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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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4 2 **Effects of Reproductive Period Duration and Number of**
5 3 **Pregnancies on Mid-Life Electrocardiographic Indices: A**
6 4 **Secondary Analysis from the Women's Health Initiative Clinical**
7 5 **Trial**
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15
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41 32

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43 34

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45 36 reproductive history, menarche, age at birth, repolarization, QTc, PR interval, electrocardiogram
46 37

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3 45
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3 **48 Abstract: (word count=259)**
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5 **49 Objective:** The effects of the hormonal milieu of pregnancy, menses and menopause on cardiac
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8 **50** electrical conduction are uncertain. Therefore, we studied the association between number of
9
10 **51** pregnancies and reproductive period duration (RD, time from menarche to menopause) with
11
12 **52** electrocardiographic intervals in the Women's Health Initiative Clinical Trial.
13

14 **53 Methods:** We employed multivariable linear regression models relating number of pregnancies
15
16 **54** and RD with millisecond (ms) changes in PR interval, P wave indices (duration and dispersion)
17
18 **55** and QTc from enrollment electrocardiogram.
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20

21 **56 Results:** Among 40,687 women (mean age=62 years), 5+ live births versus 0 prior pregnancies
22
23 **57** was associated with a 1.32 ms increase in PR interval [95% CI (0.25, 2.38)], with a graded
24
25 **58** association with longer QTc interval (ms) [none= 0.66 (-0.56, 1.88), 1= 0.15 (-0.71, 1.02), 2 to
26
27 **59** 4= 0.25 (-0.43, 0.94), and 5+ live births=1.15 (0.33, 1.98), p = 0.008]. RD was associated with
28
29 **60** longer PR interval and maximum P wave duration (but not P-wave dispersion) among never
30
31 **61** users of HT: [PR (ms) per additional RD year: 0.10 (0.04, 0.16); higher P-wave duration (ms):
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33 **62** 0.09 (0.06, 0.12)]. For every year increase in reproductive period, QTc decreased by 0.04 ms (-
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35 **63** 0.07, -0.01).
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40 **64 Limitations:** Potential misclassification of exposure due to participant recall
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42 **65 Conclusions:** An increasing number of live births are related to increased ventricular
43
44 **66** repolarization time whereas RD is related to decreased ventricular repolarization time. Both
45
46 **67** longer RD and grandmultiparity are related to increased atrial conduction time. The
47
48 **68** premenopausal hormonal milieu appears to have effects on midlife cardiac electrical conduction
49
50 **69** system remodeling in women that may modestly influence CVD risk in later life.
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56 **71 Article Summary:**
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3 72 *Strengths and Limitations of the Study.*
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- 5 73 • *A strength is the use of a well characterized multiethnic, large dataset of postmenopausal*
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7
8 74 *women representative of women in the United States.*
- 9
10 75 • *A notable limitation is that the exposure variables were acquired retrospectively.*
11
12 76 • *We were unable to adjust for pregnancy complications such as preeclampsia or*
13
14 77 *gestational diabetes.*
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17 78
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19 79 *What is already known about this subject?*
20

21 80 Clinical studies with a relatively limited number of participants suggest that there are small but
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23 81 measureable changes in electrocardiographic intervals during pregnancy.
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26 82
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28 83 *What does this study add?*
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31 84 We demonstrate for the first time in a large cohort of women with systematic research
32
33 85 electrocardiograms that number of livebirths and a longer reproductive period duration (i.e.
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35 86 longer exposure to menstrual cycling) exerts dynamic and measureable effects on ventricular
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37 87 repolarization and atrial conduction.
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42 89 *How might this impact on clinical practice?*
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45 90 Published normal reference values for electrocardiograms throughout pregnancy and the post-
46
47 91 partum period are lacking even though it is widely accepted that pregnancy has material effects
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49 92 on cardiac electrical conduction resulting to increased risks of arrhythmia.(1) There is a dearth
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51 93 of systematic studies assessing p wave parameter changes throughout the menstrual cycle. Such
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3 94 studies would be important in furthering our dual understanding of cardiac adaptations of
4
5 95 pregnancy and in our understanding of pregnancy-related arrhythmia.
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8 96

9
10 97 **Key Words:** *endogenous estrogen, sex hormones, pregnancy, menarche, menopause,*
11
12 98 *reproductive history, menarche, age at birth, repolarization, QTc, PR interval,*
13
14 99 *electrocardiogram*
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21 102 **INTRODUCTION:**

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23 103 *Electrocardiogram parameters reflect current as well as future CVD risk*

24
25 104 Electrocardiographic parameters are reflections of both current as well as future cardiovascular
26
27 105 disease risk. For example, in the Framingham Heart Study, a prolonged PR interval (> 200 ms)
28
29 106 was related to incident atrial fibrillation, all-cause mortality and to the likelihood of needing a
30
31 107 permanent pacemaker.(2) In addition to PR interval, the p wave duration more directly relates to
32
33 108 atrial size and is an antecedent of atrial fibrillation.(3) Both PR interval and p wave duration are
34
35 109 markers of left atrial size which in turn is a correlate of hypertensive heart disease(4) and
36
37 110 incident stroke.(5) It is unclear to what extent PR interval is affected by premenopausal
38
39 111 hormonal fluctuations from the menstrual cycle and childbearing.
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46 113 *Pregnancy, cardiac remodeling and the electrocardiogram*

47
48 114 Pregnancy and the post-partum period both have substantial physiologic effects on cardiac
49
50 115 electrophysiology. Physiologic studies of women during early and late pregnancy as well as early
51
52 116 post-partum suggest a shortening of the corrected QT interval (QTc) which partially reverts back
53
54 117 to pre-pregnancy values following post-partum.(6, 7) Direct pathophysiologic links connecting
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3 118 myocardial structural remodeling and cardiac electrical remodeling have been increasingly
4
5 119 recognized.(8) With regards to myocardial remodeling, pregnancy induced cardiac remodeling
6
7
8 120 does not completely revert back to pre-pregnancy levels and effects of increasing parity on
9
10 121 cardiac remodeling can be detected even in mid-life.(9) However, the extent to which an
11
12 122 increasing number of pregnancies exerts long lasting effects on the cardiac electrical conduction
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14
15 123 system is uncertain.(9)

16 17 124 18 19 125 *Estrogen exposure and the electrocardiogram*

20
21 126 In addition to the more marked hormonal fluctuations seen during pregnancy, there are also more
22
23 127 subtle, cyclic changes in estrogen and progesterone cycling that occur during menstrual cycling
24
25
26 128 in women of reproductive age. Testosterone and progesterone are recognized to decrease the
27
28 129 QTc interval.(10) Data from the Women's Health Initiative Hormone Trial (or WHI HT)
29
30 130 suggests that estrogen-only therapy modestly prolongs QTc beyond that of both estrogen-
31
32 131 progestin therapy and placebo.(11) However, it is uncertain whether the pre-menopausal
33
34 132 hormonal milieu (reflected by the length of the interval from menarche to menopause and
35
36 133 number of pregnancies) is associated with changes in QTc in the WHI HT.
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40 134
41
42 135 WHI HT represents a unique resource to study questions related to pregnancy and
43
44 136 reproductive history and ECG parameters and thus we sought to determine if there is a positive
45
46
47 137 or negative association between number of pregnancies and reproductive period duration with
48
49 138 mid-life electrocardiogram intervals (PR interval and QTc) and p wave parameters (p wave
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51 139 maximum duration, dispersion and index).
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3 **141 METHODS AND ANALYSIS PLAN:**
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5 **142** Our current study design is a secondary analysis of a previously conducted randomized
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7 **143** controlled trial.
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11 **144** *Study sample.*
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13

14 **145** The WHI recruitment began in 1991 and consisted of a set of clinical trials on HT,
15
16 **146** dietary modification and calcium/ vitamin D supplementation on cardiovascular disease, cancer
17
18 **147** and fractures and an observational study.(12) The clinicaltrial.gov identifier for the WHI is
19
20 **148** NCT00000611. This analysis drew from the cohort of women enrolled in the clinical trial.
21
22 **149** **Figure 1** shows the creation of the study sample. Of 68,132 women in WHI Studies (hormone
23
24 **150** therapy, diet and calcium/vitamin D and observational studies), we excluded 5,217 who were
25
26 **151** missing ECGs and 15,543 who had prevalent CVD. Of these, 47,372 women, 6,685 were further
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28 **152** excluded for having missing covariate data, leaving a final sample of 40,687.
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32

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34 **153** *Ascertainment of Reproductive Exposures.*
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36 **154** Information on reproductive factors was collected via questionnaire at the second screening visit
37
38 **155** in the WHI (between 1993-1998). Participants were asked how many times they had been
39
40 **156** pregnant (were given choices ranging from 0 to 9+), number of live births and how old they were
41
42 **157** at the end of the first and at the end of their last pregnancy (<20, 20-24, 25-29,30-34,35+ years).
43
44 **158** Age at menarche (<9, 10,11,12,13,14,15,>16 years) and age at menopause was asked on this
45
46 **159** screening questionnaire. Reproductive period duration (RD) was defined as the duration between
47
48 **160** age at menarche to age at menopause (in years). Detailed current and prior hormone therapy
49
50 **161** usage and hysterectomy/oophorectomy status was collected at enrollment and has been
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52 **162** previously described.(13)
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3 163 *Ascertainment of Covariates:*
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6 164 Age, income, education, self-reported race/ethnicity, geographic region of United States, history
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9 165 and duration of breastfeeding were collected at participant enrollment and second screening
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11 166 examinations. Body mass index (BMI, kg/m²) was calculated using height and weight measured
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13 167 by study staff at baseline. Women with hypertension were identified as those with a self-reported
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15 168 history of treated hypertension or blood pressure measurements meeting JNC 7 criteria for
16
17 169 hypertension.(14) Diabetes was identified by self-reported use of anti-diabetic medications and
18
19 170 hyperlipidemia by use of cholesterol lowering medications. Antianxiety and antidepressant
20
21 171 medication use was validated on enrollment by nurse examination of medication bottles.
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26 172 *Electrocardiographic parameters:*
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29 173 Standard 12-lead ECGs were recorded in all women by strictly standardized procedures in all
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31 174 clinical centers as has been described.(15) All ECGs were processed in a central laboratory
32
33 175 (EPICARE Center, University of Alberta, Edmonton, Canada, and later Wake Forest University,
34
35 176 Winston-Salem, NC), where they were visually inspected for technical errors and inadequate
36
37 177 quality. ECGs were processed with the 2001 version of the Marquette 12-SL program (GE
38
39 178 Marquette). In addition to PR and QT intervals, we also examined the maximum P wave
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41 179 duration (from all 12 leads of the ECG).(3) The QT interval was corrected using Bazett's
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43 180 formula. The Institutional Review Board of University of California San Francisco approved this
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45 181 study protocol.
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51 182 *Statistical Methods:*
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54 183 Primary Analysis
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3 184 We employed multivariable linear regression to assess the association between reproductive
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5 185 exposures (number of pregnancies and RD) with the dependent variable of ECG parameters
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7 186 (PR interval in milliseconds, QTc in milliseconds). Four primary models were fit: PR regressed
8
9 187 on number of live births, QTc regressed on number of live births, PR regressed on RD, and QTc
10
11 188 regressed on RD. Multivariable models were adjusted for a priori covariates: age, BMI,
12
13 189 hypertension status, diabetes, income, education, race/ethnicity, region, history of breastfeeding,
14
15 190 antianxiety medication, antidepressant medication, lipid medication, duration of breastfeeding,
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17 191 oophorectomy status, hormone therapy use, heart rate and QRS duration. In analyses considering
18
19 192 categories of livebirths we employed a linear trend test.
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23 193 We explored effect modification of the primary exposures, number of live births and RD, by
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25 194 hormone therapy usage and hysterectomy status. We classified hormone therapy usage into three
26
27 195 categories: women who reported current, prior or no hormone therapy usage. A statistical
28
29 196 interaction term between hormone therapy usage and the exposure (RD or number of live births)
30
31 197 was used to consider effect modification by reported hormone therapy use. When the statistical
32
33 198 interaction term was statistically significant ($p < 0.05$) according to a likelihood ratio test, we
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35 199 presented the estimates in each of the three categories of hormone therapy use and we presented
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37 200 a single estimate if there was no evidence for effect modification by hormone therapy. A similar
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39 201 approach was employed for studying RD or number of live births and hysterectomy status. To
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41 202 show sensitivity of estimates to confounders, unadjusted associations were reported as well as
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43 203 those associations adjusted for the confounders listed above.
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52 205 *Secondary Analyses:*
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3 206 In secondary analyses, we studied our exposures in relation to secondary outcomes, P wave
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5 207 duration and p wave dispersion. In an additional secondary set of analyses, we removed subjects
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7 208 who reported never being pregnant and used multivariable linear regression to model
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9 209 associations between age at first live birth and the five electrocardiographic measures. These
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11 210 models used the same covariates to adjust association as those in our primary analyses. Further,
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13 211 Subjects who had implausible secondary outcome values (i.e. all zero values or all constant
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15 212 values across all electrocardiographic measures) were removed.
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21 214 *Multiple Imputation Analyses*

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23 215 We used multiple imputation techniques to impute missing covariates and refit models from
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25 216 primary analyses to explore the sensitivity of our results to missing data. We used the PROC MI
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27 217 in SAS to construct 5 multiply imputed data sets. Missing variables were imputed via fully
28
29 218 conditional specification method in PROC MI using all variables from the analytic model. We fit
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31 219 models to each imputed data set and pooled the results. The pooled results from imputation did
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33 220 not differ appreciably from the results of the complete case analysis.
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37 221 All analyses were performed in SAS 9.4 (SAS Institute, Cary NC, USA).
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41 42 223 **RESULTS**

43
44 224 **Table 1** shows the baseline characteristics of our sample by number of pregnancies lasting at
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46 225 least 6 months. The mean age at enrollment was 62.4 years, while the mean age at menarche was
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48 226 12.6 and mean age at menopause was 50.0 years. 82.5% of women were White, 9.3% Black, 4%
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50 227 Hispanic and 2.7% Asian. Forty five percent of the study sample reported never having used
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52 228 hormone therapy prior to enrollment.
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5 230 *PR interval*
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8 231 Compared to women reporting never having been pregnant, having 5 or more pregnancies was
9
10 232 associated with a 1.3 ms longer PR interval (**Table 2**). Among women who reported never
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12 233 having used hormones, each additional year of reported reproductive period duration was
13
14 234 associated with a 0.1 ms longer PR interval (or atrial conduction velocity). Conversely, there
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16
17 235 was no significant association between RD and PR interval among women who reported prior or
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19 236 current hormone therapy use (p value for interaction < 0.01) (**Table 2**). Age at first live birth,
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21 237 was not related to PR interval (data not shown).

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24 238 *QTc*

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26 239 Compared to never having been pregnant, having 5 or more pregnancies was related to a 1.2 ms
27
28 240 longer QTc (**Table 3**). However, not carrying a pregnancy to term, or having 1 or 2-4 term
29
30 241 pregnancies (versus not being pregnant), were not related to QTc. For each additional year in
31
32 242 reproductive period duration, there was a 0.4 ms shorter QTc (**Table 3**). Restricting to women
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34 243 who had at least one live birth did not change our results (data not shown).

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37 244 *P wave duration and dispersion*

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40 245 P wave dispersion was higher for women with 2-4 live births (ms increase =0.62, 95% CI: 0.01,
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42 246 1.24) and 5 live births (0.94, 95% CI: 0.20, 1.67), compared with those who reported never
43
44 247 having been pregnant (**Table 4**). Reproductive period duration was related to maximum p wave
45
46 248 duration among women who reported never having used hormones (0.09, 95% CI: 0.06, 0.13)
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48 249 but not among those who reported prior or current hormone therapy use (p interaction < 0.01)
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50 250 (**Table 5**).

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3 **252 DISCUSSION**
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5 **253** *Summary of Findings*
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7 **254** We found that having five or more pregnancies compared to none was associated with a small
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9 **255** increase in mid-life atrial conduction time, independent of factors known to be associated with
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11 **256** this interval (PR). Number of live births among women with at least one live birth (compared to
12
13 **257** no prior pregnancies) was associated with increased atrial conduction time. Having 5 or more
14
15 **258** pregnancies was related to a small increase in ventricular repolarization time as compared to
16
17 **259** having no prior pregnancies. Among women reporting no prior exogenous hormone use, each
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19 **260** additional year of reported RD was related to a very modest (0.1 ms) longer atrial conduction
20
21 **261** time. RD was related to a very modest increase in p wave duration. RD was related to a shorter
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23 **262** ventricular repolarization time.
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30 **263**

31 **264** *Mechanisms linking pregnancy and atrial electrical remodeling*
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33 **265** The effect of cumulative pregnancies on mid-life electrocardiograms would likely result from
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35 **266** both 1) the pregnancy itself and 2) incident cardiometabolic factors that are impacted by
36
37 **267** pregnancy such as adiposity(16) and vascular stiffness,(17) and premenopausal blood
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39 **268** pressure.(18) Adiposity and blood pressure are related to increased P wave indices in a normal
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41 **269** healthy population,(19) and these P wave indices are electrocardiographic reflections of
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43 **270** increased left atrial pressure, size and potentially fibrosis. The period of pregnancy and the
44
45 **271** peripartum are characterized by hormonal changes that affect both cardiovascular hemodynamics
46
47 **272** and adaptive myocardial remodeling. Pregnancy causes increased cardiac output, increased left
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49 **273** ventricular mass, and decreased systemic vascular resistance.(20) The uterus and placenta in
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51 **274** support of the growing fetus and fetal circulatory system represent a significant vascular shunt
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3 275 which contributes to these hemodynamic adaptations in pregnancy. The sum of these changes
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5 276 result in both left atrial and left ventricular dilation. However, the effects of normal pregnancy on
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7 277 electrographic remodeling during pregnancy are not well described. A prior small clinical study
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9
10 278 has looked at P wave duration and P wave dispersion among pregnant women compared with
11
12 279 controls and found that both of these parameters are increased.(21)
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17 281 *Pregnancy and cumulative effects on ventricular repolarization*

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19 282 A prior study in 37 women in late pregnancy compared with 18 age matched controls
20
21 283 demonstrated that QTc substantially prolongs late in pregnancy and that this only partially
22
23 284 corrects back to pre-pregnancy values post-partum.(7) Our finding that having 5 or more
24
25 285 pregnancies as compared to no prior pregnancies suggests that QTc prolongation during
26
27 286 pregnancy may accumulate across successive pregnancies and will be significantly increased on
28
29 287 mid-life ECG. Furthermore, we found evidence for a dose response relationship between number
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31 288 of pregnancies and mid-life QTc.
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37 290 *Reproductive period duration and atrial conduction velocity.*

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39 291 The menstrual cycle consists of a relatively well described hormone cycling in women consisting
40
41 292 of both estrogen and progesterone as well as testosterone production. A longer reproductive
42
43 293 period duration reflects the cumulative exposure that a woman has to these endogenous
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45 294 fluctuations in sex hormone levels. Indeed, prior studies have assessed P wave parameters
46
47 295 throughout the menstrual cycle and noted that P wave duration is substantially increased in the
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49 296 luteal phase.(22) Among women who did report taking prior hormone therapy, we observed a
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51 297 very modest but significant increase in mid-life PR interval and in P wave duration. Exogenous
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3 298 hormone therapy use may obscure the relationship between endogenous hormone exposure from
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5 299 a longer reproductive period duration and P wave parameters, which would explain our findings
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7
8 300 of effect modification by hormone therapy use. An earlier age at menarche (which would be
9
10 301 related to increased reproductive period duration) has been associated with increased
11
12 302 adiposity(23) and diabetes,(24) which in turn have been linked with increased p wave
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14 303 duration(3) and, in the case of body mass index, with increased left atrial remodeling(25) and
15
16 304 thus may also partially underlie our findings.
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21 306 *Reproductive duration and decrease ventricular repolarization time*

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23
24 307 The QTc is shortened by the action of progesterone and lengthened by estrogen during normal
25
26 308 menstrual cycling. The net effect of these changes during a single menstrual cycle can result in
27
28 309 shortening of ventricular repolarization time or QTc.(26) Our finding that an increased
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30 310 reproductive duration was modestly inversely related to QTc in WHI, suggests that an increasing
31
32 311 exposure to progesterone, in particular during menstrual cycling, may have cumulative and
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34 312 measurable effects on the mid-life electrocardiogram in women.
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40 314 *Strength and Limitations*

41
42 315 The use of a well characterized multiethnic, large dataset of postmenopausal women
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44 316 representative of women in the United States is a strength of our study. A notable limitation is
45
46 317 that the exposure variables were acquired retrospectively and some are very distant events (eg
47
48 318 age at menarche occurred 40-70 years in the past). We were unable to adjust for pregnancy
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50 319 complications such as preeclampsia or gestational diabetes since these were not collected. We
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52 320 did not adjust for smoking, physical activity, and habitual consumption of alcohol and coffee
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3 321 which may have been related to the exposure variables but are not widely known to be related to
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5 322 the ECG dependent variables studied.
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10 324 *Conclusions*

11
12 325 We found that having an increasing number of pregnancies is related to significant changes in
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14 326 atrial conduction time and ventricular repolarization time. A longer reproductive period duration
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16 327 in women not exposed to exogenous hormone therapy is related to a modest increase in atrial
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18 328 conduction time and to a modest decrease in ventricular repolarization. Reproductive health
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20 329 factors reflective of endogenous sex hormone exposure may be significant determinants of
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22 330 cardiac electrical remodeling in mid-life.
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28 332 *Disclosures*

29
30 333 None
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35 335 *Author contributorship statement:*

36
37 336 Nisha I. Parikh conceived of the idea, designed the study, interpreted the analysis, drafted and
38 337 critically reviewed the manuscript. She provided final approval of the manuscript

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40 338 Kristopher Kapphahn and Haley Hedlin conducted study design, statistical analysis and critical
41 339 review of the manuscript. They provided final approval of the manuscript

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43 340 Jeffrey E. Olgin, Matthew A. Allison, Jared W. Magnani, MSc, Kelli R. Ryckman, Molly E.
44 341 Waring, Marco V. Perez assisted with study design, analysis interpretation, drafting and critical
45 342 reviewed the manuscript. They provided final approval of the manuscript

46
47 343 Barbara V. Howard assisted with study design, interpreted the analysis, drafted and critically
48 344 reviewed the manuscript. They provided final approval of the manuscript

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50 345 *Data sharing statement:*
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3 346 This was a secondary analysis of preexisting data and as such, no new data was generated by this
4 347 study. Information about data sharing for the Women's Health Initiative can be found at the
5 348 following website: <https://www.whi.org/researchers/data/Pages/Home.aspx>
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443 **Figure Legend: Creation of the Study Sample.** Clinical Trials include Hormone Trial, Dietary
444 Modification and Calcium/Vitamin D. ECG=electrocardiogram, CVD=Cardiovascular diseases.
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446 **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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449 **Table 1: Unadjusted and multivariable-adjusted association of number of pregnancies**
 450 **leading to livebirths and reproductive period duration with PR interval (ms) in N=40,687**
 451 **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 (<i>categorical with never pregnant as referent category</i>)			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 (<i>continuous, years</i>)			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)	

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

462 **Table 3: Unadjusted and multivariable-adjusted association of number of pregnancies**
 463 **leading to livebirths and reproductive period duration with QTc interval (ms) in N=40,687**
 464 **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 (<i>categorical with never pregnant as referent category</i>)			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 (<i>continuous, years</i>)	-0.09 (-0.12,-0.06)	-0.04 (-0.07, -0.01)	p value=0.01

465 *Covariates for number of livebirths analysis include age, baseline BMI, baseline hypertension
 466 status, history of diabetes, income, education, race/ethnicity, region, history/ duration of
 467 breastfeeding, antianxiety medication, antidepressant medication, lipid medication,
 468 oophorectomy status, hysterectomy status, hormone use history, heart rate and QRS duration.
 469 Covariates for reproductive period duration analysis include live births, age, baseline BMI,
 470 baseline hypertension status, history of diabetes, income, education, race/ethnicity, region,
 471 history of breastfeeding, duration of breastfeeding, antianxiety medication, antidepressant
 472 medication, lipid medication, oophorectomy status, hysterectomy status, hormone use history,
 473 and QRS duration.
 474 HT=hormone therapy
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476 **Table 4: Unadjusted and multivariable-adjusted associations between number of**
 477 **pregnancies leading to livebirths with p wave duration and p wave dispersion in N=39,338***
 478 **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)	Never Pregnant	Ref.	Ref.	p value for linear trend =0.73
	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)	Never Pregnant	Ref.	Ref.	p for linear trend =0.13
	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)	

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

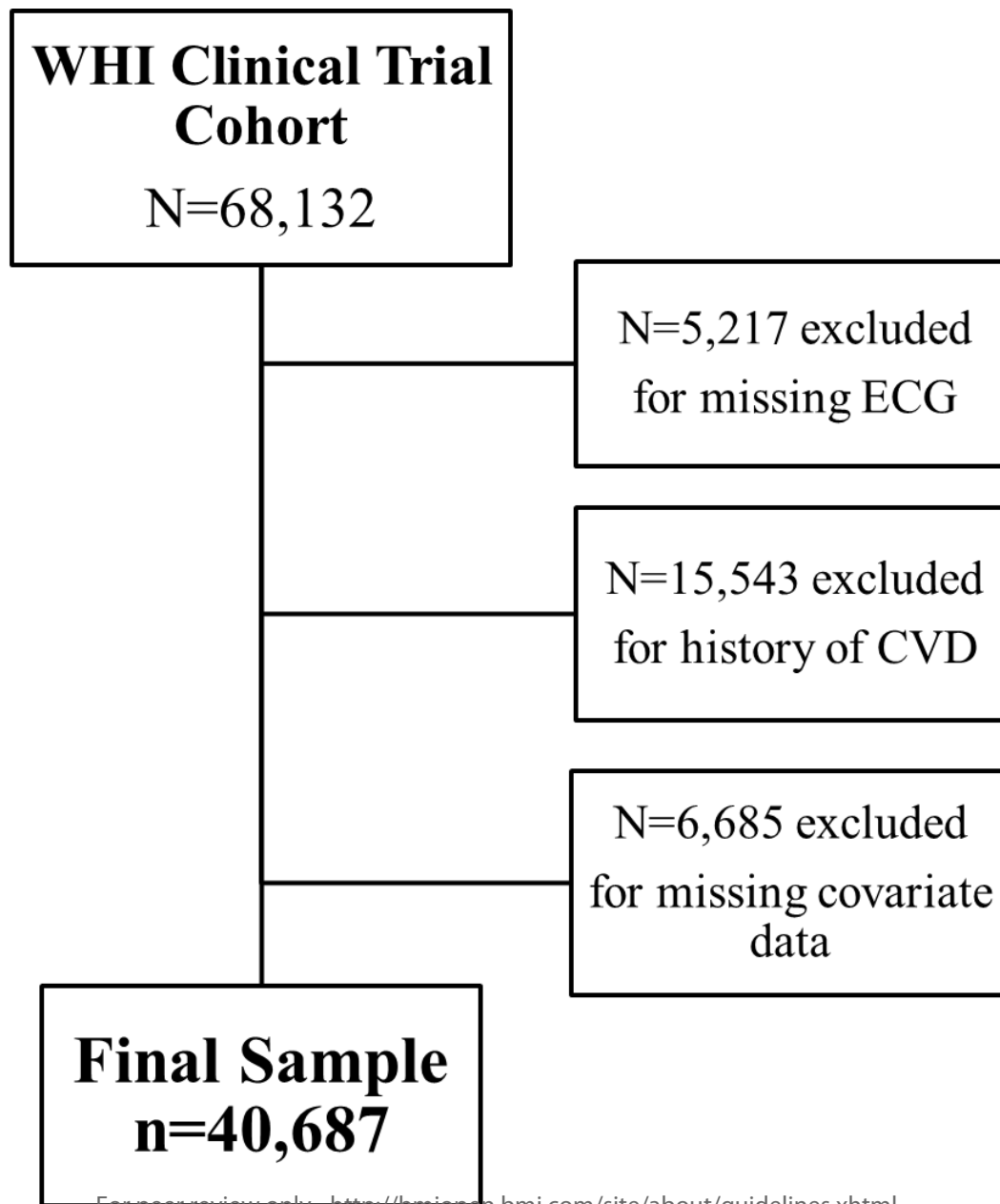
485 *n differs from main analyses due to the exclusion of women with implausible PR wave
 486 measures

490 **Table 5: Unadjusted and multivariable-adjusted associations between reproductive period**
 491 **duration and PR wave measures and PR dispersion in N= 31,538* Women in the Women's**
 492 **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)	

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

499 *n differs from main analyses due to the exclusion of women with implausible PR wave
 500 measures
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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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	7-8
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —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.

BMJ Open

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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Manuscripts

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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2
3 **46 Abstract: (word count=264)**

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5 **47 Objective:** Pregnancy, menses and menopause are related to fluctuations in endogenous sex
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7 **48** hormones in women, which cumulatively, may alter cardiac electrical conduction. Therefore, we
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9 **49** sought to study the association between number of pregnancies and reproductive period duration
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11 **50** (RD, time from menarche to menopause) with electrocardiographic intervals in the Women's
12
13 **51** Health Initiative Clinical Trials.

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15 **52 Methods:** In primary analyses, we employed multivariable linear regression models relating
16
17 **53** number of pregnancies and RD with millisecond (ms) changes in PR interval, P wave indices
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19 **54** (duration and dispersion) and QTc from enrollment electrocardiogram.

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21 **55 Results:** Among 40,687 women (mean age=62 years), 5+ live births versus 0 prior pregnancies
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23 **56** was associated with a 1.32 ms increase in PR interval [95% CI (0.25, 2.38)], with a graded
24
25 **57** association with longer QTc interval (ms) [none= 0.66 (-0.56, 1.88), 1= 0.15 (-0.71, 1.02), 2 to
26
27 **58** 4= 0.25 (-0.43, 0.94), and 5+ live births=1.15 (0.33, 1.98), p = 0.008]. RD was associated with
28
29 **59** longer PR interval and maximum P wave duration (but not P-wave dispersion) among never
30
31 **60** users of hormone therapy: [PR (ms) per additional RD year: 0.10 (0.04, 0.16); higher P-wave
32
33 **61** duration (ms): 0.09 (0.06, 0.12)]. For every year increase in reproductive period, QTc decreased
34
35 **62** by 0.04 ms (-0.07, -0.01).

36
37 **63 Limitations:** Potential misclassification of RD due to participant recall

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39 **64 Conclusions:** An increasing number of live births are related to increased ventricular
40
41 **65** repolarization time whereas RD is related to decreased ventricular repolarization time. Both
42
43 **66** longer RD and grandmultiparity are related to increased atrial conduction time. Reproductive
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45 **67** factors that alter midlife cardiac electrical conduction system remodeling in women may
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47 **68** modestly influence CVD risk in later life.

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3 **70 Article Summary:**
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5 **71 *Strengths and Limitations of the Study.***
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8 **72** • *A strength is the use of a well characterized multiethnic, large dataset of postmenopausal*
9
10 **73** *women representative of women in the United States.*
11
12 **74** • *A notable limitation is that the exposure variables were acquired retrospectively.*
13
14 **75** • *We were unable to adjust for pregnancy complications such as preeclampsia or*
15 **76** *gestational diabetes.*
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24 **79 **Key Words:** *endogenous estrogen, sex hormones, pregnancy, menarche, menopause,***
25
26 **80 *reproductive history, menarche, age at birth, repolarization, QTc, PR interval,***
27
28 **81 *electrocardiogram***
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35 **84 **INTRODUCTION:****
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37 **85 *Electrocardiogram parameters reflect current as well as future CVD risk***
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39
40 **86** Electrocardiographic parameters are reflections of both current as well as future cardiovascular
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42 **87** disease risk. For example, in the Framingham Heart Study, a prolonged PR interval (> 200 ms)
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44 **88** (which is defined as the period, measured in milliseconds, that extends from the beginning of the
45
46 **89** P wave (the onset of atrial depolarization) until the R wave), was related to incident atrial
47
48 **90** fibrillation, all-cause mortality and to the likelihood of needing a permanent pacemaker.(1) In
49
50 **91** addition to PR interval, the p wave duration (or the period in milliseconds during which the
51
52 **92** atrium depolarizes), more directly relates to atrial size and is an antecedent of atrial
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54 **93** fibrillation.(2) Both PR interval and p wave duration are markers of left atrial size which in turn
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3 94 is a correlate of hypertensive heart disease(3) and incident stroke.(4) P wave dispersion, defined
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5 95 as the difference between the maximum and the minimum P-wave duration recorded from
6
7 96 multiple different-surface ECG leads, is an additional marker of atrial remodeling and antecedent
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9
10 97 of atrial fibrillation.(5) It is unclear to what extent PR interval, p wave duration or P wave
11
12 98 dispersion are affected by premenopausal hormonal fluctuations from the menstrual cycle and
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14
15 99 childbearing.
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100

101 *Pregnancy, cardiac remodeling and the electrocardiogram*

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

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115 *Estrogen exposure and the electrocardiogram*

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3 116 In addition to the more marked hormonal fluctuations seen during pregnancy, there are also more
4
5 117 subtle, cyclic changes in estrogen and progesterone cycling that occur during menstrual cycling
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8 118 in women of reproductive age. Testosterone and progesterone are recognized to decrease the
9
10 119 QTc interval.(10) Prior data from the Women's Health Initiative (WHI) Hormone Trial suggests
11
12 120 that estrogen-only post-menopausal therapy modestly prolongs QTc beyond that of both
13
14 121 estrogen-progestin therapy and placebo.(11) However, it is uncertain whether the pre-
15
16 122 menopausal endogenous hormonal fluctuations (reflected by the length of the interval from
17
18 123 menarche to menopause, and by number of pregnancies) are associated with changes in QTc in
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21 124 the WHI.
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26 126 WHI represents a unique resource to study questions related to pregnancy and
27
28 127 reproductive history and ECG parameters and thus we sought to determine if there is a positive
29
30 128 or negative association between number of pregnancies and reproductive period duration with
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32 129 mid-life electrocardiogram intervals (PR interval and QTc) and p wave parameters (p wave
33
34 130 maximum duration and dispersion).
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39 40 132 **METHODS AND ANALYSIS PLAN:**

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42 133 Our current study design is a secondary analysis of a previously conducted set of clinical trials. .
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45 134 *Study sample.*

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49 135 The WHI recruitment began in 1991 and consisted of a set of clinical trials/ and an
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51 136 observational study on hormone therapy, dietary modification and calcium/ vitamin D
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53 137 supplementation on cardiovascular disease, cancer and fractures.(12) The clinicaltrial.gov
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55 138 identifier for the WHI is NCT00000611. At the time of enrollment, all women enrolled in the
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3 139 WHI were required to be between 50 and 79 years old, postmenopausal, and intending to reside
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5 140 in the area for at least 3 years. Other enrollment criteria have been previously described.(13) This
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7 141 analysis drew from the cohort of women enrolled in the WHI clinical trials (and not
8
9 142 observational study), as WHI clinical trial participants has ECGs performed per protocol.
10
11 143 Informed consent was obtained from all participants at study enrollment. **Figure 1** shows the
12
13 144 creation of the study sample. Of 68,132 women in WHI Studies (post- menopausal hormone
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15 145 therapy, diet and calcium/vitamin D and observational studies), we excluded 5,217 who were
16
17 146 missing ECGs and 15,543 who had prevalent CVD. Because number of pregnancies and
18
19 147 reproductive period (in particular age at menopause) are known to be associated with later CVD
20
21 148 and a history of CVD is related strongly with ECG changes including QTc and certainly
22
23 149 increased PR, we sought to exclude women with a history of CVD in order to assess associations
24
25 150 between reproductive period duration and number of pregnancies that were not directly mediated
26
27 151 through CVD. Of these, 47,372 women, 6,685 were further excluded for having missing
28
29 152 covariate data, leaving a final sample of included women =40,687. In a missing imputation
30
31 153 sensitivity analysis described below, we additionally analyzed the 6,685 women with missing
32
33 154 covariate data (total n=54,057).
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41 *Patient and Public Involvement*

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44 156 WHI was designed to address the gaps in knowledge about the major health issues in post
45
46 157 menopausal women. Patients assisted research staff in recruiting and results for all measures
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48 158 done at the study examinations were explained to each participant. Major study results are
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50 159 communicated to participants via newsletters.
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54 160 *Ascertainment of Reproductive Exposures.*

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3 161 Information on reproductive factors was collected via questionnaire at the second screening visit
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5 162 in the WHI (between 1993-1998). Participants were asked how many times they had been
6
7 163 pregnant (were given choices ranging from 0 to 8+), number of live births, and how old they
8
9 164 were at the end of the first and at the end of their last pregnancy (<20, 20-24, 25-29,30-34,35+
10
11 165 years). Age at menarche (<9, 10,11,12,13,14,15,>16 years) and age at menopause was asked on
12
13 166 this screening questionnaire. Reproductive period duration (RD) was defined as the duration
14
15 167 between age at menarche to age at menopause (in years). Detailed current and prior hormone
16
17 168 therapy (or post-menopausal hormone replacement therapy) usage and
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19 169 hysterectomy/oophorectomy status was collected at enrollment and has been previously
20
21 170 described.(14) Questions regarding the use and duration of oral contraceptive usage was also
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23 171 collected at enrollment.
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29 172 *Ascertainment of Covariates:*
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33 173 Age, income, education, self-reported race/ethnicity, geographic region of United States, history
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35 174 and duration of breastfeeding were collected at participant enrollment and second screening
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37 175 examinations. Body mass index (BMI, kg/m²) was calculated using height and weight measured
38
39 176 by study staff at baseline. Women with hypertension were identified as those with a self-reported
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41 177 history of treated hypertension or blood pressure measurements meeting JNC 7 criteria for
42
43 178 hypertension.(15) Diabetes was identified by self-reported use of anti-diabetic medications and
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45 179 hyperlipidemia by use of cholesterol lowering medications.
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50 180 *Electrocardiographic parameters:*
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53 181 Standard 12-lead ECGs were recorded in all women by strictly standardized procedures in all
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55 182 clinical centers as has been described.(16) All ECGs were processed in a central laboratory
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3 183 (EPICARE Center, University of Alberta, Edmonton, Canada, and later Wake Forest University,
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5 184 Winston-Salem, NC), where they were visually inspected for technical errors and inadequate
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8 185 quality. ECGs were processed with the 2001 version of the Marquette 12-SL program (GE
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10 186 Marquette). In addition to PR and QT intervals, we also examined the maximum P wave duration
11
12 187 and dispersion (from all 12 leads of the ECG).(2) The QT interval was corrected using Bazett's
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14 188 formula. The Institutional Review Board of University of California San Francisco approved this
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17 189 study protocol.

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20 190 *Statistical Methods:*

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23 191 *Primary Analysis*

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25 192 We employed multivariable linear regression to assess the association between reproductive
26
27 193 exposures (number of pregnancies and RD) with the dependent variable of ECG parameters
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29 194 (PR interval in milliseconds, p wave duration, p wave dispersion, QTc in milliseconds).
30
31 195 Multivariable models were adjusted for a priori covariates: age, BMI, hypertension status,
32
33 196 diabetes, income, education, race/ethnicity, region, history of breastfeeding, antianxiety
34
35 197 medication, antidepressant medication, lipid medication, duration of breastfeeding,
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37 198 oophorectomy status, hormone therapy use, heart rate and QRS duration. In analyses considering
38
39 199 categories of livebirths we employed a linear trend test.

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43 200 We explored effect modification of the primary exposures, number of live births and RD, by
44
45 201 hormone therapy usage and hysterectomy status. We classified hormone therapy usage into three
46
47 202 categories: women who reported current, prior or no hormone therapy usage. A statistical
48
49 203 interaction term between hormone therapy usage and the exposure (RD or number of live births)
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51 204 was used to consider effect modification by reported hormone therapy use. When the statistical
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53 205 interaction term was statistically significant ($p < 0.05$) according to a likelihood ratio test, we
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3 206 presented the estimates in each of the three categories of hormone therapy use and we presented
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5 207 a single estimate if there was no evidence for effect modification by hormone therapy. A similar
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7 208 approach was employed for studying RD or number of live births and hysterectomy status. To
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10 209 show sensitivity of estimates to confounders, unadjusted associations were reported as well as
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12 210 those associations adjusted for the confounders listed above.
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17 212 *Secondary Analyses:*
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19 213 In secondary analyses, we removed subjects who reported never being pregnant and used
20
21 214 multivariable linear regression to model associations between age at first live birth and the five
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23 215 electrocardiographic measures. These models used the same covariates to adjust association as
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25 216 those in our primary analyses. Subjects who had implausible secondary outcome values (i.e. all
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27 217 zero values or all constant values across all electrocardiographic measures) were removed. We
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29
30 218 additionally adjusted for covariates that we were concerned may have confounded the
31
32 219 associations between exposure and dependent variables in our study. We additionally fit
33
34 220 additional models which included both RD and number of pregnancies to ensure that one
35
36 221 exposure did not alter the other's association with the dependent variables. Given that anxiety
37
38 222 and depression could affect both exposure and dependent variables in our study, we further
39
40 223 adjusted for use of these medications. Antianxiety and antidepressant medication use (selective
41
42 224 serotonin reuptake inhibitors (or SSRI) and non-SSRI) were recorded on enrollment by nurse
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44 225 examination of medication bottles. Medications were classified according to the National Drug
45
46 226 Index classification system. We adjusted for Ca/Vitamin D status, oral contraceptive usage
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48 227 (yes/no and duration or usage). We further adjusted for menstrual irregularities/fertility
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50 228 disorders/and endometriosis, which are also related to hormonal fluctuations in women.
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229 *Multiple Imputation Analyses*

230 There were n=6,685 women in our study with missing covariate data. We used multiple
231 imputation techniques to impute missing covariates and refit models from primary analyses to
232 explore the sensitivity of our results to missing data. We used the PROC MI in SAS to construct
233 20 multiply imputed data sets. Missing variables were imputed via fully conditional specification
234 method in PROC MI using all variables from the analytic model. We fit models to each imputed
235 data set and pooled the results. The pooled results from imputation did not differ appreciably
236 from the results of the complete case analysis (data not shown).

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

238

239 **RESULTS**

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

246

247 *PR interval*

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

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2
3 252 was no significant association between RD and PR interval among women who reported prior or
4
5 253 current hormone therapy use (p value for interaction < 0.01) (**Table 2**). Age at first live birth,
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7 254 was not related to PR interval (data not shown).
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10 255 *QTc*

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12 256 Compared to never having been pregnant, having 5 or more pregnancies was related to a 1.2 ms
13
14 257 longer QTc (**Table 3**). However, not carrying a pregnancy to term, or having 1 or 2-4 term
15
16 258 pregnancies (versus not being pregnant), were not related to QTc. For each additional year in
17
18 259 reproductive period duration, there was a 0.4 ms shorter QTc (**Table 3**). Restricting to women
19
20 260 who had at least one live birth did not change our results (data not shown).
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24 261 *P wave duration and dispersion*

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26 262 P wave dispersion was higher for women with 2-4 live births (ms increase =0.62, 95% CI: 0.01,
27
28 263 1.24) and 5 live births (0.94, 95% CI: 0.20, 1.67), compared with those who reported never
29
30 264 having been pregnant (**Table 4**). Reproductive period duration was related to maximum p wave
31
32 265 duration among women who reported never having used hormones (0.09, 95% CI: 0.06, 0.13)
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34 266 but not among those who reported prior or current hormone therapy use (p interaction < 0.01)
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36 267 (**Table 5**).
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42 269 Secondary results: Models that contained both RD and number of pregnancies together were not
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44 270 materially different (data not shown). Further adjustment for antidepressants and anti-anxiety
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46 271 medications did not materially affect our results. Further adjustment for Ca and Vitamin D status
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48 272 or oral contraceptive use, and/or duration did not materially affect our results. Further adjustment
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50 273 for menstrual irregularities/fertility disorders/and endometriosis did not materially change our
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52 274 results.
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8 277 **DISCUSSION**9
10 278 *Summary of Findings*

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12 279 We found that having five or more pregnancies compared to none was associated with a small
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14 280 increase in mid-life atrial conduction time, independent of factors known to be associated with
15
16 281 this interval (PR). Number of live births among women with at least one live birth (compared to
17
18 282 no prior pregnancies) was associated with increased atrial conduction time. Having 5 or more
19
20 283 pregnancies was related to a small increase in ventricular repolarization time as compared to
21
22 284 having no prior pregnancies. Among women reporting no prior exogenous hormone use, each
23
24 285 additional year of reported RD was related to a very modest (0.1 ms) longer atrial conduction
25
26 286 time. RD was related to a very modest increase in p wave duration. RD was related to a shorter
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29
30
31 287 ventricular repolarization time.

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33 28834
35 289 *Mechanisms linking pregnancy and atrial electrical remodeling*

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37 290 The effect of cumulative pregnancies on mid-life electrocardiograms would likely result from
38
39 291 both 1) the pregnancy itself and 2) incident cardiometabolic factors that are impacted by
40
41 292 pregnancy such as adiposity(17) and vascular stiffness,(18) and premenopausal blood
42
43 293 pressure.(19) Adiposity and blood pressure are related to increased P wave indices in a normal
44
45 294 healthy population,(20) and these P wave indices are electrocardiographic reflections of
46
47 295 increased left atrial pressure, size and potentially fibrosis. The period of pregnancy and the
48
49 296 peripartum are characterized by hormonal changes that affect both cardiovascular hemodynamics
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51
52 297 and adaptive myocardial remodeling.(21) Pregnancy causes increased cardiac output, increased
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3 298 left ventricular mass, and decreased systemic vascular resistance.(22) The uterus and placenta in
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5 299 support of the growing fetus and fetal circulatory system represent a significant vascular shunt
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7
8 300 which contributes to these hemodynamic adaptations in pregnancy.(22) The sum of these
9
10 301 changes result in both left atrial and left ventricular dilation. However, the effects of normal
11
12 302 pregnancy on electrographic remodeling during pregnancy are not well described. A prior small
13
14 303 clinical study has looked at P wave duration and P wave dispersion among pregnant women
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16 304 compared with controls and found that both of these parameters are increased.(23)
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20
21 306 *Pregnancy and cumulative effects on ventricular repolarization*

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23 307 A prior study in 37 women in late pregnancy compared with 18 age matched controls
24
25 308 demonstrated that QTc substantially prolongs late in pregnancy and that this only partially
26
27 309 corrects back to pre-pregnancy values post-partum.(7) Our finding that having 5 or more
28
29 310 pregnancies as compared to no prior pregnancies suggests that QTc prolongation during
30
31 311 pregnancy may accumulate across successive pregnancies and will be significantly increased on
32
33 312 mid-life ECG. Furthermore, we found evidence for a dose response relationship between number
34
35 313 of pregnancies and mid-life QTc. Cardiac electrical remodeling often reflects myocardial
36
37 314 remodeling. We previously demonstrated that an increasing number of pregnancies were related
38
39 315 to left ventricular volume increase and increase in left ventricular mass in a multiethnic cohort of
40
41 316 women.(9) The increase in cardiac volume and mass were more marked in grandmultipara's or
42
43 317 women who had 5 or more pregnancies leading to livebirths.(9) It is important to note that
44
45 318 grandmultiparity is less common with declining parity levels in the United States.
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52
53 320 *Reproductive period duration and atrial conduction.*

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2
3 321 The menstrual cycle consists of a relatively well described hormone cycling in women consisting
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5 322 of both estrogen and progesterone as well as testosterone production. A longer reproductive
6
7 323 period duration reflects the cumulative exposure that a woman has to these endogenous
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9
10 324 fluctuations in sex hormone levels. Indeed, prior studies have assessed P wave parameters
11
12 325 throughout the menstrual cycle and noted that P wave duration is substantially increased in the
13
14 326 luteal phase.(24) Among women who did report taking prior hormone therapy, we observed a
15
16 327 very modest but significant increase in mid-life PR interval and in P wave duration. Exogenous
17
18 328 hormone therapy use may obscure the relationship between endogenous hormone exposure from
19
20 329 a longer reproductive period duration and P wave parameters, which would explain our findings
21
22 330 of effect modification by hormone therapy use. An earlier age at menarche (which would be
23
24 331 related to increased reproductive period duration) has been associated with increased
25
26 332 adiposity(25) and diabetes,(26) which in turn have been linked with increased p wave
27
28 333 duration(2) and, in the case of body mass index, with increased left atrial remodeling(27) and
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30
31 334 thus may also partially underlie our findings.
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336 *Reproductive duration and decrease ventricular repolarization time*

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39
40 337 The QTc is shortened by the action of progesterone and lengthened by estrogen during normal
41
42 338 menstrual cycling. The net effect of these changes during a single menstrual cycle can result in
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44 339 shortening of ventricular repolarization time or QTc.(28) Our finding that an increased
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46 340 reproductive duration was modestly inversely related to QTc in WHI. Underlying these findings
47
48 341 may be that increasing exposure to progesterone, in particular during menstrual cycling, may
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50 342 have cumulative and measurable effects on the mid-life electrocardiogram in women.
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3 344 *Strength and Limitations*
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5 345 The use of a well characterized multiethnic, large dataset of postmenopausal women
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7 346 representative of women in the United States is a strength of our study. A notable limitation is
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9
10 347 potential recall bias since the exposure variables were acquired retrospectively and some are very
11
12 348 distant events (eg age at menarche occurred 40-70 years in the past). We were unable to adjust
13
14 349 for pregnancy complications such as preeclampsia or gestational diabetes since these were not
15
16 350 collected. We did not adjust for smoking, physical activity, and habitual consumption of alcohol
17
18 351 and coffee which may have been related to the exposure variables but are not widely known to
19
20 352 be related to the ECG dependent variables studied. We studied number of pregnancies in a
21
22 353 categorical fashion and were unable, due to data constraints, to look at number of pregnancies as
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24 354 a continuous variables.
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31 356 *Directions for future research:*
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33 357 Future studies that disentangle specific hormonal and molecular mechanisms that underlie the
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35 358 association demonstrated in our study will help us better understand our study findings.
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37 359 Understanding which specific fertility factors alter electrical remodeling in women is an
38
39 360 important direction for future research.
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43
44 362 *Conclusions*
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46
47 363 We found that having five or more pregnancies compared to none is related to small but
48
49 364 significant changes in atrial conduction time and ventricular repolarization time. A longer
50
51 365 reproductive period duration in women not exposed to exogenous hormone therapy is related to a
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53 366 modest increase in atrial conduction time and to a modest decrease in ventricular repolarization.
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3 367 Reproductive health factors reflective of endogenous sex hormone exposure may be significant
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5 368 determinants of cardiac electrical remodeling in mid-life.
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9
10 370 *Disclosures*

11
12 371 None

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14 372

15
16 373 *Author contributorship statement:*

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18 374 Nisha I. Parikh conceived of the idea, designed the study, interpreted the analysis, drafted and
19 375 critically reviewed the manuscript. She provided final approval of the manuscript

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21 376 Kristopher Kappahn and Haley Hedlin conducted study design, statistical analysis and critical
22 377 review of the manuscript. They provided final approval of the manuscript

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25 379 Waring, Marco V. Perez assisted with study design, analysis interpretation, drafting and critical
26 380 reviewed the manuscript. They provided final approval of the manuscript

27
28 381 Barbara V. Howard assisted with study design, interpreted the analysis, drafted and critically
29 382 reviewed the manuscript. They provided final approval of the manuscript

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31 383 *Data sharing statement:*

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33 384 This was a secondary analysis of preexisting data and as such, no new data was generated by this
34 385 study. Information about data sharing for the Women's Health Initiative can be found at the
35 386 following website: <https://www.whi.org/researchers/data/Pages/Home.aspx>

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3 492 **Figure Legend: Creation of the Study Sample.** Clinical Trials include Hormone Trial, Dietary
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5 493 Modification and Calcium/Vitamin D. ECG=electrocardiogram, CVD=Cardiovascular diseases.
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495 **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

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497 HT=Hormone Therapy (or hormone replacement therapy)

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499 **Table 1: Unadjusted and multivariable-adjusted association of number of pregnancies**
 500 **leading to livebirths and reproductive period duration with PR interval (ms) in N=40,687**
 501 **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 (<i>categorical with never pregnant as referent category</i>)			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 (<i>continuous, years</i>)			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)	

502 *Covariates include age, baseline BMI, baseline hypertension status, history of diabetes, income,
 503 education, race/ethnicity, region, history/ duration of breastfeeding, lipid medication,
 504 oophorectomy status, hysterectomy status, hormone use history, heart rate and QRS duration
 505 HT=hormone therapy
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511 **Table 3: Unadjusted and multivariable-adjusted association of number of pregnancies**
 512 **leading to livebirths and reproductive period duration with QTc interval (ms) in N=40,687**
 513 **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 (<i>categorical with never pregnant as referent category</i>)			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 (<i>continuous, years</i>)	-0.09 (-0.12,-0.06)	-0.04 (-0.07, -0.01)	p value=0.01

514 *Covariates for number of livebirths analysis include age, baseline BMI, baseline hypertension
 515 status, history of diabetes, income, education, race/ethnicity, region, history/ duration of
 516 breastfeeding, lipid medication, oophorectomy status, hysterectomy status, hormone use history,
 517 heart rate and QRS duration. Covariates for reproductive period duration analysis include live
 518 births, age, baseline BMI, baseline hypertension status, history of diabetes, income, education,
 519 race/ethnicity, region, history of breastfeeding, duration of breastfeeding, lipid medication,
 520 oophorectomy status, hysterectomy status, hormone use history, and QRS duration.
 521 HT=hormone therapy
 522

523 **Table 4: Unadjusted and multivariable-adjusted associations between number of**
 524 **pregnancies leading to livebirths with p wave duration and p wave dispersion in N=39,338***
 525 **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)	Never Pregnant	Ref.	Ref.	p value for linear trend =0.73
	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)	Never Pregnant	Ref.	Ref.	p for linear trend =0.13
	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)	

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

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

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

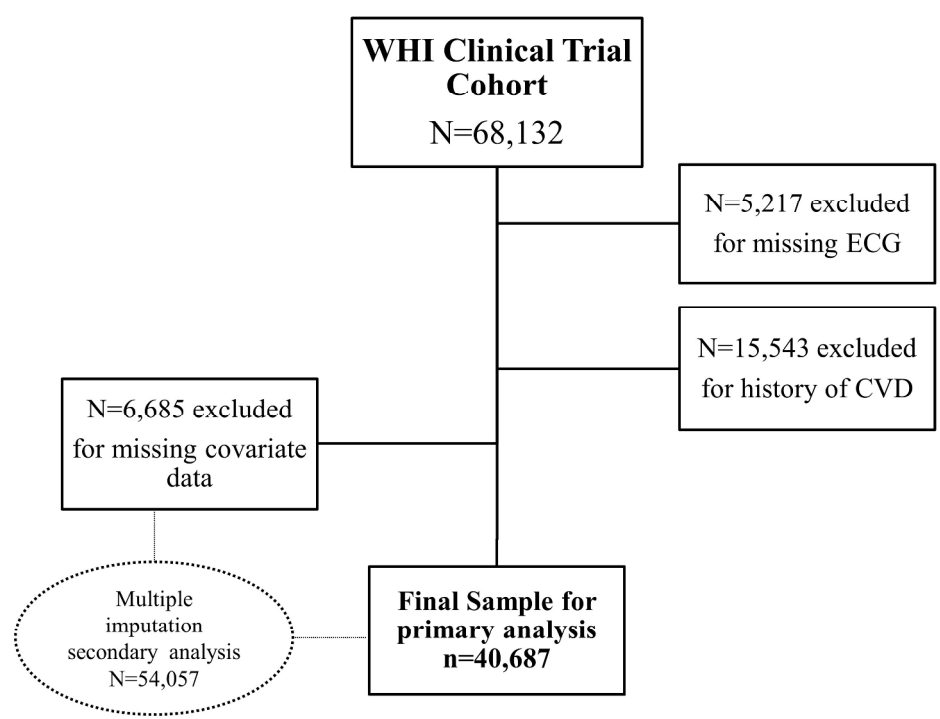
Dependent Variable	Hormone Use Status	Unadjusted Effect (95% CI)	Adjusted Effect (95% CI)	P value
P wave duration (ms)	Never User	0.07 (0.03, 0.11)	0.09 (0.06, 0.13)	p value for interaction= 0.0009
	Past	-0.04 (-0.08, 0.005)	0.01 (-0.03, 0.05)	
	Current	-0.03 (-0.06, 0.004)	0.01 (-0.02, 0.05)	
P wave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

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

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

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254x190mm (300 x 300 DPI)

Peer Review Only

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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	5-6
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	N/A
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	N/A
		<i>Cross-sectional study</i> —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.

BMJ Open

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

Journal:	<i>BMJ Open</i>
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Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Reproductive medicine, Epidemiology, Obstetrics and gynaecology
Keywords:	women, Adult cardiology < CARDIOLOGY, menopause, ECG, pregnancy

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4 2 **Effects of Reproductive Period Duration and Number of**
5 3 **Pregnancies on Mid-Life Electrocardiographic Indices: A**
6 4 **Secondary Analysis from the Women's Health Initiative Clinical**
7 5 **Trial**
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31 Running Title: Reproductive factors and electrocardiographic intervals

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

36 Word Count: 4196

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41 HHSN268201100002C, HHSN268201100003C, HHSN268201100004C
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44 *Conflict of Interest: None of the authors have any conflicts of interest in respect to this article*

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3 **46 Abstract: (word count=297)**

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5 **47 Objectives:** Pregnancy, menses and menopause are related to fluctuations in endogenous sex
6
7 **48** hormones in women, which cumulatively, may alter cardiac electrical conduction. Therefore, we
8
9 **49** sought to study the association between number of pregnancies and reproductive period duration
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11 **50** (RD, time from menarche to menopause) with electrocardiographic intervals in the Women's
12
13 **51** Health Initiative Clinical Trials.

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15 **52 Design:** Secondary Analysis of Multicenter Clinical Trial.

16
17 **53 Setting:** United States.

18
19 **54 Primary Outcome Measures:** Electrocardiographic Intervals: PR interval, P wave duration, P
20
21 **55** wave dispersion, QTc interval

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23 **56 Participants:** n=40,687 women (mean age=62 years) participating in the Women's Health
24
25 **57** Initiative Clinical Trials. 82.5% were White, 9.3% Black, 4% Hispanic and 2.7% Asian.

26
27 **58 Methods:** In primary analysis, we employed multivariable linear regression models relating
28
29 **59** number of pregnancies and RD with millisecond (ms) changes in intervals from enrollment
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31 **60** electrocardiogram. We studied effect modification by hormone therapy use.

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33 **61 Results:** Among participants, 5+ live births versus 0 prior pregnancies was associated with a
34
35 **62** 1.32 ms increase in PR interval [95% CI (0.25, 2.38)], with a graded association with longer QTc
36
37 **63** interval (ms) [none (prior pregnancy, no livebirths)= 0.66 (-0.56, 1.88), 1= 0.15 (-0.71, 1.02), 2
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39 **64** 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
40
41 **65** longer PR interval and maximum P wave duration (but not P-wave dispersion) among never
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43 **66** users of hormone therapy: [PR (ms) per additional RD year: 0.10 (0.04, 0.16); higher P-wave
44
45 **67** duration (ms): 0.09 (0.06, 0.12)]. For every year increase in reproductive period, QTc decreased
46
47 **68** by 0.04 ms (-0.07, -0.01).

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

74 **Article Summary:**

75 *Strengths and Limitations of the Study.*

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

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

88 **INTRODUCTION:**

89 *Electrocardiogram parameters reflect current as well as future CVD risk*

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

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3 93 P wave (the onset of atrial depolarization) until the R wave), was related to incident atrial
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5 94 fibrillation, all-cause mortality and to the likelihood of needing a permanent pacemaker.(1) In
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7 95 addition to PR interval, the p wave duration (or the period in milliseconds during which the
8
9 96 atrium depolarizes), more directly relates to atrial size and is an antecedent of atrial
10
11 97 fibrillation.(2) Both PR interval and p wave duration are markers of left atrial size which in turn
12
13 98 is a correlate of hypertensive heart disease(3) and incident stroke.(4) P wave dispersion, defined
14
15 99 as the difference between the maximum and the minimum P-wave duration recorded from
16
17 100 multiple different-surface ECG leads, is an additional marker of atrial remodeling and antecedent
18
19 101 of atrial fibrillation.(5) It is unclear to what extent PR interval, p wave duration or P wave
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21 102 dispersion are affected by premenopausal hormonal fluctuations from the menstrual cycle and
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23 103 childbearing.
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31 *Pregnancy, cardiac remodeling and the electrocardiogram*

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33 106 Pregnancy and the post-partum period both have substantial physiologic effects on cardiac
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35 107 electrophysiology. Physiologic studies of women during early and late pregnancy as well as early
36
37 108 post-partum suggest a shortening of the corrected QT interval (QTc) which partially reverts back
38
39 109 to pre-pregnancy values following post-partum.(6, 7) The QT interval is defined as the measure
40
41 110 of time between the onset of ventricular depolarization and completion of ventricular
42
43 111 repolarization, and because QT interval is strongly related to heart rate, the QTc is corrected for
44
45 112 heart rate. Direct pathophysiologic links connecting myocardial structural remodeling and
46
47 113 cardiac electrical remodeling have been increasingly recognized.(8) With regards to myocardial
48
49 114 remodeling, pregnancy induced cardiac remodeling does not completely revert back to pre-
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51 115 pregnancy levels and effects of increasing parity on cardiac remodeling can be detected even in
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3 116 mid-life.(9) However, the extent to which an increasing number of pregnancies exerts long
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5 117 lasting effects on the cardiac electrical conduction system is uncertain.(9)
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10 119 *Estrogen exposure and the electrocardiogram*
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12 120 In addition to the more marked hormonal fluctuations seen during pregnancy, there are also more
13
14 121 subtle, cyclic changes in estrogen and progesterone cycling that occur during menstrual cycling
15
16 122 in women of reproductive age. Testosterone and progesterone are recognized to decrease the
17
18 123 QTc interval.(10) Prior data from the Women's Health Initiative (WHI) Hormone Trial suggests
19
20 124 that estrogen-only post-menopausal therapy modestly prolongs QTc beyond that of both
21
22 125 estrogen-progestin therapy and placebo.(11) However, it is uncertain whether the pre-
23
24 126 menopausal endogenous hormonal fluctuations (reflected by the length of the interval from
25
26 127 menarche to menopause, and by number of pregnancies) are associated with changes in QTc in
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29
30 128 the WHI.
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35 130 WHI represents a unique resource to study questions related to pregnancy and
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37 131 reproductive history and ECG parameters and thus we sought to determine if there is a positive
38
39 132 or negative association between number of pregnancies and reproductive period duration with
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41 133 mid-life electrocardiogram intervals (PR interval and QTc) and p wave parameters (p wave
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43 134 maximum duration and dispersion).
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49 136 **METHODS AND ANALYSIS PLAN:**
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51 137 Our current study design is a secondary analysis of a previously conducted set of clinical trials. .
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55 138 *Study sample.*
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3 139 The WHI recruitment began in 1991 and consisted of a set of clinical trials/ and an
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5 140 observational study on hormone therapy, dietary modification and calcium/ vitamin D
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8 141 supplementation on cardiovascular disease, cancer and fractures.(12) The clinicaltrial.gov
9
10 142 identifier for the WHI is NCT00000611. At the time of enrollment, all women enrolled in the
11
12 143 WHI were required to be between 50 and 79 years old, postmenopausal, and intending to reside
13
14 144 in the area for at least 3 years. Other enrollment criteria have been previously described.(13) This
15
16 145 analysis drew from the cohort of women enrolled in the WHI clinical trials (and not
17
18 146 observational study), as WHI clinical trial participants has ECGs performed per protocol.
19
20 147 Informed consent was obtained from all participants at study enrollment. **Figure 1** shows the
21
22 148 creation of the study sample. Of 68,132 women in WHI Studies (post- menopausal hormone
23
24 149 therapy, diet and calcium/vitamin D and observational studies), we excluded 5,217 who were
25
26 150 missing ECGs and 15,543 who had prevalent CVD. Because number of pregnancies and
27
28 151 reproductive period (in particular age at menopause) are known to be associated with later CVD
29
30 152 and a history of CVD is related strongly with ECG changes including QTc and certainly
31
32 153 increased PR, we sought to exclude women with a history of CVD in order to assess associations
33
34 154 between reproductive period duration and number of pregnancies that were not directly mediated
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36 155 through CVD. Of these, 47,372 women, 6,685 were further excluded for having missing
37
38 156 covariate data, leaving a final sample of included women =40,687. In a missing imputation
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40 157 sensitivity analysis described below, we additionally analyzed the 6,685 women with missing
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42 158 covariate data (total n=54,057).
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50 159 *Patient and Public Involvement*

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53 160 WHI was designed to address the gaps in knowledge about the major health issues in post
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55 161 menopausal women. Patients assisted research staff in recruiting and results for all measures
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3 162 done at the study examinations were explained to each participant. Major study results are
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5 163 communicated to participants via newsletters.
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9 164 *Ascertainment of Reproductive Exposures.*
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11 165 Information on reproductive factors was collected via questionnaire at the second screening visit
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13 166 in the WHI (between 1993-1998). Participants were asked how many times they had been
14
15 167 pregnant (were given choices ranging from 0 to 8+), number of live births, and how old they
16
17 168 were at the end of the first and at the end of their last pregnancy (<20, 20-24, 25-29,30-34,35+
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19 169 years). In order to be able to also study women who had not experienced pregnancy and/or
20
21 170 childbirth and in an effort to make our study as representative as possible, we separately
22
23 171 categorized women who had had no prior pregnancies and women who had experienced a
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25 172 pregnancy but no livebirths (i.e. due to miscarriage, stillbirth, or abortion) as separate categories.
26
27 173 We further categorized women based on our prior work demonstrating that having 5 or more
28
29 174 pregnancies was associated with greater cardiac remodeling.⁽⁹⁾ Due to small cell sizes we
30
31 175 combined women with 5 or more pregnancies leading to livebirths into one category. Preliminary
32
33 176 data analysis reflected that 2-4 had similar effects sizes for PR and QTc and thus these categories
34
35 177 were collapsed into a single category for ease of interpretation. Therefore the exposure categories
36
37 178 for number of pregnancies leading to livebirths were as follows: no pregnancies (referent), none
38
39 179 (prior pregnancy, no livebirths), 1, 2-4, 5 or more. Age at menarche (<9, 10,11,12,13,14,15,>16
40
41 180 years) and age at menopause was asked on this screening questionnaire. Reproductive period
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43 181 duration (RD) was defined as the duration between age at menarche to age at menopause (in
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45 182 years). Detailed current and prior hormone therapy (or post-menopausal hormone replacement
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47 183 therapy) usage and hysterectomy/oophorectomy status was collected at enrollment and has been
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3 184 previously described.(14) Questions regarding the use and duration of oral contraceptive usage
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5 185 was also collected at enrollment.
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9 186 *Ascertainment of Covariates:*

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12 187 Age, income, education, self-reported race/ethnicity, geographic region of United States, history
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14 188 and duration of breastfeeding were collected at participant enrollment and second screening
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16 189 examinations. Body mass index (BMI, kg/m²) was calculated using height and weight measured
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18 190 by study staff at baseline. Women with hypertension were identified as those with a self-reported
19
20 191 history of treated hypertension or blood pressure measurements meeting JNC 7 criteria for
21
22 192 hypertension.(15) Diabetes was identified by self-reported use of anti-diabetic medications and
23
24 193 hyperlipidemia by use of cholesterol lowering medications.
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29 194 *Electrocardiographic parameters:*

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32 195 Standard 12-lead ECGs were recorded in all women by strictly standardized procedures in all
33
34 196 clinical centers as has been described.(16) All ECGs were processed in a central laboratory
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36 197 (EPICARE Center, University of Alberta, Edmonton, Canada, and later Wake Forest University,
37
38 198 Winston-Salem, NC), where they were visually inspected for technical errors and inadequate
39
40 199 quality. ECGs were processed with the 2001 version of the Marquette 12-SL program (GE
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42 200 Marquette). In addition to PR and QT intervals, we also examined the maximum P wave duration
43
44 201 and dispersion (from all 12 leads of the ECG).(2) The QT interval was corrected using Bazett's
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46 202 formula. The Institutional Review Board of University of California San Francisco approved this
47
48 203 study protocol.
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54 204 *Statistical Methods:*
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3 205 Primary Analysis
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5 206 We employed multivariable linear regression to assess the association between reproductive
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7 207 exposures (number of pregnancies and RD) with the dependent variable of ECG parameters (PR
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9 208 interval in milliseconds, p wave duration, p wave dispersion, QTc in milliseconds). Multivariable
10
11 209 models were adjusted for a priori covariates: age, BMI, hypertension status, diabetes, income,
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13 210 education, race/ethnicity, region, history of breastfeeding, antianxiety medication, antidepressant
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15 211 medication, lipid medication, duration of breastfeeding, oophorectomy status, hormone therapy
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17 212 use, heart rate and QRS duration. In analyses considering categories of livebirths we employed a
18
19 213 linear trend test.
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24 214 We explored effect modification of the primary exposures, number of live births and RD, by
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26 215 hormone therapy usage and hysterectomy status. We classified hormone therapy usage into three
27
28 216 categories: women who reported current, prior or no hormone therapy usage. A statistical
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30 217 interaction term between hormone therapy usage and the exposure (RD or number of live births)
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32 218 was used to consider effect modification by reported hormone therapy use. When the statistical
33
34 219 interaction term was statistically significant ($p < 0.05$) according to a likelihood ratio test, we
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36 220 presented the estimates in each of the three categories of hormone therapy use and we presented
37
38 221 a single estimate if there was no evidence for effect modification by hormone therapy. A similar
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40 222 approach was employed for studying RD or number of live births and hysterectomy status. To
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42 223 show sensitivity of estimates to confounders, unadjusted associations were reported as well as
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44 224 those associations adjusted for the confounders listed above.
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51 226 *Secondary Analyses:*
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3 227 In secondary analyses, we removed subjects who reported never being pregnant and used
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5 228 multivariable linear regression to model associations between age at first live birth and the five
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7 229 electrocardiographic measures. These models used the same covariates to adjust association as
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10 230 those in our primary analyses. Subjects who had implausible secondary outcome values (i.e. all
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12 231 zero values or all constant values across all electrocardiographic measures) were removed. We
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14 232 additionally adjusted for covariates that we were concerned may have confounded the
15
16 233 associations between exposure and dependent variables in our study. We additionally fit
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18 234 additional models which included both RD and number of pregnancies to ensure that one
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20 235 exposure did not alter the other's association with the dependent variables. Given that anxiety
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22 236 and depression could affect both exposure and dependent variables in our study, we further
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24 237 adjusted for use of these medications. Antianxiety and antidepressant medication use (selective
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26 238 serotonin reuptake inhibitors (or SSRI) and non-SSRI) were recorded on enrollment by nurse
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28 239 examination of medication bottles. Medications were classified according to the National Drug
29
30 240 Index classification system. We adjusted for Ca/Vitamin D status, oral contraceptive usage
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32 241 (yes/no and duration or usage). We further adjusted for menstrual irregularities/fertility
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34 242 disorders/and endometriosis, which are also related to hormonal fluctuations in women.
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40 243 *Multiple Imputation Analyses*

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42 244 There were n=6,685 women in our study with missing covariate data. We used multiple
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44 245 imputation techniques to impute missing covariates and refit models from primary analyses to
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46 246 explore the sensitivity of our results to missing data. We used the PROC MI in SAS to construct
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48 247 20 multiply imputed data sets. Missing variables were imputed via fully conditional specification
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50 248 method in PROC MI using all variables from the analytic model. We fit models to each imputed
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3 249 data set and pooled the results. The pooled results from imputation did not differ appreciably
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5 250 from the results of the complete case analysis (data not shown).

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7 251 All analyses were performed in SAS 9.4 (SAS Institute, Cary NC, USA).

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11 12 253 **RESULTS**

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14 254 **Table 1** shows the baseline characteristics of our sample including women who were included in
15
16 255 our study and those excluded from analysis for missing variables. Data is displayed by number of
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18 256 pregnancies lasting at least 6 months. The mean age at enrollment was 62.4 years, while the
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20 257 mean age at menarche was 12.6 and mean age at menopause was 50.0 years. 82.5% of women
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22 258 were White, 9.3% Black, 4% Hispanic and 2.7% Asian. Forty five percent of the study sample
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24 259 reported never having used hormone therapy prior to enrollment.
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30 31 261 *PR interval*

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33 262 Compared to women reporting never having been pregnant, having 5 or more pregnancies was
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35 263 associated with a 1.3 ms longer PR interval (**Table 2**). Among women who reported never
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37 264 having used hormones, each additional year of reported reproductive period duration was
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39 265 associated with a 0.1 ms longer PR interval (or atrial conduction velocity). Conversely, there
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41 266 was no significant association between RD and PR interval among women who reported prior or
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43 267 current hormone therapy use (p value for interaction < 0.01) (**Table 2**). Age at first live birth,
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45 268 was not related to PR interval (data not shown).

46 47 269 *QTc*

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50 270 Compared to never having been pregnant, having 5 or more pregnancies was related to a 1.2 ms
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52 271 longer QTc (**Table 3**). However, not carrying a pregnancy to term, or having 1 or 2-4 term
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3 272 pregnancies (versus not being pregnant), were not related to QTc. For each additional year in
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5 273 reproductive period duration, there was a 0.4 ms shorter QTc (**Table 3**). Restricting to women
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7 274 who had at least one live birth did not change our results (data not shown).
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10 275 *P wave duration and dispersion*

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12 276 P wave dispersion was higher for women with 2-4 live births (ms increase =0.62, 95% CI: 0.01,
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14 277 1.24) and 5 live births (0.94, 95% CI: 0.20, 1.67), compared with those who reported never
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16 278 having been pregnant (**Table 4**). Reproductive period duration was related to maximum p wave
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18 279 duration among women who reported never having used hormones (0.09, 95% CI: 0.06, 0.13)
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20 280 but not among those who reported prior or current hormone therapy use (p interaction < 0.01)
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22 281 (**Table 5**).
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28 283 Secondary results: Models that contained both RD and number of pregnancies together were not
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30 284 materially different (data not shown). Further adjustment for antidepressants and anti-anxiety
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32 285 medications did not materially affect our results. Further adjustment for Ca and Vitamin D status
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34 286 or oral contraceptive use, and/or duration did not materially affect our results. Further adjustment
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36 287 for menstrual irregularities/fertility disorders/and endometriosis did not materially change our
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38 288 results.
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46 291 **DISCUSSION**

47 292 *Summary of Findings*

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49 293 We found that having five or more pregnancies compared to none was associated with a small
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51 294 increase in mid-life atrial conduction time, independent of factors known to be associated with
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3 295 this interval (PR). Number of live births among women with at least one live birth (compared to
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5 296 no prior pregnancies) was associated with increased atrial conduction time. Having 5 or more
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7 297 pregnancies was related to a small increase in ventricular repolarization time as compared to
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9 298 having no prior pregnancies. Among women reporting no prior exogenous hormone use, each
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11 299 additional year of reported RD was related to a very modest (0.1 ms) longer atrial conduction
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13 300 time. RD was related to a very modest increase in p wave duration. RD was related to a shorter
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15 301 ventricular repolarization time.
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303 *Mechanisms linking pregnancy and atrial electrical remodeling*

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

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3 317 clinical study has looked at P wave duration and P wave dispersion among pregnant women
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5 318 compared with controls and found that both of these parameters are increased.(23)
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10 320 *Pregnancy and cumulative effects on ventricular repolarization*
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12 321 A prior study in 37 women in late pregnancy compared with 18 age matched controls
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14 322 demonstrated that QTc substantially prolongs late in pregnancy and that this only partially
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16 323 corrects back to pre-pregnancy values post-partum.(7) Our finding that having 5 or more
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18 324 pregnancies as compared to no prior pregnancies suggests that QTc prolongation during
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20 325 pregnancy may accumulate across successive pregnancies and will be significantly increased on
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22 326 mid-life ECG. Furthermore, we found evidence for a dose response relationship between number
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24 327 of pregnancies and mid-life QTc. Cardiac electrical remodeling often reflects myocardial
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26 328 remodeling. We previously demonstrated that an increasing number of pregnancies were related
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28 329 to left ventricular volume increase and increase in left ventricular mass in a multiethnic cohort of
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30 330 women.(9) The increase in cardiac volume and mass were more marked in grandmultipara's or
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32 331 women who had 5 or more pregnancies leading to livebirths.(9) It is important to note that
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34 332 grandmultiparity is less common with declining parity levels in the United States.
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42 334 *Reproductive period duration and atrial conduction.*
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44 335 The menstrual cycle consists of a relatively well described hormone cycling in women consisting
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46 336 of both estrogen and progesterone as well as testosterone production. A longer reproductive
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48 337 period duration reflects the cumulative exposure that a woman has to these endogenous
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50 338 fluctuations in sex hormone levels. Indeed, prior studies have assessed P wave parameters
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52 339 throughout the menstrual cycle and noted that P wave duration is substantially increased in the
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3 340 luteal phase.(24) Among women who did report taking prior hormone therapy, we observed a
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5 341 very modest but significant increase in mid-life PR interval and in P wave duration. Exogenous
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7 342 hormone therapy use may obscure the relationship between endogenous hormone exposure from
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9 343 a longer reproductive period duration and P wave parameters, which would explain our findings
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11 344 of effect modification by hormone therapy use. An earlier age at menarche (which would be
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13 345 related to increased reproductive period duration) has been associated with increased
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15 346 adiposity(25) and diabetes,(26) which in turn have been linked with increased p wave
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17 347 duration(2) and, in the case of body mass index, with increased left atrial remodeling(27) and
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19 348 thus may also partially underlie our findings.
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26 350 *Reproductive duration and decrease ventricular repolarization time*

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28 351 The QTc is shortened by the action of progesterone and lengthened by estrogen during normal
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30 352 menstrual cycling. The net effect of these changes during a single menstrual cycle can result in
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32 353 shortening of ventricular repolarization time or QTc.(28) Our finding that an increased
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34 354 reproductive duration was modestly inversely related to QTc in WHI. Underlying these findings
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36 355 may be that increasing exposure to progesterone, in particular during menstrual cycling, may
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38 356 have cumulative and measurable effects on the mid-life electrocardiogram in women.
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44 358 *Clinical relevance of our findings.*

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46 359 The PR interval normally ranges from 120 to 200 ms in duration. Therefore our finding that
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48 360 having 5 or more livebirths versus never having been pregnant was associated with an adjusted
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50 361 increase in PR interval of 1.32 ms, has modest clinical significance. For an individual with a PR
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52 362 interval at the upper limits of normal, 1.32 ms may be more clinically relevant in terms of the
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3 363 increased risks of later cardiovascular diseases with PR >200 ms. (1) The association of number
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5 364 of pregnancies leading to livebirths with QTc (with 5 or more pregnancies leading to livebirths
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7 365 having a 1.15 ms increase in QTc compared to nulligravid women) is similarly modest with a
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10 366 normal QTc ranging from ~350 to 460 ms in women. The effect sizes for reproductive duration
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12 367 were even more modest in size than those for P wave indices and therefore likely have more
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14 368 relevance in terms of uncovering novel biologic mechanisms related to cardiac electrical
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16 369 remodeling rather than reflecting clinically significant differences among individuals.
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21 371 *Strength and Limitations*

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24 372 The use of a well characterized multiethnic, large dataset of postmenopausal women
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26 373 representative of women in the United States is a strength of our study. A notable limitation is
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28 374 potential recall bias since the exposure variables were acquired retrospectively and some are very
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30 375 distant events (eg age at menarche occurred 40-70 years in the past). We were unable to adjust
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32 376 for pregnancy complications such as preeclampsia or gestational diabetes since these were not
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34 377 collected. We did not adjust for smoking, physical activity, and habitual consumption of alcohol
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36 378 and coffee which may have been related to the exposure variables but are not widely known to
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38 379 be related to the ECG dependent variables studied. We studied number of pregnancies in a
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40 380 categorical fashion and were unable, due to data constraints, to look at number of pregnancies as
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42 381 a continuous variables.
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49 383 *Directions for future research:*

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51 384 Future studies that disentangle specific hormonal and molecular mechanisms that underlie the
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53 385 association demonstrated in our study will help us better understand our study findings.
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3 386 Understanding which specific fertility factors alter electrical remodeling in women is an
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5 387 important direction for future research.
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10 389 *Conclusions*

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12 390 We found that having five or more pregnancies leading to livebirths compared to never having
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14 391 been pregnant is related to small but significant changes in atrial conduction time and ventricular
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16 392 repolarization time. A longer reproductive period duration in women not exposed to exogenous
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18 393 hormone therapy is related to a modest increase in atrial conduction time and to a modest
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20 394 decrease in ventricular repolarization. Reproductive health factors reflective of endogenous sex
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22 395 hormone exposure may be significant determinants of cardiac electrical remodeling in mid-life.
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28 397 *Disclosures*

29
30 398 None
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35 400 *Author contributorship statement:*

36
37 401 Nisha I. Parikh conceived of the idea, designed the study, interpreted the analysis, drafted and
38 402 critically reviewed the manuscript. She provided final approval of the manuscript

39
40 403 Kristopher Kapphahn and Haley Hedlin conducted study design, statistical analysis and critical
41 404 review of the manuscript. They provided final approval of the manuscript

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44 405 Jeffrey E. Olgin, Matthew A. Allison, Jared W. Magnani, MSc, Kelli R. Ryckman, Molly E.
45 406 Waring, Marco V. Perez assisted with study design, analysis interpretation, drafting and critical
46 407 reviewed the manuscript. They provided final approval of the manuscript

47
48
49 408 Barbara V. Howard assisted with study design, interpreted the analysis, drafted and critically
50 409 reviewed the manuscript. They provided final approval of the manuscript

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52 410 *Data sharing statement:*
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3 411 This was a secondary analysis of preexisting data and as such, no new data was generated by this
4 412 study. Information about data sharing for the Women's Health Initiative can be found at the
5 413 following website: <https://www.whi.org/researchers/data/Pages/Home.aspx>
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10 415 **References**
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519 **Figure Legend: Creation of the Study Sample.** Clinical Trials include Hormone Trial, Dietary
520 Modification and Calcium/Vitamin D. ECG=electrocardiogram, CVD=Cardiovascular diseases.
521

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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

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524 HT=Hormone Therapy (or hormone replacement therapy)

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526 **Table 1: Unadjusted and multivariable-adjusted association of number of pregnancies**
 527 **leading to livebirths and reproductive period duration with PR interval (ms) in N=40,687**
 528 **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 (<i>categorical with never pregnant as referent category</i>)			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 (<i>continuous, years</i>)			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)	

529 *Covariates include age, baseline BMI, baseline hypertension status, history of diabetes, income,
 530 education, race/ethnicity, region, history/ duration of breastfeeding, lipid medication,
 531 oophorectomy status, hysterectomy status, hormone use history, heart rate and QRS duration
 532 HT=hormone therapy
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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 (<i>categorical with never pregnant as referent category</i>) Never Pregnant None (prior pregnancy, no livebirths)	Ref. 0.54 (-0.76,1.83)	Ref. 0.66 (-0.56, 1.88)	p value for linear trend=0.008
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 (<i>continuous, years</i>)	-0.09 (-0.12,-0.06)	-0.04 (-0.07, -0.01)	

541 *Covariates for number of livebirths analysis include age, baseline BMI, baseline hypertension
 542 status, history of diabetes, income, education, race/ethnicity, region, history/ duration of
 543 breastfeeding, lipid medication, oophorectomy status, hysterectomy status, hormone use history,
 544 heart rate and QRS duration. Covariates for reproductive period duration analysis include live
 545 births, age, baseline BMI, baseline hypertension status, history of diabetes, income, education,
 546 race/ethnicity, region, history of breastfeeding, duration of breastfeeding, lipid medication,
 547 oophorectomy status, hysterectomy status, hormone use history, and QRS duration.
 548 HT=hormone therapy
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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)	Never Pregnant None (prior pregnancy, no livebirths)	Ref. 0.09 (-0.73, 0.92)	Ref. 0.09 (-0.69, 0.87)	p value for linear trend =0.73
	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)	Never Pregnant None (prior pregnancy, no livebirths)	Ref. 0.67 (-0.42, 1.77)	Ref. 0.64 (-0.45, 1.72)	p for linear trend =0.13
	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

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

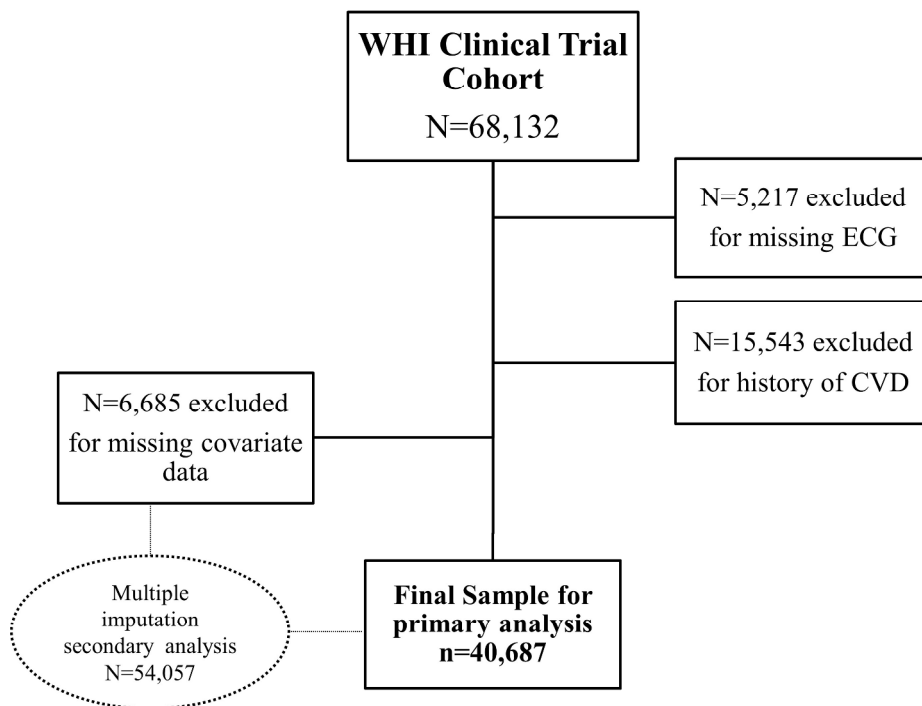
Dependent Variable	Hormone Use Status	Unadjusted Effect (95% CI)	Adjusted Effect (95% CI)	P value
Due to the fact that there was statistically significant effect modification by HT use upon the association between reproductive period and P wave duration in linear regression models, we present the model estimates <i>by strata</i> 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)	

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

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

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Pre-view only

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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	5-6
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	N/A
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	N/A
		<i>Cross-sectional study</i> —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.