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Effects of Reproductive Period Duration and Number of Pregnancies on Mid-Life Electrocardiographic Indices: A Secondary Analysis from the Women's Health Initiative Clinical Trial

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Abstract: (word count=259)

Objective: The effects of the hormonal milieu of pregnancy, menses and menopause on cardiac electrical conduction are uncertain. Therefore, we studied the association between number of pregnancies and reproductive period duration (RD, time from menarche to menopause) with electrocardiographic intervals in the Women's Health Initiative Clinical Trial.

Methods: We employed multivariable linear regression models relating number of pregnancies and RD with millisecond (ms) changes in PR interval, P wave indices (duration and dispersion) and QTc from enrollment electrocardiogram.

Results: Among 40,687 women (mean age=62 years), 5+ live births versus 0 prior pregnancies was associated with a 1.32 ms increase in PR interval [95% CI (0.25, 2.38)], with a graded association with longer QTc interval (ms) [none= 0.66 (-0.56, 1.88), 1= 0.15 (-0.71, 1.02), 2 to 4= 0.25 (-0.43, 0.94), and 5+ live births=1.15 (0.33, 1.98), p = 0.008]. RD was associated with longer PR interval and maximum P wave duration (but not P-wave dispersion) among never users of HT: [PR (ms) per additional RD year: 0.10 (0.04, 0.16); higher P-wave duration (ms): 0.09 (0.06, 0.12)]. For every year increase in reproductive period, QTc decreased by 0.04 ms (-0.07, -0.01).

Limitations: Potential misclassification of exposure due to participant recall

Conclusions: An increasing number of live births are related to increased ventricular repolarization time whereas RD is related to decreased ventricular repolarization time. Both longer RD and grandmultiparity are related to increased atrial conduction time. The premenopausal hormonal milieu appears to have effects on midlife cardiac electrical conduction system remodeling in women that may modestly influence CVD risk in later life.

Article Summary:

Strengths and Limitations of the Study.

- *A strength is the use of a well characterized multiethnic, large dataset of postmenopausal women representative of women in the United States.*
- *A notable limitation is that the exposure variables were acquired retrospectively.*
- *We were unable to adjust for pregnancy complications such as preeclampsia or gestational diabetes.*

What is already known about this subject?

Clinical studies with a relatively limited number of participants suggest that there are small but measureable changes in electrocardiographic intervals during pregnancy.

What does this study add?

We demonstrate for the first time in a large cohort of women with systematic research electrocardiograms that number of livebirths and a longer reproductive period duration (i.e. longer exposure to menstrual cycling) exerts dynamic and measureable effects on ventricular repolarization and atrial conduction.

How might this impact on clinical practice?

Published normal reference values for electrocardiograms throughout pregnancy and the post-partum period are lacking even though it is widely accepted that pregnancy has material effects on cardiac electrical conduction resulting to increased risks of arrhythmia.(1) There is a dearth of systematic studies assessing p wave parameter changes throughout the menstrual cycle. Such

studies would be important in furthering our dual understanding of cardiac adaptations of pregnancy and in our understanding of pregnancy-related arrhythmia.

Key Words: *endogenous estrogen, sex hormones, pregnancy, menarche, menopause, reproductive history, menarche, age at birth, repolarization, QTc, PR interval, electrocardiogram*

INTRODUCTION:

Electrocardiogram parameters reflect current as well as future CVD risk

Electrocardiographic parameters are reflections of both current as well as future cardiovascular disease risk. For example, in the Framingham Heart Study, a prolonged PR interval (> 200 ms) was related to incident atrial fibrillation, all-cause mortality and to the likelihood of needing a permanent pacemaker.(2) In addition to PR interval, the p wave duration more directly relates to atrial size and is an antecedent of atrial fibrillation.(3) Both PR interval and p wave duration are markers of left atrial size which in turn is a correlate of hypertensive heart disease(4) and incident stroke.(5) It is unclear to what extent PR interval is affected by premenopausal hormonal fluctuations from the menstrual cycle and childbearing.

Pregnancy, cardiac remodeling and the electrocardiogram

Pregnancy and the post-partum period both have substantial physiologic effects on cardiac electrophysiology. Physiologic studies of women during early and late pregnancy as well as early post-partum suggest a shortening of the corrected QT interval (QTc) which partially reverts back to pre-pregnancy values following post-partum.(6, 7) Direct pathophysiologic links connecting

myocardial structural remodeling and cardiac electrical remodeling have been increasingly recognized.(8) With regards to myocardial remodeling, pregnancy induced cardiac remodeling does not completely revert back to pre-pregnancy levels and effects of increasing parity on cardiac remodeling can be detected even in mid-life.(9) However, the extent to which an increasing number of pregnancies exerts long lasting effects on the cardiac electrical conduction system is uncertain.(9)

Estrogen exposure and the electrocardiogram

In addition to the more marked hormonal fluctuations seen during pregnancy, there are also more subtle, cyclic changes in estrogen and progesterone cycling that occur during menstrual cycling in women of reproductive age. Testosterone and progesterone are recognized to decrease the QTc interval.(10) Data from the Women's Health Initiative Hormone Trial (or WHI HT) suggests that estrogen-only therapy modestly prolongs QTc beyond that of both estrogen-progestin therapy and placebo.(11) However, it is uncertain whether the pre-menopausal hormonal milieu (reflected by the length of the interval from menarche to menopause and number of pregnancies) is associated with changes in QTc in the WHI HT.

WHI HT represents a unique resource to study questions related to pregnancy and reproductive history and ECG parameters and thus we sought to determine if there is a positive or negative association between number of pregnancies and reproductive period duration with mid-life electrocardiogram intervals (PR interval and QTc) and p wave parameters (p wave maximum duration, dispersion and index).

METHODS AND ANALYSIS PLAN:

Our current study design is a secondary analysis of a previously conducted randomized controlled trial.

Study sample.

The WHI recruitment began in 1991 and consisted of a set of clinical trials on HT, dietary modification and calcium/ vitamin D supplementation on cardiovascular disease, cancer and fractures and an observational study.(12) The clinicaltrial.gov identifier for the WHI is NCT00000611. This analysis drew from the cohort of women enrolled in the clinical trial. **Figure 1** shows the creation of the study sample. Of 68,132 women in WHI Studies (hormone therapy, diet and calcium/vitamin D and observational studies), we excluded 5,217 who were missing ECGs and 15,543 who had prevalent CVD. Of these, 47,372 women, 6,685 were further excluded for having missing covariate data, leaving a final sample of 40,687.

Ascertainment of Reproductive Exposures.

Information on reproductive factors was collected via questionnaire at the second screening visit in the WHI (between 1993-1998). Participants were asked how many times they had been pregnant (were given choices ranging from 0 to 9+), number of live births and how old they were at the end of the first and at the end of their last pregnancy (<20, 20-24, 25-29,30-34,35+ years). Age at menarche (<9, 10,11,12,13,14,15,>16 years) and age at menopause was asked on this screening questionnaire. Reproductive period duration (RD) was defined as the duration between age at menarche to age at menopause (in years). Detailed current and prior hormone therapy usage and hysterectomy/oophorectomy status was collected at enrollment and has been previously described.(13)

Ascertainment of Covariates:

Age, income, education, self-reported race/ethnicity, geographic region of United States, history and duration of breastfeeding were collected at participant enrollment and second screening examinations. Body mass index (BMI, kg/m^2) was calculated using height and weight measured by study staff at baseline. Women with hypertension were identified as those with a self-reported history of treated hypertension or blood pressure measurements meeting JNC 7 criteria for hypertension.(14) Diabetes was identified by self-reported use of anti-diabetic medications and hyperlipidemia by use of cholesterol lowering medications. Antianxiety and antidepressant medication use was validated on enrollment by nurse examination of medication bottles.

Electrocardiographic parameters:

Standard 12-lead ECGs were recorded in all women by strictly standardized procedures in all clinical centers as has been described.(15) All ECGs were processed in a central laboratory (EPICARE Center, University of Alberta, Edmonton, Canada, and later Wake Forest University, Winston-Salem, NC), where they were visually inspected for technical errors and inadequate quality. ECGs were processed with the 2001 version of the Marquette 12-SL program (GE Marquette). In additional to PR and QT intervals, we also examined the maximum P wave duration (from all 12 leads of the ECG).(3) The QT interval was corrected using Bazett's formula. The Institutional Review Board of University of California San Francisco approved this study protocol.

Statistical Methods:

Primary Analysis

We employed multivariable linear regression to assess the association between reproductive exposures (number of pregnancies and RD) with the dependent variable of ECG parameters (PR interval in milliseconds, QTc in milliseconds). Four primary models were fit: PR regressed on number of live births, QTc regressed on number of live births, PR regressed on RD, and QTc regressed on RD. Multivariable models were adjusted for a priori covariates: age, BMI, hypertension status, diabetes, income, education, race/ethnicity, region, history of breastfeeding, antianxiety medication, antidepressant medication, lipid medication, duration of breastfeeding, oophorectomy status, hormone therapy use, heart rate and QRS duration. In analyses considering categories of livebirths we employed a linear trend test.

We explored effect modification of the primary exposures, number of live births and RD, by hormone therapy usage and hysterectomy status. We classified hormone therapy usage into three categories: women who reported current, prior or no hormone therapy usage. A statistical interaction term between hormone therapy usage and the exposure (RD or number of live births) was used to consider effect modification by reported hormone therapy use. When the statistical interaction term was statistically significant ($p < 0.05$) according to a likelihood ratio test, we presented the estimates in each of the three categories of hormone therapy use and we presented a single estimate if there was no evidence for effect modification by hormone therapy. A similar approach was employed for studying RD or number of live births and hysterectomy status. To show sensitivity of estimates to confounders, unadjusted associations were reported as well as those associations adjusted for the confounders listed above.

Secondary Analyses:

In secondary analyses, we studied our exposures in relation to secondary outcomes, P wave duration and p wave dispersion. In an additional secondary set of analyses, we removed subjects who reported never being pregnant and used multivariable linear regression to model associations between age at first live birth and the five electrocardiogaphic measures. These models used the same covariates to adjust association as those in our primary analyses. Further,. Subjects who had implausible secondary outcome values (i.e. all zero values or all constant values across all electrocardiographic measures) were removed.

Multiple Imputation Analyses

We used multiple imputation techniques to impute missing covariates and refit models from primary analyses to explore the sensitivity of our results to missing data. We used the PROC MI in SAS to construct 5 multiply imputed data sets. Missing variables were imputed via fully conditional specification method in PROC MI using all variables from the analytic model. We fit models to each imputed data set and pooled the results. The pooled results from imputation did not differ appreciably from the results of the complete case analysis.

All analyses were performed in SAS 9.4 (SAS Institute, Cary NC, USA).

RESULTS

Table 1 shows the baseline characteristics of our sample by number of pregnancies lasting at least 6 months. The mean age at enrollment was 62.4 years, while the mean age at menarche was 12.6 and mean age at menopause was 50.0 years. 82.5% of women were White, 9.3% Black, 4% Hispanic and 2.7% Asian. Forty five percent of the study sample reported never having used hormone therapy prior to enrollment.

PR interval

Compared to women reporting never having been pregnant, having 5 or more pregnancies was associated with a 1.3 ms longer PR interval (**Table 2).** Among women who reported never having used hormones, each additional year of reported reproductive period duration was associated with a 0.1 ms longer PR interval (or atrial conduction velocity). Conversely, there was no significant association between RD and PR interval among women who reported prior or current hormone therapy use (p value for interaction < 0.01) (**Table 2**). Age at first live birth, was not related to PR interval (data not shown).

QTc

Compared to never having been pregnant, having 5 or more pregnancies was related to a 1.2 ms longer QTc (**Table 3**). However, not carrying a pregnancy to term, or having 1 or 2-4 term pregnancies (versus not being pregnant), were not related to QTc. For each additional year in reproductive period duration, there was a 0.4 ms shorter QTc (**Table 3**). Restricting to women who had at least one live birth did not change our results (data not shown).

P wave duration and dispersion

P wave dispersion was higher for women with 2-4 live births (ms increase =0.62, 95% CI: 0.01, 1.24) and 5 live births (0.94, 95% CI: 0.20, 1.67), compared with those who reported never having been pregnant (**Table 4**). Reproductive period duration was related to maximum p wave duration among women who reported never having used hormones (0.09, 95% CI: 0.06, 0.13) but not among those who reported prior or current hormone therapy use (p interaction < 0.01) (**Table 5**).

DISCUSSION

Summary of Findings

We found that having five or more pregnancies compared to none was associated with a small increase in mid-life atrial conduction time, independent of factors known to be associated with this interval (PR). Number of live births among women with at least one live birth (compared to no prior pregnancies) was associated with increased atrial conduction time. Having 5 or more pregnancies was related to a small increase in ventricular repolarization time as compared to having no prior pregnancies. Among women reporting no prior exogenous hormone use, each additional year of reported RD was related to a very modest (0.1 ms) longer atrial conduction time. RD was related to a very modest increase in p wave duration. RD was related to a shorter ventricular repolarization time.

Mechanisms linking pregnancy and atrial electrical remodeling

The effect of cumulative pregnancies on mid-life electrocardiograms would likely result from both 1) the pregnancy itself and 2) incident cardiometabolic factors that are impacted by pregnancy such as adiposity(16) and vascular stiffness,(17) and premenopausal blood pressure.(18) Adiposity and blood pressure are related to increased P wave indices in a normal healthy population,(19) and these P wave indices are electrocardiographic reflections of increased left atrial pressure, size and potentially fibrosis. The period of pregnancy and the peripartum are characterized by hormonal changes that affect both cardiovascular hemodynamics and adaptive myocardial remodeling. Pregnancy causes increased cardiac output, increased left ventricular mass, and decreased systemic vascular resistance.(20) The uterus and placenta in support of the growing fetus and fetal circulatory system represent a significant vascular shunt

which contributes to these hemodynamic adaptations in pregnancy. The sum of these changes result in both left atrial and left ventricular dilation. However, the effects of normal pregnancy on electrographic remodeling during pregnancy are not well described. A prior small clinical study has looked at P wave duration and P wave dispersion among pregnant women compared with controls and found that both of these parameters are increased.(21)

Pregnancy and cumulative effects on ventricular repolarization

A prior study in 37 women in late pregnancy compared with 18 age matched controls demonstrated that QTc substantially prolongs late in pregnancy and that this only partially corrects back to pre-pregnancy values post-partum.(7) Our finding that having 5 or more pregnancies as compared to no prior pregnancies suggests that QTc prolongation during pregnancy may accumulate across successive pregnancies and will be significantly increased on mid-life ECG. Furthermore, we found evidence for a dose response relationship between number of pregnancies and mid-life QTc.

Reproductive period duration and atrial conduction velocity.

The menstrual cycle consists of a relatively well described hormone cycling in women consisting of both estrogen and progesterone as well as testosterone production. A longer reproductive period duration reflects the cumulative exposure that a woman has to these endogenous fluctuations in sex hormone levels. Indeed, prior studies have assessed P wave parameters throughout the menstrual cycle and noted that P wave duration is substantialy increased in the luteal phase.(22) Among women who did report taking prior hormone therapy, we observed a very modest but significant increase in mid-life PR interval and in P wave duration. Exogenous

hormone therapy use may obscure the relationship between endogenous hormone exposure from a longer reproductive period duration and P wave parameters, which would explain our findings of effect modification by hormone therapy use. An earlier age at menarche (which would be related to increased reproductive period duration) has been associated with increased adiposity(23) and diabetes,(24) which in turn have been linked with increased p wave duration(3) and, in the case of body mass index, with increased left atrial remodeling(25) and thus may also partially underlie our findings.

Reproductive duration and decrease ventricular repolarization time

The QTc is shortened by the action of progesterone and lengthened by estrogen during normal menstrual cycling. The net effect of these changes during a single menstrual cycle can result in shortening of ventricular repolarization time or QTc.(26) Our finding that an increased reproductive duration was modestly inversely related to QTc in WHI, suggests that an increasing exposure to progesterone, in particular during menstrual cycling, may have cumulative and measurable effects on the mid-life electrocardiogram in women.

Strength and Limitations

The use of a well characterized multiethnic, large dataset of postmenopausal women representative of women in the United States is a strength of our study. A notable limitation is that the exposure variables were acquired retrospectively and some are very distant events (eg age at menarche occurred 40-70 years in the past). We were unable to adjust for pregnancy complications such as preeclampsia or gestational diabetes since these were not collected. We did not adjust for smoking, physical activity, and habitual consumption of alcohol and coffee

which may have been related to the exposure variables but are not widely known to be related to the ECG dependent variables studied.

Conclusions

We found that having an increasing number of pregnancies is related to significant changes in atrial conduction time and ventricular repolarization time. A longer reproductive period duration in women not exposed to exogenous hormone therapy is related to a modest increase in atrial conduction time and to a modest decrease in ventricular repolarization. Reproductive health factors reflective of endogenous sex hormone exposure may be significant determinants of cardiac electrical remodeling in mid-life.

Disclosures

None

Author contributorship statement:

Nisha I. Parikh conceived of the idea, designed the study, interpreted the analysis, drafted and critically reviewed the manuscript. She provided final approval of the manuscript

Kristopher Kapphahn and Haley Hedlin conducted study design, statistical analysis and critical review of the manuscript. They provided final approval of the manuscript

Jeffrey E. Olgin, Matthew A. Allison, Jared W. Magnani, MSc, Kelli R. Ryckman, Molly E. Waring, Marco V. Perez assisted with study design, analysis interpretation, drafting and critical reviewed the manuscript. They provided final approval of the manuscript

Barbara V. Howard assisted with study design, interpreted the analysis, drafted and critically reviewed the manuscript. They provided final approval of the manuscript

Data sharing statement:

This was a secondary analysis of preexisting data and as such, no new data was generated by this study. Information about data sharing for the Women's Health Initiative can be found at the following website: https://www.whi.org/researchers/data/Pages/Home.aspx

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Figure Legend: Creation of the Study Sample. Clinical Trials include Hormone Trial, Dietary Modification and Calcium/Vitamin D. ECG=electrocardiogram, CVD=Cardiovascular diseases.

Table 1: Baseline Characteristics of the Study Sample

Distribution of Covariates by Number of Live Births	Never pregnant	0	1	2-4	5+
n (%)	3296 (8.1)	1082 (2.7)	3536 (8.7)	26599 (65.4)	6174 (15.2)
Age (y), Mean (SD)	61.95 (7.43)	60.4 (7.36)	61.14 (7.32)	62.23 (6.87)	64.14 (5.97)
BMI, Median (IQR)	27.39 (7.72)	27.74 (8.02)	27.69 (7.56)	27.73 (7.34)	28.78 (7.45)
Age at menopause (y), Median (IQR)	48 (8)	49 (7)	49 (8)	50 (7)	50 (8)
Age at menarche (y), Mean (SD)	12.54 (1.48)	12.55 (1.57)	12.58 (1.54)	12.58 (1.47)	12.67 (1.46)
Duration of reproductive period (y), Median (IQR)	36 (8)	36 (8)	36 (8)	37 (8)	37 (7)
Race/Ethnicity, N (%)					
American Indian/Alaskan Native	3 (0.1)	3 (0.3)	13 (0.4)	96 (0.4)	32 (0.5)
Asian/PI	121 (3.7)	33 (3)	102 (2.9)	728 (2.7)	106 (1.7)
African-American	263 (8)	203 (18.8)	604 (17.1)	2126 (8)	598 (9.7)
Hispanic	106 (3.2)	50 (4.6)	160 (4.5)	1005 (3.8)	300 (4.9)
White	2767 (84)	780 (72.1)	2601 (73.6)	22352 (84)	5056 (81.9)
Other	36 (1.1)	13 (1.2)	56 (1.6)	292(1.1)	82 (1.3)
Education Level, N (%)					
No high school diploma	73 (2.2)	41 (3.8)	157 (4.4)	1033 (3.9)	543 (8.8)
High school diploma	1352 (41)	490 (45.3)	1872 (52.9)	15726 (59.1)	4096 (66.3)
Bachelor's degree	802 (24.3)	250 (23.1)	801 (22.7)	5859 (22)	1087 (17.6)
Graduate degree	1069 (32.4)	301 (27.8)	706 (20)	3981 (15)	448 (7.3)
Household Income, N (%)					
< $50,000	2071 (62.8)	639 (59.1)	2136 (60.4)	15872 (59.7)	4543 (73.6)
$50 to 100,000	974 (29.6)	333 (30.8)	1090 (30.8)	8260 (31.1)	1330 (21.5)
> $100,000	251 (7.6)	110 (10.2)	310 (8.8)	2467 (9.3)	301 (4.9)

Hypertension, N (%)					
Treated	712 (21.6)	240 (22.2)	819 (23.2)	6141 (23.1)	1638 (26.5)
Untreated	276 (8.4)	100 (9.2)	278 (7.9)	2045 (7.7)	491 (8)
History of Diabetes, N (%)	150 (4.6)	57 (5.3)	179 (5.1)	1377 (5.2)	434 (7)
History of Breastfeeding, N (%)	6 (0.2)	36 (3.3)	1476 (41.7)	15375 (57.8)	4258 (69)
History of HT, N (%)					
Never used	1520 (46.1)	478 (44.2)	1601 (45.3)	11660 (43.8)	3324 (53.8)
Past user	561 (17)	191 (17.7)	575 (16.3)	4780 (18)	1147 (18.6)
Current user	1215 (36.9)	413 (38.2)	1360 (38.5)	10159 (38.2)	1703 (27.6)

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Table 1: Unadjusted and multivariable-adjusted association of number of pregnancies leading to livebirths and reproductive period duration with PR interval (ms) in N=40,687 women in the Womens Health Initiative Clinical Trials

	Unadjusted Effect (95% CI)	**Multivariable Adjusted Effect* (95% CI)**	
Number of live births and reproductive period duration are each in their own separate multivariable models.			
Number of Live Births *(categorical with never pregnant as referent category)*			p value for linear trend= 0.11
Never Pregnant	Ref.	Ref.	
None	1.44 (-0.18,3.06)	1.15 (-0.43, 2.74)	
1	1.16 (0.04,2.28)	0.54 (-0.57, 1.66)	
2-4	1.20 (0.34,2.05)	0.59 (-0.301, 1.48)	
5+	3.06 (2.07,4.06)	1.32 (0.25, 2.39)	
Reproductive period duration *(continuous, years)*			p value for interaction = 0.009
Never HT User	0.05 (-0.01, 0.11)	0.10 (0.04, 0.16)	
Past HT use	0.002 (-0.07, 0.08)	0.08 (-0.00,0.15)	
Current HT use	-0.09 (-0.15, -0.03)	-0.02 (-0.08, 0.04)	

*Covariates include age, baseline BMI, baseline hypertension status, history of diabetes, income, education, race/ethnicity, region, history/ duration of breastfeeding, antianxiety medication, antidepressant medication, lipid medication, oophorectomy status, hysterectomy status, hormone use history, heart rate and QRS duration

HT=hormone therapy

Table 3: Unadjusted and multivariable-adjusted association of number of pregnancies leading to livebirths and reproductive period duration with QTc interval (ms) in N=40,687 women in the Womens Health Initiative and Clinical Trials

	Unadjusted Effect (95% CI)	**Multivariable Adjusted Effect* (95% CI)**	**P value**
Number of live births and reproductive period duration are each in their own multivariable models			
Number of Live Births *(categorical with never pregnant as referent category)*			p value for linear trend=0.008
Never Pregnant	Ref.	Ref.	
None	0.54 (-0.76,1.83)	0.66 (-0.56, 1.88)	
1	0.29 (-0.60,1.18)	0.15 (-0.71, 1.02)	
2-4	0.63 (-0.05,1.31)	0.25 (-0.43, 0.94)	
5+	2.39 (1.59,3.19)	1.15 (0.33, 1.98)	
Reproductive period duration *(continuous, years)*	-0.09 (-0.12,-0.06)	-0.04 (-0.07, -0.01)	p value=0.01

*Covariates for number of livebirths analysis include age, baseline BMI, baseline hypertension status, history of diabetes, income, education, race/ethnicity, region, history/ duration of breastfeeding, antianxiety medication, antidepressant medication, lipid medication, oophorectomy status, hysterectomy status, hormone use history, heart rate and QRS duration. Covariates for reproductive period duration analysis include live births, age, baseline BMI, baseline hypertension status, history of diabetes, income, education, race/ethnicity, region, history of breastfeeding, duration of breastfeeding, antianxiety medication, antidepressant medication, lipid medication, oophorectomy status, hysterectomy status, hormone use history, and QRS duration.

HT=hormone therapy

Table 4: Unadjusted and multivariable-adjusted associations between number of pregnancies leading to livebirths with p wave duration and p wave dispersion in N=39,338* women in the Women's Health Initiative and Clinical Trials

Dependent Variable	Number of Live Births	Unadjusted Effect (95% CI)	Adjusted Effect (95% CI)	
PR wave duration (ms)				p value for linear trend =0.73
	Never Pregnant	Ref.	Ref.	
	None	0.09 (-0.73, 0.92)	0.09 (-0.69, 0.87)	
	1	-0.06 (-0.63, 0.51)	-0.20 (-0.76, 0.35)	
	2-4	-0.03 (-0.47, 0.40)	-0.26 (-0.70, 0.18)	
	5+	0.99 (0.49, 1.50)	-0.22 (-0.74, 0.31)	
PR wave dispersion (ms)				p for linear trend =0.13
	Never Pregnant	Ref.	Ref.	
	None	0.67 (-0.42, 1.77)	0.64 (-0.45, 1.72)	
	1	0.44 (-0.32, 1.20)	0.34 (-0.42, 1.11)	
	2-4	0.72 (0.15, 1.30)	0.62 (0.01, 1.24)	
	5+	1.49 (0.82, 2.17)	0.94 (0.20, 1.67)	

Effect estimates correspond to expected ms increase in the specified interval measure for each parity group relative to the never pregnant group. Fully adjusted models were adjusted for age, baseline BMI, baseline hypertension status, history of diabetes, income, education, race/ethnicity, region, history of breastfeeding, antianxiety medication, antidepressant medication, lipid medication, duration of breastfeeding, oophorectomy status, hysterectomy status, hormone use history, heart rate, and QRS duration.

*n differs from main analyses due to the exclusion of women with implausible PR wave measures

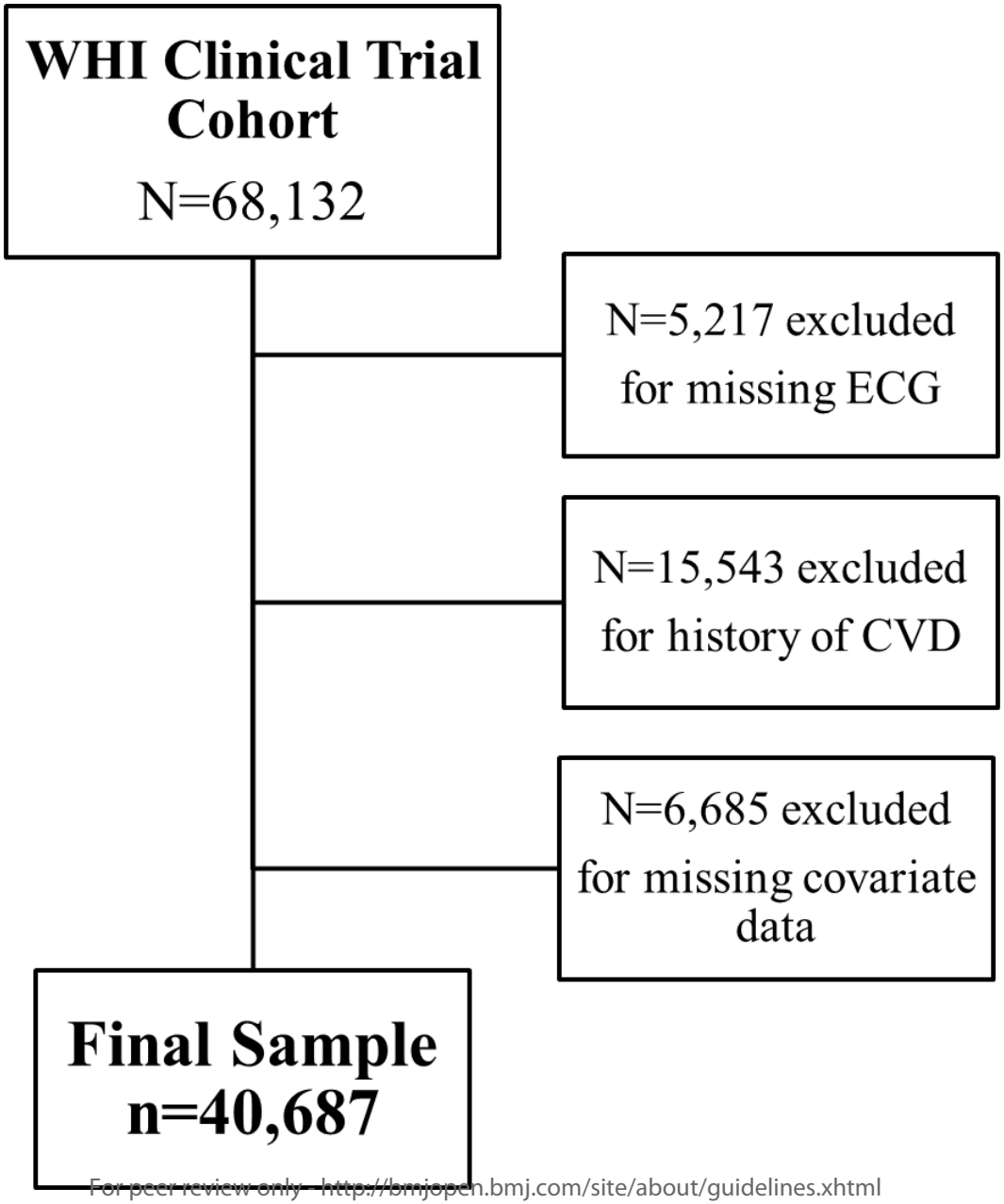
Table 5: Unadjusted and multivariable-adjusted associations between reproductive period duration and PR wave measures and PR dispersion in N= 31,538* Women in the Women's Health Initiative Clinical Trial.

Dependent Variable	Hormone Use Status	Unadjusted Effect (95% CI)	Adjusted Effect (95% CI)	
PR wave max (ms)	Never User	0.07 (0.03, 0.11)	0.09 (0.06, 0.13)	p value for interaction= 0.0009
PR wave max (ms)	Past	-0.04 (-0.08, 0.005)	0.01 (-0.03, 0.05)	
PR wave max (ms)	Current	-0.03 (-0.06, 0.004)	0.01 (-0.02, 0.05)	
PR wave dispersion (ms)	Never User	0.002 (-0.04, 0.05)	0.01 (-0.03, 0.06)	p value for interaction= 0.65
PR wave dispersion (ms)	Past	-0.03 (-0.09, 0.02)	-0.01 (-0.06, 0.05)	
PR wave dispersion (ms)	Current	-0.04 (-0.08, 0.003)	-0.02 (-0.06, 0.03)	

Effect estimates correspond to expected ms increase in PR measure. These models contained an interaction term for reproductive period duration hormone use status. Fully adjusted models were adjusted for number of live births, age, baseline BMI, baseline hypertension status, history of diabetes, income, education, race/ethnicity, region, history of breastfeeding, duration of breastfeeding, antianxiety medication, antidepressant medication, lipid medication, oophorectomy status, hysterectomy status, hormone use history, heart rate, and QRS duration.

*n differs from main analyses due to the exclusion of women with implausible PR wave measures

Figure.



STROBE Statement—checklist of items that should be included in reports of observational studies with page number in manuscript.

	Item No	Recommendation	Page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-8
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	7-8
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*7-10*
Bias	9	Describe any efforts to address potential sources of bias	7-10
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-10
		(*b*) Describe any methods used to examine subgroups and interactions	8-10
		(*c*) Explain how missing data were addressed	10
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	7
		(*e*) Describe any sensitivity analyses	10

Continued on next page

Results			**Page number**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	7
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	*7*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	20-21
		(*b*) Report category boundaries when continuous variables were categorized	20-21
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10-11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10-11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14-15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Effects of Reproductive Period Duration and Number of Pregnancies on Mid-Life Electrocardiographic Indices: A Secondary Analysis from the Women's Health Initiative Clinical Trial

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Effects of Reproductive Period Duration and Number of Pregnancies on Mid-Life Electrocardiographic Indices: A Secondary Analysis from the Women's Health Initiative Clinical Trial

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Running Title: Reproductive factors and electrocardiographic intervals

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Conflict of Interest: None of the authors have any conflicts of interest in respect to this article

Abstract: (word count=264)

Objective: Pregnancy, menses and menopause are related to fluctuations in endogenous sex hormones in women, which cumulatively, may alter cardiac electrical conduction. Therefore, we sought to study the association between number of pregnancies and reproductive period duration (RD, time from menarche to menopause) with electrocardiographic intervals in the Women's Health Initiative Clinical Trials.

Methods: In primary analyses, we employed multivariable linear regression models relating number of pregnancies and RD with millisecond (ms) changes in PR interval, P wave indices (duration and dispersion) and QTc from enrollment electrocardiogram.

Results: Among 40,687 women (mean age=62 years), 5+ live births versus 0 prior pregnancies was associated with a 1.32 ms increase in PR interval [95% CI (0.25, 2.38)], with a graded association with longer QTc interval (ms) [none= 0.66 (-0.56, 1.88), 1= 0.15 (-0.71, 1.02), 2 to 4= 0.25 (-0.43, 0.94), and 5+ live births=1.15 (0.33, 1.98), p = 0.008]. RD was associated with longer PR interval and maximum P wave duration (but not P-wave dispersion) among never users of hormone therapy: [PR (ms) per additional RD year: 0.10 (0.04, 0.16); higher P-wave duration (ms): 0.09 (0.06, 0.12)]. For every year increase in reproductive period, QTc decreased by 0.04 ms (-0.07, -0.01).

Limitations: Potential misclassification of RD due to participant recall

Conclusions: An increasing number of live births are related to increased ventricular repolarization time whereas RD is related to decreased ventricular repolarization time. Both longer RD and grandmultiparity are related to increased atrial conduction time. Reproductive factors that alter midlife cardiac electrical conduction system remodeling in women may modestly influence CVD risk in later life.

Article Summary:

Strengths and Limitations of the Study.

- *A strength is the use of a well characterized multiethnic, large dataset of postmenopausal women representative of women in the United States.*
- *A notable limitation is that the exposure variables were acquired retrospectively.*
- *We were unable to adjust for pregnancy complications such as preeclampsia or gestational diabetes.*

Key Words: *endogenous estrogen, sex hormones, pregnancy, menarche, menopause, reproductive history, menarche, age at birth, repolarization, QTc, PR interval, electrocardiogram*

INTRODUCTION:

Electrocardiogram parameters reflect current as well as future CVD risk

Electrocardiographic parameters are reflections of both current as well as future cardiovascular disease risk. For example, in the Framingham Heart Study, a prolonged PR interval (> 200 ms) (which is defined as the period, measured in milliseconds, that extends from the beginning of the P wave (the onset of atrial depolarization) until the R wave), was related to incident atrial fibrillation, all-cause mortality and to the likelihood of needing a permanent pacemaker.(1) In addition to PR interval, the p wave duration (or the period in milliseconds during which the atrium depolarizes), more directly relates to atrial size and is an antecedent of atrial fibrillation.(2) Both PR interval and p wave duration are markers of left atrial size which in turn

is a correlate of hypertensive heart disease(3) and incident stroke.(4) P wave dispersion, defined as the difference between the maximum and the minimum P-wave duration recorded from multiple different-surface ECG leads, is an additional marker of atrial remodeling and antecedent of atrial fibrillation.(5) It is unclear to what extent PR interval, p wave duration or P wave dispersion are affected by premenopausal hormonal fluctuations from the menstrual cycle and childbearing.

Pregnancy, cardiac remodeling and the electrocardiogram

Pregnancy and the post-partum period both have substantial physiologic effects on cardiac electrophysiology. Physiologic studies of women during early and late pregnancy as well as early post-partum suggest a shortening of the corrected QT interval (QTc) which partially reverts back to pre-pregnancy values following post-partum.(6, 7) The QT interval is defined as the measure of time between the onset of ventricular depolarization and completion of ventricular repolarization, and because QT interval is strongly related to heart rate, the QTc is corrected for heart rate. Direct pathophysiologic links connecting myocardial structural remodeling and cardiac electrical remodeling have been increasingly recognized.(8) With regards to myocardial remodeling, pregnancy induced cardiac remodeling does not completely revert back to pre-pregnancy levels and effects of increasing parity on cardiac remodeling can be detected even in mid-life.(9) However, the extent to which an increasing number of pregnancies exerts long lasting effects on the cardiac electrical conduction system is uncertain.(9)

Estrogen exposure and the electrocardiogram

In addition to the more marked hormonal fluctuations seen during pregnancy, there are also more subtle, cyclic changes in estrogen and progesterone cycling that occur during menstrual cycling in women of reproductive age. Testosterone and progesterone are recognized to decrease the QTc interval.(10) Prior data from the Women's Health Initiative (WHI) Hormone Trial suggests that estrogen-only post-menopausal therapy modestly prolongs QTc beyond that of both estrogen-progestin therapy and placebo.(11) However, it is uncertain whether the pre-menopausal endogenous hormonal fluctuations (reflected by the length of the interval from menarche to menopause, and by number of pregnancies) are associated with changes in QTc in the WHI.

WHI represents a unique resource to study questions related to pregnancy and reproductive history and ECG parameters and thus we sought to determine if there is a positive or negative association between number of pregnancies and reproductive period duration with mid-life electrocardiogram intervals (PR interval and QTc) and p wave parameters (p wave maximum duration and dispersion).

METHODS AND ANALYSIS PLAN:

Our current study design is a secondary analysis of a previously conducted set of clinical trials. .

Study sample.

The WHI recruitment began in 1991 and consisted of a set of clinical trials/ and an observational study on hormone therapy, dietary modification and calcium/ vitamin D supplementation on cardiovascular disease, cancer and fractures.(12) The clinicaltrial.gov identifier for the WHI is NCT00000611. At the time of enrollment, all women enrolled in the

WHI were required to be between 50 and 79 years old, postmenopausal, and intending to reside in the area for at least 3 years. Other enrollment criteria have been previously described.(13) This analysis drew from the cohort of women enrolled in the WHI clinical trials (and not observational study), as WHI clinical trial participants has ECGs performed per protocol. Informed consent was obtained from all participants at study enrollment. **Figure 1** shows the creation of the study sample. Of 68,132 women in WHI Studies (post- menopausal hormone therapy, diet and calcium/vitamin D and observational studies), we excluded 5,217 who were missing ECGs and 15,543 who had prevalent CVD. Because number of pregnancies and reproductive period (in particular age at menopause) are known to be associated with later CVD and a history of CVD is related strongly with ECG changes including QTc and certainly increased PR, we sought to exclude women with a history of CVD in order to assess associations between reproductive period duration and number of pregnancies that were not directly mediated through CVD. Of these, 47,372 women, 6,685 were further excluded for having missing covariate data, leaving a final sample of included women =40,687. In a missing imputation sensitivity analysis described below, we additionally analyzed the 6,685 women with missing covariate data (total n=54,057).

Patient and Public Involvement

WHI was designed to address the gaps in knowledge about the major health issues in post menopasual women. Patients assisted research staff in recruiting and results for all measures done at the study examinations were explained to each participant. Major study results are communicated to participants via newsletters.

Ascertainment of Reproductive Exposures.

Information on reproductive factors was collected via questionnaire at the second screening visit in the WHI (between 1993-1998). Participants were asked how many times they had been pregnant (were given choices ranging from 0 to 8+), number of live births, and how old they were at the end of the first and at the end of their last pregnancy (<20, 20-24, 25-29,30-34,35+ years). Age at menarche (<9, 10,11,12,13,14,15,>16 years) and age at menopause was asked on this screening questionnaire. Reproductive period duration (RD) was defined as the duration between age at menarche to age at menopause (in years). Detailed current and prior hormone therapy (or post-menopausal hormone replacement therapy) usage and hysterectomy/oophorectomy status was collected at enrollment and has been previously described.(14) Questions regarding the use and duration of oral contraceptive usage was also collected at enrollment.

Ascertainment of Covariates:

Age, income, education, self-reported race/ethnicity, geographic region of United States, history and duration of breastfeeding were collected at participant enrollment and second screening examinations. Body mass index (BMI, kg/m^2) was calculated using height and weight measured by study staff at baseline. Women with hypertension were identified as those with a self-reported history of treated hypertension or blood pressure measurements meeting JNC 7 criteria for hypertension.(15) Diabetes was identified by self-reported use of anti-diabetic medications and hyperlipidemia by use of cholesterol lowering medications.

Electrocardiographic parameters:

Standard 12-lead ECGs were recorded in all women by strictly standardized procedures in all clinical centers as has been described.(16) All ECGs were processed in a central laboratory

(EPICARE Center, University of Alberta, Edmonton, Canada, and later Wake Forest University, Winston-Salem, NC), where they were visually inspected for technical errors and inadequate quality. ECGs were processed with the 2001 version of the Marquette 12-SL program (GE Marquette). In addition to PR and QT intervals, we also examined the maximum P wave duration and dispersion (from all 12 leads of the ECG).(2) The QT interval was corrected using Bazett's formula. The Institutional Review Board of University of California San Francisco approved this study protocol.

Statistical Methods:

Primary Analysis

We employed multivariable linear regression to assess the association between reproductive exposures (number of pregnancies and RD) with the dependent variable of ECG parameters (PR interval in milliseconds, p wave duration, p wave dispersion, QTc in milliseconds). Multivariable models were adjusted for a priori covariates: age, BMI, hypertension status, diabetes, income, education, race/ethnicity, region, history of breastfeeding, antianxiety medication, antidepressant medication, lipid medication, duration of breastfeeding, oophorectomy status, hormone therapy use, heart rate and QRS duration. In analyses considering categories of livebirths we employed a linear trend test.

We explored effect modification of the primary exposures, number of live births and RD, by hormone therapy usage and hysterectomy status. We classified hormone therapy usage into three categories: women who reported current, prior or no hormone therapy usage. A statistical interaction term between hormone therapy usage and the exposure (RD or number of live births) was used to consider effect modification by reported hormone therapy use. When the statistical interaction term was statistically significant ($p < 0.05$) according to a likelihood ratio test, we

presented the estimates in each of the three categories of hormone therapy use and we presented a single estimate if there was no evidence for effect modification by hormone therapy. A similar approach was employed for studying RD or number of live births and hysterectomy status. To show sensitivity of estimates to confounders, unadjusted associations were reported as well as those associations adjusted for the confounders listed above.

Secondary Analyses:

In secondary analyses, we removed subjects who reported never being pregnant and used multivariable linear regression to model associations between age at first live birth and the five electrocardiogaphic measures. These models used the same covariates to adjust association as those in our primary analyses. Subjects who had implausible secondary outcome values (i.e. all zero values or all constant values across all electrocardiographic measures) were removed. We additionally adjusted for covariates that we were concerned may have confounded the associations between exposure and dependent variables in our study. We additionally fit additional models which included both RD and number of pregnancies to ensure that one exposure did not alter the other's association with the dependent variables. Given that anxiety and depression could affect both exposure and dependent variables in our study, we further adjusted for use of these medications. Antianxiety and antidepressant medication use (selective serotonin reuptake inhibitors (or SSRI) and non-SSRI) were recorded on enrollment by nurse examination of medication bottles. Medications were classified according to the National Drug Index classification system. We adjusted for Ca/Vitamin D status, oral contraceptive usage (yes/no and duration or usage). We further adjusted for menstrual irregularities/fertility disorders/and endometriosis, which are also related to hormonal fluctuations in women.

Multiple Imputation Analyses

There were n=6,685 women in our study with missing covariate data. We used multiple imputation techniques to impute missing covariates and refit models from primary analyses to explore the sensitivity of our results to missing data. We used the PROC MI in SAS to construct 20 multiply imputed data sets. Missing variables were imputed via fully conditional specification method in PROC MI using all variables from the analytic model. We fit models to each imputed data set and pooled the results. The pooled results from imputation did not differ appreciably from the results of the complete case analysis (data not shown).

All analyses were performed in SAS 9.4 (SAS Institute, Cary NC, USA).

RESULTS

Table 1 shows the baseline characteristics of our sample including women who were included in our study and those excluded from analysis for missing variables. Data is displayed by number of pregnancies lasting at least 6 months. The mean age at enrollment was 62.4 years, while the mean age at menarche was 12.6 and mean age at menopause was 50.0 years. 82.5% of women were White, 9.3% Black, 4% Hispanic and 2.7% Asian. Forty five percent of the study sample reported never having used hormone therapy prior to enrollment.

PR interval

Compared to women reporting never having been pregnant, having 5 or more pregnancies was associated with a 1.3 ms longer PR interval (**Table 2).** Among women who reported never having used hormones, each additional year of reported reproductive period duration was associated with a 0.1 ms longer PR interval (or atrial conduction velocity). Conversely, there

was no significant association between RD and PR interval among women who reported prior or current hormone therapy use (p value for interaction < 0.01) (**Table 2**). Age at first live birth, was not related to PR interval (data not shown).

QTc

Compared to never having been pregnant, having 5 or more pregnancies was related to a 1.2 ms longer QTc (**Table 3**). However, not carrying a pregnancy to term, or having 1 or 2-4 term pregnancies (versus not being pregnant), were not related to QTc. For each additional year in reproductive period duration, there was a 0.4 ms shorter QTc (**Table 3**). Restricting to women who had at least one live birth did not change our results (data not shown).

P wave duration and dispersion

P wave dispersion was higher for women with 2-4 live births (ms increase =0.62, 95% CI: 0.01, 1.24) and 5 live births (0.94, 95% CI: 0.20, 1.67), compared with those who reported never having been pregnant (**Table 4**). Reproductive period duration was related to maximum p wave duration among women who reported never having used hormones (0.09, 95% CI: 0.06, 0.13) but not among those who reported prior or current hormone therapy use (p interaction < 0.01) (**Table 5**).

Secondary results: Models that contained both RD and number of pregnancies together were not materially different (data not shown). Further adjustment for antidepressants and anti-anxiety medications did not materially affect our results. Further adjustment for Ca and Vitamin D status or oral contraceptive use, and/or duration did not materially affect our results. Further adjustment for menstrual irregularities/fertility disorders/and endometriosis did not materially change our results.

DISCUSSION

Summary of Findings

We found that having five or more pregnancies compared to none was associated with a small increase in mid-life atrial conduction time, independent of factors known to be associated with this interval (PR). Number of live births among women with at least one live birth (compared to no prior pregnancies) was associated with increased atrial conduction time. Having 5 or more pregnancies was related to a small increase in ventricular repolarization time as compared to having no prior pregnancies. Among women reporting no prior exogenous hormone use, each additional year of reported RD was related to a very modest (0.1 ms) longer atrial conduction time. RD was related to a very modest increase in p wave duration. RD was related to a shorter ventricular repolarization time.

Mechanisms linking pregnancy and atrial electrical remodeling

The effect of cumulative pregnancies on mid-life electrocardiograms would likely result from both 1) the pregnancy itself and 2) incident cardiometabolic factors that are impacted by pregnancy such as adiposity(17) and vascular stiffness,(18) and premenopausal blood pressure.(19) Adiposity and blood pressure are related to increased P wave indices in a normal healthy population,(20) and these P wave indices are electrocardiographic reflections of increased left atrial pressure, size and potentially fibrosis. The period of pregnancy and the peripartum are characterized by hormonal changes that affect both cardiovascular hemodynamics and adaptive myocardial remodeling.(21) Pregnancy causes increased cardiac output, increased

left ventricular mass, and decreased systemic vascular resistance.(22) The uterus and placenta in support of the growing fetus and fetal circulatory system represent a significant vascular shunt which contributes to these hemodynamic adaptations in pregnancy.(22) The sum of these changes result in both left atrial and left ventricular dilation. However, the effects of normal pregnancy on electrographic remodeling during pregnancy are not well described. A prior small clinical study has looked at P wave duration and P wave dispersion among pregnant women compared with controls and found that both of these parameters are increased.(23)

Pregnancy and cumulative effects on ventricular repolarization

A prior study in 37 women in late pregnancy compared with 18 age matched controls demonstrated that QTc substantially prolongs late in pregnancy and that this only partially corrects back to pre-pregnancy values post-partum.(7) Our finding that having 5 or more pregnancies as compared to no prior pregnancies suggests that QTc prolongation during pregnancy may accumulate across successive pregnancies and will be significantly increased on mid-life ECG. Furthermore, we found evidence for a dose response relationship between number of pregnancies and mid-life QTc. Cardiac electrical remodeling often reflects myocardial remodeling. We previously demonstrated that an increasing number of pregnancies were related to left ventricular volume increase and increase in left ventricular mass in a multiethnic cohort of women.(9) The increase in cardiac volume and mass were more marked in grandmultipara's or women who had 5 or more pregnancies leading to livebirths.(9) It is important to note that grandmultiparity is less common with declining parity levels in the United States.

Reproductive period duration and atrial conduction.

The menstrual cycle consists of a relatively well described hormone cycling in women consisting of both estrogen and progesterone as well as testosterone production. A longer reproductive period duration reflects the cumulative exposure that a woman has to these endogenous fluctuations in sex hormone levels. Indeed, prior studies have assessed P wave parameters throughout the menstrual cycle and noted that P wave duration is substantially increased in the luteal phase.(24) Among women who did report taking prior hormone therapy, we observed a very modest but significant increase in mid-life PR interval and in P wave duration. Exogenous hormone therapy use may obscure the relationship between endogenous hormone exposure from a longer reproductive period duration and P wave parameters, which would explain our findings of effect modification by hormone therapy use. An earlier age at menarche (which would be related to increased reproductive period duration) has been associated with increased adiposity(25) and diabetes,(26) which in turn have been linked with increased p wave duration(2) and, in the case of body mass index, with increased left atrial remodeling(27) and thus may also partially underlie our findings.

Reproductive duration and decrease ventricular repolarization time

The QTc is shortened by the action of progesterone and lengthened by estrogen during normal menstrual cycling. The net effect of these changes during a single menstrual cycle can result in shortening of ventricular repolarization time or QTc.(28) Our finding that an increased reproductive duration was modestly inversely related to QTc in WHI. Underlying these findings may be that increasing exposure to progesterone, in particular during menstrual cycling, may have cumulative and measurable effects on the mid-life electrocardiogram in women.

Strength and Limitations

The use of a well characterized multiethnic, large dataset of postmenopausal women representative of women in the United States is a strength of our study. A notable limitation is potential recall bias since the exposure variables were acquired retrospectively and some are very distant events (eg age at menarche occurred 40-70 years in the past). We were unable to adjust for pregnancy complications such as preeclampsia or gestational diabetes since these were not collected. We did not adjust for smoking, physical activity, and habitual consumption of alcohol and coffee which may have been related to the exposure variables but are not widely known to be related to the ECG dependent variables studied. We studied number of pregnancies in a categorical fashion and were unable, due to data constraints, to look at number of pregnancies as a continuous variables.

Directions for future research:

Future studies that disentangle specific hormonal and molecular mechanisms that underlie the association demonstrated in our study will help us better understand our study findings. Understanding which specific fertility factors alter electrical remodeling in women is an important direction for future research.

Conclusions

We found that having five or more pregnancies compared to none is related to small but significant changes in atrial conduction time and ventricular repolarization time. A longer reproductive period duration in women not exposed to exogenous hormone therapy is related to a modest increase in atrial conduction time and to a modest decrease in ventricular repolarization.

Reproductive health factors reflective of endogenous sex hormone exposure may be significant determinants of cardiac electrical remodeling in mid-life.

Disclosures

None

Author contributorship statement:

Nisha I. Parikh conceived of the idea, designed the study, interpreted the analysis, drafted and critically reviewed the manuscript. She provided final approval of the manuscript

Kristopher Kapphahn and Haley Hedlin conducted study design, statistical analysis and critical review of the manuscript. They provided final approval of the manuscript

Jeffrey E. Olgin, Matthew A. Allison, Jared W. Magnani, MSc, Kelli R. Ryckman, Molly E. Waring, Marco V. Perez assisted with study design, analysis interpretation, drafting and critical reviewed the manuscript. They provided final approval of the manuscript

Barbara V. Howard assisted with study design, interpreted the analysis, drafted and critically reviewed the manuscript. They provided final approval of the manuscript

Data sharing statement:

This was a secondary analysis of preexisting data and as such, no new data was generated by this study. Information about data sharing for the Women's Health Initiative can be found at the following website: https://www.whi.org/researchers/data/Pages/Home.aspx

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Figure Legend: Creation of the Study Sample. Clinical Trials include Hormone Trial, Dietary Modification and Calcium/Vitamin D. ECG=electrocardiogram, CVD=Cardiovascular diseases.

Table 1: Baseline Characteristics of the Study Sample- Analytic and Excluded

Distribution of Covariates by Number of Live Births	Never pregnant	None	1	2-4	5+	Analytic	Excluded
Sample Size	3296	1082	3536	26599	6174	40687	6685
Age, N (%)							
50 to 54	598 (18.1)	265 (24.5)	745 (21.1)	3718 (14)	329 (5.3)	5655 (13.9)	834 (12.5)
55 to 59	768 (23.3)	295 (27.3)	869 (24.6)	6282 (23.6)	1054 (17.1)	9268 (22.8)	1204 (18)
60 to 69	1323 (40.1)	371 (34.3)	1363 (38.5)	12189 (45.8)	3580 (58)	18826 (46.3)	3146 (47.1)
70 to 79	607 (18.4)	151 (14)	559 (15.8)	4410 (16.6)	1211 (19.6)	6938 (17.1)	1501 (22.5)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Race/Ethnicity, N (%)							
American Indian/Alaskan Native	3 (0.1)	3 (0.3)	13 (0.4)	96 (0.4)	32 (0.5)	147 (0.4)	39 (0.6)
Asian/PI	121 (3.7)	33 (3)	102 (2.9)	728 (2.7)	106 (1.7)	1090 (2.7)	143 (2.1)
African-American	263 (8)	203 (18.8)	604 (17.1)	2126 (8)	598 (9.7)	3794 (9.3)	1017 (15.2)
Hispanic	106 (3.2)	50 (4.6)	160 (4.5)	1005 (3.8)	300 (4.9)	1621 (4)	471 (7)
White	2767 (84)	780 (72.1)	2601 (73.6)	22352 (84)	5056 (81.9)	33556 (82.5)	4808 (71.9)
Other	36 (1.1)	13 (1.2)	56 (1.6)	292 (1.1)	82 (1.3)	479 (1.2)	98 (1.5)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	109 (1.6)
Education Level, N (%)							
No high school diploma	73 (2.2)	41 (3.8)	157 (4.4)	1033 (3.9)	543 (8.8)	1847 (4.5)	644 (9.6)
High school diploma	1352 (41)	490 (45.3)	1872 (52.9)	15726 (59.1)	4096 (66.3)	23536 (57.8)	3752 (56.1)
Bachelor's degree	802 (24.3)	250 (23.1)	801 (22.7)	5859 (22)	1087 (17.6)	8799 (21.6)	1171 (17.5)
Graduate degree	1069 (32.4)	301 (27.8)	706 (20)	3981 (15)	448 (7.3)	6505 (16)	789 (11.8)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	329 (4.9)
Household Income, N (%)							

<$50,000	2071 (62.8)	639 (59.1)	2136 (60.4)	15872 (59.7)	4543 (73.6)	25261 (62.1)	2809 (42)
$50 to 100,000	974 (29.6)	333 (30.8)	1090 (30.8)	8260 (31.1)	1330 (21.5)	11987 (29.5)	874 (13.1)
>$100,000	251 (7.6)	110 (10.2)	310 (8.8)	2467 (9.3)	301 (4.9)	3439 (8.5)	208 (3.1)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2794 (41.8)
Region, N (%)							
Northeast	763 (23.1)	202 (18.7)	694 (19.6)	5830 (21.9)	1554 (25.2)	9043 (22.2)	1484 (22.2)
Midwest	719 (21.8)	192 (17.7)	709 (20.1)	5793 (21.8)	1792 (29)	9205 (22.6)	1422 (21.3)
South	778 (23.6)	321 (29.7)	1012 (28.6)	6559 (24.7)	1205 (19.5)	9875 (24.3)	2081 (31.1)
West	1036 (31.4)	367 (33.9)	1121 (31.7)	8417 (31.6)	1623 (26.3)	12564 (30.9)	1698 (25.4)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
BMI, N (%)							
Underweight (< 18.5)	34 (1)	8 (0.7)	26 (0.7)	103 (0.4)	15 (0.2)	186 (0.5)	25 (0.4)
Normal (18.5 - 24.9)	1001 (30.4)	309 (28.6)	1041 (29.4)	7557 (28.4)	1341 (21.7)	11249 (27.6)	1582 (23.7)
Overweight (25.0 - 29.9)	1118 (33.9)	377 (34.8)	1234 (34.9)	9660 (36.3)	2262 (36.6)	14651 (36)	2262 (33.8)
Obese (30+)	1143 (34.7)	388 (35.9)	1235 (34.9)	9279 (34.9)	2556 (41.4)	14601 (35.9)	2579 (38.6)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	237 (3.5)
Hypertension, N (%)							
Never hypertensive	2308 (70)	742 (68.6)	2439 (69)	18413 (69.2)	4045 (65.5)	27947 (68.7)	4236 (63.4)
Treated hypertensive	712 (21.6)	240 (22.2)	819 (23.2)	6141 (23.1)	1638 (26.5)	9550 (23.5)	1684 (25.2)
Untreated hypertensive	276 (8.4)	100 (9.2)	278 (7.9)	2045 (7.7)	491 (8)	3190 (7.8)	516 (7.7)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	249 (3.7)
History of Diabetes, N (%)							
Yes	150 (4.6)	57 (5.3)	179 (5.1)	1377 (5.2)	434 (7)	2197 (5.4)	459 (6.9)
No	3146 (95.4)	1025 (94.7)	3357 (94.9)	25222 (94.8)	5740 (93)	38490 (94.6)	6217 (93)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	9 (0.1)

History of Breastfeeding, N (%)							
Yes	6 (0.2)	36 (3.3)	1476 (41.7)	15375 (57.8)	4258 (69)	21151 (52)	3309 (49.5)
No	3290 (99.8)	1046 (96.7)	2060 (58.3)	11224 (42.2)	1916 (31)	19536 (48)	2942 (44)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	434 (6.5)
History of Oophorectomy, N (%)							
No	2253 (68.4)	711 (65.7)	2462 (69.6)	19371 (72.8)	4704 (76.2)	29501 (72.5)	4404 (65.9)
Yes, part of an ovary was taken out	32 (1)	20 (1.8)	42 (1.2)	225 (0.8)	41 (0.7)	360 (0.9)	85 (1.3)
Yes, one was taken out	203 (6.2)	127 (11.7)	285 (8.1)	1718 (6.5)	356 (5.8)	2689 (6.6)	645 (9.6)
Yes, both were taken out	782 (23.7)	207 (19.1)	716 (20.2)	5082 (19.1)	1019 (16.5)	7806 (19.2)	759 (11.4)
Yes, unknown number taken out	26 (0.8)	17 (1.6)	31 (0.9)	203 (0.8)	54 (0.9)	331 (0.8)	159 (2.4)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	633 (9.5)
History of HT, N (%)							
Never used	1520 (46.1)	478 (44.2)	1601 (45.3)	11660 (43.8)	3324 (53.8)	18583 (45.7)	4372 (65.4)
Past user	561 (17)	191 (17.7)	575 (16.3)	4780 (18)	1147 (18.6)	7254 (17.8)	821 (12.3)
Current user	1215 (36.9)	413 (38.2)	1360 (38.5)	10159 (38.2)	1703 (27.6)	14850 (36.5)	1450 (21.7)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	42 (0.6)
Age (y), Mean (SD)	61.95 (7.43)	60.4 (7.36)	61.14 (7.32)	62.23 (6.87)	64.14 (5.97)	62.36 (6.9)	63.46 (7.16)
Missing (%)	0	0	0	0	0	0	0
BMI, Median (IQR)	27.39 (7.72)	27.74 (8.02)	27.69 (7.56)	27.73 (7.34)	28.78 (7.45)	27.85 (7.46)	28.39 (7.86)
Missing (%)	0	0	0	0	0	0	3.55
QTc wave duration (ms), Mean (SD)	417.95 (18.38)	418.49 (19.6)	418.24 (19.2)	418.58 (18.7)	420.34 (19.33)	418.76 (18.85)	419.82 (19.84)
Missing (%)	0	0	0	0	0	0	0
PR wave dispersion	63.86 (19.42)	64.43 (19.43)	64.51 (19.43)	64.79 (19.57)	65.77 (19.57)	64.83 (19.55)	64.87 (20.56)

(ms), Mean (SD)							
Missing (%)	0	0	0	0	0	0	0
PR interval duration (ms), Median (IQR)	156 (30)	158 (28)	158 (30)	158 (30)	160 (30)	158 (30)	158 (30)
Missing (%)	0	0	0	0	0	0	0
P wave duration (ms), Mean (SD)	106.81 (12.85)	107.13 (12.33)	106.96 (12.18)	106.88 (12.34)	107.93 (12.44)	107.05 (12.39)	106.61 (16.09)
Missing (%)	0	0	0	0	0	0	0
Age at menopause (y), Median (IQR)	48 (8)	49 (7)	49 (8)	50 (7)	50 (8)	50 (7)	49 (7)
Missing (%)	0	0	0	0	0	0	32.3
Age at menarche (y), Mean (SD)	12.54 (1.48)	12.55 (1.57)	12.58 (1.54)	12.58 (1.47)	12.67 (1.46)	12.59 (1.48)	12.65 (1.53)
Missing (%)	0	0	0	0	0	0	1.78
Duration of reproductive period (y), Median (IQR)	36 (8)	36 (8)	36 (8)	37 (8)	37 (7)	37 (8)	36 (8)
Missing (%)	0	0	0	0	0	0	33.63

HT=Hormone Therapy (or hormone replacement therapy)

Table 1: Unadjusted and multivariable-adjusted association of number of pregnancies leading to livebirths and reproductive period duration with PR interval (ms) in N=40,687 women in the Womens Health Initiative Clinical Trials

	Unadjusted Effect (95% CI)	**Multivariable Adjusted Effect* (95% CI)**	
Number of live births and reproductive period duration are each in their own separate multivariable models.			
Number of Live Births *(categorical with never pregnant as referent category)*			p value for linear trend= 0.11
Never Pregnant	Ref.	Ref.	
None	1.44 (-0.18,3.06)	1.15 (-0.43, 2.74)	
1	1.16 (0.04,2.28)	0.54 (-0.57, 1.66)	
2-4	1.20 (0.34,2.05)	0.59 (-0.301, 1.48)	
5+	3.06 (2.07,4.06)	1.32 (0.25, 2.39)	
Reproductive period duration *(continuous, years)*			p value for interaction = 0.009
Never HT User	0.05 (-0.01, 0.11)	0.10 (0.04, 0.16)	
Past HT use	0.002 (-0.07, 0.08)	0.08 (-0.00,0.15)	
Current HT use	-0.09 (-0.15, -0.03)	-0.02 (-0.08, 0.04)	

*Covariates include age, baseline BMI, baseline hypertension status, history of diabetes, income, education, race/ethnicity, region, history/ duration of breastfeeding, lipid medication, oophorectomy status, hysterectomy status, hormone use history, heart rate and QRS duration
HT=hormone therapy

Table 3: Unadjusted and multivariable-adjusted association of number of pregnancies leading to livebirths and reproductive period duration with QTc interval (ms) in N=40,687 women in the Womens Health Initiative and Clinical Trials

	Unadjusted Effect (95% CI)	**Multivariable Adjusted Effect* (95% CI)**	**P value**
Number of live births and reproductive period duration are each in their own multivariable models			
Number of Live Births *(categorical with never pregnant as referent category)*			p value for linear trend=0.008
Never Pregnant	Ref.	Ref.	
None	0.54 (-0.76,1.83)	0.66 (-0.56, 1.88)	
1	0.29 (-0.60,1.18)	0.15 (-0.71, 1.02)	
2-4	0.63 (-0.05,1.31)	0.25 (-0.43, 0.94)	
5+	2.39 (1.59,3.19)	1.15 (0.33, 1.98)	
Reproductive period duration *(continuous, years)*	-0.09 (-0.12,-0.06)	-0.04 (-0.07, -0.01)	p value=0.01

*Covariates for number of livebirths analysis include age, baseline BMI, baseline hypertension status, history of diabetes, income, education, race/ethnicity, region, history/ duration of breastfeeding, lipid medication, oophorectomy status, hysterectomy status, hormone use history, heart rate and QRS duration. Covariates for reproductive period duration analysis include live births, age, baseline BMI, baseline hypertension status, history of diabetes, income, education, race/ethnicity, region, history of breastfeeding, duration of breastfeeding, lipid medication, oophorectomy status, hysterectomy status, hormone use history, and QRS duration.
HT=hormone therapy

Table 4: Unadjusted and multivariable-adjusted associations between number of pregnancies leading to livebirths with p wave duration and p wave dispersion in N=39,338* women in the Women's Health Initiative and Clinical Trials

Dependent Variable	Number of Live Births	Unadjusted Effect (95% CI)	Adjusted Effect (95% CI)	p-value
P wave duration (ms)				p value for linear trend =0.73
	Never Pregnant	Ref.	Ref.	
	None	0.09 (-0.73, 0.92)	0.09 (-0.69, 0.87)	
	1	-0.06 (-0.63, 0.51)	-0.20 (-0.76, 0.35)	
	2-4	-0.03 (-0.47, 0.40)	-0.26 (-0.70, 0.18)	
	5+	0.99 (0.49, 1.50)	-0.22 (-0.74, 0.31)	
P wave dispersion (ms)				p for linear trend =0.13
	Never Pregnant	Ref.	Ref.	
	None	0.67 (-0.42, 1.77)	0.64 (-0.45, 1.72)	
	1	0.44 (-0.32, 1.20)	0.34 (-0.42, 1.11)	
	2-4	0.72 (0.15, 1.30)	0.62 (0.01, 1.24)	
	5+	1.49 (0.82, 2.17)	0.94 (0.20, 1.67)	

Effect estimates correspond to expected ms increase in the specified interval measure for each parity group relative to the never pregnant group. Fully adjusted models were adjusted for age, baseline BMI, baseline hypertension status, history of diabetes, income, education, race/ethnicity, region, history of breastfeeding, antianxiety medication, antidepressant medication, lipid medication, duration of breastfeeding, oophorectomy status, hysterectomy status, hormone use history, heart rate, and QRS duration.

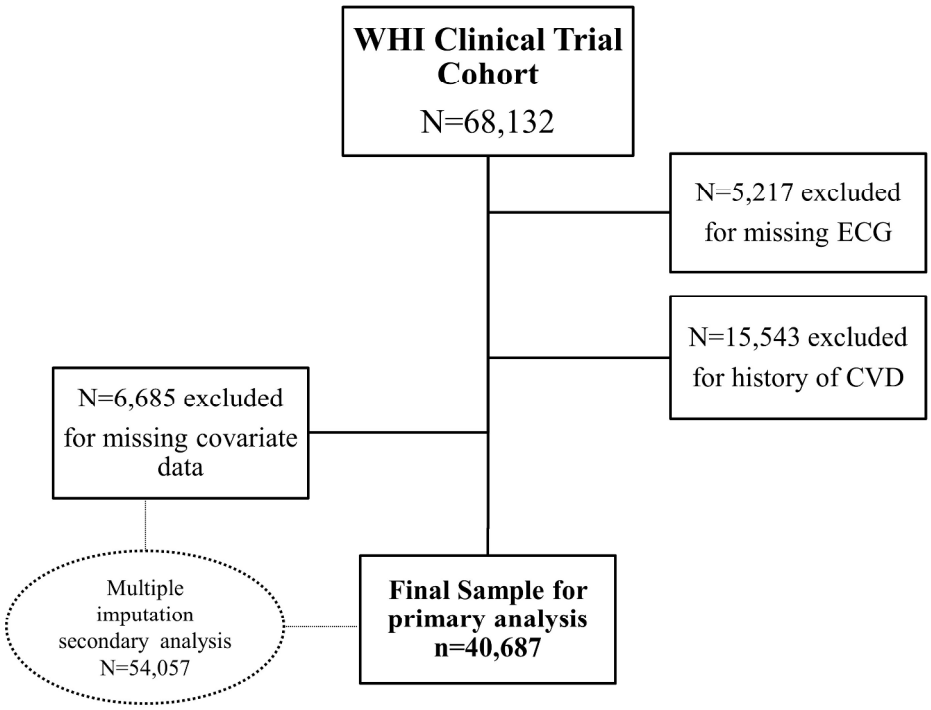
*n differs from main analyses due to the exclusion of women with implausible PR wave measures

Table 5: Reproductive Duration and P wave Duration and Dispersion by hormone use status. in N= 31,538* Women in the Women's Health Initiative Clinical Trial.

Dependent Variable	Hormone Use Status	Unadjusted Effect (95% CI)	Adjusted Effect (95% CI)	P value
P wave duration (ms)	Never User	0.07 (0.03, 0.11)	0.09 (0.06, 0.13)	p value for interaction= 0.0009
	Past	-0.04 (-0.08, 0.005)	0.01 (-0.03, 0.05)	
	Current	-0.03 (-0.06, 0.004)	0.01 (-0.02, 0.05)	
P wave dispersion (ms)	Never User	0.002 (-0.04, 0.05)	0.01 (-0.03, 0.06)	p value for interaction= 0.65
	Past	-0.03 (-0.09, 0.02)	-0.01 (-0.06, 0.05)	
	Current	-0.04 (-0.08, 0.003)	-0.02 (-0.06, 0.03)	

Effect estimates correspond to expected ms increase in PR measure. These models contained an interaction term for reproductive period duration hormone use status. Fully adjusted models were adjusted for number of live births, age, baseline BMI, baseline hypertension status, history of diabetes, income, education, race/ethnicity, region, history of breastfeeding, duration of breastfeeding, antianxiety medication, antidepressant medication, lipid medication, oophorectomy status, hysterectomy status, hormone use history, heart rate, and QRS duration.

*n differs from main analyses due to the exclusion of women with implausible PR wave measures



254x190mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies with page number in manuscript.

	Item No	Recommendation	Page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	1, 5-10
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5-6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6-7*
Bias	9	Describe any efforts to address potential sources of bias	8-9
Study size	10	Explain how the study size was arrived at	6-7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-9
		(*b*) Describe any methods used to examine subgroups and interactions	8-9
		(*c*) Explain how missing data were addressed	9-10
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	5-7
		(*e*) Describe any sensitivity analyses	9-10

Continued on next page

Results			**Page number**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5-6
		(b) Give reasons for non-participation at each stage	5-6
		(c) Consider use of a flow diagram	5-6, 19
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5-6, 20-23
		(b) Indicate number of participants with missing data for each variable of interest	19, 20-23
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*N/A*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*N/A*
		Cross-sectional study—Report numbers of outcome events or summary measures	*5-7*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	24-27
		(*b*) Report category boundaries when continuous variables were categorized	20-23
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10-11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10-11
Discussion			
Key results	18	Summarise key results with reference to study objectives	11-12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14-15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Effects of Reproductive Period Duration and Number of Pregnancies on Mid-Life Electrocardiographic Indices: A Secondary Analysis from the Women's Health Initiative Clinical Trial

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Effects of Reproductive Period Duration and Number of Pregnancies on Mid-Life Electrocardiographic Indices: A Secondary Analysis from the Women's Health Initiative Clinical Trial

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Key Words: endogenous estrogen, sex hormones, pregnancy, menarche, menopause, reproductive history, menarche, age at birth, repolarization, QTc, PR interval, electrocardiogram

Word Count: 4196

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Conflict of Interest: None of the authors have any conflicts of interest in respect to this article

Abstract: (word count=297)

Objectives: Pregnancy, menses and menopause are related to fluctuations in endogenous sex hormones in women, which cumulatively, may alter cardiac electrical conduction. Therefore, we sought to study the association between number of pregnancies and reproductive period duration (RD, time from menarche to menopause) with electrocardiographic intervals in the Women's Health Initiative Clinical Trials.

Design: Secondary Analysis of Multicenter Clinical Trial.

Setting: United States.

Primary Outcome Measures: Electrocardiographic Intervals: PR interval, P wave duration, P wave dispersion, QTc interval

Participants: n=40,687 women (mean age=62 years) participating in the Women's Health Initiative Clinical Trials. 82.5% were White, 9.3% Black, 4% Hispanic and 2.7% Asian.

Methods: In primary analysis, we employed multivariable linear regression models relating number of pregnancies and RD with millisecond (ms) changes in intervals from enrollment electrocardiogram. We studied effect modification by hormone therapy use.

Results: Among participants, 5+ live births versus 0 prior pregnancies was associated with a 1.32 ms increase in PR interval [95% CI (0.25, 2.38)], with a graded association with longer QTc interval (ms) [none (prior pregnancy, no livebirths)= 0.66 (-0.56, 1.88), 1= 0.15 (-0.71, 1.02), 2 to 4= 0.25 (-0.43, 0.94), and 5+ live births=1.15 (0.33, 1.98), p = 0.008]. RD was associated with longer PR interval and maximum P wave duration (but not P-wave dispersion) among never users of hormone therapy: [PR (ms) per additional RD year: 0.10 (0.04, 0.16); higher P-wave duration (ms): 0.09 (0.06, 0.12)]. For every year increase in reproductive period, QTc decreased by 0.04 ms (-0.07, -0.01).

Conclusions: An increasing number of live births is related to increased and RD to decreased ventricular repolarization time. Both grandmultiparity and longer RD are related to increased atrial conduction time. Reproductive factors that alter midlife cardiac electrical conduction system remodeling in women may modestly influence CVD risk in later life.

Article Summary:

Strengths and Limitations of the Study.

- *A strength is the use of a well characterized multiethnic, large dataset of postmenopausal women representative of women in the United States.*
- *A notable limitation is that the exposure variables were acquired retrospectively.*
- *We were unable to adjust for pregnancy complications such as preeclampsia or gestational diabetes.*

Key Words: *endogenous estrogen, sex hormones, pregnancy, menarche, menopause, reproductive history, menarche, age at birth, repolarization, QTc, PR interval, electrocardiogram*

INTRODUCTION:

Electrocardiogram parameters reflect current as well as future CVD risk

Electrocardiographic parameters are reflections of both current as well as future cardiovascular disease risk. For example, in the Framingham Heart Study, a prolonged PR interval (> 200 ms) (which is defined as the period, measured in milliseconds, that extends from the beginning of the

P wave (the onset of atrial depolarization) until the R wave), was related to incident atrial fibrillation, all-cause mortality and to the likelihood of needing a permanent pacemaker.(1) In addition to PR interval, the p wave duration (or the period in milliseconds during which the atrium depolarizes), more directly relates to atrial size and is an antecedent of atrial fibrillation.(2) Both PR interval and p wave duration are markers of left atrial size which in turn is a correlate of hypertensive heart disease(3) and incident stroke.(4) P wave dispersion, defined as the difference between the maximum and the minimum P-wave duration recorded from multiple different-surface ECG leads, is an additional marker of atrial remodeling and antecedent of atrial fibrillation.(5) It is unclear to what extent PR interval, p wave duration or P wave dispersion are affected by premenopausal hormonal fluctuations from the menstrual cycle and childbearing.

Pregnancy, cardiac remodeling and the electrocardiogram

Pregnancy and the post-partum period both have substantial physiologic effects on cardiac electrophysiology. Physiologic studies of women during early and late pregnancy as well as early post-partum suggest a shortening of the corrected QT interval (QTc) which partially reverts back to pre-pregnancy values following post-partum.(6, 7) The QT interval is defined as the measure of time between the onset of ventricular depolarization and completion of ventricular repolarization, and because QT interval is strongly related to heart rate, the QTc is corrected for heart rate. Direct pathophysiologic links connecting myocardial structural remodeling and cardiac electrical remodeling have been increasingly recognized.(8) With regards to myocardial remodeling, pregnancy induced cardiac remodeling does not completely revert back to pre-pregnancy levels and effects of increasing parity on cardiac remodeling can be detected even in

mid-life.(9) However, the extent to which an increasing number of pregnancies exerts long lasting effects on the cardiac electrical conduction system is uncertain.(9)

Estrogen exposure and the electrocardiogram

In addition to the more marked hormonal fluctuations seen during pregnancy, there are also more subtle, cyclic changes in estrogen and progesterone cycling that occur during menstrual cycling in women of reproductive age. Testosterone and progesterone are recognized to decrease the QTc interval.(10) Prior data from the Women's Health Initiative (WHI) Hormone Trial suggests that estrogen-only post-menopausal therapy modestly prolongs QTc beyond that of both estrogen-progestin therapy and placebo.(11) However, it is uncertain whether the pre-menopausal endogenous hormonal fluctuations (reflected by the length of the interval from menarche to menopause, and by number of pregnancies) are associated with changes in QTc in the WHI.

WHI represents a unique resource to study questions related to pregnancy and reproductive history and ECG parameters and thus we sought to determine if there is a positive or negative association between number of pregnancies and reproductive period duration with mid-life electrocardiogram intervals (PR interval and QTc) and p wave parameters (p wave maximum duration and dispersion).

METHODS AND ANALYSIS PLAN:

Our current study design is a secondary analysis of a previously conducted set of clinical trials. .

Study sample.

The WHI recruitment began in 1991 and consisted of a set of clinical trials/ and an observational study on hormone therapy, dietary modification and calcium/ vitamin D supplementation on cardiovascular disease, cancer and fractures.(12) The clinicaltrial.gov identifier for the WHI is NCT00000611. At the time of enrollment, all women enrolled in the WHI were required to be between 50 and 79 years old, postmenopausal, and intending to reside in the area for at least 3 years. Other enrollment criteria have been previously described.(13) This analysis drew from the cohort of women enrolled in the WHI clinical trials (and not observational study), as WHI clinical trial participants has ECGs performed per protocol. Informed consent was obtained from all participants at study enrollment. **Figure 1** shows the creation of the study sample. Of 68,132 women in WHI Studies (post- menopausal hormone therapy, diet and calcium/vitamin D and observational studies), we excluded 5,217 who were missing ECGs and 15,543 who had prevalent CVD. Because number of pregnancies and reproductive period (in particular age at menopause) are known to be associated with later CVD and a history of CVD is related strongly with ECG changes including QTc and certainly increased PR, we sought to exclude women with a history of CVD in order to assess associations between reproductive period duration and number of pregnancies that were not directly mediated through CVD. Of these, 47,372 women, 6,685 were further excluded for having missing covariate data, leaving a final sample of included women =40,687. In a missing imputation sensitivity analysis described below, we additionally analyzed the 6,685 women with missing covariate data (total n=54,057).

Patient and Public Involvement

WHI was designed to address the gaps in knowledge about the major health issues in post menopausal women. Patients assisted research staff in recruiting and results for all measures

done at the study examinations were explained to each participant. Major study results are communicated to participants via newsletters.

Ascertainment of Reproductive Exposures.

Information on reproductive factors was collected via questionnaire at the second screening visit in the WHI (between 1993-1998). Participants were asked how many times they had been pregnant (were given choices ranging from 0 to 8+), number of live births, and how old they were at the end of the first and at the end of their last pregnancy (<20, 20-24, 25-29,30-34,35+ years). In order to be able to also study women who had not experienced pregnancy and/or childbirth and in an effort to make our study as representative as possible, we separately categorized women who had had no prior pregnancies and women who had experienced a pregnancy but no livebirths (i.e. due to miscarriage, stillbirth, or abortion) as separate categories. We further categorized women based on our prior work demonstrating that having 5 or more pregnancies was associated with greater cardiac remodeling.(9) Due to small cell sizes we combined women with 5 or more pregnancies leading to livebirths into one category. Preliminary data analysis reflected that 2-4 had similar effects sizes for PR and QTc and thus these categories were collapsed into a single category for ease of interpretation. Therefore the exposure categories for number of pregnancies leading to livebirths were as follows: no pregnancies (referent), none (prior pregnancy, no livebirths), 1, 2-4, 5 or more. Age at menarche (<9, 10,11,12,13,14,15,>16 years) and age at menopause was asked on this screening questionnaire. Reproductive period duration (RD) was defined as the duration between age at menarche to age at menopause (in years). Detailed current and prior hormone therapy (or post-menopausal hormone replacement therapy) usage and hysterectomy/oophorectomy status was collected at enrollment and has been

previously described.(14) Questions regarding the use and duration of oral contraceptive usage was also collected at enrollment.

Ascertainment of Covariates:

Age, income, education, self-reported race/ethnicity, geographic region of United States, history and duration of breastfeeding were collected at participant enrollment and second screening examinations. Body mass index (BMI, kg/m^2) was calculated using height and weight measured by study staff at baseline. Women with hypertension were identified as those with a self-reported history of treated hypertension or blood pressure measurements meeting JNC 7 criteria for hypertension.(15) Diabetes was identified by self-reported use of anti-diabetic medications and hyperlipidemia by use of cholesterol lowering medications.

Electrocardiographic parameters:

Standard 12-lead ECGs were recorded in all women by strictly standardized procedures in all clinical centers as has been described.(16) All ECGs were processed in a central laboratory (EPICARE Center, University of Alberta, Edmonton, Canada, and later Wake Forest University, Winston-Salem, NC), where they were visually inspected for technical errors and inadequate quality. ECGs were processed with the 2001 version of the Marquette 12-SL program (GE Marquette). In addition to PR and QT intervals, we also examined the maximum P wave duration and dispersion (from all 12 leads of the ECG).(2) The QT interval was corrected using Bazett's formula. The Institutional Review Board of University of California San Francisco approved this study protocol.

Statistical Methods:

Primary Analysis

We employed multivariable linear regression to assess the association between reproductive exposures (number of pregnancies and RD) with the dependent variable of ECG parameters (PR interval in milliseconds, p wave duration, p wave dispersion, QTc in milliseconds). Multivariable models were adjusted for a priori covariates: age, BMI, hypertension status, diabetes, income, education, race/ethnicity, region, history of breastfeeding, antianxiety medication, antidepressant medication, lipid medication, duration of breastfeeding, oophorectomy status, hormone therapy use, heart rate and QRS duration. In analyses considering categories of livebirths we employed a linear trend test.

We explored effect modification of the primary exposures, number of live births and RD, by hormone therapy usage and hysterectomy status. We classified hormone therapy usage into three categories: women who reported current, prior or no hormone therapy usage. A statistical interaction term between hormone therapy usage and the exposure (RD or number of live births) was used to consider effect modification by reported hormone therapy use. When the statistical interaction term was statistically significant ($p < 0.05$) according to a likelihood ratio test, we presented the estimates in each of the three categories of hormone therapy use and we presented a single estimate if there was no evidence for effect modification by hormone therapy. A similar approach was employed for studying RD or number of live births and hysterectomy status. To show sensitivity of estimates to confounders, unadjusted associations were reported as well as those associations adjusted for the confounders listed above.

Secondary Analyses:

In secondary analyses, we removed subjects who reported never being pregnant and used multivariable linear regression to model associations between age at first live birth and the five electrocardiogaphic measures. These models used the same covariates to adjust association as those in our primary analyses. Subjects who had implausible secondary outcome values (i.e. all zero values or all constant values across all electrocardiographic measures) were removed. We additionally adjusted for covariates that we were concerned may have confounded the associations between exposure and dependent variables in our study. We additionally fit additional models which included both RD and number of pregnancies to ensure that one exposure did not alter the other's association with the dependent variables. Given that anxiety and depression could affect both exposure and dependent variables in our study, we further adjusted for use of these medications. Antianxiety and antidepressant medication use (selective serotonin reuptake inhibitors (or SSRI) and non-SSRI) were recorded on enrollment by nurse examination of medication bottles. Medications were classified according to the National Drug Index classification system. We adjusted for Ca/Vitamin D status, oral contraceptive usage (yes/no and duration or usage). We further adjusted for menstrual irregularities/fertility disorders/and endometriosis, which are also related to hormonal fluctuations in women.

Multiple Imputation Analyses

There were n=6,685 women in our study with missing covariate data. We used multiple imputation techniques to impute missing covariates and refit models from primary analyses to explore the sensitivity of our results to missing data. We used the PROC MI in SAS to construct 20 multiply imputed data sets. Missing variables were imputed via fully conditional specification method in PROC MI using all variables from the analytic model. We fit models to each imputed

data set and pooled the results. The pooled results from imputation did not differ appreciably from the results of the complete case analysis (data not shown).

All analyses were performed in SAS 9.4 (SAS Institute, Cary NC, USA).

RESULTS

Table 1 shows the baseline characteristics of our sample including women who were included in our study and those excluded from analysis for missing variables. Data is displayed by number of pregnancies lasting at least 6 months. The mean age at enrollment was 62.4 years, while the mean age at menarche was 12.6 and mean age at menopause was 50.0 years. 82.5% of women were White, 9.3% Black, 4% Hispanic and 2.7% Asian. Forty five percent of the study sample reported never having used hormone therapy prior to enrollment.

PR interval

Compared to women reporting never having been pregnant, having 5 or more pregnancies was associated with a 1.3 ms longer PR interval (**Table 2).** Among women who reported never having used hormones, each additional year of reported reproductive period duration was associated with a 0.1 ms longer PR interval (or atrial conduction velocity). Conversely, there was no significant association between RD and PR interval among women who reported prior or current hormone therapy use (p value for interaction < 0.01) (**Table 2**). Age at first live birth, was not related to PR interval (data not shown).

QTc

Compared to never having been pregnant, having 5 or more pregnancies was related to a 1.2 ms longer QTc (**Table 3**). However, not carrying a pregnancy to term, or having 1 or 2-4 term

pregnancies (versus not being pregnant), were not related to QTc. For each additional year in reproductive period duration, there was a 0.4 ms shorter QTc (**Table 3**). Restricting to women who had at least one live birth did not change our results (data not shown).

P wave duration and dispersion

P wave dispersion was higher for women with 2-4 live births (ms increase =0.62, 95% CI: 0.01, 1.24) and 5 live births (0.94, 95% CI: 0.20, 1.67), compared with those who reported never having been pregnant (**Table 4**). Reproductive period duration was related to maximum p wave duration among women who reported never having used hormones (0.09, 95% CI: 0.06, 0.13) but not among those who reported prior or current hormone therapy use (p interaction < 0.01) (**Table 5**).

Secondary results: Models that contained both RD and number of pregnancies together were not materially different (data not shown). Further adjustment for antidepressants and anti-anxiety medications did not materially affect our results. Further adjustment for Ca and Vitamin D status or oral contraceptive use, and/or duration did not materially affect our results. Further adjustment for menstrual irregularities/fertility disorders/and endometriosis did not materially change our results.

DISCUSSION

Summary of Findings

We found that having five or more pregnancies compared to none was associated with a small increase in mid-life atrial conduction time, independent of factors known to be associated with

this interval (PR). Number of live births among women with at least one live birth (compared to no prior pregnancies) was associated with increased atrial conduction time. Having 5 or more pregnancies was related to a small increase in ventricular repolarization time as compared to having no prior pregnancies. Among women reporting no prior exogenous hormone use, each additional year of reported RD was related to a very modest (0.1 ms) longer atrial conduction time. RD was related to a very modest increase in p wave duration. RD was related to a shorter ventricular repolarization time.

Mechanisms linking pregnancy and atrial electrical remodeling

The effect of cumulative pregnancies on mid-life electrocardiograms would likely result from both 1) the pregnancy itself and 2) incident cardiometabolic factors that are impacted by pregnancy such as adiposity(17) and vascular stiffness,(18) and premenopausal blood pressure.(19) Adiposity and blood pressure are related to increased P wave indices in a normal healthy population,(20) and these P wave indices are electrocardiographic reflections of increased left atrial pressure, size and potentially fibrosis. The period of pregnancy and the peripartum are characterized by hormonal changes that affect both cardiovascular hemodynamics and adaptive myocardial remodeling.(21) Pregnancy causes increased cardiac output, increased left ventricular mass, and decreased systemic vascular resistance.(22) The uterus and placenta in support of the growing fetus and fetal circulatory system represent a significant vascular shunt which contributes to these hemodynamic adaptations in pregnancy.(22) The sum of these changes result in both left atrial and left ventricular dilation. However, the effects of normal pregnancy on electrographic remodeling during pregnancy are not well described. A prior small

clinical study has looked at P wave duration and P wave dispersion among pregnant women compared with controls and found that both of these parameters are increased.(23)

Pregnancy and cumulative effects on ventricular repolarization

A prior study in 37 women in late pregnancy compared with 18 age matched controls demonstrated that QTc substantially prolongs late in pregnancy and that this only partially corrects back to pre-pregnancy values post-partum.(7) Our finding that having 5 or more pregnancies as compared to no prior pregnancies suggests that QTc prolongation during pregnancy may accumulate across successive pregnancies and will be significantly increased on mid-life ECG. Furthermore, we found evidence for a dose response relationship between number of pregnancies and mid-life QTc. Cardiac electrical remodeling often reflects myocardial remodeling. We previously demonstrated that an increasing number of pregnancies were related to left ventricular volume increase and increase in left ventricular mass in a multiethnic cohort of women.(9) The increase in cardiac volume and mass were more marked in grandmultipara's or women who had 5 or more pregnancies leading to livebirths.(9) It is important to note that grandmultiparity is less common with declining parity levels in the United States.

Reproductive period duration and atrial conduction.

The menstrual cycle consists of a relatively well described hormone cycling in women consisting of both estrogen and progesterone as well as testosterone production. A longer reproductive period duration reflects the cumulative exposure that a woman has to these endogenous fluctuations in sex hormone levels. Indeed, prior studies have assessed P wave parameters throughout the menstrual cycle and noted that P wave duration is substantially increased in the

luteal phase.(24) Among women who did report taking prior hormone therapy, we observed a very modest but significant increase in mid-life PR interval and in P wave duration. Exogenous hormone therapy use may obscure the relationship between endogenous hormone exposure from a longer reproductive period duration and P wave parameters, which would explain our findings of effect modification by hormone therapy use. An earlier age at menarche (which would be related to increased reproductive period duration) has been associated with increased adiposity(25) and diabetes,(26) which in turn have been linked with increased p wave duration(2) and, in the case of body mass index, with increased left atrial remodeling(27) and thus may also partially underlie our findings.

Reproductive duration and decrease ventricular repolarization time

The QTc is shortened by the action of progesterone and lengthened by estrogen during normal menstrual cycling. The net effect of these changes during a single menstrual cycle can result in shortening of ventricular repolarization time or QTc.(28) Our finding that an increased reproductive duration was modestly inversely related to QTc in WHI. Underlying these findings may be that increasing exposure to progesterone, in particular during menstrual cycling, may have cumulative and measurable effects on the mid-life electrocardiogram in women.

Clinical relevance of our findings.

The PR interval normally ranges from 120 to 200 ms in duration. Therefore our finding that having 5 or more livebirths versus never having been pregnant was associated with an adjusted increase in PR interval of 1.32 ms, has modest clinical significance. For an individual with a PR interval at the upper limits of normal, 1.32 ms may be more clinically relevant in terms of the

increased risks of later cardiovascular diseases with PR >200 ms. (1) The association of number of pregnancies leading to livebirths with QTc (with 5 or more pregnancies leading to livebirths having a 1.15 ms increase in QTc compared to nulligravid women) is similarly modest with a normal QTc ranging from ~350 to 460 ms in women. The effect sizes for reproductive duration were even more modest in size than those for P wave indices and therefore likely have more relevance in terms of uncovering novel biologic mechanisms related to cardiac electrical remodeling rather than reflecting clinically significant differences among individuals.

Strength and Limitations

The use of a well characterized multiethnic, large dataset of postmenopausal women representative of women in the United States is a strength of our study. A notable limitation is potential recall bias since the exposure variables were acquired retrospectively and some are very distant events (eg age at menarche occurred 40-70 years in the past). We were unable to adjust for pregnancy complications such as preeclampsia or gestational diabetes since these were not collected. We did not adjust for smoking, physical activity, and habitual consumption of alcohol and coffee which may have been related to the exposure variables but are not widely known to be related to the ECG dependent variables studied. We studied number of pregnancies in a categorical fashion and were unable, due to data constraints, to look at number of pregnancies as a continuous variables.

Directions for future research:

Future studies that disentangle specific hormonal and molecular mechanisms that underlie the association demonstrated in our study will help us better understand our study findings.

Understanding which specific fertility factors alter electrical remodeling in women is an important direction for future research.

Conclusions

We found that having five or more pregnancies leading to livebirths compared to never having been pregnant is related to small but significant changes in atrial conduction time and ventricular repolarization time. A longer reproductive period duration in women not exposed to exogenous hormone therapy is related to a modest increase in atrial conduction time and to a modest decrease in ventricular repolarization. Reproductive health factors reflective of endogenous sex hormone exposure may be significant determinants of cardiac electrical remodeling in mid-life.

Disclosures

None

Author contributorship statement:

Nisha I. Parikh conceived of the idea, designed the study, interpreted the analysis, drafted and critically reviewed the manuscript. She provided final approval of the manuscript

Kristopher Kapphahn and Haley Hedlin conducted study design, statistical analysis and critical review of the manuscript. They provided final approval of the manuscript

Jeffrey E. Olgin, Matthew A. Allison, Jared W. Magnani, MSc, Kelli R. Ryckman, Molly E. Waring, Marco V. Perez assisted with study design, analysis interpretation, drafting and critical reviewed the manuscript. They provided final approval of the manuscript

Barbara V. Howard assisted with study design, interpreted the analysis, drafted and critically reviewed the manuscript. They provided final approval of the manuscript

Data sharing statement:

This was a secondary analysis of preexisting data and as such, no new data was generated by this study. Information about data sharing for the Women's Health Initiative can be found at the following website: https://www.whi.org/researchers/data/Pages/Home.aspx

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Figure Legend: Creation of the Study Sample. Clinical Trials include Hormone Trial, Dietary Modification and Calcium/Vitamin D. ECG=electrocardiogram, CVD=Cardiovascular diseases.

522 **Table 1: Baseline Characteristics of the Study Sample- Analytic and Excluded**

Distribution of Covariates by Number of Live Births	Never pregnant	None (prior pregnancy, no livebirths)	1	2-4	5+	Analytic	Excluded
Sample Size	3296	1082	3536	26599	6174	40687	6685
Age, N (%)							
50 to 54	598 (18.1)	265 (24.5)	745 (21.1)	3718 (14)	329 (5.3)	5655 (13.9)	834 (12.5)
55 to 59	768 (23.3)	295 (27.3)	869 (24.6)	6282 (23.6)	1054 (17.1)	9268 (22.8)	1204 (18)
60 to 69	1323 (40.1)	371 (34.3)	1363 (38.5)	12189 (45.8)	3580 (58)	18826 (46.3)	3146 (47.1)
70 to 79	607 (18.4)	151 (14)	559 (15.8)	4410 (16.6)	1211 (19.6)	6938 (17.1)	1501 (22.5)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Race/Ethnicity, N (%)							
American Indian/Alaskan Native	3 (0.1)	3 (0.3)	13 (0.4)	96 (0.4)	32 (0.5)	147 (0.4)	39 (0.6)
Asian/PI	121 (3.7)	33 (3)	102 (2.9)	728 (2.7)	106 (1.7)	1090 (2.7)	143 (2.1)
African-American	263 (8)	203 (18.8)	604 (17.1)	2126 (8)	598 (9.7)	3794 (9.3)	1017 (15.2)
Hispanic	106 (3.2)	50 (4.6)	160 (4.5)	1005 (3.8)	300 (4.9)	1621 (4)	471 (7)
White	2767 (84)	780 (72.1)	2601 (73.6)	22352 (84)	5056 (81.9)	33556 (82.5)	4808 (71.9)
Other	36 (1.1)	13 (1.2)	56 (1.6)	292 (1.1)	82 (1.3)	479 (1.2)	98 (1.5)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	109 (1.6)
Education Level, N (%)							
No high school diploma	73 (2.2)	41 (3.8)	157 (4.4)	1033 (3.9)	543 (8.8)	1847 (4.5)	644 (9.6)
High school diploma	1352 (41)	490 (45.3)	1872 (52.9)	15726 (59.1)	4096 (66.3)	23536 (57.8)	3752 (56.1)
Bachelor's degree	802 (24.3)	250 (23.1)	801 (22.7)	5859 (22)	1087 (17.6)	8799 (21.6)	1171 (17.5)
Graduate degree	1069 (32.4)	301 (27.8)	706 (20)	3981 (15)	448 (7.3)	6505 (16)	789 (11.8)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	329 (4.9)
Household							

Income, N (%)							
<$50,000	2071 (62.8)	639 (59.1)	2136 (60.4)	15872 (59.7)	4543 (73.6)	25261 (62.1)	2809 (42)
$50 to 100,000	974 (29.6)	333 (30.8)	1090 (30.8)	8260 (31.1)	1330 (21.5)	11987 (29.5)	874 (13.1)
>$100,000	251 (7.6)	110 (10.2)	310 (8.8)	2467 (9.3)	301 (4.9)	3439 (8.5)	208 (3.1)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2794 (41.8)
Region, N (%)							
Northeast	763 (23.1)	202 (18.7)	694 (19.6)	5830 (21.9)	1554 (25.2)	9043 (22.2)	1484 (22.2)
Midwest	719 (21.8)	192 (17.7)	709 (20.1)	5793 (21.8)	1792 (29)	9205 (22.6)	1422 (21.3)
South	778 (23.6)	321 (29.7)	1012 (28.6)	6559 (24.7)	1205 (19.5)	9875 (24.3)	2081 (31.1)
West	1036 (31.4)	367 (33.9)	1121 (31.7)	8417 (31.6)	1623 (26.3)	12564 (30.9)	1698 (25.4)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
BMI, N (%)							
Underweight (< 18.5)	34 (1)	8 (0.7)	26 (0.7)	103 (0.4)	15 (0.2)	186 (0.5)	25 (0.4)
Normal (18.5 - 24.9)	1001 (30.4)	309 (28.6)	1041 (29.4)	7557 (28.4)	1341 (21.7)	11249 (27.6)	1582 (23.7)
Overweight (25.0 - 29.9)	1118 (33.9)	377 (34.8)	1234 (34.9)	9660 (36.3)	2262 (36.6)	14651 (36)	2262 (33.8)
Obese (30+)	1143 (34.7)	388 (35.9)	1235 (34.9)	9279 (34.9)	2556 (41.4)	14601 (35.9)	2579 (38.6)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	237 (3.5)
Hypertension, N (%)							
Never hypertensive	2308 (70)	742 (68.6)	2439 (69)	18413 (69.2)	4045 (65.5)	27947 (68.7)	4236 (63.4)
Treated hypertensive	712 (21.6)	240 (22.2)	819 (23.2)	6141 (23.1)	1638 (26.5)	9550 (23.5)	1684 (25.2)
Untreated hypertensive	276 (8.4)	100 (9.2)	278 (7.9)	2045 (7.7)	491 (8)	3190 (7.8)	516 (7.7)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	249 (3.7)
History of Diabetes, N (%)							
Yes	150 (4.6)	57 (5.3)	179 (5.1)	1377 (5.2)	434 (7)	2197 (5.4)	459 (6.9)
No	3146 (95.4)	1025 (94.7)	3357 (94.9)	25222 (94.8)	5740 (93)	38490 (94.6)	6217 (93)

MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	9 (0.1)
History of Breastfeeding, N (%)							
Yes	6 (0.2)	36 (3.3)	1476 (41.7)	15375 (57.8)	4258 (69)	21151 (52)	3309 (49.5)
No	3290 (99.8)	1046 (96.7)	2060 (58.3)	11224 (42.2)	1916 (31)	19536 (48)	2942 (44)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	434 (6.5)
History of Oophorectomy, N (%)							
No	2253 (68.4)	711 (65.7)	2462 (69.6)	19371 (72.8)	4704 (76.2)	29501 (72.5)	4404 (65.9)
Yes, part of an ovary was taken out	32 (1)	20 (1.8)	42 (1.2)	225 (0.8)	41 (0.7)	360 (0.9)	85 (1.3)
Yes, one was taken out	203 (6.2)	127 (11.7)	285 (8.1)	1718 (6.5)	356 (5.8)	2689 (6.6)	645 (9.6)
Yes, both were taken out	782 (23.7)	207 (19.1)	716 (20.2)	5082 (19.1)	1019 (16.5)	7806 (19.2)	759 (11.4)
Yes, unknown number taken out	26 (0.8)	17 (1.6)	31 (0.9)	203 (0.8)	54 (0.9)	331 (0.8)	159 (2.4)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	633 (9.5)
History of HT, N (%)							
Never used	1520 (46.1)	478 (44.2)	1601 (45.3)	11660 (43.8)	3324 (53.8)	18583 (45.7)	4372 (65.4)
Past user	561 (17)	191 (17.7)	575 (16.3)	4780 (18)	1147 (18.6)	7254 (17.8)	821 (12.3)
Current user	1215 (36.9)	413 (38.2)	1360 (38.5)	10159 (38.2)	1703 (27.6)	14850 (36.5)	1450 (21.7)
MISSING	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	42 (0.6)
Age (y), Mean (SD)	61.95 (7.43)	60.4 (7.36)	61.14 (7.32)	62.23 (6.87)	64.14 (5.97)	62.36 (6.9)	63.46 (7.16)
Missing (%)	0	0	0	0	0	0	0
BMI, Median (IQR)	27.39 (7.72)	27.74 (8.02)	27.69 (7.56)	27.73 (7.34)	28.78 (7.45)	27.85 (7.46)	28.39 (7.86)
Missing (%)	0	0	0	0	0	0	3.55
QTc wave duration (ms), Mean (SD)	417.95 (18.38)	418.49 (19.6)	418.24 (19.2)	418.58 (18.7)	420.34 (19.33)	418.76 (18.85)	419.82 (19.84)
Missing (%)	0	0	0	0	0	0	0
PR wave	63.86	64.43	64.51	64.79	65.77	64.83	64.87

dispersion (ms), Mean (SD)	(19.42)	(19.43)	(19.43)	(19.57)	(19.57)	(19.55)	(20.56)
Missing (%)	0	0	0	0	0	0	0
PR interval duration (ms), Median (IQR)	156 (30)	158 (28)	158 (30)	158 (30)	160 (30)	158 (30)	158 (30)
Missing (%)	0	0	0	0	0	0	0
P wave duration (ms), Mean (SD)	106.81 (12.85)	107.13 (12.33)	106.96 (12.18)	106.88 (12.34)	107.93 (12.44)	107.05 (12.39)	106.61 (16.09)
Missing (%)	0	0	0	0	0	0	0
Age at menopause (y), Median (IQR)	48 (8)	49 (7)	49 (8)	50 (7)	50 (8)	50 (7)	49 (7)
Missing (%)	0	0	0	0	0	0	32.3
Age at menarche (y), Mean (SD)	12.54 (1.48)	12.55 (1.57)	12.58 (1.54)	12.58 (1.47)	12.67 (1.46)	12.59 (1.48)	12.65 (1.53)
Missing (%)	0	0	0	0	0	0	1.78
Duration of reproductive period (y), Median (IQR)	36 (8)	36 (8)	36 (8)	37 (8)	37 (7)	37 (8)	36 (8)
Missing (%)	0	0	0	0	0	0	33.63

HT=Hormone Therapy (or hormone replacement therapy)

Table 1: Unadjusted and multivariable-adjusted association of number of pregnancies leading to livebirths and reproductive period duration with PR interval (ms) in N=40,687 women in the Womens Health Initiative Clinical Trials

	Unadjusted Effect (95% CI)	**Multivariable Adjusted Effect* (95% CI)**	
Number of live births and reproductive period duration are each in their own separate multivariable models.			
Number of Live Births *(categorical with never pregnant as referent category)*			p value for linear trend= 0.11
Never Pregnant	Ref.	Ref.	
None (prior pregnancy, no livebirths)	1.44 (-0.18,3.06)	1.15 (-0.43, 2.74)	
1	1.16 (0.04,2.28)	0.54 (-0.57, 1.66)	
2-4	1.20 (0.34,2.05)	0.59 (-0.301, 1.48)	
5+	3.06 (2.07,4.06)	1.32 (0.25, 2.39)	
Due to the fact that there was statistically significant effect modification by HT use upon the association between reproductive period and PR interval in linear regression models, we present the model estimates *by* strata of HT use.			
Reproductive period duration *(continuous, years)*			p value for interaction = 0.009
Never HT User	0.05 (-0.01, 0.11)	0.10 (0.04, 0.16)	
Past HT use	0.002 (-0.07, 0.08)	0.08 (-0.00,0.15)	
Current HT use	-0.09 (-0.15, -0.03)	-0.02 (-0.08, 0.04)	

*Covariates include age, baseline BMI, baseline hypertension status, history of diabetes, income, education, race/ethnicity, region, history/ duration of breastfeeding, lipid medication, oophorectomy status, hysterectomy status, hormone use history, heart rate and QRS duration
HT=hormone therapy

Table 3: Unadjusted and multivariable-adjusted association of number of pregnancies leading to livebirths and reproductive period duration with QTc interval (ms) in N=40,687 women in the Womens Health Initiative and Clinical Trials

	Unadjusted Effect (95% CI)	**Multivariable Adjusted Effect* (95% CI)**	**P value**
Number of live births and reproductive period duration are each in their own multivariable models			
Number of Live Births *(categorical with never pregnant as referent category)*			p value for linear trend=0.008
Never Pregnant	Ref.	Ref.	
None (prior pregnancy, no livebirths)	0.54 (-0.76,1.83)	0.66 (-0.56, 1.88)	
1	0.29 (-0.60,1.18)	0.15 (-0.71, 1.02)	
2-4	0.63 (-0.05,1.31)	0.25 (-0.43, 0.94)	
5+	2.39 (1.59,3.19)	1.15 (0.33, 1.98)	
Reproductive period duration *(continuous, years)*	-0.09 (-0.12,-0.06)	-0.04 (-0.07, -0.01)	p value=0.01

*Covariates for number of livebirths analysis include age, baseline BMI, baseline hypertension status, history of diabetes, income, education, race/ethnicity, region, history/ duration of breastfeeding, lipid medication, oophorectomy status, hysterectomy status, hormone use history, heart rate and QRS duration. Covariates for reproductive period duration analysis include live births, age, baseline BMI, baseline hypertension status, history of diabetes, income, education, race/ethnicity, region, history of breastfeeding, duration of breastfeeding, lipid medication, oophorectomy status, hysterectomy status, hormone use history, and QRS duration.
HT=hormone therapy

Table 4: Unadjusted and multivariable-adjusted associations between number of pregnancies leading to livebirths with p wave duration and p wave dispersion in N=39,338* women in the Women's Health Initiative and Clinical Trials

Dependent Variable	Number of Live Births	Unadjusted Effect (95% CI)	Adjusted Effect (95% CI)	p-value
P wave duration (ms)				p value for linear trend =0.73
	Never Pregnant	Ref.	Ref.	
	None (prior pregnancy, no livebirths)	0.09 (-0.73, 0.92)	0.09 (-0.69, 0.87)	
	1	-0.06 (-0.63, 0.51)	-0.20 (-0.76, 0.35)	
	2-4	-0.03 (-0.47, 0.40)	-0.26 (-0.70, 0.18)	
	5+	0.99 (0.49, 1.50)	-0.22 (-0.74, 0.31)	
P wave dispersion (ms)				p for linear trend =0.13
	Never Pregnant	Ref.	Ref.	
	None (prior pregnancy, no livebirths)	0.67 (-0.42, 1.77)	0.64 (-0.45, 1.72)	
	1	0.44 (-0.32, 1.20)	0.34 (-0.42, 1.11)	
	2-4	0.72 (0.15, 1.30)	0.62 (0.01, 1.24)	
	5+	1.49 (0.82, 2.17)	0.94 (0.20, 1.67)	

Effect estimates correspond to expected ms increase in the specified interval measure for each parity group relative to the never pregnant group. Fully adjusted models were adjusted for age, baseline BMI, baseline hypertension status, history of diabetes, income, education, race/ethnicity, region, history of breastfeeding, antianxiety medication, antidepressant medication, lipid medication, duration of breastfeeding, oophorectomy status, hysterectomy status, hormone use history, heart rate, and QRS duration.

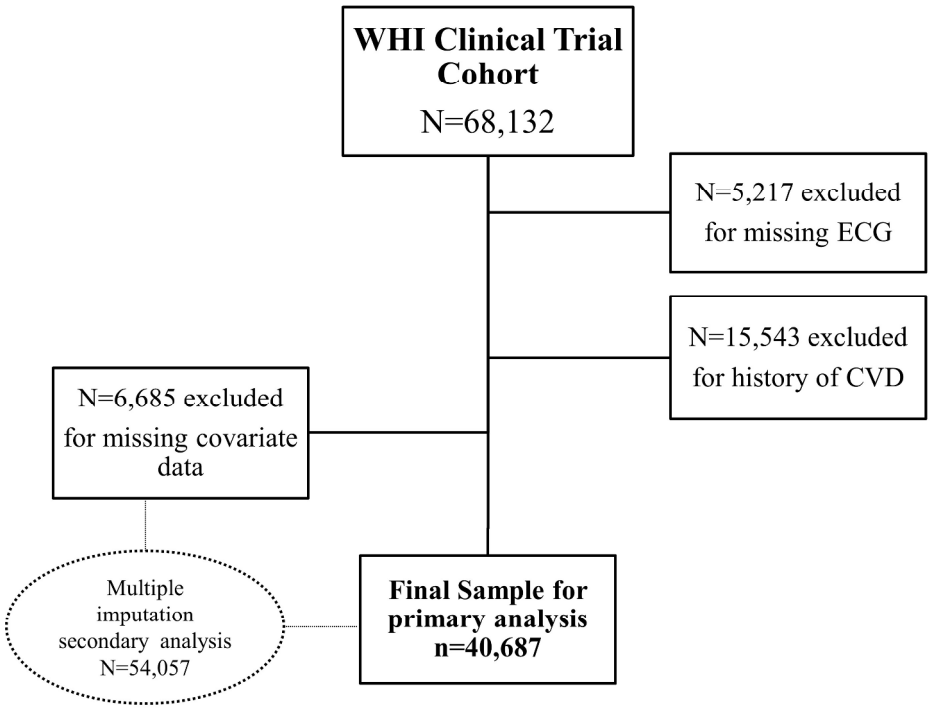
*n differs from main analyses due to the exclusion of women with implausible PR wave measures

Table 5: Reproductive Duration and P wave Duration and Dispersion by hormone use status. in N= 31,538* Women in the Women's Health Initiative Clinical Trial.

Dependent Variable	Hormone Use Status	Unadjusted Effect (95% CI)	Adjusted Effect (95% CI)	P value
Due to the fact that there was statistically significant effect modification by HT use upon the association between reproductive period and P wave duration in linear regression models, we present the model estimates *by* strata of HT use.				
P wave duration (ms)	Never User	0.07 (0.03, 0.11)	0.09 (0.06, 0.13)	p value for interaction= 0.0009
	Past	-0.04 (-0.08, 0.005)	0.01 (-0.03, 0.05)	
	Current	-0.03 (-0.06, 0.004)	0.01 (-0.02, 0.05)	
P wave dispersion (ms)	Never User	0.002 (-0.04, 0.05)	0.01 (-0.03, 0.06)	p value for interaction= 0.65
	Past	-0.03 (-0.09, 0.02)	-0.01 (-0.06, 0.05)	
	Current	-0.04 (-0.08, 0.003)	-0.02 (-0.06, 0.03)	

Effect estimates correspond to expected ms increase in PR measure. These models contained an interaction term for reproductive period duration hormone use status. Fully adjusted models were adjusted for number of live births, age, baseline BMI, baseline hypertension status, history of diabetes, income, education, race/ethnicity, region, history of breastfeeding, duration of breastfeeding, antianxiety medication, antidepressant medication, lipid medication, oophorectomy status, hysterectomy status, hormone use history, heart rate, and QRS duration.

*n differs from main analyses due to the exclusion of women with implausible PR wave measures



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STROBE Statement—checklist of items that should be included in reports of observational studies with page number in manuscript.

	Item No	Recommendation	Page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	1, 5-10
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5-6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6-7*
Bias	9	Describe any efforts to address potential sources of bias	8-9
Study size	10	Explain how the study size was arrived at	6-7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-9
		(*b*) Describe any methods used to examine subgroups and interactions	8-9
		(*c*) Explain how missing data were addressed	9-10
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	5-7
		(*e*) Describe any sensitivity analyses	9-10

Continued on next page

Results			**Page number**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5-6
		(b) Give reasons for non-participation at each stage	5-6
		(c) Consider use of a flow diagram	5-6, 19
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5-6, 20-23
		(b) Indicate number of participants with missing data for each variable of interest	19, 20-23
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*N/A*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*N/A*
		Cross-sectional study—Report numbers of outcome events or summary measures	*5-7*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	24-27
		(*b*) Report category boundaries when continuous variables were categorized	20-23
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10-11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10-11
Discussion			
Key results	18	Summarise key results with reference to study objectives	11-12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14-15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.