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Hormone replacement therapy affects iron status more than endometrial bleeding in older US women: A role for estrogen in iron homeostasis?

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ABSTRACT

High iron levels in women of post-reproductive age may be related to their increased risk of chronic disease as they become older, but the causes of this rise in iron in late life is unclear. Recently estrogen has been implicated in non-human models of iron homeostasis. Studying iron in women who take hormone replacement therapy (HRT) may provide insight into the relationship between iron status and hormonal status in older women. This study examines the association between HRT and iron status in women aged 50+ who took part in the 1999–2000 National Health and Nutrition Examination Survey (NHANES). Data were analyzed using multiple imputation, which corrects for missing data, and complex survey regression, which adjusts for NHANES sampling. Current HRT use was associated with lower ferritin ($\beta = -34.13$, $p = 0.0002$), controlling for potential breakthrough bleeding with a hysterectomy variable. HRT was associated with lower iron stores in women of post-reproductive in the absence of uterine blood loss, indicating potential homeostatic hormonal control of iron status. This research demonstrates the utility of studying clinical hormonal therapy to advance new understandings about the basic biology of iron homeostasis in women.

1. Introduction

The "iron hypothesis" proposed by Sullivan [1], states that higher levels of iron in post-reproductive aged women compared to reproductive-aged women put these groups at increased risk of chronic illnesses such as cardiovascular disease. In women, the iron hypothesis was proposed in contrast to the "estrogen hypothesis," which suggests that increased risk of cardiovascular risk in post-reproductive aged women is related to decreases in estrogen post-menopause [2]. Certainly, iron is higher and estrogen is lower in post-reproductive aged women compared to reproductive aged women [3,4]. The estrogen hypothesis has largely been disproven by data demonstrating that medical estrogen use in the form of HRT may in fact increase risk for some chronic illnesses [5], and many indicators of chronic disease risk may be attributed simply to increasing age, rather than menopausal status [6]. Further, the iron hypothesis largely suffers from lack of empirical investigation [7]. Few researchers have questioned whether the increases in iron status associated with menopause are directly associated with to concomitant changes in reproductive hormone levels. If they are related, it will demonstrate that (1) both iron and reproductive hormones may play a role in the increased risk of chronic illness for post-reproductive aged women, and (2) there are implications for the role of estrogen in the maintenance of iron homeostasis across women's lifespans.

Higher iron post-menopause has traditionally been attributed to reduced menstrual bleeding and lack of iron loss from pregnancy that women experience with menopause [3]. Reproductive aged women have a well-known susceptibility to perturbations in iron status due to reproductive events such as pregnancy [8]. Repeated menstrual periods have also been cited as the reason for lower iron levels in reproductive aged women [3,9], although this finding has been challenged by researchers who have found improved iron status in women with thicker endometriums, a proxy for menstrual blood loss [10]. However, evidence from mouse models [11,12] and animal cells [13] suggests a connection between the iron-regulating protein hepcidin and the reproductive hormone estradiol, but this association has yet to be tested explicitly among humans [14]. Post-reproductive women are an excellent model for determining the differential effects of reproductive-related iron loss and reproductive hormones, specifically estradiol and progesterone, in women of all ages. First, they are not currently losing blood due to pregnancy or menstruation but may have a history of reproductive-related

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iron loss. Second, they are not subject to menstrual-cycle related hormonal fluctuations, but may take HRT, which provides an opportunity to assess the effects of hormones in a setting largely divorced from reproductive-related fluctuations in iron status.

This study tests the hypothesis that the reproductive hormones in HRT impact women's iron status, using post-reproductive aged women as a model. Specifically, does HRT alter post-reproductive aged women's hemoglobin and ferritin levels? The prediction is that hormone replacement will alter women's iron status, particularly ferritin levels, but based on previous literature [3,15], the directionality of the association is unclear. This variable will be controlled for using a hysterectomy variable, to assess the potential role of breakthrough bleeding on iron status.

2. Methods

2.1. Study population

The NHANES is a nationally-representative cross-sectional study of the health and nutrition of the US population, conducted by the Centers for Disease Control and Prevention on a continuous basis since 1999. The NHANES employs a complex statistical survey design in which populations are weighted according to census data [16]. The sample for this study included women aged 50 and older who completed the physical examination during survey years 1999–2000. Pregnant women (n = 3) were excluded. This lead to a potential sample size of 1090 women, although most variables had missing values (Table 1). This study uses a multiple imputation (MI) analysis to correct for missing data bias.

This project is the result of secondary analysis on a deidentified data set and is exempt from local IRB approval. The original study was approved by the National Center for Health Statistics Research Ethics Review Board and participants gave informed consent.

2.2. Variables

2.2.1. Iron status variables

This study used two main indicators of iron status available in the 1999–2000 data for women ages 50 and above: hemoglobin and ferritin.

1. Hemoglobin is an iron-containing molecule found in red blood cells and is responsible for oxygen transport from the lungs to tissues throughout the body. Measuring hemoglobin levels are the simplest method of diagnosing iron-deficiency anemia, with levels below 12–12.5 g/dL indicating anemia in women [17]. Hemoglobin was determined using the Coulter HMX Hematology Analyzer and was part of complete blood count data set [18].
2. Ferritin is a protein that stores iron and is indicative of general iron levels in the body, with ferritin levels of less than 12 ng/mL considered iron-deficient [17]. Ferritin levels are increased during acute-phase inflammatory responses [19], so ferritin levels must be controlled for using CRP levels. Ferritin levels were assayed for all age groups in the 1999–2000 survey release. Across survey years, NHANES staff switched ferritin analysis methods, necessitating the use of a piecewise linear equation to normalize the 1999–2003 ferritin values to the 2004–2010 ferritin values [20]. While the current study uses only the BioRad Laboratories' two-site immunoradiometric assay to generate ferritin values [20], these values were converted to the later Roche/Hitachi immunoturbidity values so that they could be evaluated with respect to later NHANES survey years [8].

2.2.2. Reproductive hormone (HRT) variables

The following variables from the reproductive health questionnaire were included in multivariate models as independent variables.

1. Women were asked if they were currently using estrogen-only HRT, progesterone-only HRT, and/or estrogen/progesterone combined HRT. Because there was considerable overlap between categories, these data were combined into one dichotomous variable. Women's reported reasons for ever taking HRT can be found in Table 2. Women were most likely to report hysterectomy or menopause-related symptoms as reasons for taking HRT.
2. Women were asked if they had ever had any type of hysterectomy, coded into a dichotomous variable. This variable was used to control for the potential for bleeding, as women with hysterectomies cannot have endometrial bleeding.

2.2.3. Control variables

Control variables were assessed using stepwise regression in non-weighted models to assess their inclusion in either model (inclusion criteria set at 0.15), and included BMI, C-reactive protein (CRP), age, 24-h dietary intake of iron, race/ethnicity (Hispanic including Mexican Americans, black, and other with white as the reference category) age at menarche, parity, history of hormonal contraceptive use, history of HRT use, and menopausal status. Based on this regression, final models were controlled for using BMI, CRP, age, 24-h dietary intake of iron, race/ethnicity, parity, and menopausal status.

2.3. Statistical analysis

When individuals who have missing values for variables are excluded from multivariate analyses, biased results can occur [21]. Multiple imputation is a method for replacing missing variables, using a probability model on both complete and missing variables to generate likely values for the missing variables [21]. Multiple imputation creates multiple data sets, and the statistical test is performed on each set. After the statistical analyses, results from each set are combined in a way that adjusts for the variance introduced by the imputation process [22].

Prior to multiple imputation, NHANES data were inspected to analyze patterns of missing data. Although missing data appeared to have an arbitrary pattern, the addition of categorical data in can confound the imputation [22]. For the purposes of this analysis, multiple imputation was performed using Markov chain Monte Carlo (MCMC) methods, as this approach is robust for linear regression. Categorical variables were not rounded to 0 or 1, as it has been shown that rounding imputed categorical variables can lead to bias, and that unrounded imputed categorical variables perform well in linear regression after MCMC imputation [23].

To complement the multiple imputation analyses, unimputed, weighted, descriptive statistics were performed using PROC SURVEYMEANS for non-pregnant women aged 50 and older who participated in the exam. An unimputed, weighted regression (using PROC SURVEYREG) was performed on complete cases (individuals with no missing variables). There were two statistical models performed, with hemoglobin and ferritin as dependent variables. The independent variables were the same across both models: ever used HRT and currently use HRT (adjusted for hysterectomy). Each model was adjusted for reproductive history, ethnicity, BMI, CRP, age, and 24-h dietary iron intake. In all descriptive statistics and models, the sample was weighted for the complex sample structure using the two-year examination weights provided with the data. Statistical significance was assessed at $\alpha = 0.05$.