High-sensitivity C-reactive protein is not independently associated with self-reported infertility in National Health and Nutrition Examination Survey 2015–2018 data

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Objective: To study the association between high-sensitivity C-reactive protein (hs-CRP) and infertility among reproductive-age women while controlling for obesity and other metabolic markers. Previous studies found a link between infertility and cardiovascular diseases (CVDs). C-reactive protein is a sensitive marker of CVDs, and its levels are affected by obesity.
Design/Setting: We conducted a cross-sectional study using national data from 2015 through 2018.
Patients: A total of 940 women aged 20–45 years who self-reported infertility, had hs-CRP values measured, and did not have CRP >10 mg/L, asthma, arthritis, bronchitis, thyroid disease, bilateral oophorectomy, hysterectomy, and who were not breastfeeding or pregnant, premenarchal at the time of study or had menarche after the age of 20.
Interventions: N/A.
Main outcome measure(s): Infertility status (ever reporting inability to conceive with 12 months of trying to become pregnant).
Results: In comparison to noninfertile women, self-reported infertile women had higher mean of hs-CRP (3.11 mg/L vs. 2.40 mg/L) and higher percentage of moderate/high hs-CRP values (77.0% vs 58.8%). However, after adjusting for metabolic markers, there was a nonsignificant association between moderate/high hs-CRP and self-reported infertility in the multivariable logistic regression analysis. Odds ratio estimates of the association between hs-CRP and infertility increased over 40% after removing obesity measures and/or high-density lipoprotein from regression models.
Conclusion: There was no association between hs-CRP and self-reported infertility after controlling for obesity measures and other risk factors for CVDs in a sample of U.S. women aged 20–45 years. (Fertil Steril Rep® 2022;3:63–70. ©2021 by American Society for Reproductive Medicine.)
Key Words: CRP, infertility, obesity, cardiovascular disease

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C-reactive protein (CRP) is a nonspecific but sensitive marker of inflammation that is synthesized by the liver under the influence of various stimuli, with interleukin 6 being a dominant regulator (1). C-reac-

tive protein can modulate the immune response and inflammation by affecting the function of the innate and adaptive immune systems, and the complement system (1). C-reactive protein is associated with and predicts

the development of cardiovascular diseases (CVDs) mainly by mediating inflammation-induced endothelium injury (2–4). Levels of CRP are highly correlated with obesity (5). In response to chronic low-grade inflammation associated with obesity, the liver increases the synthesis of various acute phase reactants, including CRP (6). Specifically, in women, CRP is associated with a high body mass index (BMI) and can predict the development of CVDs (7). Obesity, especially central obesity, is more closely associated with CRP levels in women than men

(8). Further, CRP levels were shown to increase with increasing BMI in premenopausal women across different phases of menstrual cycles (6, 9).

Women with infertility were at higher risk of chronic metabolic diseases, including CVDs (10–13). The association between fertility impairment and CVDs was postulated to lie in the presence of shared alterations in hormones and inflammatory mechanisms mediated by obesity. Infertility can be associated with disruption of stress hormones linked to sympathetic stimulation and immune disturbance that could subsequently lead to the development of CVDs (12, 14). For example, disruption of the normal physiology of the hypothalamic-pituitary-adrenal axis, which is found in some women with impaired fertility, has been linked to the development of CVDs (15–18). Activation of the hypothalamic-pituitary-adrenal axis leads to high cortisol levels, which may subsequently lead to high blood pressure, fat redistribution with truncal obesity, and disruption of the normal menstrual cycle, as manifested in Cushing syndrome (15). In addition to increasing the risk of CVDs, the chronic inflammatory state in obesity could impair women's ovarian function and fertility (19). Infertile women have higher inflammatory cytokines partly mediated by obesity (20, 21).

To date, no study tested the association between CRP and infertility status in reproductive-age women using a national sample of the U.S. Therefore, we sought to conduct a cross-sectional study to test the association between high-sensitivity C-reactive protein (hs-CRP) and infertility status in reproductive-age women (20–45 years) using a sample from National Health and Nutrition Examination Survey (NHANES) for the period 2015–2018. Since CRP level is mainly determined by obesity and metabolic markers in the general population and premenopausal women, we controlled obesity, serum lipids, anthropometric measures, hypertension, and diabetes in our analyses.

MATERIALS AND METHODS
Data Source and Sample Design

We used NHANES data which is a national cross-sectional study conducted every two years using a complex, multistage and stratified sampling method to represent civilian and noninstitutionalized residents of the U.S from all age groups. The surveys gather information using personal interviews, physical examinations, and laboratory investigations. National Health and Nutrition Examination Survey cycle 2015–2016 and 2017–2018 were used for this study since these two cycles contain complete information about hs-CRP, infertility, and most metabolic variables. Our study was deemed exempt from the Institutional Review Board of The University of Texas Health Science Center at Houston.

Main Dependent and Independent Variables

Our outcome was the self-reported infertility categorical variable (questionnaire RHQ074) "Have you or your partner ever attempted to become pregnant over at least a year without becoming pregnant?." Women who responded "yes" were labeled as "ever-infertile,"" and those who responded "no"

were labeled as "noninfertile."" (12). The main independent variable was hs-CRP measured in mg/L. We categorized hs-CRP according to the American Heart Association risk groups (low < 1.0 mg/L, moderate 1.0–3.0 mg/L, and high >3.0 mg/L) (22). Because of limited subjects with high hs-CRP values, we combined moderate and high hs-CRP (1–10 mg/L) into one category. Values below the lower limit of detection (<0.11 mg/L) were not included in our sample domain.

Covariates. variables that have known association with hs-CRP or infertility were included (e.g., hypertension, diabetes, serum lipids, obesity measures, and demographic factors including age, race, and smoking) (6, 12, 23). Hypertension and diabetes were defined according to previous studies using NHANES data (23–25). Hypertension categorical variable was created if systolic blood pressure was ≥140 mmHg or diastolic blood pressure was ≥ 90 mmHg based on average systolic blood pressure and diastolic blood pressure from four readings recorded for each subject. In addition, the subject was labeled as having hypertension if the subject reported taking antihypertensive medications (responded "yes" to the question "now taking medicine for high blood pressure"). Diabetes categorical variable was created if the subject had fasting blood glucose ≥126 mg/dL or HbA1c ≥6.5 or reported taking insulin or oral hypoglycemic medication (responded "yes" to questions "now taking pills to lower blood glucose," "now taking insulin"). Hip circumference was not found in the 2015–2016 cycle, and therefore it was not included. Because of a limited number of subjects who responded to the infertility question and had nonobese BMI values, we dichotomized BMI into nonobese (BMI <30) and obese (BMI ≥30). We dichotomized waist circumference as high (≥88 cm) and low (<88 cm), total cholesterol as high (≥ 200 mg/dL) and low (<200 mg/dL), and high-density lipoprotein (HDL) as low (<50 mg/dL) and high (≥ 50 mg/dL) in accordance with previous studies (26, 27). Trunk percent fat was included as a continuous variable. Smoking respondents were defined as "ever smokers" if they reported smoking at least 100 cigarettes in their entire life (12). We recoded health insurance coverage as private, Medicaid, other, and none (12).

Subjects

The sample included female respondents aged 20–45 years who responded to the question RHQ074 and had a valid lab value hs-CRP in two cycles of NHANES for the period 2015–2018 (Fig. 1). A sample domain was specified that excluded subjects with missing values on the main dependent and independent variables or high missing percentage (>20%) in the covariates. Our sample domain did not include pregnant women (by positive urine pregnancy test), breastfeeding, self-reported having a hysterectomy or bilateral oophorectomy, having thyroid disease, was premenarchal, or began menarche after the age of 20 at the time of the study. Current pregnancy, breastfeeding, history of hysterectomy, or bilateral oophorectomy may not indicate the current presence of infertility and thus were excluded. Thyroid diseases can include autoimmune thyroid disorders and might affect the levels of hs-CRP and therefore were excluded. Primary

FIGURE 1
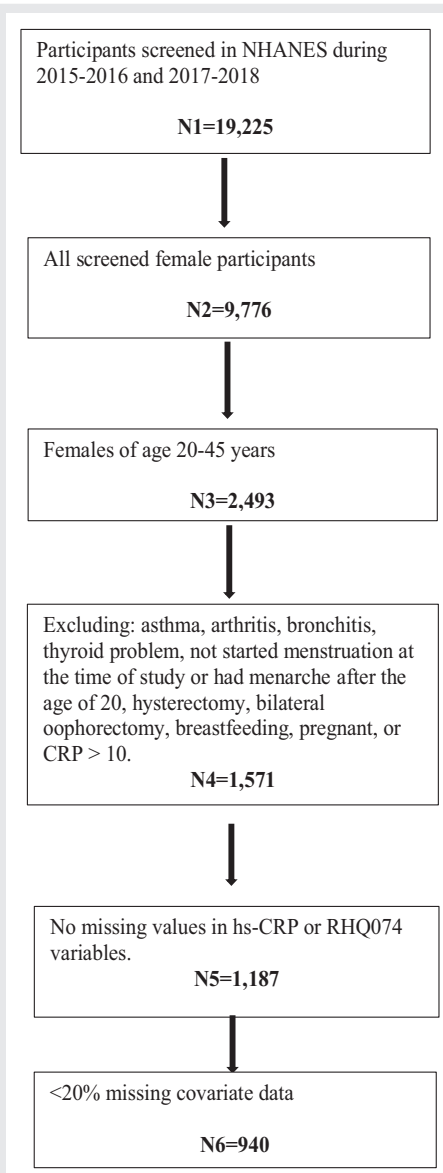
Flow chart of sample selection. CRP = C-reactive protein; hs-CRP = high-sensitivity C-reactive protein; NHANES = National Health and Nutrition Examination Survey.

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amenorrhea may indicate chromosomal aneuploidy and genetic disorders that have an increased risk of CVDs than the general population (e.g., Turner syndrome) (28). No variable in NHANES reflects the definition of primary amenorrhea (absence of secondary sexual characteristics by the age of 13 years or absence of menstruation by the age of 15 years despite the presence of secondary sexual characteristics). Therefore, we excluded premenarchal women or women who began menarche after 20 years at the time of the study. Patients with asthma, chronic bronchitis, or arthritis may use steroid or non-steroidal anti-inflammatory drugs (which

could affect the CRP levels) and, therefore, were excluded. High-sensitivity C-reactive protein levels higher than 10 mg/L most likely indicated infection and were excluded, in accordance with previous studies on CRP in premenopausal women (9).

Statistical Analysis

SAS version 9.4 (SAS Institute v.9.4) was used to conduct all analyses. All analyses were performed on weighted sample responses to account for differential probabilities of participant selection and nonresponse. The use of sample weights in NHANES allows for unbiased parameter estimates to be calculated and generalized to the U.S. population (29). Rao-Scott F adjusted χ^2 and t tests were used to report the differences in independent variables between self-reported infertile and noninfertile women in the unadjusted analysis as recommended by NHANES (29). P value of $< .05$ was used to determine the statistical significance.

In the multivariable logistic regression models, we controlled for variables that showed significant differences between noninfertile and infertile women in the unadjusted analysis (age, BMI, waist circumference, and trunk % fat, Table 1) as well as known associated factors with hs-CRP or infertility (hypertension, diabetes, total cholesterol, and HDL) (6). We tested the correlation between the covariates and found that BMI, waist circumference, and trunk % fat were highly correlated (r=0.70 – 0.93). Therefore, we included BMI, % trunk fat, and waist circumference in separate models of multivariable logistic regression analyses (Table 2). We further examined the change of hs-CRP adjusted odds ratio estimates after removing some of the metabolic covariates that had significant associations with infertility in the multivariable logistic regression analysis.

RESULTS

2,493 women between the ages 20 and 45 participated in NHANES from 2015 to 2018, of whom 2,102 answered the RHQ074 question (response rate 84.32%). The analytic sample included 940 subjects (20–45 years of age) with no missing values on the selected variables (Fig. 1). Of the 940 subjects, 79 and 861 women were self-reported having or not having infertility, respectively. Because of NHANES weight, 940 subjects in our sample reflect an estimate of 21,800,049 women aged 20–45 years in the U.S population, 79 infertile women in our sample correspond to 1,735,244 861 noninfertile women correspond to 20,064,805 subjects in the U.S population. The estimated prevalence of self-reported infertility among women aged 20–45 years in the U.S was 7.96% from 2015 to 2018.

Women with self-reported infertility were older (34.28 years vs. 31.12 years), had a higher hs-CRP mean (3.11 mg/L vs. 2.40 mg/L), and higher moderate-high hs-CRP percentages (77.0% vs. 58.8%) than women who did not report infertility. Women with infertility were more obese (56.6% vs. 29.2%), had higher waist circumference (72.4% vs. 51.8%), more mean trunk percent fat (38.7 vs. 33.8) and lower HDL mean (54.06 mg/dL vs. 59.63 mg/dL) than noninfertile women. There were no significant differences between

TABLE 1

General characteristics of women age 20–45 years with self-reported infertility and non-infertility. N = 940, weighted n = 21,800,049.

Variable	Ever-infertile women (Mean or %, SE)	Noninfertile women (Mean or %, SE)	Weighted *P* value
Hs-CRP (mg/L)	3.11, 0.29	2.40, 0.08	**.015**
Hs-CRP categories			
Low (<1 mg/L)	23.0%, 7.18	41.2%, 2.45	**.0326**
Moderate/high (1–10 mg/L)	77.0%, 7.18	58.8%, 2.45	
Age at the time of interview (years)	34.28, 0.69	31.12, 0.25	**<.0001**
Age categories			
20–25	11.8%, 5.17	29.6%, 2.14	**.0013**
26–30	14.7%, 3.86	21.3%, 1.64	
31–35	32.0%, 5.34	18.2%, 1.61	
≥36	41.5%, 5.53	30.9%, 1.91	
Race categories			
NHW	25.3%, 0.99	27.6%, 3.59	.623
NHB	19.0%, 0.21	20.6%, 2.05	
Asian	15.2%, 0.16	16.7%, 1.07	
Other	40.5%, 0.72	35.1%, 2.72	
Smoking categories			
No	63.5%, 0.80	75.2%, 2.74	.136
Yes	36.5%, 0.77	24.8%, 2.70	
Insurance categories			
None	21.5%, 0.43	22.7%, 1.58	.948
Other	8.8%, 0.33	11.2%, 0.99	
Private	53.2%, 0.83	49.5%, 2.41	
Medicaid	16.5%, 0.39	16.6%, 1.75	
BMI kg/m^2	30.87, 0.88	27.39, 0.23	**<.001**
BMI categories (kg/m^2)			
Nonobese (<30)	43.4%, 7.52	70.8%, 2.33	**.0004**
Obese (≥30)	56.6%, 7.52	29.2%, 2.33	
Waist circumference categories			
Low (normal) <88cm	27.6%, 6.11	48.2%, 2.54	**.008**
High ≥88cm	72.4%, 6.11	51.8%, 2.54	
Trunk % fat	38.70, 0.79	33.80, 0.28	**<.0001**
Total cholesterol (mg/dL)	181.6, 4.10	176.9, 1.08	.221
Total cholesterol (mg/dL)			
Low (normal) <200	70.9%, 6.27	78.3%, 1.47	.200
High ≥200	29.1%, 6.27	21/7%, 1.47	
HDL (mg/dL)	54.06, 1.35	59.63, 0.53	**.0002**
HDL (mg/dL)	—	—	—
Low <50	26.5%, 5.86	28.4%, 2.04	.744
High (normal) ≥50	73.5%, 5.86	71.6%, 2.04	
Hypertension categories			
No	86.87%, 0.88	95.0%, 1.21	**.045**
Yes	13.13%, 0.50	5.0%, 0.89	
Diabetes categories			
No	93.7%, 0.99	97.9%, 1.20	.099
Yes	6.3%, 0.37	2.1%, 0.89	

Bolded values indicate statistical significance at α <0.05
BMI = body mass index; HDL = high-density lipoprotein; Hs-CRP = high-sensitivity C-reactive protein; NHB = non-Hispanic Black; NHW = non-Hispanic White; SE = standard error.

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infertile and noninfertile women by race, smoking status, health insurance coverage status, total cholesterol, hypertension, and diabetes (Table 1).

High-sensitivity C-reactive protein was no longer significantly associated with infertility in the multivariable logistic regression analyses after adjusting for metabolic factors (Table 2). In the model that included BMI but not waist circumference or trunk % fat as measures of obesity (model[a] from Table 2), women of age group 31–35 years had 3.95 times higher odds of self-reported infertility compared with women of age group 20–25 years (95% CI [confidence interval] 1.33, 11.67). In the same model, obese women had 2.86 times higher odds of self-reported infertility compared

to nonobese women (95% CI 1.52, 5.34), and women with high HDL (≥50 mg/dL) had almost 50% lower odds of self-reported infertility in comparison to women with low HDL values (<50 mg/dL). In the model that included waist circumference but not BMI or trunk % fat as measures of obesity (model[b] from Table 2), women of age group 31–35 years had 3.90 times higher odds of self-reported infertility compared with women of age group 20–25 years (95% CI 1.32, 11.55). While in the model that included trunk % fat but not BMI or waist circumference as measures of obesity (model[c] from Table 2), women of age group 31–35 years had 3.78 times higher odds of self-reported infertility compared with women of age group 20–25 years (95% CI

TABLE 2

Multivariable analyses with adjusted odds ratios and confidence intervals of the association between hs-CRP and self-reported infertility among women 20–45 years of age; NHANES 2015–2018. The outcome is (ever-infertile women).

	Odds Ratio (95% Confidence Interval)		
Variable	Model [a] BMI binary	Model [b] Waist circumference	Model [c] Trunk % fat
High-sensitivity CRP*			
Low hs-CRP[d]	REF	REF	REF
Moderate/high hs-CRP[d]	1.48 (0.66–3.31)	1.83 (0.86–3.90)	1.27 (0.61–2.63)
Age (years)			
20–25	REF	REF	REF
26–30	1.64 (0.52–5.17)	1.62 (0.54–4.84)	1.55 (0.49–4.89)
31–35	**3.95 (1.34–11.67)**	**3.90 (1.32–11.55)**	**3.78 (1.32–10.85)**
≥36	2.81 (0.95–8.29)	2.65 (0.89–7.88)	2.68 (0.91–7.85)
BMI categories (kg/m^2)			
Nonobese (<30)	REF	N/A	N/A
Obese (≥30)	**2.86 (1.52–5.38)**		
Waist circumference			
<88cm	N/A	REF	N/A
≥88cm		1.73 (0.83–3.64)	
Trunk % fat	N/A	N/A	**1.09 (1.02–1.15)**
Total cholesterol (mg/dL)			
<200	REF	REF	REF
≥200	1.01 (0.53–1.93)	1.05 (0.54–2.03)	1.05 (0.55–2.01)
HDL (mg/dL)			
<50	REF	REF	REF
≥50	**0.50 (0.27–0.96)**	0.59 (0.31–1.10)	**0.51 (0.28–0.90)**
Hypertension			
No	REF	REF	REF
Yes	1.33 (0.51–3.49)	1.66 (0.61–4.58)	1.39 (0.56–3.49)
Diabetes			
No	REF	REF	REF
Yes	1.94 (0.46–8.20)	1.90 (0.49–7.45)	1.65 (0.41–6.59)

BMI = body mass index; CRP = C-reactive protein; HDL = high-density lipoprotein; hs-CRP = high-sensitivity C-reactive protein; N/A = not applicable; NHANES = National Health and Nutrition Examination Survey; REF = Reference group.
Model [a] Adjusted for age, **BMI**, total cholesterol, HDL, hypertension, and diabetes.
Model [b] Adjusted for age, **waist circumference,** total cholesterol, HDL, hypertension, and diabetes.
Model [c] Adjusted for age, **trunk % fat,** total cholesterol, HDL, hypertension, and diabetes.
Bolded values indicate statistical significance at α <0.05
[d] Low: <1 mg/L, Moderate/High: 1–10 mg/L.

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1.32, 10.85). In the same model, women who had one unit increase in trunk % fat had about 10% higher odds of self-reported infertility (95% CI 1.02, 1.15), and women with high HDL (≥50 mg/dL) had almost 50% lower odds of self-reported infertility in comparison to women with low HDL values (<50 mg/dL).

We further explored the effect of the metabolic covariates that were significantly associated with infertility in the adjusted multivariable logistic regression analysis (obesity, trunk % fat, and HDL). Removing obesity, trunk % fat, or HDL variables from model[a] and model[c] resulted in >40% increase in odds ratio estimates of the association between hs-CRP and self-reported infertility. However, it was not statistically significant. Taken together, these data indicate that obesity measures confounded the association between hs-CRP and self-reported infertility.

DISCUSSION

This is the first national study that tested the association between hs-CRP and self-reported infertility in women aged 20–45 years in the U.S. Using the weighted analysis, the prevalence of self-reported infertility among women aged 20–45 years was 7.96% for the period 2015–2018 which was within the range of the reported national prevalence from 2002 to 2017 (6%–8.8%). (30) We found that self-reported infertility among women aged 20–45 increased with increasing age and obesity. (31–34) At the national level of the U.S., we found that women with self-reported infertility had higher hs-CRP (a sensitive cardiometabolic marker) values than noninfertile women. In the multivariable logistic regression analysis, obese women and women with increasing trunk percent fat had higher odds of self-reported infertility. On the other hand, high HDL levels were associated with lower odds of self-reported infertility. These findings are consistent with previous reports on the association between metabolic syndrome, markers of obesity, and infertility (12, 13, 31). However, the association between hs-CRP and self-reported infertility was not significant after adjustment for metabolic markers, highlighting the possible superior roles of obesity and metabolic pathways in infertility pathogenesis. Our findings remained consistent across the models that separately tested obesity measures (BMI, waist circumference, and trunk % fat). Obesity and low HDL are also associated with high hs-CRP and higher risk of CVDs (6, 22), which could

delineate the shared mechanism between infertility and CVDs pathogenesis (or high CRP levels). Our study supported the possible confounding effects of metabolic dysfunction on the association between hs-CRP and infertility by showing increased estimates of moderate/high hs-CRP odds ratio (although not significant) after removing obesity and/or HDL from the models in our adjusted multivariable logistic regression analysis.

Most published studies have tested the association of infertility with CVDs and not with hs-CRP (12, 35–37). Our results are consistent with the subgroup analysis of NHANES data by Gleason and colleagues (12), where they reported no increased odds of CVDs among self-reported infertile women who gave birth but not with their results of overall higher odds of CVDs (83%) among infertile women aged 20–59 years. Gleason and colleagues (12) included a sample of women up to 59 years of age when testing the overall association between infertility and CVDs. Further, they defined CVDs as self-reported "congestive heart failure," "coronary heart disease," "heart attack," or "stroke" rather than using a biomarker (e.g., hs-CRP) to estimate long-term CVDs risk. Our finding of non-statistically significant association between hs-CRP and infertility in the adjusted analysis was not consistent with a single-center Turkish study that reported an association between unexplained infertility and high hs-CRP, high triglycerides, and low HDL in a group of normal-weight women (of age 20–35 years) with similar exclusion criteria as ours (13). Criteria of women in the U.S may differ from Turkish women, and our study sample included obese infertile women (mean BMI = 30.87 kg/m^2) and overweight noninfertile women (mean BMI = 27.39 kg/m^2). Parikh et al. (35) found an increased risk of CVDs among Swedish women who self-reported subfertility for five or more years but not in women who experienced subfertility for four or fewer years. Our NHANES sample likely included women who experienced infertility for a short period as we did not have information on the duration of experienced infertility in NHANES data. Further, Parikh et al. (35) included normal-weight women in all of their groups, and they controlled for hypertension and diabetes in their adjusted multivariable analysis but not for other metabolic markers. Yldrm et al. (38) found no association between CRP an primary ovarian insufficiency (POI) in Turkish women aged 20–40 years. However, our sample mainly included women with regular menstrual cycles. Some causes of infertility have been directly attributed to inflammatory damage to the ovary (19, 38, 39). Although Yldrm et al (38) did not find an association between CRP and POI, a neutrophil-to-lymphocyte ratio of less than 1.5 was an independent factor associated with POI. Therefore, it is important to consider the inflammatory mechanisms in infertility pathogenesis. Further, our results may highlight the findings from survival analyses of two large-scale studies, Stentz et al. (36) and Parikh et al. (37) who found no significant difference in the risk of death from CVDs between infertile and noninfertile women (mean age= 62.5 years) over 10 years and no association between history of infertility and CVDs in postmenopausal women (mean age= 63.2 years), respectively. Stentz (36) accounted for BMI in their disease risk score-adjusted survival

analysis, while Parikh (37) did not control for metabolic markers or BMI in their survival analysis.

In defined samples, infertility due to polycystic ovarian syndrome, endometriosis, or menstrual irregularities showed significant association with CRP and CVDs (40–44). These conditions can be associated with chronic low-grade inflammation and may exhibit high CRP values, explaining the associated increased risk of CVDs (40, 45–47). Our final analytic sample included only 4.8% of the subjects with irregular menstrual cycles and thus, might not include a large proportion of women with polycystic ovarian syndrome. However, our sample consisted of obese infertile women and overweight noninfertile women, which could explain the non-statistically significant association between infertility and hs-CRP in the models adjusted for metabolic markers and obesity but higher hs-CRP values in infertile compared to noninfertile women. Low-grade inflammation (particularly high hs-CRP) can be majorly driven by metabolic dysfunction and obesity (6, 27). Adipocytes can release various adipocytokines, augment interleukin 6 induced hepatic synthesis of CRP (48). In addition, adipocytes can secrete leptin, altering the hypothalamic-pituitary-ovarian axis and disrupting reproductive function (48). Moreover, obesity can disrupt the ovarian immune microenvironment, oocyte quality, and various cellular mechanisms in the oocytes (19, 49). Collectively, increased fat mass can impair reproductive function and increase the risk of CVDs through, at least in part, inducing inflammation. Further, obese women (BMI $\geq$ 30) were found to have significantly lower odds of implantation, clinical pregnancy, and having a live birth after conception via the first assisted reproductive technology cycle (50). Obese women seeking fertility services have a higher chance of becoming pregnant after losing weight (51). Since most women with infertility in the U.S would seek fertility services (60%), it is important to consider counseling women with infertility on weight loss (52, 53). Our results underscore the need to counsel women about behavioral changes that could impact their future development of CVDs and reproductive health. Primary care providers should also counsel all women seeking conception on the negative impact of obesity on reproductive health and encourage weight loss.

The results of our study should be interpreted within the scope of its limitations and strengths. Since infertility was assessed through self-reporting, women may not precisely recall how long they tried to become pregnant, resulting in misclassification bias of including women in the noninfertile group who were otherwise infertile. Similarly, our study could have missed women who might be infertile but have not tried yet to conceive. We did not have information on the duration of infertility or the time interval to conceive. Thus, we could have missed women over the age of 35 years who could otherwise be infertile after six months of unsuccessful attempts to conceive. Since this is a cross-sectional study, we could not derive a causal inference on the association between hs-CRP and infertility in reproductive-age women. Hormonal contraception could modulate hs-CRP values (54). It is unknown how many reproductive-age women in our sample were currently on hormonal contraception since NHANES data do not provide such information. Therefore, it is possible

that we included self-reported infertile women who were using hormonal contraception on the day of the interview and thus on the day of hs-CRP assessment. Lastly, we did not include women older than 45 years of age; while the risk of CVDs increases significantly with increasing age, NHANES data do not provide enough information on CVDs risk factors (e.g., comprehensive family history of CVDs, triglycerides, and low-density lipoprotein levels).

Despite these limitations, this study has several strengths. This is the first national study to test the association between a highly sensitive cardiometabolic marker (hs-CRP) and self-reported infertility in reproductive-age women of the U.S using weighted analysis. We accounted for various metabolic markers and removed various conditions that could modulate hs-CRP values or infertility. We defined our covariates using biomarkers, thus avoiding recall bias. Although we did not have information on causes of infertility, we included non-pregnant and non-breastfeeding women aged 20–45 years and excluded various inflammatory conditions. Thus, it is reasonable to generalize the results of this study on a similar population of infertile women aged 20-45 years in the U.S.

Our results are important in view of recent studies that found an association between infertility and risk of CVDs or hs-CRP (12, 13, 35). At the national level of the U.S., we found that the hs-CRP was not independently associated with self-reported infertility after adjustment for metabolic markers and obesity. Prospective studies are warranted to test the causal relationship between infertility and cardiometabolic markers like hs-CRP.

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