4,437 (57.5%)	1070223.3 (54.0%)	1,641 (59.1%)	3780.28.3 (55.1%)	
BMI (Kg/m^2)									
<20.00 (underweight)	60 (4.8%)	8147.3 (4.9%)	185 (5.2%)	64558.1 (6.6%)	350 (4.5%)	91122.5 (4.6%)	115 (4.1%)	23985.8 (0.6%)	0.0298
20.0-24.99 (normal weight)	418 (33.1%)	127351.8 (34.2%)	1,274 (35.7%)	302902.2 (31.2%)	2,882 (37.3%)	763054.0 (38.5%)	907 (32.7%)	228406.0 (33.3%)	
25.0-29.99 (overweight)	429 (34.0%)	121776.7 (32.7%)	1,221 (34.2%)	302902.2 (31.2%)	2,589 (33.5%)	635393.1 (32.1%)	1,014 (36.5%)	255393.6 (37.2%)	
> 30.0 (obese)	340 (26.9%)	97155.8 (26.1%)	873 (24.5%)	240370.9 (24.7%)	1,860 (24.1%)	473272.3 (23.9%)	731 (26.3%)	175894.5 (25.7%)	
Cancer									
Yes	235 (18.6%)	63005.2 (16.9%)	507 (14.2%)	126764.3 (13.15)	1,018 (13.2%)	224387.7 (11.3%)	395 (14.2%)	88408.9 (12.9%)	0.0133
No	1,026 (81.2%)	308152.5 (82.8%)	3,054 (85.6%)	843804.7 (86.9%)	6,693 (86.7%)	1755792.3 (88.6%)	2,377 (85.6%)	596854.6 (87.15%)	
Reproductive Factors									
Duration of Oral Contraceptive (years)									
0-3	423 (33.5%)	106675.2 (28.7%)	1,148 (32.2%)	287192.2 (29.6%)	2,591 (33.6%)	645990.8 (32.6%)	990 (35.7%)	244338.6 (35.6%)	<0.001
4-7	174 (13.8%)	41944.4 (14.0%)	466 (13.1%)	119490.9 (12.3%)	1,226 (15.9%)	312997.1 (15.8%)	404 (14.6%)	97067.0 (14.2%)	
8-11	145 (11.5%)	42448.3 (11.4%)	453 (12.7%)	136796.3 (14.1%)	995 (12.9%)	275277.0 (13.9%)	360 (13.0%)	71849.3 (10.5%)	
≥12	229 (18.1%)	72698.1 (19.5%)	768 (21.5%)	316667.4 (22.3%)	1,642 (21.3%)	460801.5 (23.3%)	553 (19.9%)	160751.2 (23.45%)	
Number of births									
0	210 (16.6%)	54105.6 (14.5%)	640 (17.9%)	137635.5 (14.2%)	1,287 (16.7%)	267574.3 (13.5%)	343 (12.4%)	64172.0 (93.6%)	<0.001
1	165 (13.1%)	37178.4 (10.%)	402 (11.3%)	90622.8 (9.3%)	945 (12.2%)	221588.3 (11.25%)	324 (11.7%)	69835.8 (10.2%)	
2	375 (29.7%)	115788.6 (31.1%)	1,097 (30.8%)	308150.5 (31.7%)	2,630 (34.1%)	707586.7 (35.75%)	989 (35.6%)	246396.4 (35.9%)	
3	194 (15.4%)	56711.5 (15.2%)	634 (17.8%)	167612.8 (17.3%)	1,388 (18.0%)	379652.1 (19.2%)	548 (19.7%)	126245.8 (18.4%)	
≥4	124 (9.8%)	28298.9 (7.6%)	336 (9.4%)	104249.7 (10.7%)	766 (9.9%)	184576.6 (9.3%)	338 (12.2%)	101145.2 (14.8%)	
Age at Menarche									
≤11	192 (15.2%)	56931.7 (15.3%)	476 (13.3%)	129317.0 (13.3%)	1,070 (13.9%)	264808.7 (13.4%)	350 (12.6%)	87658.2 (12.8%)	<0.001
12-14	612 (48.5%)	158552.4 (42.6%)	1,951 (54.7%)	486512.88 (50.1%)	4,580 (59.3%)	1122828.5 (56.7%)	1,624 (58.5%)	351505.8 (51.3%)	
≥15	127 (10.1%)	36778.9 (9.9%)	326 (9.1%)	95682.9 (9.8%)	720 (9.3%)	212485.0 (10.7%)	315 (11.3%)	93660.3 (13.7%)	
Duration of use of any HRT (years)									
Never	659 (52.2%)	223925.7 (60.2%)	2,274 (63.7%)	661035.6 (68.1%)	5,458 (70.7%)	1482563.5 (74.8%)	1,899 (68.4%)	483370.9 (70.5%)	<0.001
<1	71 (5.6%)	17781.2 (4.8%)	206 (5.8%)	53912.6 (5.6%)	412 (5.3%)	90719.2 (4.6%)	145 (5.2%)	40234.8 (5.9%)	
1-2	102 (8.1%)	23889.7 (6.4%)	246 (6.9%)	53049.0 (5.5%)	460 (5.9%)	120403.2 (60.8%)	199 (7.2%)	40523.2 (5.9%)	
3-4	49 (3.9%)	14584.1 (3.9%)	143 (4.0%)	43127.3 (4.4%)	305 (4.0%)	65117.2 (3.3%)	113 (4.1%)	23619.3 (3.4%)	
≥5	370 (29.3%)	88301.0 (23.7%)	667 (18.7%)	153043.8 (15.8%)	1,037(13.4%)	211434.7 (10.7%)	393 9(14.2%)	92425.7 (13.5%)	
Type of HRT									

None	659 (52.2%)	223925.7 (60.2%)	2,274 (63.7%)	661035.6 (68.1%)	5,458 (70.7%)	1482563.5 (74.8%)	1,899 (68.4%)	483370.9 (70.5%)	<0.001
estrogen & progesterone	228 (18.1%)	50362.9 (13.5%)	538 (15.1%)	119874.8 (12.3%)	1,034 (13.4%)	204445.7 (10.3%)	368 (13.3%)	76864.1 (11.2%)	
Estrogen	241 (19.1%)	67869.6 (18.2%)	489 (13.7%)	119474.2 (12.3%)	727 (9.4%)	169287.7 (8.5%)	279 (10.1%)	69314.573 (10.1%)	
Progestrone	35 (2.8%)	9322.5 (2.5%)	76 (2.1%)	25648.4 (2.6%)	171 (2.2%)	41700.6 (2.1%)	58 (2.1%)	10842.2 (2.2%)	

BMI, body mass index; CAD, Canadian dollars; 95% CI, 95% confidence intervals; HRT, hormone replacement therapy
All variables were obtained from baseline expect for number of births which was obtained from follow-up 1, age at menarche which was obtained from follow-up 2, and duration of oral contraceptives which was obtained from follow-ups 1 & 2.
[a]Estimated using inflation weights.
[b]Other included South Asian, Chinese, Filipino, Latin American, Japanese, Southeast Asian, Korean, Arab, West Asian, Black, and Other North American Origins
Table is showing column percentages. Totals may not sum to 100% due to missing data

TABLE 4. Unadjusted and adjusted hazard ratios (HRs) and 95% confidence intervals (CI) between age at natural menopause and risk of hypothyroidism

Variables	**Unadjusted HR (95% CI)[a]**	**Adjusted HR (95% CI) [ab]**
Age at Natural menopause (years)		
40-44	0.9 (0.8-1.1)	0.9 (0.8-1.1)
45-49	1.0 (0.9-1.2)	1.0 (0.9-1.1)
50-54 (ref)	1	1
≥55	1.1 (0.9-1.3)	1.1 (0.9-1.2)
Sociodemographic		
Ethnicity		
White	1	1
Other [c]	1.1 (0.8-1.4)	1.1 (0.9-1.4)
Education Level		
Less than high school	0.8 (0.7-1.0)	0.8 (0.7-1.0)
High school – some college	0.9 (0.9-1.0)	0.9 (0.8-1.0)
Bachelors or higher	1	1
Health-related Factors		
Smoking		
Never	1	1
Current	0.8 (0.6-1.0)	0.8 (0.7-1.0)
Former	1.1 (0.9-1.2)	1.1 (1.0-1.2)
Alcohol consumption		
Never	1	1
Less than once weekly	1.2 (0.9-1.3)	0.8 (0.9-1.3)
More than once weekly	1.0 (0.9-1.2)	1.0 (0.8-1.2)
Physical activity		
Non regular	1	1
regular	1.0 (0.9-1.1)	1.00 (0.9-1.1)
BMI (Kg/m^2)		
<20.00 (underweight)	1.0 (0.8-1.3)	1.0 (0.8-1.3)
20.0-24.99 (normal weight)	1	1
25.0-29.99 (overweight)	**1.2 (1.1-1.4)**	**1.2 (1.1-1.4)**
> 30.0 (obese)	**1.4 (1.2-1.5)**	**1.4 (1.2-1.6)**
Cancer		

No	1	1
yes	1.1 (0.9-1.2)	1.0 (0.9-1.2)
Reproductive Factors		
Duration of Oral Contraceptive (years)		
0-3	1	1
4-7	1.0 (0.9-1.2)	1.0 (0.9-1.2)
8-11	1.0 (0.9-1.2)	1.0 (0.9-1.2)
≥12	1.0 (0.8-1.1)	1.0 (0.8-1.1)
Number of births		
0	1	1
1	1.0 (0.8-1.2)	1.0 (0.8-1.2)
2	1.0 (0.8-1.1)	1.0 (0.8-1.1)
3	0.9 (0.8-1.1)	1.0 (0.8-1.1)
≥4	1.0 (0.8-1.2)	1.0 (0.9-1.2)
Age at Menarche		
≤11	1.0 (0.9-1.2)	1.0 (0.9-1.2)
12-14	1	1
≥15	1.0 (0.8-1.2)	1.0 (0.9-1.2)
Duration of use of any HRT (years)		
Never	1	1
<1	1.0 (0.8-1.3)	1.0 (0.7-1.4)
1-2	1.1 (0.9-1.3)	1.1 (0.8-1.5)
3-4	1.2 (0.9-1.5)	1.1 (0.8-1.6)
≥ 5	**1.2 (1.1-1.4)**	1.2 (0.9-1.6)
Type of HRT		
None	1	1
Combined estrogen and progesterone	1.2 (1.0-1.3)	1.1 (0.8-1.5)
Estrogen	1.2(1.0-1.3)	1.1 (0.8-1.5)
Progestrone	1.1 (0.8-1.5)	1.0 (0.6-1.5)

BMI, body mass index; CAD, Canadian dollars; CES-D, the Center for Epidemiological Studies Depression Scale; 95% CI, 95% confidence intervals; HR, hazard ratio; HRT, hormone replacement therapy.

[a] Calculated HR and 95% CI using survey analytical weights and robust standard errors

[b]Other included South Asian, Chinese, Filipino, Latin American, Japanese, Southeast Asian, Korean, Arab, West Asian, Black & other North American Origins .

Regression analysis was performed after implementing multiple imputation chained equation.

Bold numbers indicate the significant results with p-value <0.05.

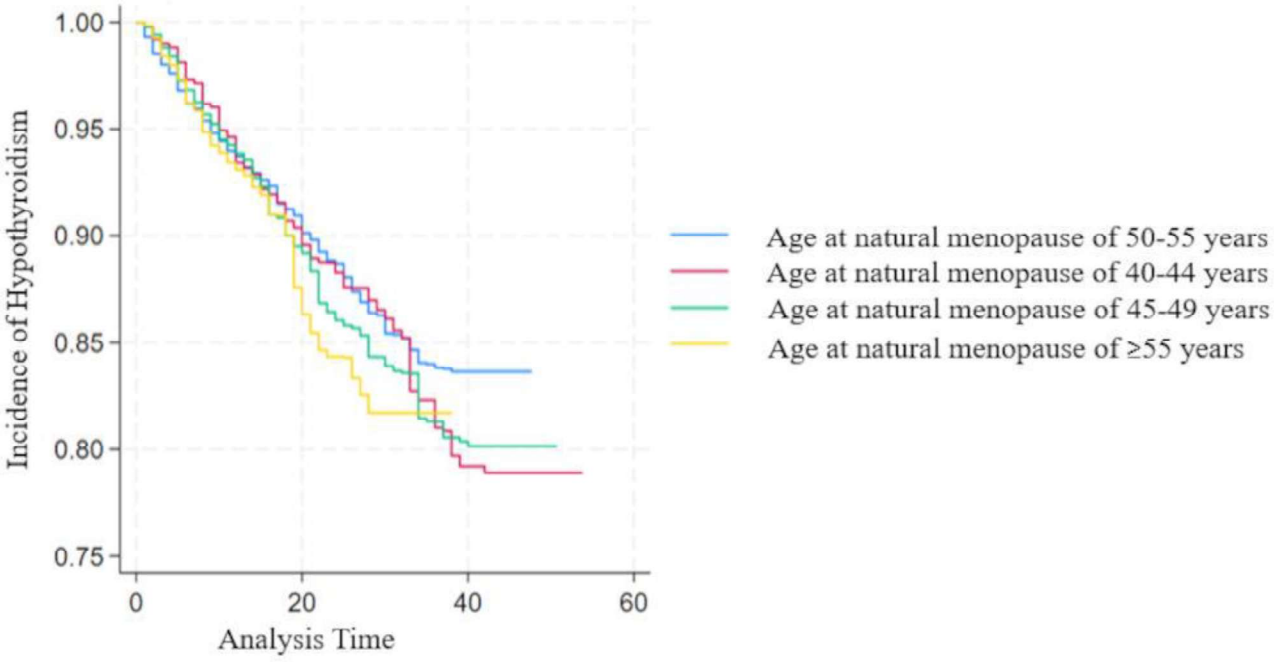

FIG. 5: Kaplan Meier survival curves by ANM groups

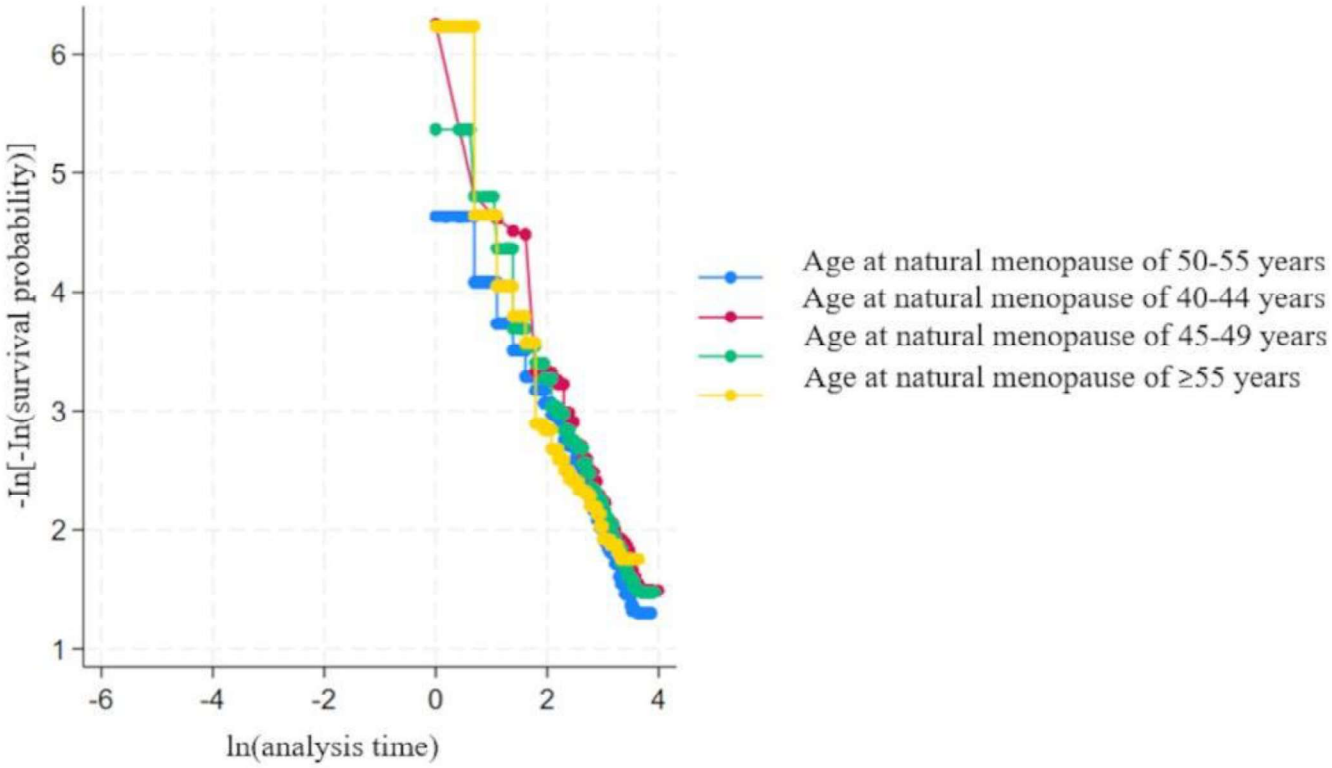

FIG. 6: Cox Proportional Hazard assumption using adjusted log-log plots

Discussion

This study examined the association between ANM and risk of hypothyroidism over a 10-year period among postmenopausal women. The multivariable Cox regression model found no evidence of a statistically significant association between ANM and incidence of hypothyroidism. Although no previous study has examined the association between ANM and hypothyroidism, we can compare our results to studies that looked at subclinical hypothyroidism, a milder form of hypothyroidism. Our results align with findings of a cross-sectional study by Kotopouli et al.[17] which reported no significant difference in age at menopause in women with or without subclinical hypothyroidism. In contrast, a cross-sectional study by Monterrosa-Castro et al.[14] on 643 Colombian women found increased likelihood of subclinical hypothyroidism in women with age at menopause between 40-44 with OR of 3.37 (95% CI 1.40-8.10), and in women with age at menopause before 40 years with an OR of 4.31 (95% CI 1.24-14.97). The study also reported an increased risk in the combined group of age at menopause of less than 45 years with an OR of 3.57 (95% CI 1.57-8.10), compared to women with age at menopause of 45 years or older.[14] However, the study was focused on Colombian women and did not account for the average age at menopause which is typically between 50-54 years. The study also included older age at menopause of up to 69 years in the reference and included women with surgical menopause, which has been found to increase risk of thyroid cancer.[6] These differences in the reference group, inclusion criteria, and the authors' use of a cross-sectional study design, could explain the contrasting findings.

Estrogen has been found to negatively affect women with existing hypothyroidism by significantly increasing hypothyroidism markers.[32,33] Studies of estrogen administration in women without existing hypothyroidism found no significant changes in hypothyroidism

markers,[7,34] or found a significant increase in hypothyroidism markers that remained within normal ranges.[35,36] Estrogen's effect on hypothyroidism is mainly due to its ability to increase TBG levels. Studies on HRT and hypothyroidism markers showed a significant increase in TBG levels after estrogen administration, this was not necessarily observed for combined therapy.[7,32,33,35,36] Estrogen increases TBG levels through reducing its clearance and enhancing its biosynthesis.[32] Higher TBG levels in the blood lead to an increase of its binding with free T4 leading to slower entry of T4 into cells. This signals for more production of TSH which consequently leads to an increase of the production of thyroid hormones.[37] It seems that this cycle increases levels of bound T4 but not free T4 thus not affecting hypothyroidism because healthy women are able to produce free T4 to compensate. However, women with existing hypothyroidism are not able to compensate for this effect and thus are at an increased risk of increased disease severity.[32]

Hypothyroidism is correlated with higher BMI and obesity in our study and in others.[38,39] Two studies have shown a significant positive association between higher BMI levels and higher TSH levels.[40,41] Thyroid hormones play an important role in regulating thermogenesis, energy consumption, enzymes involved in lipid metabolism, and salt and water retention.[42] Moreover, a small increase in TSH has been found to be associated with deficiency in resting energy consumption.[42] Furthermore, low levels of thyroid hormones may lead to fat accumulation and reduced lipolysis.[43] This suggests that obesity in hypothyroid women may be a consequence of the condition rather than a cause.

This study had a high degree of generalizability given the large sample size and use of weighting to represent the Canadian population. Validity and precision were enhanced by the inclusion of detailed information on sociodemographic, health-related, and reproductive factors.

Also, this the first study to investigate incidence of overt and not subclinical hypothyroidism by ANM categories. Nonetheless, there were several limitations to this study. Self-reported incidence of hypothyroidism and ANM could lead to recall and misclassification biases. A study by Verschoor & Tamim[27] indicated that self-reported age at menopause is reliable with a high degree of accuracy. Furthermore, hypothyroidism after menopause is difficult to diagnose given the similarities of symptoms between menopause and hypothyroidism.[3,6] Also, typical symptoms of hypothyroidism are less evident in the older population, and their symptoms are sometimes confused for other co-morbid conditions[44] or to aging.[45] It is also possible that some women with subclinical hypothyroidism may have reported it as overt hypothyroidism. These factors may have resulted in over or underestimation of the true incidence of hypothyroidism. In the Tracking cohort, BMI was self-reported. Overweight and obese populations tend to underestimate their BMI levels.[46] However, having accurate BMI results would probably have strengthen the association between higher BMI levels and hypothyroidism. The study would have been stronger if it had included actual measurements of thyroid and sex hormone levels, rather than relying solely on self-reported doctor diagnosis. Furthermore, the generalizability of the findings may be impacted by the CLSA exclusion criteria and the fact that 94.4% of the sample being of White ethnicity. Additionally, there is risk of some violations of the proportional hazard assumption, but this was minimized by using robust standard errors.

Conclusion

Our study found no evidence of any statistically significant associations between ANM and risk of hypothyroidism in postmenopausal women. Higher BMI levels have shown to be associated with hypothyroidism. A longitudinal examination of hypothyroidism and BMI could offer deeper insight into the hormonal and metabolic factors involved in the disease

development. Future studies could benefit from incorporating actual thyroid and sex hormone measurements for clinical confirmation of the disease to provide a more accurate assessment of the relationship between age at menopause and hypothyroidism.

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Chapter 4

A cohort study on the associations between age at natural menopause and rheumatoid arthritis in postmenopausal women from the Canadian Longitudinal Study on Aging

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Abstract

Menopause represents a significant phase in a woman's life, marked by profound physiological changes. An early onset of menopause has been associated with a variety of negative outcomes. Estrogen has been shown to be protective of bone and joint health. Hormonal links to rheumatoid arthritis have been found; previous studies exploring age at natural menopause (ANM) and Rheumatoid arthritis have produced conflicting results. This study investigated the association between ANM and incidence of rheumatoid arthritis among postmenopausal Canadian women. The study included women between the ages of 45–85 years from the Canadian Longitudinal Study on Aging followed over a 10-year period. Analysis was restricted to naturally postmenopausal women that did not have rheumatoid arthritis prior to menopause. ANM was examined using the following categories ≤ 44 (reference), 45–49, and ≥50. Survival analysis was used to determine time to onset of rheumatoid arthritis. Unadjusted and adjusted multivariable Cox regression models were used to examine the relationship between ANM and incidence of rheumatoid arthritis. The adjusted multivariable Cox regression model showed significantly lower risk of rheumatoid arthritis in women with an older ANM of ≥50 years and who have been on hormone replacement therapy for ≥8 years with a hazard ratio of 0.2 (95 % CI: 0.1–0.7) compared to women with an ANM ≤ 44 who have never used hormone replacement therapy. Our findings suggest a potential beneficial effect of longer estrogen exposure on the risk of developing rheumatoid arthritis.

Introduction

Menopause is a major event in women's lives marked by considerable decreases in estrogen.[1] It is defined as the cessation of menstruation for 12 consecutive months with an average age at menopause of approximately 51 years.[2] An earlier age at menopause has been associated with increased risk of several diseases including such as cardiovascular diseases (CVD), strokes, cognitive decline,[2] and lupus erythematosus.[3] Further, estrogen and progesterone, whose levels decrease during menopause, have a protective effect on bones and joints.[4] Rheumatoid arthritis (RA) is an autoimmune disease that targets synovial joints and can lead to chronic inflammation of one or more joints in the body.[4] It is characterized by painful, swollen, and stiff joints and is usually associated with fever and fatigue[4] and systemic inflammation.[5] RA is four times more prevalent in females compared to males with an incidence ratio of 2.3.[6] Women typically develop the first RA symptoms around the time of menopause with the peak age of RA onset typically coinciding with menopause.[6]

The exact etiology of RA is unknown;[7,8] however, it is believed to be a result of genetic, environmental,[7] and hormonal[8] factors. The elevated prevalence of RA in women is believed to be associated with reproductive hormones[9]. Specifically, other observations including the diminishing of RA symptoms during pregnancy, and their flare-ups during periods of postpartum, perimenopause and post menopause[10] have led researchers to assume a hormonal link between sex hormones and RA in females. Furthermore, both pre- and post-menopausal women showed increased risk of RA after being given aromatase inhibitors, which interferes with the production of estrogen.[5] Moreover, a decrease in estrogen levels leads to an increase in Interleukin-1 beta (IL-1β) cytokine which is an important proinflammatory cytokine associated with the development of RA.[11]

Few studies have explored the relationship between age at natural menopause (ANM) and incidence of RA, and the findings have been inconsistent. Some research suggests an increased risk[7,9,12] and others report no association.[13-15] Only one study excluded cases of surgical menopause,[7] and no study looked at the potential interaction with hormone replacement therapy (HRT). Finally, there is a lack of Canadian data on this topic, highlighting the need for further research in this area. Given these gaps and inconclusive findings in the literature, this study will investigate the association between ANM (excluding surgical and medically induced menopause) and risk of RA on postmenopausal women using data from the Canadian Longitudinal Study on Aging (CLSA) while accounting for HRT use.

Methods

Study design and sample

This study is a secondary analysis of the CLSA, a national and longitudinal study on 51,338 participants from across the 10 Canadian provinces. At baseline, participants between 45–85 years were enrolled in one of two cohorts. The Tracking cohort consisted of 21,241 individuals (10,406 men and 10,835 women) who were randomly selected from across the Canadian provinces and were interviewed by telephone. In contrast, the Comprehensive cohort included 30,097 participants (14,777 men and 15,320 women) who were randomly selected from within 25–50 km of one of 11 data collection sites in seven provinces, interviewed in person, and underwent a physical assessment. Data collection started in 2011 with new cycle of data collection every three years. Data collection is ongoing and expected to continue for a total of 20 years or until the participant's death. Around 21.4 % of participants were lost to follow-up or deceased by follow-up 2. Further details on the CLSA methodology are available elsewhere.[16,17]

This study was a historical cohort analysis of the Tracking cohort including three cycles of data collection (baseline, follow-up 1, and follow-up 2). Data from the Comprehensive cohort were not used, as it did not include information on age at rheumatoid arthritis. The CLSA study design excluded residents of Canadian territories and some remote regions, people on Federal or provincial First Nations reserves and settlements, full-time members of the Canadian Armed Forces, and institutionalized persons, at recruitment, including those in long-term care. The CLSA also excluded those unable to speak English or French, those who were physically or cognitively impaired, or unable to participate on their own at recruitment. The CLSA received ethical approval from McMaster University Health Integrated Research Ethics Board and by research ethics boards at all collaborating Canadian institutions This study involved a secondary analysis of fully de-identified CLSA data and was approved by the York University Office of Research Ethics (ORE) under protocol number STU 2022–114. Additional informed consent was not required, as all CLSA participants provided informed consent during primary data collection to have their de-identified data in future research.

Study participants

This study excluded all male participants. Women were also excluded if they had missing information on menopause status, had not yet reached menopause, or had undergone surgical or medically induced menopause. Additionally, women with missing ANM were also excluded, as were those with ANM under 40 or over 67 years as this is outside the normal range of ANM.[18] The study also excluded women with missing age at RA. To determine incidence, women who reported RA prior to menopause were excluded from the study. A detailed breakdown of participant selection is provided in Figure 7.

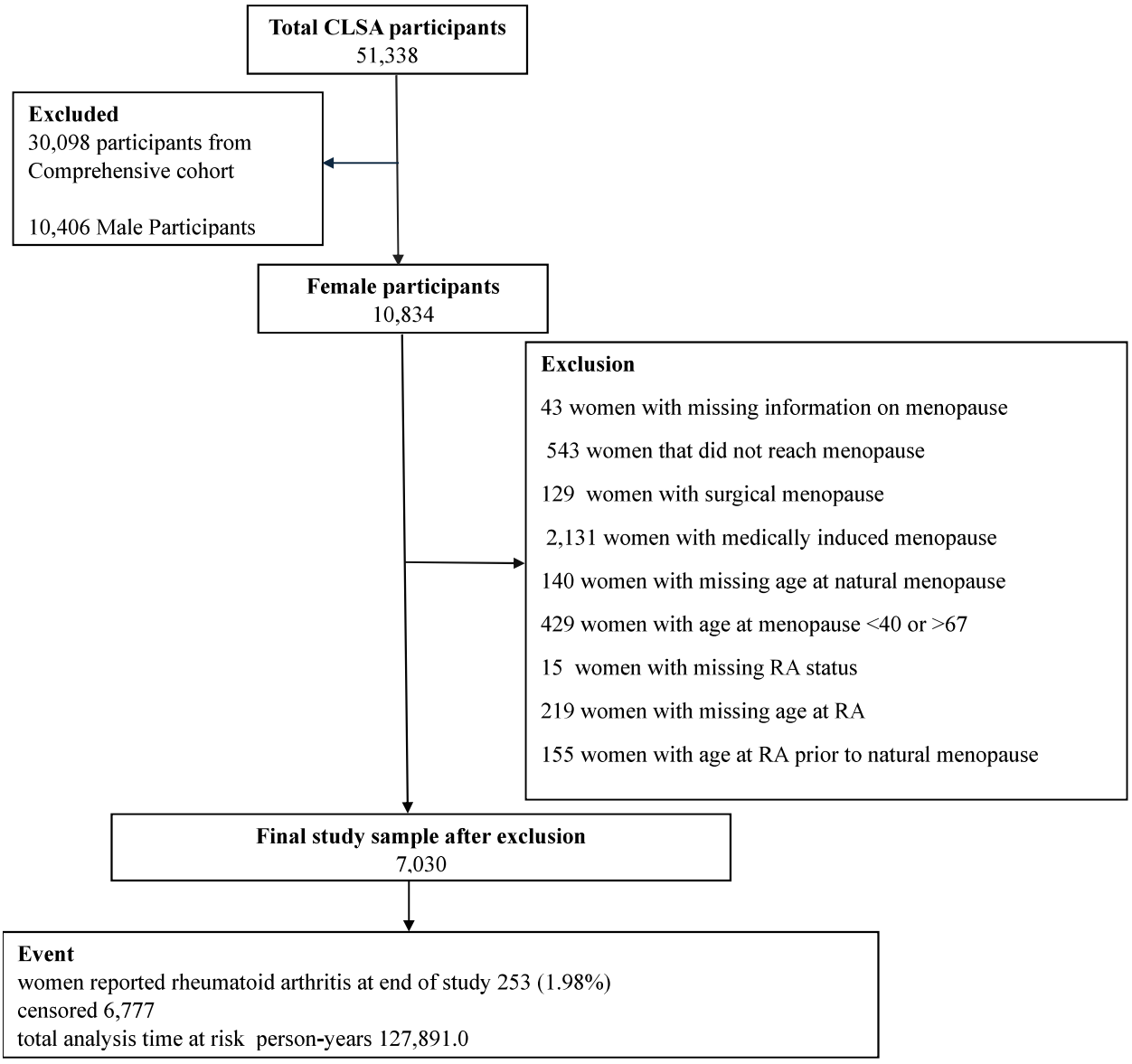

FIG. 7: Canadian Longitudinal Study on Aging (CLSA) participant chart.

Exposure assessment: ANM

This study included women with available data on ANM. ANM refers exclusively to menopause that occurred naturally, excluding surgical or medically induced menopause. The exposure variable, self-reported ANM, was measured from all three cycles (baseline, follow-up 1

& follow-up 2) using the question: "*Have you gone through menopause, meaning that your menstrual periods stopped for at least one year and did not restart?*" answers were recorded as, "Yes" or "No". Women who answered "Yes" were then asked about their ANM: "*How old were you when your menstrual periods stopped for at least one year and did not re-start?*" answers were reported in years of age. ANM was categorized into the following groups: ≤ 44 (reference), 45–49, and ≥50, similar to categories presented in Bengtsson et al., 2017.[7]

Outcome assessment (incidence of RA)

Self-reported RA was the primary outcome measure. Women who reported RA prior to entering menopause were excluded. Information on RA incidence was collected during follow-ups 1 and 2. RA was measured as a dichotomous variable using the interviewer's question: "*Has a doctor ever told you that you have rheumatoid arthritis?*" answered by "Yes" or "No". Women who responded "Yes" were then asked to specify their age at diagnosis with the question: "*At what age or in what year were you first told you had rheumatoid arthritis*?" with answers recorded in years. Women who answered "No" were asked again in the next cycle.

Covariates

Sociodemographic factors included ethnicity (white, other), where "other" included, South Asian, Chinese, Filipino, Latin American, Japanese, Southeast Asian, Korean, Arab, West Asian, Black, and other North American origins, level of education (less than high school, high school to some college, bachelor's degree or higher), and total annual household income in Canadian dollars (<$20,000, $20,000- $50,000, $50,000-$100,000, >$100,000). Health-related factors including: smoking status (never, former, current), alcohol consumption (never, less than once weekly, at least once weekly), frequency of leisure time physical activity in the past year

(nonregular, weekly), with weekly defined as participation in physical activity at least once a week and diabetes status (yes, no) were also recorded. Self-reported height and weight were used to calculate body mass index (BMI) in kg/m^2. The BMI cut-off for underweight was increased from the standard 18.5 kg/m^2 because very few participants had that low of a BMI.[19] BMI level was categorized as, underweight < 20.0 kg/m^2, normal weight = 20.0–24.99 kg/m^2, overweight = 25.0–29.99 kg/m^2, and obese >30.0 kg/m^2. Depressive symptomology reported as (yes, no) was measured using "The Center for Epidemiological Studies short Depression scale", with a score of ≥10 on the scale suggesting presence of depressive symptomology.[20] Reproductive factors including age at menarche in years (≤11, 12–14, ≥15), duration of oral contraceptive use in years (never, <5, ≥5), number of births (0, 1, ≥2), and duration of HRT in years (never, <4, 4–7, ≥8) were also included. All covariates were measured at baseline, except for number of births which was obtained from follow-up 1, age at menarche which was obtained from follow-up 2, and duration of oral contraceptive use which was obtained from follow-ups 1 & 2.

Censoring

Women who did not develop RA during the 10-year study period were considered censored observations, contributing person-years to the analysis. Follow-up ended at the first occurrence of one of the following three events: a) incidence of RA b) loss to follow-up or death or c) end of the study period.

Statistical analysis

This study used survival analysis to determine time to onset of RA. Descriptive statistics by ANM categories are presented using frequencies and percentages. Missing data were addressed using multiple imputation by chained equations (MICE).[21] Three variables collected at

later cycles rather than at baseline - oral contraceptive duration, number of births, and age at menarche showed moderate rates of missingness of 17.7 %, 15.8 %, 26.5 %, respectively. This prompted our adoption of the missing-not-at-random assumptions and multiple imputation strategy. The MICE procedure was implemented using the 'mi impute chained' command in Stata version 18, generating 20 imputed data sets, which were combined using Rubin's rule.[21] All variables in the analysis were imputed for missing values. Logistic regression was used to predict binary variables and ordered logistic regression as applied to categorical variables.[21]

For the bivariate analysis, Kaplan-Meier survival curves were used to determine time-onset of RA, and a log-rank test was used to compare survival curves. Unadjusted and adjusted Cox proportional hazard regression models were used to estimate the hazard ratios (HR) and their 95 % confidence intervals (*CIs*) for the association between ANM (in years: ≤ 44 (reference), 45–49, or ≥50) and incidence of RA. Smoking, BMI, and HRT were considered in the model as interaction terms with ANM. While no significant interaction was found for smoking and BMI, a significant interaction was found between ANM and HRT. The Cox proportional hazard assumption was evaluated using log-log plots. Inverse probability weights provided by the CLSA were used to make results generalizable to the Canadian population.[22] A p-value of < 0.05 (two-sided) was considered statistically significant. All analyses were performed using Stata statistical software (version 18, StataCorp LLC, College Station, TX, USA).

Results

Table 5 shows descriptive characteristics of the 7,030 women in the analysis, representing a weighted sample of (*N*: 3321,187). Among them, 253 women developed RA during the follow-up period of 127,891.0 person-years, resulting in an incidence of 1.98 cases of RA per 1000

person-years. The average age at baseline was 60.5 years with 68.6 % of participants between 45–64 years. The average and median ANM was 50.2 and 50.0 years, respectively. The majority of the sample was of white ethnicity (96.6 %). Only 13.2 % of the sample were current smokers, while 54.3 % were former smokers. A quarter of the sample reported an annual income of over $100,000. More than half had high school to college education, engaged in weekly physical activity, and consumed alcohol at least once a week. Normal BMI levels were reported by 38.0 % of the sample, while 20.0 % reported experiencing depressive symptomology and 12.0 % reported diabetes. In addition, 71.0 % had never used HRT, while 12.0 % have taken it for less than four years. Half the sample reported an age at menarche between 12–14 years, and 74.1 % had two or more children. Nearly half of the sample had used oral contraceptives for >5 years. Details of study exclusions are presented in Figure 7.

Table 6 shows the unadjusted and adjusted Cox regression model with HRs and their 95 % *CIs*. Figure 8 includes the bivariate analysis using Kaplan-Meier survival curves to illustrate time to onset of RA across ANM category. No evidence of a statistically significant difference between ANM categories and incidence of RA were found. A statistically significant association was found between the interaction between ANM and HRT use. Women with ANM of 50 or older who used HRT for eight or more years showed a reduced risk of developing RA compared to women with ANM of <44 who never used HRT with a hazard ratio (HR) of 0.2 (95 % CI: 0.1–0.7). Education level was also significantly associated with RA risk. Women with less than a high school education, or with high school to some college education, had increased risk for the development of RA compared to those with a bachelor's or higher, with HRs of 1.9 (95 % CI: 1.0–3.2) and 1.5 (95 % CI: 1.1–2.3), respectively. Figure 9 shows the Cox proportional hazard assumption using log-log plots. The overlapping lines on the plot suggest a potential violation of

the proportional hazard assumption. To address this, robust standard errors were applied to maintain the model's reliability despite potential violation of the proportional hazard's assumption.

TABLE 5: Characteristics of study population according to age at natural menopause

Variables	Age at Natural Menopause					
	≤ 44		45-49		≥50	
	Unweighted *N* (%)	Weighted[a] *N* (%)	Unweighted *N* (%)	Weighted[a] *N* (%)	Unweighted *N* (%)	Weighted[a] *N* (%)
N (%)	625 (8.9)	302230.9 (9.1)	1671 (23.8)	824011.7 (24.8)	4735 (67.3)	2194944.8 (66.1)
Ethnicity						
White	605 (96.8)	296256.5 (98)	1582 (94.7)	778581.9 (94.5)	4531 (95.7)	2076514 (94.6)
Other[a]	15 (2.4)	4557.8 (1.5)	55 (3.3)	26420.1 (3.2)	124 (2.6)	79813 (3.9)
Education Level						
Less than high school	77 (12.3)	77658.9 (25.7)	132 (7.9)	176995.6 (21.5)	353 (7.5)	410403.7 (18.7)
High school – some college	410 (65.6)	196723.6 (65.1)	1004 (60.1)	503706.1 (61.1)	2691 (56.8)	1373829 (62.6)
Bachelor's or higher	134 (21.4)	26640.1 (8.8)	527 (31.5)	136005.8 (16.5)	1677 (35.4)	405369 (18.5)
Income						
<20,000	66 (10.6)	43513.6 (14.4)	138 (8.3)	66275.6 (8.0)	295 (6.2)	135556.8 (6.2)
$20,000-$50,000	211 (33.8)	87244.5 (28.8)	491 (29.4)	217712.0 (26.4)	1347 (28.5)	605995.1 (27.6)
$50,000-$100,000	196 (31.4)	98451.4 (32.6)	525 (31.4)	265598.1 (32.2)	1558 (32.9)	733361.7 (33.4)
≥$100,000	94 (15.0)	51514.4 (17.0)	382 (22.0)	221782.4 (26.9)	1150 (24.3)	569658.5 (26.0)
Health-related Factors						
Smoking						
Never	198 (31.7)	80630.5 (26.7)	516 (30.9)	212780.2 (25.8)	1729 (36.5)	767819.3 (35.0)
Current	100 (6.0)	61177.9 (20.2)	234 (14.0)	163730.2 (19.9)	378 (9.0)	214638.7 (6.4)
Former	325 (52.0)	158286.9 (52.4)	915 (54.8)	445002.7 (54.0)	2601 (54.9)	1201405.3 (54.7)
Alcohol consumption						
Never	87 (13.9)	36985.8 (12.2)	199 (11.9)	95571.3 (11.6)	511 (10.8)	258873.7 (11.8)
Less than once weekly	263 (42.1)	121581.2 (40.2)	649 (38.8)	313576.6 (38.0)	1828 (38.6)	809038.5 (36.9)
More than once weekly	242 (28.7)	130047.2 (43.0)	734 (43.9)	376976.5 (45.7)	2209 (46.7)	1043042.9 (47.5)
Leisure time physical activity						
Non-regular	342 (54.7)	157485.5 (52.1)	830 (49.7)	420463.0 (51.0)	2160 (45.6)	1011898.8 (46.1)
Weekly	282 (45.1)	144663.6 (47.9)	836 (50.0)	401704.9 (48.7)	2568 (54.2)	1181492.5 (53.8)
BMI (kg/m^2)						
<20.00 (underweight)	33 (5.3)	14573.4 (4.8)	95 (5.7)	58645.4 (7.1)	232 (4.9)	95917.6 (4.4)
20.0-24.99 (normal weight)	214 (34.2)	1076340 (35.6)	642 (38.4)	306606.5 (37.2)	1777 (37.5)	833642.5 (38.0)
25.0-29.99 (overweight)	204 (32.6)	92056.3 (30.5)	531 (31.8)	244614.1 (29.7)	1601 (33.8)	719589.6 (32.8)
≥ 30.0 (obese)	167 (26.7)	82225.5 (27.2)	390 (23.3)	204509.3 (24.8)	1092 (23.1)	524746.6 (24.0)
Depression symptomology						
Yes	129 (20.6)	59460.1 (19.6)	323 (19.3)	163381.6 (19.8)	822 (17.4)	408777.1 (18.6)

No	495 (79.2)	242748.4 (80.3)	1,344 (80.4)	659258.3 (80.0)	3902 (82.4)	1781799.7 (81.2)
Diabetes						
Yes	99 (15.8)	40138.5 (13.3)	211 (12.6)	104568.5 (12.7)	598 (12.6)	284648.0 (13.0)
No	392 (62.7)	183058.9 (60.6)	1,135 (67.9)	554309.1 (67.3)	3494 (73.8)	1619683.8 (73.8)
Reproductive Factors						
Duration of Oral Contraceptive (years)						
Never	113 (18.1)	41699.3 (13.8)	254 (15.2)	106329.8 (12.9)	814 (17.2)	336290.4 (15.3)
<5	112 (17.9)	56135.7 (18.6)	328 (19.6)	143990.9 (17.5)	990 (20.9)	458266.8 (20.9)
≥5	252 (40.3)	123538.1 (40.9)	723 (43.3)	383965.0 (46.6)	2,201 (46.5)	1078071 (49.1)
Number of births						
0	101 (16.2)	40567.1 (13.4)	261 (15.6)	105451.8 (12.8)	640 (13.5)	250243.3 (11.4)
1	61 (9.8)	25476.4 (8.4)	172 (10.3)	67866.4 (8.2)	516 (10.9)	233883.9 (10.7)
≥2	463 (74.1)	236187.4 (78.1)	1238 (74.1)	50693 (79.0)	3579 (75.6)	1710818.6 (77.9)
Age at Menarche						
≤11	84 (13.4)	46082.2 (15.2)	193 (11.6)	104680.1 (12.7)	601 (12.7)	290787.5 (13.2)
12-14	272 (43.5)	125589.7 (41.6)	830 (49.7)	392892 (47.7)	2563 (54.1)	1168606.4 (53.2)
≥15	55 (8.8)	24265.4 (8.0)	136 (8.1)	75630.6 (9.1)	432 (9.1)	234474.8 (10.7)
Duration of use of any HRT (years)						
Never	342 (54.7)	189804.1 (62.8)	1078 (64.5)	556722.8 (67.5)	3330 (70.3)	1626329.8 (74.1)
<4	82 (13.1)	31978.3 (10.6)	243 (14.5)	115830.7 (14.1)	660 (13.9)	283760.3 (12.9)
4-7	62 (9.9)	31891.0 (10.6)	127 (7.6)	61330.4 (7.4)	345 (7.3)	149220.4 (6.7)
≥8	139 (22.2)	48557.5 (16.0)	223 (13.4)	90127.9 (10.9)	400 (8.5) (6.2)	135634.3

BMI, body mass index; CAD, Canadian dollars; the Center for Epidemiological Studies Depression Scale; 95% CI, 95% confidence intervals; HRT, hormone replacement therapy

All variables were obtained from baseline expect for number of births which was obtained from follow-up 1, age at menarche which was obtained from follow-up 2, and duration of oral contraceptives which was obtained from follow-ups 1 & 2.

[a]Estimated using survey weights.

[b]Other included South Asian, Chinese, Filipino, Latin American, Japanese, Southeast Asian, Korean, Arab, West Asian, Black, and Other North American Origins

Totals may not sum to 100% due to missing data and rounding. Table is showing column percentages.

TABLE 6. Unadjusted and adjusted hazard ratios and 95% confidence intervals between ANM and risk of RA

Variables	Unadjusted HR (95% CI)[a]	Adjusted HR (95% CI)[a]
ANM (years)		
≤ 44	1	1
45-49	1.3 (0.8-2.2)	1.1 (0.8-2.9)
≥50	1.2 (0.7-1.9)	1.6 (0.9-2.8)
Sociodemographic		
Ethnicity		
White	1	1
Other [b]	1.3 (0.6-2.8)	1.5 (0.7-3.3)
Education Level		
Less than high school	1.8 (1.1-2.9)	**1.8 (1.0-3.2)**
High school – some college	1.6 (1.1-2.2)	**1.5 (1.1-2.3)**
Bachelor's or higher	1	1

Income		
<20,000	1.2 (0.6-2.7)	1.1 (0.5-2.5)
$20,000-$50,000	1.3 (0.8-2.2)	1.2 (0.7-2.0)
$50,000-$100,000	1.5 (0.9-2.4)	1.5 (0.9-2.5)
≥$100,000	1	1
Health-related Factors		
Smoking		
Never	1	1
Current	0.8 (0.4-1.8)	0.8 (0.4-1.7)
Former	1.2 (0.8-1.6)	1.2 (0.8-1.7)
Alcohol consumption		
Never	1	1
Less than once weekly	1.2 (0.6-1.7)	1.1 (0.6-1.8)
More than once weekly	0.8 (0.5-1.3)	0.9 (0.5-1.5)
Physical activity		
Non regular	1	1
regular	1.2 (0.9-1.7)	1.3 (0.9-1.8)
BMI (Kg/m^2)		
<20.00 (underweight)	0.5 (0.2-1.2)	0.5 (0.2-1.3)
20.0-24.99 (normal weight)	1	1
25.0-29.99 (overweight)	1.0 (0.7-1.6)	1.0 (0.7-1.6)
> 30.0 (obese)	**1.5 (1.0-2.3)**	1.4 (0.9-2.3)
Depressive symptomology		
Yes	0.8 (0.5-1.3)	0.8 (0.5-1.3)
No	1	1
Diabetes		
Yes	**1.4 (0.9-2.0)**	1.2 (0.8-1.9)
No	1	1
Reproductive Factors		
Duration of Oral Contraceptive (years)		
Never	1	1
<5	0.9 (0.6-1.5)	0.9 (0.6-1.5)
≥5	1.2 (0.8-1.7)	1.2 (0.8-1.9)
Number of births		
0	1	1
1	1.3 (0.6-2.0)	0.9 (0.6-1.5)
≥2	0.9 (0.6-1.5)	1.1 (0.8-1.9)
Age at Menarche		
≤11	1.3 (0.7-1.9)	1.2 (0.6-2.0)
12-14	1	1
≥15	0.9 (0.5-1.6)	1.0 (0.6-1.7)
Duration of HRT (years)		
Never	1	1
<4	0.8 (0.5-1.2)	0.8 (0.5-1.2)
4-7	0.7 (0.3-1.5)	0.7 (0.3-1.5)
≥8	0.9 (0.6-1.5)	0.9 (0.6-1.5)

Interaction: ANM x Duration of HRT (years)		
≤ 44 & never	1	1
45-49 & <4	1.1 (0.2-5.7)	1.1 (0.2-5.6)
45-49 & 4-7	6.6 (0.8-50.4)	5.6 (0.7-43.2)
45-49 & ≥8	0.6 (0.2-2.0)	0.5 (0.1-1.8)
≥50 & <4	0.9 (0.2-3.3)	0.8 (0.2-3.2)
≥50 & <4-7	2.6 (0.4-16.2)	2.3 (0.4-14.8)
≥50 & ≥8	**0.2 (0.8-0.8)**	**0.2 (0.1-0.7)**

BMI, body mass index; CAD, Canadian dollars; CES-D, the Center for Epidemiological Studies Depression Scale; 95% CI, 95% confidence intervals; HR, hazard ratio; HRT, hormone replacement therapy.

[a] Calculated HR and 95% CI using survey analytical weights and robust standard errors

[b]Other included South Asian, Chinese, Filipino, Latin American, Japanese, Southeast Asian, Korean, Arab, West Asian, Black & other North American Origins .

Regression analysis was performed after implementing multiple imputation chained equation.

Bold numbers indicate the significant results with p-value <0.05.

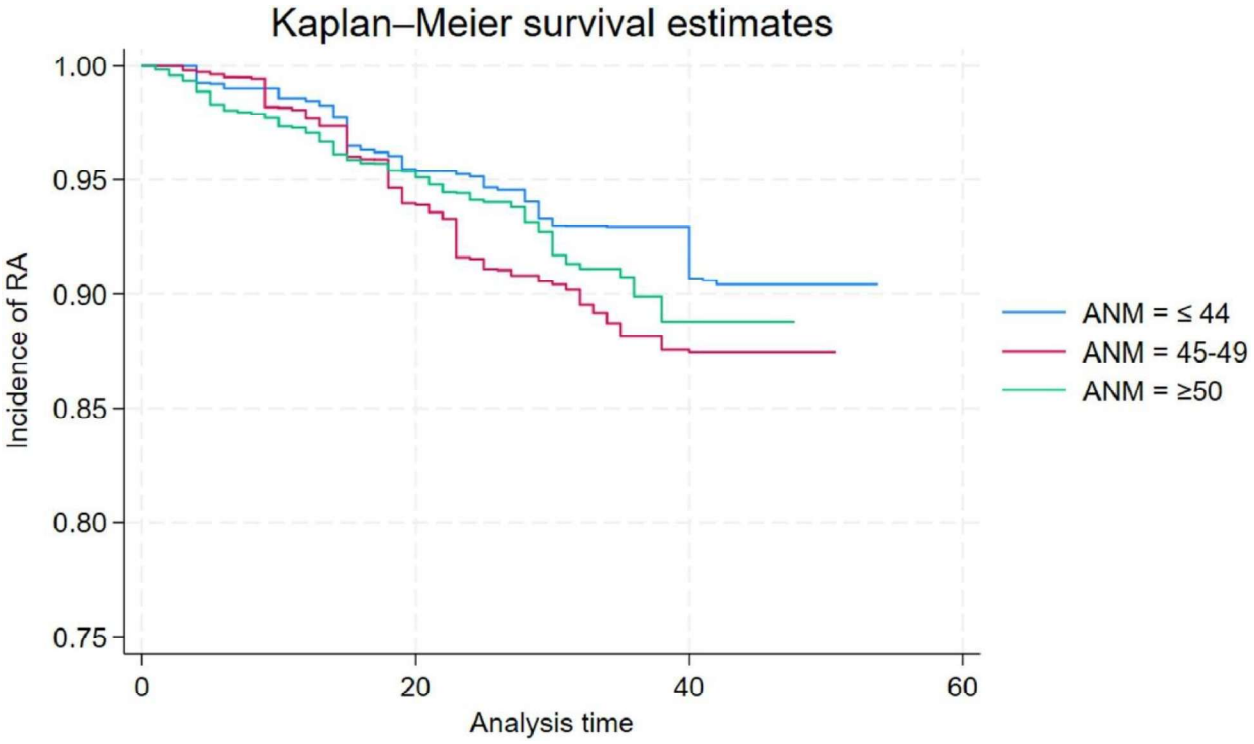

FIG. 8: Kaplan Meier survival curves by ANM groups.

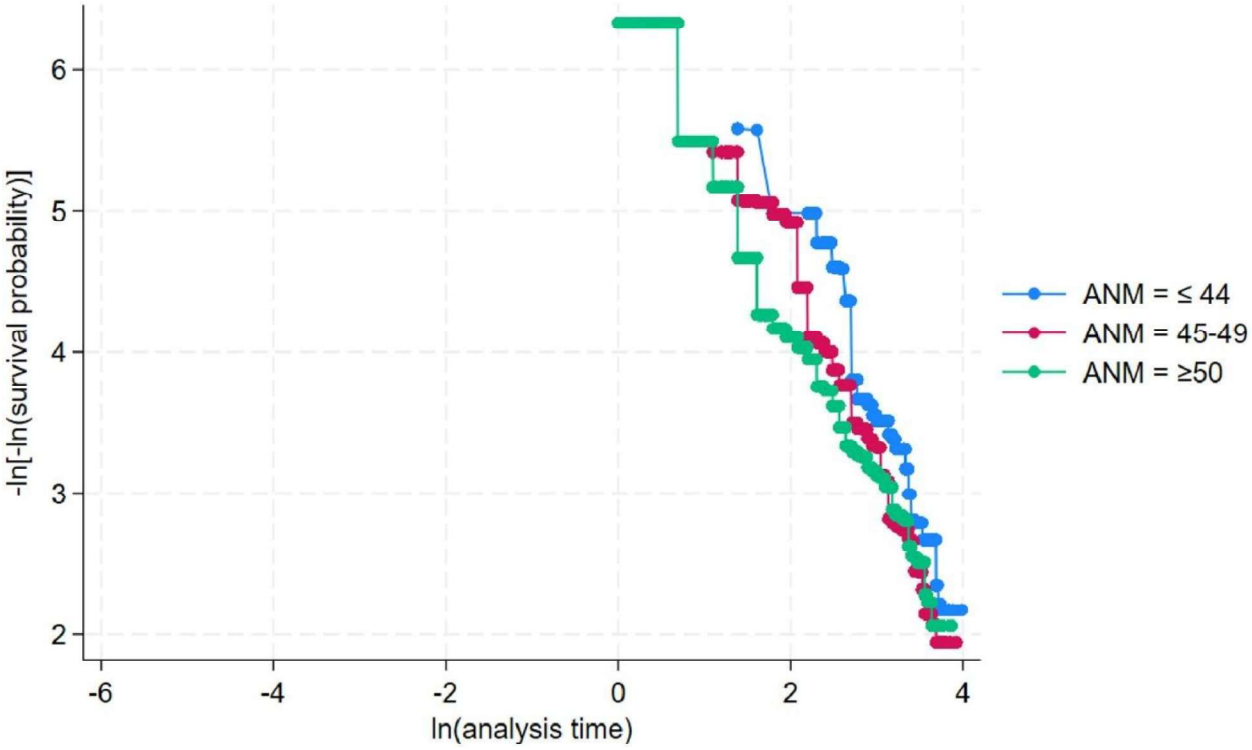

FIG. 8: Cox Proportional Hazard assumption using adjusted log-log plots.

Discussion

This study examined the association between ANM and risk of RA in postmenopausal women from the CLSA. Of note, our findings demonstrated a significant interaction between ANM with HRT. After controlling for other factors, women with an older ANM ≥50 years and who have been on HRT for ≥8 years experienced an 80.0 % reduced risk of developing RA compared to women with an ANM ≤ 44 who have never used HRT. Our findings suggest the potential beneficial effect of longer estrogen exposure on the risk of RA.

Previous cross-sectional studies found contradicting results with some finding significantly increased risk,[10,23] while others found no evidence of any statistically significant differences.[8,24] Compared to cohort studies our results are consistent with a recent prospective study of the UK biobank which found increased risk of RA in the adjusted model for women

with early age at menopause.[9] Similarly, a nested case-control study from the Malmö Diet and Cancer cohort reported a significantly higher risk of RA in women with an early age at menopause.[12] Only one study explicitly excluded women with surgical menopause like we did. Bengtsson et al.[7] used pooled data from the Nurses' Health Study cohorts and found higher risk of RA with earlier ANM compared to later ANM; however, the reference used was premenopausal women, and the results were only significant for seropositive RA.[7] Contrasting this, a prospective analysis of the Iowa Women's Health Study cohort found a decreased RA risk with older age at menopause, but it was not statistically significant.[13] Similarly, an Iranian and South Korean cohort found no significant association between ANM and RA.[14,15] Of note, the studies showing statistical significance had appropriate adjustment for confounders and included reproductive, hormonal and lifestyle variables that are important to consider for the association between age at menopause and RA. Finally, a Mendelian randomization study also found no association between ANM and risk of RA, but the authors noted that the study was limited by examining only genetic markers and not overt RA disease.[25]

Our study found that in the interaction model of HRT duration and ANM, longer HRT use combined with a later ANM was associated with decreased risk of RA. To our knowledge, no prior study has examined this interaction and incidence of RA; however, there is some research on the associations between HRT duration and RA risk. In contrast to our findings, two studies reported an increased risk of seropositive RA,[7,15] and another found an increased risk of RA[9] with longer duration of HRT use; while other studies found no significant association between longer HRT duration and RA risk.[13,26] It appears that later ANM modified this relationship. Women with later ANM experience prolonged exposure to estrogen, which may cause immune-modulating effects, thereby reducing RA risk.[27]

Our results suggest that longer duration of estradiol exposure is protective against RA disease. It has been suggested that the loss of estradiol can trigger immune responses related to Th1[9,28] and Th17[9,23], which drive autoimmune and inflammatory processes. Estradiol has a role in modulating cytokine production, thus its decreasing levels in postmenopausal women may cause an increase in pro-inflammatory markers such as TNF-a and IL-6.[9,23] Furthermore, menopause leads to a rapid decline of estrogen levels, leading to long-term activation of the immune system and changes of cytokines and immune cell profiles negatively affecting fibroblasts and damaging the skeletal system.[9] Also, before menopause, estradiol is the principal estrogen in the body; however, after menopause estrone becomes the main estrogen which is proinflammatory and when it interacts with TNF-a, it activates Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-kB) signalling pathway which is a critical contributor to RA development.[29]

This study found an increased risk of RA with lower education levels, and several previous studies have supported this finding. Bergström et al.[30] found that individuals with elementary school education showed a significantly higher odds of RA compared to those with university level education. Similarly, women with university degrees demonstrated a reduced risk of RA compared to those with only elementary school education.[8] In another study, those without a university degree were at higher risk of developing RA, compared to those with a university degree.[7] In a large nationally representative study, those with high school or more than high school education had significantly reduced risk of RA as compared to those without high school.[10] The increased risk with lower education may be attributed to several factors such as the job fields associated with lower education levels which could lead to exposure to environmental factors that are known risks to RA development.[30] Additionally, individuals with lower education

levels may have less access to health care or delays in seeking medical attention.[8,30] Furthermore, people with higher education levels are more likely to lead healthier lifestyles, which may contribute to their reduced risk of RA.[8]

This study had several strengths, including a high degree of generalizability due to its large sample size and the use of sample weights to represent the Canadian population. The inclusion of detailed socio-demographic, health-related, and reproductive factors further enhanced validity and precision. To our knowledge this is also the first study to examine the interaction of ANM with HRT duration on risk of RA. Nonetheless, there were several limitations to the study, specifically the self-reported incidence of RA and ANM which could lead to recall and misclassification biases, although self-reported age at menopause has be found to have a high degree of accuracy.[18] Furthermore, the generalizability of the findings may be limited due to the CLSA exclusion criteria and the predominance of participants of white ethnicity (94.4 %), and a tendency toward higher socio-economic status within the sample. Additionally, our results do not include adjustments for multiple testing and we note potential violations of the proportional hazard assumption, but this was mitigated by using robust standard errors.

Conclusion

Results from this study underscore the protective effect of the combination of later ANM and long-term use of HRT on risk of development of RA. Future studies are needed to confirm these findings and underlying mechanisms.

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Chapter 5

General Discussion

Major findings

This dissertation examined the associations between ANM and incidence of three immune-mediated diseases: asthma, hypothyroidism, and RA- in postmenopausal women from CLSA. The key findings were that women with an earlier ANM had a reduced risk of asthma, while women with a later ANM and longer duration of HRT use had a reduced risk of RA. No significant association between ANM and hypothyroidism was found.

Differential effects of estrogen

The varying results of ANM on incidence of asthma and RA may be explained by the different biological effects of estrogen on these diseases. Although the term "estrogen" is used broadly here, it is important to note that estradiol is the primary form of estrogen before menopause, while estrone predominates after menopause.[1] These forms differ in potency and effects,[2] which may influence disease pathways differently. Although both diseases are immune-mediated, they exhibit different inflammatory responses that interact differently with estrogen. Estrogen receptors alpha and beta (ERα and ERβ) are expressed in various immune cells, and estrogen can exert both pro-inflammatory and anti-inflammatory effects depending on the cell type involved, such as B cells or T cells of the immune system.[3] This effect is mediated through the activation of genes that regulate immune responses, particularly those that control Th1 and Th2 cytokines.[4] B cells promote Th2 immune response,[5] while Th1 cells promote Th1 immune response.[6]

Rheumatoid arthritis is an autoimmune disease characterized by Th1 type proinflammatory response.[6] Estrogen has been found to supress Th1 mediated inflammation,[2]

therefore reducing the risk of RA. Furthermore, estrogen has a dose-dependent effect meaning its impact varies based on both its duration and concentration.[3,4] The biological effect of estrogen also differs based on whether it is exogenous or endogenous.[2] Therefore, prolonged exposure to endogenous estrogen in women with a later age at menopause, combined with exogenous estrogen from HRT, may result in a cumulative dose sufficient to exert a protective effect against RA.

In contrast, asthma falls under the category of an allergic disorders which are represented by Th2 immune response.[7] Estrogen is known to enhance Th2 inflammation,[8] which underlies the T2-high asthma phenotype.[9] This is represented by B cells which estrogen has a pro-inflammatory effect on.[2] Furthermore, a study found that women with more severe asthma had more Th2 cells and it is suggested that this is because estrogen may help Th2 cells live longer and produce more T2 cytokines which are key to T2- high asthma.[9] Therefore, earlier menopause resulting in lower lifetime estrogen exposure may reduce the risk of Th2-driven asthma.

There was no association found between ANM and hypothyroidism. One hypothesis is that there are less estrogen receptors on the thyroid as compared to other organs making the thyroid less sensitive to estrogen changes.[10]

Clinical & Public Health Implications

Findings from this research are of great importance for women as they provide better understanding of disease risks associated with menopausal aging. Based on these findings, health care providers should provide improved risk assessments tailored to women based on their ANM. For example, women with later ANM should be monitored for asthma symptoms, while women with earlier ANM should be monitored for symptoms of RA.

Strengths and limitations

This dissertation is the first to examine the effect of ANM on hypothyroidism, the second to investigate its association with asthma, and the first to explore the interaction between ANM and HRT in relation to RA. The large sample size and use of survey weights enhances the generalizability of the findings to the Canadian population. Additionally, the inclusion of numerous sociodemographic, health-related, and reproductive covariates strengthens the precision of the results. However, the study is limited by the largely white participant population and by the self-reported nature of both the exposure (ANM), outcomes (asthma, hypothyroidism, and RA), and some of the covariates.

We adjusted for urban versus rural residence in the asthma paper but could not control for more specific environmental exposures such as aero-allergens, pesticides, or plastic-derived endocrine disruptors, because these variables were not measured or available in the CLSA files we requested. Unmeasured environmental factors could therefore still influence the observed association between ANM and asthma incidence. Finally, we handled missing data with MICE, initially assuming a Missing-Not-at-Random mechanism, but diagnostic logistic-regression tests of missingness indicators showed significant associations with observed variables, supporting a Missing-at-Random assumption. Although we cannot definitively rule out Missing-Not-at-Random, our diagnostics justify the Missing-at-Random assumption, making MICE appropriate across all three papers.

Future Directions

Future research should consider use of medical records to validate both disease diagnoses and ANM. Important covariates such as HRT use and BMI should also be more rigorously

measured. In particular, for hypothyroidism, objective clinical measures should be used instead of self-report. Prospective study designs are recommended for future work to better establish temporal relationships. Moreover, future research should explore the mechanistic role of estrogen in immune system modulation related to these diseases. As genetic data and environmental exposures continue to become available, incorporation of this information may also offer deeper insights into the complex etiologies involved. Future studies could also investigate the association of surgical menopause as compared to natural menopause on the risk of these diseases.

Conclusion

Given the rising life expectancy and the projected global increase in the population of postmenopausal women,[9] a better understanding of the health risks associated with different ages at menopause is essential for improving health assessments and enhancing quality of life for women worldwide. Specifically, careful monitoring of women with later ANM for asthma and those with earlier ANM for RA can support early detection and management of these diseases. Individually tailored monitoring of women based on ANM may reduce misdiagnosis and improve disease outcomes, particularly in the case of RA, where early detection is crucial for slowing disease progression.

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Appendix A

Appendix A1. copyright/license, funding, data use, conflict of interest, and acknowledgements for Chapter 2

This chapter is reproduced from the following open access article published under a Creative Commons Attribution-NonCommercial-NoDerivatives License (CC BY-NC-ND):

Kesibi, D., Rotondi, M., Edgell, H., et al. (2024). The association between age at natural menopause and risk of asthma among postmenopausal women from the Canadian Longitudinal Study on Aging. *Menopause.* https://doi.org/xxxxx

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Data are available from the CLSA (www.clsa-elcv.ca) for researchers who meet the criteria for access to deidentified CLSA data.

Appendix A2. copyright/license, funding, data use, conflict of interest, and acknowledgements for Chapter 3

This chapter is adapted from the following open access article published under a Creative Commons Attribution License (CC BY 4.0):

Kesibi, D., Rotondi, M., Edgell, H., & Tamim, H. (2025). Associations between age at natural menopause and risk of hypothyroidism among postmenopausal women from the Canadian Longitudinal Study on Aging (CLSA). *PLoS ONE, 20*(5), e0324635. https://doi.org/10.1371/journal.pone.0324635

authors and source are credited. This chapter includes minor adaptations such as formatting and table renumbering.

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Competing interests: The authors have declared that no competing interests exist.

“This research has been conducted using the CLSA Baseline Tracking Dataset Version 4.0, Baseline Comprehensive Dataset Version 7.0, Follow-up 1 Tracking Dataset Version 2.3, Follow-up 1 Comprehensive Dataset Version 3.2, Follow-up 2 Tracking Dataset Version 1.0, Follow-up 2 Comprehensive Dataset Version 1.0 and participant status version 3.0, under Application Number 2206014.”

‘The opinions expressed in this manuscript are the author's own and do not reflect the views of the Canadian Longitudinal Study on Aging.’

Appendix A3. copyright/license, funding, data use, conflict of interest, and acknowledgements for Chapter 4

Kesibi, D., Rotondi, M., Edgell, H., & Tamim, H. (2025). A cohort study on the associations between age at natural menopause and rheumatoid arthritis in postmenopausal women from the Canadian Longitudinal Study on Aging. *Seminars in Arthritis and Rheumatism, 73*, 152747. https://doi.org/10.1016/j.semarthrit.2025.152747

‘The opinions expressed in this manuscript are the author's own and do not reflect the views of the Canadian Longitudinal Study on Aging.’

Data are available from the Canadian Longitudinal Study on Aging (www.clsa-elcv.ca) for researchers who meet the criteria for access to de-identified CLSA data.

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Appendix B – Cumulative Incidence Curves

This appendix presents cumulative incidence curves for asthma, hypothyroidism, and rheumatoid arthritis by age at natural menopause group. These plots complement the Kaplan–Meier survival curves presented in the main text and illustrate the cumulative probability of disease occurrence over the follow-up period for each outcome.

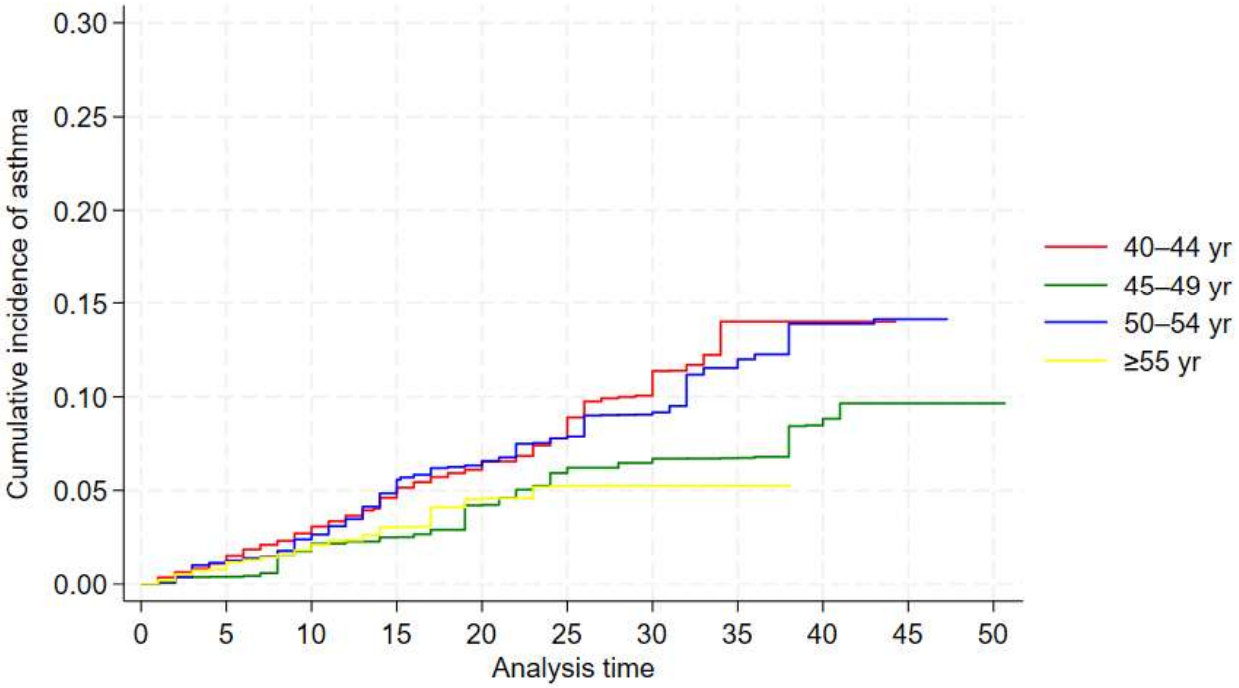

FIG. 10: Cumulative incidence of asthma over time by ANM

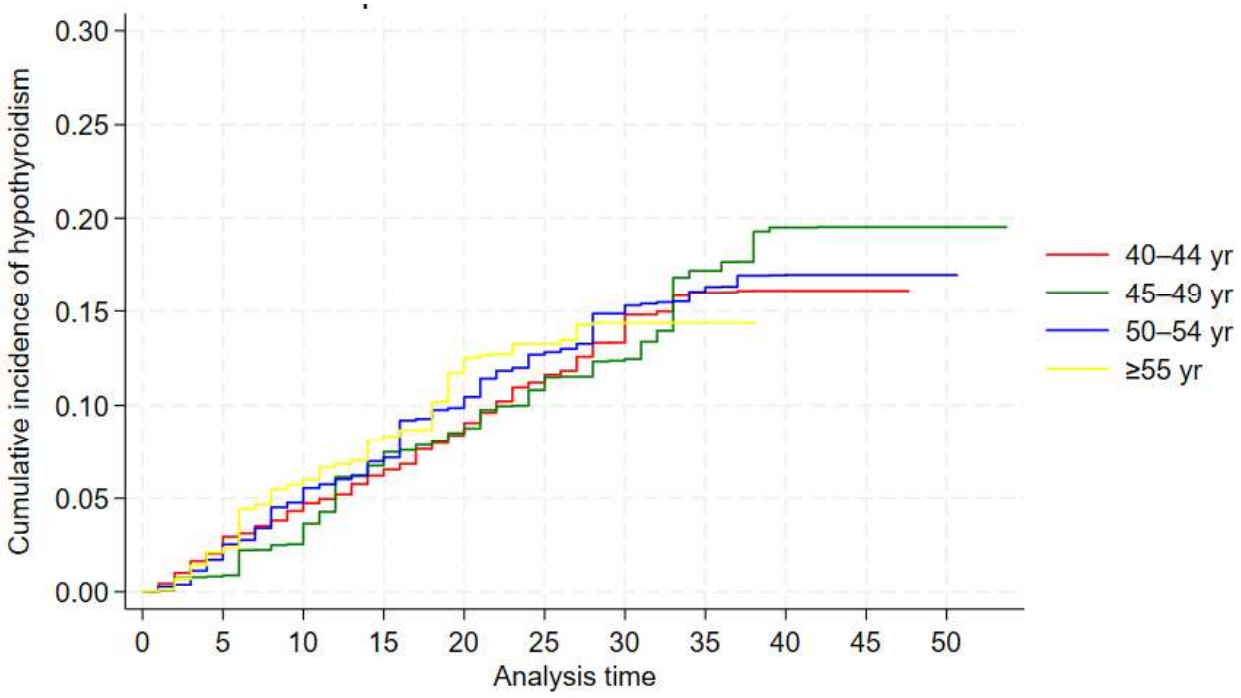

FIG. 11: Cumulative incidence of hypothyroidism over time by ANM

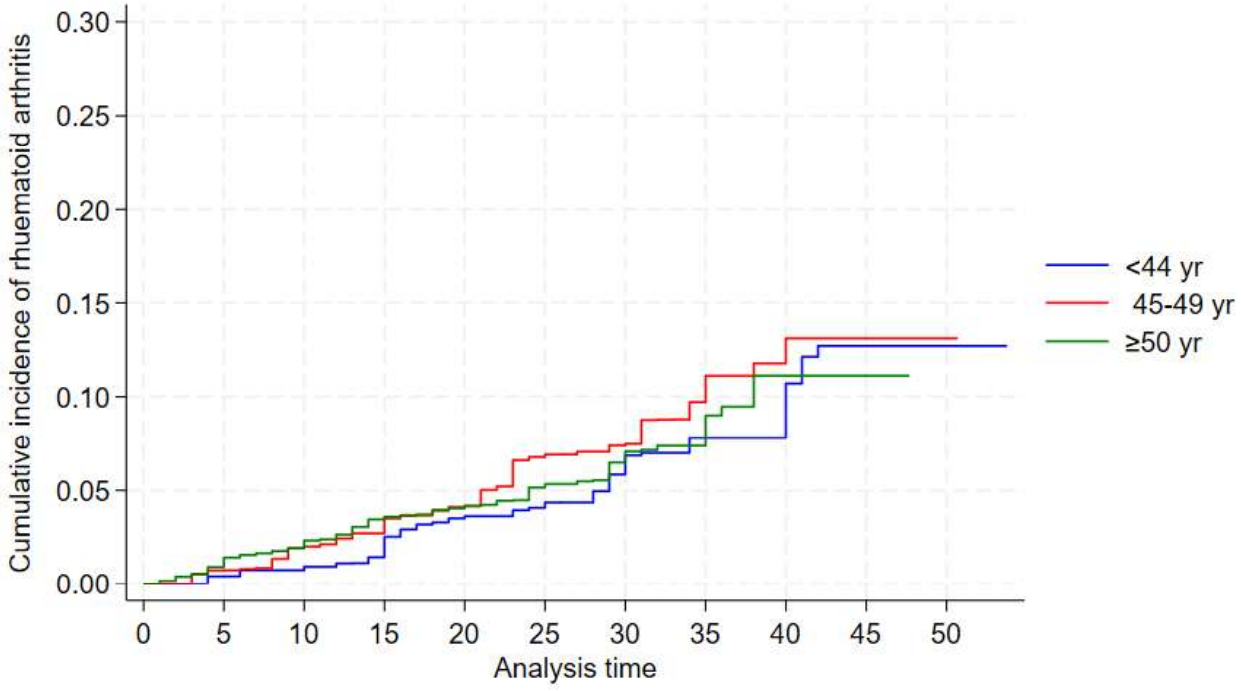

FIG. 12: Cumulative incidence of Rheumatoid arthritis over time by ANM