# ASSOCIATION BETWEEN HORMONE REPLACEMENT THERAPY (HRT) USE AND RADIOGRAPHIC EMPHYSEMA RISK

by

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Abstract

Emphysema, a form of chronic obstructive pulmonary disease (COPD) is a disease of significant public health importance that remains a major cause of morbidity and mortality within the US population and elsewhere in the industrialized world. An emerging body of literature appears to suggest gender differences in susceptibility to emphysema, with women showing significantly less emphysematous changes on high resolution Computed Tomography radiography compared to men. A pertinent but yet to be addressed question in the literature however, is whether these apparent differences in emphysematous structural lung disease result from differential exposures and or lifestyle choices on the one hand, or if on the other hand, these gender differences in CT-emphysema are related to certain biological mechanisms such as female hormonal influences or genetic differences in toxin metabolism.

The present study evaluated differences in CT-emphysema status among 1834 women with different hormone replacement therapy (HRT) exposure profiles. In an unadjusted generalized logistic regression model, current use of HRT was statistically significantly associated with a 38% reduction in the risk of moderate to severe CT-emphysema, OR 0.62 (95% CI=0.39-0.99). After adjusting for the effects of age and smoking dose intensity, current HRT-users had a 34% reduction in the risk of mild to moderate CT-emphysema compared to

never-users. Although this effect is quite sizable, it was marginally statistically insignificant, OR=0.66 (95% CI=0.41-1.07). We conclude that current use of hormone replacement therapy may be associated with a lower risk of moderate to severe CT-emphysema.

# TABLE OF CONTENTS

## LIST OF TABLES

## 1.0 INTRODUCTION AND STATEMENT OF PUBLIC HEALTH SIGNIFICANCE

Emphysema is a lower respiratory disease defined as an abnormal enlargement of air spaces distal to the terminal bronchioles and destruction of alveolar walls[1,2]. As a distinct clinical entity, the onset of emphysema is often insidious but in the long term, manifests as airflow limitation that is not fully reversible. Other common clinical manifestations in the course of the natural history of the disease include breathlessness, wheezing, and cough, generally progressing over a number of years to a disabling impairment of physical and psychological functioning[1]. Without adequate treatment, death from emphysema often results from respiratory and or other systemic complications[2,3].

As a disease of public health importance, emphysema which often coexists with chronic bronchitis is generally considered in most epidemiological studies under the composite term chronic obstructive pulmonary disease (COPD) which includes both diseases[3]. Tobacco smoking is the strongest known risk factor for the diseases, although some studies have implicated genetic factors, certain occupations and advancing age in a relatively small minority of cases[1,3]. As such emphysema is considered a largely preventable disease. Because it is a silent disease especially at the early stages, the population prevalence of COPD is difficult to estimate[3]. Most cases become apparent only when moderate to severe respiratory problems resulting from the disease cause individuals with the condition to seek care. Even when typical clinical symptoms and signs are present, the diagnosis of emphysema tends to be difficult, given that until recently, a

definitive diagnosis of the disease could only be made through lung biopsies, further worsening case ascertainment.

In spite of the difficulties with case identification, emphysema remains a disease with a substantial socio-economic, morbidity and mortality profile. Annual healthcare expenditure on emphysema is estimated at more than $2.5 billion in the US alone. This disease also causes or contributes to 100,000 deaths in the U.S. annually[4]. Emphysema and other COPD forms also exert a significant burden of disability, second only to coronary artery disease as the commonest cause of disability in the United States. Similarly, data from the third National Health and Nutrition Examination Survey (NHANES-III) suggest that about 7% of individuals aged 25-70 years in the United States currently live with significant airflow limitation. Notably, whereas COPD-related mortality has shown a decline among US men, it has increased 150% among women over the last twenty years, reflecting likely changes in smoking behavior among the female population[5].

## 1.1 GENDER DIFFERENCES IN CT-EMPHYSEMA RISK

Recent advancements in computed tomography imaging technology have led to an increasingly useful application of Computed Tomography (CT) radiography in emphysema diagnosis, shedding more light on structural involvement of lung tissues in emphysema while eliminating often-associated lung tissue biopsy complications. An emerging body of pulmonary literature appears to show significant differences in the prevalence and patterns of CT-emphysema on the basis of gender. In a multichannel helical CT scan evaluation of 396 patients enrolled in the National Lung Screening Trial (NLST), Dransfeild et al concluded that at all stages of COPD

male participants showed more extensive emphysema damage compared to females[6], suggesting a possible emphysema protective effect for females in the study. Similarly, in a study of 1053 patients on the National Emphysema Treatment Trial, Martinez at al demonstrated using high resolution computed tomography imaging, that women with advanced COPD had significantly less emphysematous changes but more extensive airway thickening compared to men, even at similar levels of $FEV_1$[7]. In a recent report on 688 patients on the International COPD Genetics Network (ICGN), Camp and his colleagues reported similarly increased emphysema damage among male participants in the study, adding that, while female participants tended to have increased airway damage relative to emphysema, there were no significant differences in airway modification between men and women in the study[8]. Studies in populations with no spirometric evidence of COPD have yielded similar results. In a sample of 957 smokers with no detectable airflow obstruction, Sverzellati et al[9] found that compared to males, females in the study exhibited less extensive emphysematous damage on CT. They added that females were more likely to show worsening CT emphysema with age which correlated significantly with FEV1 values.

## 1.2 GENDER DIFFERENCES IN EMPHYSEMA: EVALUATING THE POSSIBLE ROLE OF FEMALE REPRODUCTIVE HORMONES

A growing body of evidence currently points to decreased prevalence and severity of emphysematous structural lung damage on CT-scan among women relative to men. Why this differential risk exists remains to be substantiated. A number of hypotheses have been put forward by commentators[7,10] as probable but yet to be tested explanations for these observations.

They include the possibility that these findings may in fact be a reflection of the normal anatomical differences between adult male and female lungs, or the effect of environmental/socio-cultural exposures, genes, hormones or an interaction between any number of factors. Given that the differences in emphysematous structural lung damage continue to exist on CT even after statistically adjusting for indices of tobacco smoking exposures, age and body weight[9] it is conceivable that differences in environmental exposures may not be sufficient to explain these CT findings.

A line of inquiry focusing on female reproductive hormonal exposure and CT-radiographic outcome may be relevant in the light of current knowledge regarding the role of estrogen in lung development, growth, maturation and response to inflammatory stimuli across the lifespan. Early in-utero studies in animal models have demonstrated an earlier maturation of the lung alveoli in female fetuses relative to male fetuses[11,12], possibly explaining findings of increased risk of respiratory distress syndrome in male newborns relative to female newborns. Subsequent research studies in animal models established that while high androgens levels was associated with delayed fetal lung maturation[13], administration of estrogens to pregnant females accelerated production of surfactants in their fetuses leading to earlier fetal lung alveolar cell maturation, irrespective of the gender of those fetuses[14,15]. Trotter et al further demonstrated the in-utero effect of estrogen on lung development, when they showed that prenatal deprivation of estrogen led to impairment of alveolar development in newborn piglets[16].

Estrogen has also been shown to play a role in the observed gender differences in alveolar cell size and numbers in pubescent rats. Pubescent female rat lungs have smaller and more numerous lung alveoli – ideal for gaseous exchange - compared to age-matched male rats.

Massaro et al demonstrated that rats ovariectomized on the 21st day of life had larger - and less efficient - alveoli as well as smaller alveolar surface areas after 5-6 weeks compared to sham-ovariectomized rats[17]. They also showed that estrogen was required in the maintenance of alveolar cells and more importantly, that the administration of exogenous estrogen induced alveolar cell regeneration in ovariectomized mice following alveolar cell loss[18,19].

The role of estrogen in alveolar cell response to inflammation has also been documented. Speyer et al have demonstrated the inflammation-suppressing actions of estrogen in rat lung models[20]. More recently, Vegeto and her co-workers have put forward a hypothesis designating the estrogen receptor alpha (ERα) subtype a potential target for anti-inflammatory drugs, after they demonstrated its role in the reduction of lung inflammatory response to noxious agents[21]. This finding was further buttressed recently by the findings of Chen et al which showed that lungs of rat offspring were hypoplastic and structurally deficient when exposed to in-utero nitrofen injury, but nonetheless well developed, if their pregnant mothers received exogenous estrogen after the initial nitrofen insult[22].

Taken together, studies in animal models strongly suggest that exposure to estrogen at various life stages may confer beneficial effects expressed as earlier lung development, increased alveolar cell numbers, accelerated growth and maturation, protection of alveolar tissues from noxious exposures, as well as alveolar cellular regeneration following lethal or near-lethal insults. At present, it is not clear if these effects apply to human lungs and alveolar cells. If they do, then it may help explain the findings of decreased radiographic emphysema severity in females compared to males and the accelerated decline in alveolar integrity with advancing age among post-menopausal females noted in some studies[9].

This present study will therefore aim to understand if exposure to female reproductive hormones, specifically, use of hormone replacement therapy has any effect on t he risk of emphysema among female participants in a lung cancer screening study.

# 2.0 STUDY POPULATION, EXPOSURE AND OUTCOME VARIABLES

## 2.1 STUDY POPULATION

Participants in this study are females enrolled in the Pittsburgh Lung Screening Study (PLuSS) cohort. Conceived and conducted as one of the sub-projects of the University of Pittsburgh's Lung Cancer Specialized Program of Research Excellence (SPORE), the PLuSS is a community-based lung cancer screening study using low dose multi-detector helical CT. To be eligible, participants had to meet the following selection criteria: Age between 50-79 years at the time of entry into the study, be a current or former smoker with at least a 25 year history of smoking of at least half a pack of cigarettes a day on the average, if quit, has not been quit for more than 10 years, no history of chest CT scans in the previous 12 months, not a participant in another ongoing cancer screening study, body weight less than 400 pounds and must have signed an informed consent form.

A total of 3755 individuals who met the above eligibility criteria were recruited into the study. Gender distribution in the study was fairly balanced with 1836 women (~49%) and 1919 men (~51%). In addition to the multi-detector CT scan, participants also received spirometric Pulmonary Function Tests (PFTs), donated biological tissue samples, including saliva, sputum and blood, and filled out detailed questionnaires on their smoking behavior, cancer history and other factors of interest. Of particular importance to this study, female participants also filled out

a reproductive history questionnaire that detailed such variables as age at menarche, menopause status and exogenous hormone use. Further details of subject demographics, study design and methodology have been published elsewhere[23,24]. Participant follow up in the PLuSS is currently ongoing.

## 2.2 EXPOSURE

### 2.2.1 MEASURES OF EXPOSURE

Exposure to hormone replacement therapy is the major exposure variable in this study. Specific measures of exposure include the following: HRT-use classed as (1) never-use, (2) ever-use and (3) current use. Other measures of exposure include HRT use duration (among ever and never users) and duration of current use class (less than 1 year, 2-3 years, 4-5 years, 6-9 years and greater than 10 years).

### 2.2.2 DETERMINANTS OF EXPOSURE - HORMONE REPLACEMENT THERAPY

Of the 1836 women in the PLuSS, two participants with missing information on hor mone replacement therapy (HRT) status were excluded leaving a total of 1834 women eligible for analysis. Of this number of women with HRT-use history, 611 (33.3%) self-identified as never-users, 755 (41.1%) self-identified as former-users, while 468 (25.5%) reported current use. In order to gain a better understanding of the distribution of participants in these classes of HRT-use, we used a frequency procedure and chi-square statistics to evaluate the relationship between

certain important predictor variables and HRT status. These include age at completion of the baseline questionnaire, smoking intensity (cigarettes per day) and physician diagnosed emphysema (MD-Emphysema).

The entire study population distributed according to age in the following manner: 30.5% 50-54 years, 28.6% 55-59 years, 20.4% 60-64 years and 20.5% 65 years and older. There was a significant relationship between age and HRT status ($p<0.0001$). The never-HRT category contained a relatively higher proportion (38.8%) of women in the youngest (50-54 years) age group and relatively lower proportions of women (21.9%, 15.4% and 23.9%) in the intermediate (55-59 and 60-64 years) and older (65 years and older) age groups respectively. The current user group contained a relatively higher proportion of women, 31.8% and 30.3% in the 50-54 years and 55-59 years age groups categories respectively, with lower proportions, 22.2% and 15.6% were in the 60-64 years and 65 years and older age groups respectively. This may be due to the fact that while a relatively larger proportion of younger women may not have reached menopause (and therefore have no menopausal symptoms requiring treatment), older women are more likely to have transitioned through menopause and thus not likely to require current hormonal treatment.

A significant relationship was also demonstrated between age group category and duration of use among ever-HRT users ($p<0.0001$). Among ever HRT users of one year duration or less, the proportion of women in the younger (50-54) age group was 39.7% which is higher than the proportion of 50-54 year-old women (10.2%) in the 10+ year ever HRT use category. Also, only 13.1% of ever-users of ≤1 year duration fell in the oldest (65+ year) age group, while

a higher proportion, 30.5% of ever users greater than or equal to 10 years were women in the oldest (65 years and older) age group category. This association may be explained by the fact that older women are more likely to have had the onset of menopause for much longer than younger women. A similar distribution pattern is shown in the current-user table. The current-user less than one year category, was entirely composed of women 50-54 years (69%) and 55-59 years (31%), while the current users of ten years or more were distributed in this manner; 50-54 year old group 13.7%, 55-59 year old group 22.6%, 60-64 year old group (36.8%) and 65 years and older 26.9%. (Please see Table 1.)

There was no relationship demonstrated between either smoking intensity or physician-diagnosed emphysema and HRT status ($p=0.88$ and 0.07 respectively). However, it is worthy of note that whereas the prevalence of physician diagnosed emphysema is 6.6% among all current HRT users, the prevalence was about 50% higher among never users and former users (9.3% and 10.5% respectively). This observation may suggest an inverse association between current HRT use and physician diagnosed emphysema but this relationship may possibly be confounded by a number of other variables.

**Table 1: Association between selected characteristics and hormone replacement therapy history**

| | | | HRT status | | | | Ever users, by duration of use in years | | | | | | Current user, by duration of use in years | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Characteristic | | ALL | Never | Former | Current | p-value | <=1 | 2-3 | 4-5 | 6-9 | 10+ | p-value | <=1 | 2-3 | 4-5 | 6-9 | 10+ | p-value |
| ALL | N | 1834 | 611 | 755 | 468 | | 237 | 195 | 157 | 222 | 410 | | 29 | 71 | 55 | 101 | 212 | |
| AgeGroup (years) | | | | | | <0.0001 | | | | | | <0.0001 | | | | | | <0.0001 |
| 50-54 | % | 30.5 | 38.8 | 22.9 | 31.8 | | 39.7 | 39.5 | 31.8 | 26.1 | 10.2 | | 69.0 | 63.4 | 43.6 | 30.7 | 13.7 | |
| 55-59 | % | 28.6 | 21.9 | 33.0 | 30.3 | | 34.2 | 33.3 | 38.2 | 42.8 | 22.0 | | 31.0 | 22.5 | 45.5 | 43.6 | 22.6 | |
| 60-64 | % | 20.4 | 15.4 | 23.3 | 22.2 | | 13.1 | 12.8 | 17.8 | 18.9 | 37.3 | | 0.0 | 9.9 | 5.5 | 15.8 | 36.8 | |
| 65+ | % | 20.5 | 23.9 | 20.8 | 15.6 | | 13.1 | 14.4 | 12.1 | 12.2 | 30.5 | | 0.0 | 4.2 | 5.5 | 9.9 | 26.9 | |
| Cigarettes per day | | | | | | 0.8787 | | | | | | 0.7516 | | | | | | 0.8288 |
| 1 to 9 | % | 3.7 | 4.6 | 2.9 | 3.8 | | 3.0 | 3.1 | 1.3 | 3.6 | 4.1 | | 3.4 | 4.2 | 0.0 | 3.0 | 5.2 | |
| 10 to 19 | % | 33.6 | 32.6 | 33.8 | 34.6 | | 34.2 | 36.4 | 33.1 | 30.6 | 35.1 | | 37.9 | 38.0 | 36.4 | 28.7 | 35.4 | |
| 20 to 29 | % | 43.8 | 43.5 | 44.1 | 43.6 | | 42.6 | 45.1 | 48.4 | 45.9 | 41.5 | | 44.8 | 46.5 | 45.5 | 47.5 | 40.1 | |
| 30 to 39 | % | 13.2 | 13.6 | 13.6 | 12.0 | | 13.5 | 12.8 | 10.2 | 14.4 | 12.9 | | 6.9 | 9.9 | 10.9 | 14.9 | 12.3 | |
| 40 or more | % | 5.7 | 5.7 | 5.6 | 6.0 | | 6.8 | 2.6 | 7.0 | 5.4 | 6.3 | | 6.9 | 1.4 | 7.3 | 5.9 | 7.1 | |
| MD-Emphysema | | | | | | 0.0742 | | | | | | 0.5811 | | | | | | 0.7808 |
| no | % | 90.9 | 90.7 | 89.5 | 93.4 | | 89.9 | 89.2 | 92.4 | 93.2 | 90.7 | | 93.1 | 93.0 | 96.4 | 91.1 | 93.9 | |
| yes | % | 9.1 | 9.3 | 10.5 | 6.6 | | 10.1 | 10.8 | 7.6 | 6.8 | 9.3 | | 6.9 | 7.0 | 3.6 | 8.9 | 6.1 | |

## 2.3 OUTCOME

### 2.3.1 MEASURES OF OUTCOME

Measurement of CT-emphysema outcomes was based on the standards developed by the National Emphysema Treatment Trial criteria. This is essentially a five-level semi-quantitative CT image scoring system graded as (1) no, (2) trace, (3) mild, (4) moderate, and (5) severe emphysema. The trace, mild, moderate and severe categories roughly correspond to emphysema affecting less than 10, 1 0–25, 25–50%, and greater than 50% of the lung, respectively. This grading system was applied in the scoring of participants' CT scans by three readers including two radiologists and a pulmonologist.

### 2.3.2 DETERMINANTS OF CT-EMPHYSEMA SCORE – DEMOGRAPHIC VARIABLES

Of the 1834 participants with HRT use information, 66 had no baseline CT-Emphysema score and were excluded, leaving 1768 women eligible for the final analysis. Owing to the sparse data in the moderate and severe CT-Emphysema score groups, both groups were merged to form the "greater than mild" group. Of the 1768 w omen not missing CT-emphysema scores, 1070 (60.5%) had no emphysema on their baseline CT scan, while 306 (17.3%), 233 (13.2%) and 159 (8.9%) had trace, mild and greater than mild emphysema on their CT scan images. The relationship between and CT-Emphysema scores and various demographic variables including

age, educational status, BMI and history of physician diagnosed emphysema were evaluated using simple chi-square statistic.

The analysis (See Table 2 be low) demonstrated that increasing age was significantly associated with CT-emphysema ($p<0.0001$). The entire 1768 w omen in the study were age distributed as follows: 30% 50-54 years, 28.5% 55-59 years, 20.6% 60-64 years and 20.9% 65 years and over. Among women with no CT-emphysema, a relatively higher proportion (32.6%) was aged 50-54 years whereas lower proportions (30.9%, 18.8% and 17.7%) were in the 55-59 years, 60-64 years and 65 years and over age groups. In the moderate to severe emphysema, group, only 18.2% fell in the 50-54 age group while 36.5% fell in the the oldest (65 years and over) age group. This suggests that increasing age was associated with the presence of CT-emphysema.

Body mass index (BMI) also showed a strong association with CT-emphysema ($p<0.0001$). Among women with moderate to severe emphysema, a higher proportion, 57.6% belonged to the lower BMI (16-24.99) category with lower proportions 31.0% and 11.4%, belonging to the overweight and obese categories respectively. This contrasts with the no-emphysema group where only 24.6% belonged to the 1ower BMI category while higher proportions, 36.3% and 39.0 belonged to the overweight and obese categories respectively.

A statistically significant relationship was also established between CT-emphysema and physician diagnosed emphysema ($p<0.0001$). It is important to note that the prevalence of physician diagnosed emphysema was six-fold more common (31.4%) among the mild to

moderate CT-emphysema group compared to the no CT-emphysema. There was no association between educational attainment and CT-emphysema (p=0.15).

**Table 2: Association between selected risk factors and CT emphysema**

**(missing observations shown in parenthesis)**

| | | | CT Emphysema | | | | |
|---|---|---|---|---|---|---|---|
| Characteristic | | ALL | None | Trace | Mild | >Mild | p-value |
| ALL | N | 1768 | 1070 | 306 | 233 | 159 | |
| Age | | | | | | | <.0001 |
| 50-54 years | % | 30.0 | 32.6 | 32.7 | 22.3 | 18.2 | |
| 55-59 years | % | 28.5 | 30.9 | 27.1 | 24.9 | 19.5 | |
| 60-64 years | % | 20.6 | 18.8 | 21.9 | 24.0 | 25.8 | |
| 65 years and above | % | 20.9 | 17.7 | 18.3 | 28.8 | 36.5 | |
| Education | | (1) | (1) | | | | 0.15 |
| High School or Less | % | 27.4 | 26.0 | 27.1 | 30.5 | 32.7 | |
| Post High School | % | 41.9 | 42.4 | 38.6 | 44.2 | 41.5 | |
| College or Postgraduate | % | 30.7 | 31.6 | 34.3 | 25.3 | 25.8 | |
| BMI ($kg/m^2$) | | (4) | (2) | (1) | | (1) | <.0001 |
| 16 - 24.99 | % | 31.9 | 24.6 | 38.0 | 39.5 | 57.6 | |
| 25-29.99 | % | 36.6 | 36.3 | 35.7 | 42.9 | 31.0 | |
| 30 or Greater | % | 31.5 | 39.0 | 26.2 | 17.6 | 11.4 | |
| History of emphysema | | | | | | | <.0001 |
| no | % | 90.8 | 94.8 | 92.5 | 85.8 | 68.6 | |
| yes | % | 9.2 | 5.2 | 7.5 | 14.2 | 31.4 | |

### 2.3.3 DETERMINANTS OF CT-EMPHYSEMA SCORE - SMOKING RELATED FACTORS

As shown in the table below, most measures of tobacco smoking including average number of cigarettes smoked per day, number of pack years smoked, smoking duration, and type of cigarette smoked all showed strong statistical relationships with CT emphysema ($p<.0001$). Current smoking was also associated with CT-emphysema class ($p=0.0072$). An interesting observation is that the prevalence of quitting (current smoking = no) appears similar for women with no CT-emphysema (39.6%) and those with moderate to severe emphysema (39.0%). It is possible that the higher than expected prevalence of quitting among those with moderate to severe emphysema may be explained by possible increased severity of emphysema symptoms among these women that may have prompted them to quit. Age at onset of regular tobacco smoking showed no relationship with CT-emphysema class (0.3884).

**Table 3: Association between smoking-related factors and CT-Emphysema**

| | | | CT Emphysema | | | | |
|---|---|---|---|---|---|---|---|
| Characteristic | | ALL | None | Trace | Mild | >Mild | p-value |
| ALL | N | 1768 | 1070 | 306 | 233 | 159 | |
| Current smoking status | | | | | | | 0.0072 |
| Yes | % | 63.1 | 60.4 | 69.9 | 67.8 | 61.0 | |
| No | % | 36.9 | 39.6 | 30.1 | 32.2 | 39.0 | |
| Cigarettes per day | | | | | | | <.0001 |
| 1 to 9 cigarettes/day | % | 3.6 | 4.8 | 2.3 | 2.1 | 0.6 | |
| 10 to 19 cigarettes/day | % | 33.3 | 36.2 | 33.3 | 28.8 | 20.8 | |
| 20 to 29 cigarettes/day | % | 44.0 | 42.3 | 44.8 | 45.5 | 51.6 | |
| 30 to 39 cigarettes/day | % | 13.3 | 11.0 | 14.1 | 17.6 | 20.8 | |
| 40 or more cigarettes/day | % | 5.8 | 5.7 | 5.6 | 6.0 | 6.3 | |
| Pack-years | | | | | | | <.0001 |
| Less than 30 | % | 22.0 | 26.6 | 21.9 | 12.0 | 5.7 | |
| 30-39 | % | 20.1 | 21.1 | 19.3 | 19.3 | 15.7 | |
| 40-49 | % | 19.9 | 19.6 | 19.3 | 20.6 | 21.4 | |
| 50-59 | % | 16.7 | 15.2 | 16.3 | 20.2 | 22.0 | |
| 60 or more | % | 21.4 | 17.4 | 23.2 | 27.9 | 41.5 | |
| Smoking duration (yrs) | | | | | | | <.0001 |
| Less than 40 years | % | 60.8 | 65.4 | 62.4 | 49.4 | 43.4 | |
| 40 years or more | % | 39.2 | 34.6 | 37.6 | 50.6 | 56.6 | |
| Type of cigarette smoked | | | | | | | <.0001 |
| Filter cigarette | % | 92.0 | 94.0 | 90.2 | 90.6 | 83.6 | |
| Non-filter cigarette | % | 2.8 | 1.6 | 5.6 | 3.0 | 5.0 | |
| Both about equally | % | 5.3 | 4.4 | 4.2 | 6.4 | 11.3 | |
| Age started smoking | | | | | | | 0.3884 |
| Less than 16 years | % | 23.0 | 21.4 | 24.8 | 26.2 | 25.8 | |
| 16-20 years | % | 58.7 | 59.8 | 55.6 | 59.2 | 56.0 | |
| 21 years or over | % | 18.3 | 18.8 | 19.6 | 14.6 | 18.2 | |

### 2.3.4 DETERMINANTS OF CT-EMPHYSEMA SCORE – FACTORS RELATED TO REPRODUCTIVE HORMONE

As shown in the table below, most female reproductive hormone related-factors including age at onset of menarche, menopausal status, age at menopause, age at first pregnancy and number of pregnancies all showed no statistical relationship with CT-emphysema. However, use of oral contraceptive pills (OCP) showed a weak statistical relationship with CT-emphysema ($p=0.053$) while age at onset of oral contraceptive use among ever OCP users showed a strong relationship with CT-emphysema ($p<.0001$). Among 1240 participants with non-missing history of OCP use, the proportion of those who reported starting OCP use at age less than 20 years was 20.5%, 67.9% reported starting OCP use at age 22-29 years while 11.6% reported starting OCP use aged 30 years or more. Among women with moderate to severe CT-emphysema in the study, a lower proportion (10.2%) started OCP use at age less than 20 years, while a higher proportion started OCP use at thirty years or later (27.8%). This suggests that earlier age at onset of OCP use may be associated with a reduced risk of moderate to severe CT-emphysema, although this relationship may be confounded by other variables including age.

**Table 4: Association between reproductive-related factors and CT emphysema**

**(missing observations shown in parenthesis)**

| | | | CT Emphysema | | | | |
|---|---|---|---|---|---|---|---|
| Characteristic | | ALL | None | Trace | Mild | >Mild | p-value |
| ALL | N | 1768 | 1070 | 306 | 233 | 159 | |
| Age at menarche | | (2) | (1) | (1) | | | 0.0607 |
| Less than 12 years | % | 22.7 | 23.7 | 18.7 | 24.6 | 20.8 | |
| 12-13 years | % | 54.6 | 53.4 | 59.7 | 55.6 | 51.6 | |
| 14-15 years | % | 18.4 | 19.5 | 16.7 | 13.8 | 20.8 | |
| 16 years or older | % | 4.3 | 3.4 | 4.9 | 6.0 | 6.9 | |
| Menopausal | | | | | | | 0.0904 |
| Yes | % | 94.9 | 94.0 | 95.1 | 96.6 | 98.1 | |
| No | % | 5.1 | 6.0 | 4.9 | 3.4 | 1.9 | |
| Age at menopause* | | (8) | (4) | (2) | (1) | (1) | 0.1963 |
| Less than 40 years | % | 20.2 | 20.4 | 20.1 | 19.6 | 20.6 | |
| 40-44 years | % | 18.0 | 16.3 | 19.7 | 21.4 | 20.6 | |
| 45-49 years | % | 29.8 | 29.2 | 30.8 | 31.3 | 29.0 | |
| 50-54 years | % | 28.0 | 30.8 | 24.6 | 21.9 | 25.2 | |
| 55 years or older | % | 4.0 | 3.3 | 4.8 | 5.8 | 4.5 | |
| Ever pregnant | | | | | | | 0.3569 |
| No | % | 14.0 | 14.4 | 16.0 | 11.6 | 11.3 | |
| Yes | % | 86.0 | 85.6 | 84.0 | 88.4 | 88.7 | |
| Age first pregnant** | | (2) | | (1) | (1) | | 0.7280 |
| Less than 19 years | % | 28.1 | 27.7 | 27.3 | 27.3 | 32.6 | |
| 20-24 years | % | 43.1 | 42.9 | 45.7 | 41.0 | 43.3 | |
| 25-29 years | % | 20.7 | 21.8 | 18.0 | 21.5 | 17.0 | |
| 30-34 years | % | 5.9 | 5.2 | 7.0 | 6.8 | 6.4 | |
| 35-44 years | % | 2.2 | 2.3 | 2.0 | 3.4 | 0.7 | |
| Number of pregnancies | | (2) | | (1) | (1) | | 0.2664 |
| 0 | % | 14.0 | 14.4 | 16.1 | 11.6 | 11.3 | |
| 1 to 2 | % | 34.9 | 36.6 | 30.8 | 33.6 | 33.3 | |
| 3 to 4 | % | 34.8 | 34.6 | 34.8 | 34.9 | 36.5 | |
| 5 or more | % | 16.2 | 14.4 | 18.4 | 19.8 | 18.9 | |
| OCP Use | | (4) | (1) | (1) | (1) | (1) | 0.053 |
| None | % | 29.9 | 28.4 | 27.5 | 38.4 | 32.3 | |
| One year or less | % | 16.8 | 16.7 | 21.0 | 12.1 | 17.1 | |
| 2-9 years | % | 39.2 | 40.8 | 37.7 | 34.1 | 38.6 | |
| 10 years or more | % | 14.1 | 14.1 | 13.8 | 15.5 | 12.0 | |
| Age OCP use started*** | | | | | | | <.0001 |
| Less than 20 years | % | 18.9 | 20.5 | 22.0 | 12.2 | 10.2 | |
| 20-29 years | % | 67.7 | 67.9 | 67.9 | 70.3 | 62.0 | |
| 30 years or more | % | 13.5 | 11.6 | 10.1 | 17.6 | 27.8 | |

*Analysis restricted to menopausal participants
**Analysis restricted to ever-pregnant participants
***Analysis restricted to ever-OCP use

## 3.0 HORMONE REPLACEMENT THERAPY AND CT-EMPHYSEMA

In order to understand the relationship between hormone replacement therapy and CT-emphysema, a frequency procedure with a simple chi-square test was conducted to assess the relationship between the two variables. Globally, there was no statistically significant relationship between CT-emphysema class and hormone replacement therapy status (p=0.43). As earlier stated and shown in the table 5 below, 66 women with no baseline CT were excluded from the final analysis. The rest of the study group was distributed according to CT-Emphysema status in the following manner: 60.5% no CT-emphysema, 17.3% trace, 13.2% mild and 9.0% moderate to severe CT-emphysema. Of note, the prevalence of moderate to severe CT-emphysema was about 60% higher (10.2%) among never HRT-users compared to the prevalence among current HRT-users (6.4%).

There were no statistically significant relationships between CT-emphysema and duration of use among ever-users (p=0.38) or current users (p=0.28) respectively.

**Table 5: Association between measures of hormone replacement therapy exposure and CT emphysema**

**(missing observations shown in parenthesis)**

| | | | HRT status | | | | Ever users, by duration of use in years | | | | | | Current user, by duration of use in years | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Characteristic | | ALL | Never | Former | Current | p-value | <=1 | 2-3 | 4-5 | 6-9 | 10+ | p-value | <=1 | 2-3 | 4-5 | 6-9 | 10+ | p-value |
| ALL | N | 1834 | 611 | 755 | 468 | | 237 | 195 | 157 | 222 | 410 | | 29 | 71 | 55 | 101 | 212 | |
| CT emphysema | | (66) | (32) | (22) | (12) | 0.43 | (11) | (5) | (4) | (6) | (8) | 0.38 | (2) | (1) | (0) | (3) | (6) | 0.28 |
| none | % | 60.5 | 60.3 | 60.6 | 60.7 | | 64.6 | 60.5 | 66.0 | 55.6 | 59.2 | | 66.7 | 60.0 | 74.5 | 51.0 | 61.2 | |
| trace | % | 17.3 | 17.8 | 16.0 | 18.9 | | 15.0 | 14.2 | 19.6 | 20.4 | 16.7 | | 18.5 | 17.1 | 18.2 | 25.5 | 16.5 | |
| mild | % | 13.2 | 11.7 | 13.8 | 14.0 | | 11.9 | 16.3 | 9.8 | 15.3 | 14.7 | | 11.1 | 18.6 | 3.6 | 15.3 | 15.0 | |
| moderate or severe | % | 9.0 | 10.2 | 9.7 | 6.4 | | 8.4 | 8.9 | 4.6 | 8.8 | 9.5 | | 3.7 | 4.3 | 3.6 | 8.2 | 7.3 | |

# 4.0 ADJUSTED RELATIONSHIP BETWEEN HORMONE REPLACEMENT THERAPY AND CT-EMPHYSEMA

## 4.1 LOGISTIC REGRESSION MODEL

A generalized logistic regression analysis was conducted with CT-emphysema score as the outcome variable and HRT use as the main predictor variable. Age and tobacco smoking dose intensity were sequentially added to the model, thus controlling for these possible confounders. Each unadjusted odds ratios (OR1) compares the odds of CT-emphysema among former HRT users and current HRT users respectively to never users for each of the three CT-emphysema classes (trace, mild, greater than mild). These odds ratios were then sequentially adjusted for age (OR2) and smoking dose intensity (OR3). All statistical analyses were conducted using SAS (version 9.2, Cary North Carolina).

## 4.2 RESULTS

As shown in Table 6 below, there is no global statistically significant relationship detected between CT-emphysema and hormone replacement therapy status (p=0.27), although a closer review of the results appear very instructive. Compared to never-users, the unadjusted odds ratio (and 95% confidence interval) values for trace, mild and moderate to severe CT-emphysema

among former users of HRT, were as follows: 0.89 (0.66-1.20); 1.17 (0.83-1.64) and 0.95 (0.65-1.37) respectively. Sequential adjustments for age and smoking dose intensity did not significantly change these values (p=0.39 and 0.41 respectively). (See table 6).

There was a significant relationship between current HRT use and the unadjusted odds of mild to moderate CT-emphysema, but there was no association between current use and the odds of trace or mild CT-emphysema. For current HRT-users, the unadjusted odds ratio for moderate to severe CT-emphysema was about 40% lower compared to never users, OR=0.62 (95%CI=0.39-0.99). After adjusting for age and smoking intensity, the odds of moderate to severe CT-emphysema among current HRT-users was 34% lower among current HRT-users compared to non-users OR=0.66 (95%CI=0.41-1.07).

The unadjusted odds ratio (and 95% confidence limits) for trace and mild CT-emphysema for current users compared to never users were 1.05 ( 95% CI=0.76-1.46) and 1.19 (95% CI=0.89-1.73) respectively. After adjustments for age and smoking intensity, the odds ratio showed very little changes, OR trace CT-emphysema=1.06 (95% CI=0.76-1.47); OR mild CT-emphysema=1.23 (95% CI=0.89-1.80).

**Table 6: Association between hormone replacement therapy history and CT emphysema**

unadjusted (OR1, p-value=0.27), adjusted for age (OR2, p-value=0.39), and adjusted for age and cigarette dose intensity (OR3, p-value=0.41).

| | | CT emphysema | | | |
|---|---|---|---|---|---|
| HRT | | None | Trace | Mild | >Mild |
| Never | N | 349 | 103 | 68 | 59 |
| Former | N | 444 | 117 | 101 | 71 |
| | OR1 | | 0.89 | 1.17 | 0.95 |
| | 95% CI | | 0.66-1.20 | 0.83-1.64 | 0.65-1.37 |
| | OR2 | | 0.89 | 1.13 | 0.91 |
| | 95% CI | | 0.66-1.21 | 0.80-1.59 | 0.62-1.34 |
| | OR3 | | 0.89 | 1.13 | 0.92 |
| | 95% CI | | 0.66-1.21 | 0.80-1.60 | 0.63-1.35 |
| Current | N | 277 | 86 | 64 | 29 |
| | OR1 | | 1.05 | 1.19 | 0.62 |
| | 95% CI | | 0.76-1.46 | 0.81-1.73 | 0.39-0.99 |
| | OR2 | | 1.05 | 1.21 | 0.65 |
| | 95% CI | | 0.76-1.46 | 0.83-1.78 | 0.40-1.05 |
| | OR3 | | 1.06 | 1.23 | 0.66 |
| | 95% CI | | 0.76-1.47 | 0.84-1.80 | 0.41-1.07 |

Legend: OR1 – crude odds ratio, OR2 – age-adjusted odds ratio, OR3 – age- and cigarette dose intensity-adjusted odds ratio, CI – confidence interval

Notes: A ll odds ratio referenced to HRT never users without CT emphysema. Odds ratios adjusted by means of generalized logit models with age modeled as a four level categorical variable (50-54, 55-59, 60-64, and 65+ years) and cigarette dose intensity modeled as an ordinal variable (integer index assigned to cigarettes/day expressed as five ordered categories, 1-9, 10-19, 20-29, 30-39, 40+ cigarettes/day).

# 5.0 DISCUSSION AND LIMITATIONS

## 5.1 DISCUSSION

In this analysis, no global statistically significant relationship was established between hormone replacement therapy (HRT) use and CT-emphysema. There are two potentially valid conclusions to be drawn from the results of this analysis. The first, possibly demonstrated by the global statistical significance values above is that no r elationship exists between HRT use and CT-emphysema.

Alternatively, the study group may have been too small to detect a statistically significant inverse association between current HRT use and greater than mild CT-emphysema, a relationship suggested by the observed lower age- and smoking intensity-adjusted odds of greater than mild emphysema (vs. no emphysema, OR 0.66, 95% CI 0.41-1.07) in current HRT vs. never HRT users (Table 6). First, as shown in table 6, current use of HRT was associated with a large (38%) and statistically significant reduction in the unadjusted risk of moderate to severe emphysema. This large effect size persisted even after adjustments for age and smoking intensity, although statistically, the relationship became marginally insignificant. Given that the current HRT-users with moderate to severe CT-emphysema represent a very small proportion of the total sample size; 29 of the 1768 women (1.64%), it is possible that we did not have enough

power to detect a statistical significance, even in the presence of a very large effect size (35-38%). Finally, it is important to note that the large risk reduction demonstrated among current HRT-users may be clinically or radiographically significant and agrees with current knowledge regarding the effects of estrogen on alveolar cell development, growth, and regeneration in animal models[12,16,20-22].

## 5.2 LIMITATIONS

This study is a cross sectional study with some noteworthy limitations. First, the aphorism "association does not imply causation" rings true, given that a proof of causation (or the lack of it) as it applies to HRT use and CT-emphysema cannot be established by this study alone. Further research in this area is therefore encouraged

The second is that the exposure variables including age, indices of tobacco smoking behavior and reproductive history including HRT use exposure information are accurate to the extent that they are self-reports. It is possible that case classification or sub-classification may have been affected by the degree of accuracy of the provided self report information.

Third, determination of CT-emphysema outcome status was made using a semi-quantitative procedure. Given the element of subjectivity introduced in the CT-reading process, it is possible that discrepancies in CT reading and subsequent case allocation may have affected the classification of CT-emphysema. This uncertainty may be eliminated in future studies by using machines with fully quantitative and standardized CT-emphysema assessment system based on percent low attenuation areas (% LAA) scores.

Fourth, it is possible that our findings in this study may have been confounded by other variables not accounted for in the logistic regression model. These include other reproductive hormone variables, particularly use and duration of use of oral contraceptive pills (OCPs), as well as body mass index (BMI) which showed strong statistical relationships with CT-emphysema in univariate models (see tables 2 and 4). Future studies will explore the effects of these variables on CT-emphysema outcome.

In the future, further studies with larger sample sizes may help to firmly establish or disprove an association between HRT use and CT-emphysema.

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