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Iron, Inflammation and Atherosclerosis Risk in Men vs. Perimenopausal Women

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Abstract

Objective—Age at first atherosclerotic event is typically older for women vs. men; monthly iron loss has been postulated to contribute to this advantage. We investigated the relationship between an MRI-based arterial wall biomarker and the serum inflammatory biomarker high-sensitivity C-reactive protein (hsCRP) in perimenopausal women vs. men.

Methods and Results—Women without evident atherosclerotic disease were prospectively enrolled and observed over 24 months of menopause transition, indicated by hormone levels and reduction in median number of menstrual cycles from 4 [3 – 6] per year to 0 [0 – 1] per year ($P < 0.01$). Higher hsCRP predicted shorter carotid artery wall T2* in women entering the menopause transition ($r = -0.3139$, $P = 0.0014$); this relationship weakened after 24 months of perimenopause in women ($r = -0.1718$, $P = 0.0859$) and was not significant in a cohort of men matched for age and cardiovascular risk category ($r = -0.0310$, $P = 0.8362$). Serum ferritin increased from baseline to 24-month follow-up during women's menopause transition (37 [20–79] to 67 [36–97] ng/mL, $P < 0.01$), but still remained lower compared to men (111 [45–220] ng/mL, $P < 0.01$). Circulating ferritin levels correlated with arterial wall T2* values in women at baseline ($r = -0.3163$, $P = 0.0013$) but not in women after 24 months ($r = -0.0730$, $P = 0.4684$) of menopause transition nor in men ($r = 0.0862$, $P = 0.5644$).

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Conclusions—An arterial wall iron-based imaging biomarker reflects degree of systemic inflammation in younger women, whereas this relationship is lost as women transition through menopause to become more similar to men. Iron homeostasis and inflammation in the arterial wall microenvironment warrants further investigation as a potential early target for interventions that mitigate atherosclerosis risk.

Keywords

atherosclerosis; women; magnetic resonance imaging; iron; risk factors

Introduction

Atherosclerosis causes the vast majority of acute coronary and cerebrovascular syndromes. While it affects both men and women, heart disease and stroke statistics have consistently shown a 10–20 year lag in first atherosclerotic event for women compared to men¹. Furthermore, the incidence of heart attack and stroke increases after menopause beyond that predicted by chronologic age². Both the Framingham Heart Study and the Multi-Ethnic Study of Atherosclerosis (MESA) indicate that early menopause increases atherosclerotic events^{3, 4}.

Women experience a rapid decline in sex steroid exposure with menopause. However, randomised trials have shown that hormone therapy provides inconsistent cardiovascular risk reduction^{5–7} and even increased risk of stroke^{8–10}. A less-explored alternate hypothesis to explain relative lag in events for women vs. men is that monthly iron loss prior to menopause is cardioprotective¹¹. Iron excess accelerates, while iron restriction attenuates, atheroma formation in animals^{12, 13} and humans¹⁴. Iron-generated free radicals oxidise LDL via Fenton chemistry^{15, 16}, and LDL modification leads to foam cell production¹⁷.

Just as carotid ultrasound-based intima medial thickness (cIMT) has been widely used in studies of atherosclerosis risk¹⁹, non-contrast magnetic resonance imaging (MRI) of the carotid artery wall offers high reproducibility²⁰, agreement with cIMT^{21, 22} and prognostic value²³ while offering unique tissue characterization approaches. For instance, the MRI relaxation parameter T2* can accurately estimate iron in liver and myocardium^{24, 25}, whereas serum measures such as ferritin do not reliably predict tissue iron content.²⁶ Our group has developed T2* mapping for the carotid artery wall and applied it to analyze both disease-free as well as atherosclerotic arteries, the latter providing histopathological evidence of T2* as a marker of arterial wall iron^{27–29}. In this study, we incorporated this arterial wall imaging biomarker into a prospective study of perimenopausal women with atherosclerosis risk factors. We hypothesized that i) shorter carotid artery T2* correlates with higher levels of high-sensitivity C-reactive protein (hsCRP) in men and women at risk of atherosclerosis and ii) iron serologies do not consistently predict tissue-specific iron-based imaging measures.

Methods

Study Population

Women over the age of 40 reporting 1 to 6 menstrual cycles in the year prior to enrolment were prospectively identified via community advertisements as well as through a primary care-targeted, electronic health record (EHR) query. A group of men matched by age and Framingham risk score category (low, intermediate, high³⁰) was identified through a similar primary care EHR query. Recorded risk factors for perimenopausal women and age/risk-matched men included: hyperlipidaemia (total cholesterol ≥ 240 mg/dL), hypertension (diastolic blood pressure ≥ 90 mmHg, systolic blood pressure ≥ 140 mmHg or on medications for hypertension), smoking, and treated diabetes. Excluded from enrolment were individuals with clinically-apparent atherosclerotic cardiovascular disease, prior acute coronary syndrome, exertional angina or equivalent, prior coronary revascularization, anaemia, bleeding disorder, regular blood donation, known abnormality of iron metabolism, or unwillingness to not donate blood during the study. To avoid the confounding effect of chronic kidney disease on iron homeostasis, subjects with GFR < 40 mL/min/m² were excluded. Presence of any MRI contraindication (e.g. incompatible implant) also precluded enrolment. Female subjects who were pregnant or taking hormone therapy were not enrolled. The university's Institutional Review Board approved the study, compliant with the Declaration of Helsinki, and all subjects provided written informed consent to participate.

Clinical and Laboratory Data

Laboratory measurement at baseline for men and women and again for women after 2 years included serum creatinine, hsCRP, lipid levels (total cholesterol, high-density lipoprotein [HDL], triglycerides, calculated low-density lipoprotein [LDL]), follicle-stimulating hormone (FSH), and iron serologies (ferritin, total iron binding capacity, transferrin and iron saturation). Subjects' 10-year atherosclerotic cardiovascular disease (ASCVD) risk scores were computed based on clinical and laboratory values per recently published guidelines³¹. Estradiol was quantified in serum by radioimmuno-assay (RIA) methods^{32, 33}. Analysis of sex hormone-binding globulin (SHBG) used a chemiluminescent immunometric assay. Free estradiol was calculated using the measured total estradiol levels and SHBG concentrations as well as an average assumed concentration for albumin^{34–36}.

Non-contrast Carotid Artery MRI

Female subjects underwent non-contrast MRI of bilateral carotid arteries at baseline and 2-year follow-up on the identical 3 Tesla scanner (Verio, Siemens) using a custom built 8-element (4 left and 4 right) carotid coil for carotid imaging (Massachusetts General Hospital); male subjects underwent the same imaging protocol. Three-dimensional (3D) time-of-flight imaging was used to localise the carotid arteries followed by 5–7 minutes of 3D T1-weighted (T1W) dark blood imaging using a 3D turbo spin echo (TSE) sequence³⁷ optimised for carotid artery wall imaging³⁸. Multi-slice, multi-echo gradient echo acquisitions for T2* mapping were subsequently obtained perpendicular to the vessel axis in both common carotid arteries (CCA) just proximal to the carotid bulb and then in both

internal carotid arteries (ICA) at 5 mm and 2.5 mm distal to the bifurcation (6 slices per MRI)³⁹.

Image Analysis

Carotid artery wall volume was measured from the 3D T1W TSE scan, from which contiguous multi-planar reformatted images were generated perpendicular to the right and left CCA and ICA (Fig. 1). Five consecutive 1 mm-thick slices were generated with the first slice starting at just below the carotid bulb (for CCA) and at the carotid bifurcation (for ICA). From the T2* acquisition, custom software (MATLAB) was used to fit a monoexponential decay curve at each pixel location using a neighborhood weighted least squares model and compute arterial wall T2* for each slice²⁸. T2* values were then averaged across all 6 slices for each subject's MRI. Repeat vessel wall T2* computation in 80 studies by two independent observers confirmed excellent inter-reader reliability (Fig. 2). Vessel wall area was calculated by subtracting lumen area from the outer border enclosed area. Carotid wall volume for each of four locations (right CCA/ICA, left CCA/ICA) was calculated by multiplying the sum of the five slice wall areas by slice thickness, and total carotid artery wall volume was the sum of these 4 volumes. Wall volume normalized to height was also computed. Repeat wall volume measurements in 70 studies by two independent observers showed excellent agreement (Fig. 2).

Statistical Analysis

Median values of continuous clinical and laboratory characteristics were compared between women and men using the Wilcoxon rank-sum tests. Chi-squared and Fisher's exact tests were applied for categorical variables. Furthermore, changes in clinical characteristics over 2-year follow-up were also analysed for women using Wilcoxon signed rank tests for continuous outcomes. The correlation between continuous variables was computed with the Pearson's correlation coefficient. Data were analysed using Stata 13.0 (Stata Corporation, College Station, TX).

Results

Of 127 women enrolled, 24 did not complete 2-year follow-up visits and 2 additional women had excessive motion artefact precluding use of their carotid images. Of 50 men enrolled, artefact precluded use of carotid images in 3. Table 1 summarises the characteristics of the final study cohort that consisted of 101 women and 47 men. Laboratory findings are summarised in Table 2.

Number of menstrual cycles in the prior 12 months significantly decreased from a median of 4 [3 – 6] at baseline to 0 [0 – 1] at 24-month follow-up ($P<0.$). FSH increased significantly over 24 months (Table 2). The frequency of major risk factors did not change significantly over the follow-up period. Serum ferritin increased over 24 months for women, and was significantly lower in women vs. men at baseline ($P<0.01$).

In women, baseline carotid artery T2* was inversely related to hsCRP ($r=-0.3139$, $P=0.0014$). This relationship weakened after 24 months of menopause transition ($r=-0.1718$, $P=0.0859$), and was not present in men ($r=-0.0310$, $P=0.8362$, Fig. 3). Similarly, higher

levels of serum ferritin predicted shorter carotid artery T2* in women at baseline ($r = -0.3163$, $P = 0.0013$); this relationship attenuated over 24 months of menopause transition ($r = -0.0730$, $P = 0.4684$) and was not significant in men of similar age and risk category ($r = 0.0862$, $P = 0.5644$, Fig. 4).

Higher ASCVD risk scores predicted higher hsCRP levels only in women at 24 months of menopause transition ($r = 0.2083$, $P = 0.0476$), but not in women ($r = 0.0053$, $P = 0.9600$) or men ($r = 0.1127$, $P = 0.4946$) at baseline. The correlation between ASCVD risk scores and arterial wall T2* values was not significant in any group ($P > 0.47$ in women or men at baseline as well as women at 24 months).

Discussion

This study represents the first examination of iron homeostasis using *in vivo* arterial wall biomarkers in perimenopausal women in comparison to age- and risk-matched men. We found that in women entering the menopause transition, an iron-based non-invasive arterial wall MRI biomarker correlated with hsCRP, an established systemic marker of inflammation and atherosclerosis risk. This relationship diminished over 2 years of perimenopause, and was absent in men of similar age and cardiovascular risk profile. An increase in iron balance in women transitioning through menopause was supported by an increase in ferritin levels. Arterial wall volume, previously shown to be increased in the presence of atherosclerosis and predictive for cardiovascular events, was lower in perimenopausal women compared to men.

The association of hsCRP with shortened T2*, traditionally used to indicate increased iron content in myocardium and hepatic tissue, underscores the link between iron and inflammation. Considerable experimental evidence supports this link, from iron's role in LDL oxidation via Fenton chemistry to accelerated uptake of oxidised LDL by macrophages to form foam cells that, in turn, promote atheroma progression^{40, 41}. In a recent post-hoc analysis of the Iron in Atherosclerosis Study (FeAST), ferritin reduction –not lipid changes– with statin therapy plus phlebotomy paralleled reduced mortality, non-fatal myocardial infarction and stroke in patients with established peripheral arterial disease⁴², suggesting that statin's pleiotropic effects may occur via iron and inflammation⁴³.

In a randomised clinical trial, chelation therapy reduced total mortality and atherosclerotic events such as myocardial infarction and stroke in patients with established coronary heart disease⁴⁴. Dietary modification may be a more feasible intervention for at-risk individuals, noting that consumption of red meat, a large source of dietary iron, increases atherosclerosis and cardiovascular events^{45, 46}. Collectively, these data make it difficult to dismiss iron in the plaque microenvironment as a reasonable target for cardiovascular risk reduction.

While ferritin increased, its correlation with arterial wall T2* values diminished over the menopause transition. These findings suggest that iron homeostasis may have a role via inflammation early in subclinical arterial wall dynamics in patients at risk, but less so as women's iron profiles become similar to those of men. Prior studies of serum ferritin, transferrin and TIBC have shown an inconsistent correlation with atherosclerosis risk⁴⁷;

similarly, serum ferritin is an unreliable predictor of tissue iron by T2* quantification in patients with myocardial iron overload⁴⁸. Together, these data remind us that changes in total body iron vs. local tissue iron homeostasis reflect distinct processes.

While data from Multi-Ethnic Study of Atherosclerosis did not find an association between estradiol levels and measures of subclinical arterial disease such as cIMT and coronary artery calcium score⁴⁹, other studies suggest that HT favorably impacts subclinical coronary calcification⁵. Mechanistic data also suggest that calcium may defend against plaque destabilization by excluding intra-lesion iron⁵⁰. Heterogeneity in hormone therapy trial results plus our findings behoove an integrated approach that considers iron balance as well as ovarian hormones in understanding sex differences in atherosclerosis.

Limitations

A direct mechanistic link between iron accrual and atherosclerosis initiation cannot be established with these observational data. However, the distinct sex-specific correlations between systemic inflammation and changing iron homeostasis through perimenopause make a compelling case to further investigate iron accrual's role in atherosclerosis initiation. Measuring oxidised LDL might further illuminate potential mechanistic links between iron and excess atherosclerosis. Carotid IMT was not included in this protocol in favor of a volumetric approach to measure arterial wall disease; several studies comparing cIMT to MRI measurements indicate similar measurements with greater reproducibility using the MR-based volumetric approach^{20–22}.

Conclusions

An iron-sensitive tissue biomarker measured in the arterial wall reflects systemic inflammation early in perimenopausal women but less so as menopause transition progresses and not in men. Further preclinical and clinical studies that investigate iron and inflammation in atherosclerosis initiation and progression may help identify novel interventions to reduce the burden of atherosclerosis in both women and men at risk.

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- Iron balance differences may influence atherosclerosis incidence in women vs. men.
- A magnetic resonance arterial wall biomarker sensitive to endogenous iron was used.
- Arterial wall MRI-T2* paralleled hsCRP in early but not later menopause nor in men.
- Ferritin's link to MRI-T2* in women was lost after 2 years and was absent in men.
- Targeting iron homeostasis to lower incident atherosclerosis warrants further study.

Figure 1. Carotid Artery Wall Volume Measurement

3D T1W-TSE magnetic resonance images of the right internal (A-1) and common (A-2) carotid artery are shown. Orthogonal sections through the arterial wall are generated (B) for wall volume measurement. Manually delineated inner and outer vessel wall contours are shown (C) for the same common carotid artery.

Figure 2. Interobserver Agreement in Carotid Artery Wall T2* and Wall Volume
 Bland-Altman plots of arterial wall T2* (top) and wall volume (bottom) measurements show good agreement between 2 reviewers. The Bland-Altman mean interobserver difference in T2* was 0.1 ms (95% confidence interval [CI], -3.7 to 3.9 ms), and this difference was 8.2 mL for wall volume (95% confidence interval [CI], -37.4 to 53.9).

Figure 3. Arterial Wall MRI vs. Systemic Inflammatory Biomarkers in Perimenopausal Women vs. Men

The magnetic resonance imaging biomarker T2* measured in the carotid artery wall of women entering perimenopause shows a significant correlation with serum levels of hsCRP ($r=-0.3139$, $P=0.0014$). This relationship is weaker as women transition through perimenopause ($r=-0.1718$, $P=0.0859$) and is insignificant in men of similar age and risk factor profile ($r=-0.0310$, $P=0.8362$).

Figure 4.

Table 1

Baseline Clinical and Laboratory Characteristics of the Study Cohort

Variable ¹	Women (N=101)	Men (N=47)	P-value Women vs. men
Age, years	50 [48 – 52]	49 [47 – 52]	0.59 ²
Race, n (%)			
White	82 (81.2)	40 (85.1)	
Black	18 (17.8)	5 (10.6)	0.19 ³
Asian	1 (1.0)	2 (4.3)	
Body mass index, kg/m ²	30.2 [25.6 – 37.1]	29.8 [26.5 – 32.7]	0.54 ²
Hypertension	74 (73.3)	27 (57.4)	0.05 ⁴
Diabetes	28 (27.7)	14 (29.8)	0.80 ⁴
Statin therapy	34 (33.7)	17 (36.2)	0.77 ⁴
Framingham Risk Score			
Low	36 (35.6)	17 (36.2)	
Intermediate	58 (57.4)	25 (53.2)	0.72 ⁴
High	7 (6.9)	5 (10.6)	

¹ Median [inter-quartile range] reported for continuous variables and n (%) reported for categorical variables.² Based on Wilcoxon rank-sum tests.³ Based on Fisher's exact tests.⁴ Based on chi-squared tests.

Table 2

Blood Measurements

Variable ¹	Women at baseline	Women at 2-year follow-up (P-value ²)	Men at baseline (P-value ³)
High-sensitivity CRP, mg/L	2.5 [1.0 – 5.5]	2.2 [0.9 – 5.4] (0.25)	1.7 [0.8 – 4.3] (0.21)
Total cholesterol, mg/dL	209 [180 – 233]	206 [169 – 232] (0.40)	185 [146 – 212] (<0.01)
HDL, mg/dL	49 [41 – 65]	53 [44 – 66] (0.04)	42 [37 – 49] (<0.01)
LDL, mg/dL	122.5 [86 – 153]	113 [82 – 138] (0.03)	96.5 [67 – 123] (<0.01)
Triglycerides, mg/dL	125 [82 – 206]	139 [95 – 215] (0.07)	183 [135 – 233] (<0.01)
Hematocrit, %	39.7 [37.3 – 41.1]	40.2 [38.5 – 41.7] (0.08)	42.6 [41.0 – 45.6] (<0.01)
Ferritin, ng/mL	37 [20 – 79]	67 [36 – 96] (<0.01)	111 [45 – 220] (<0.01)
TIBC, ug/dL	411 [373 – 447]	404 [371 – 432] (0.75)	392 [353 – 450] (0.22)
Transferrin, mg/dL	276 [250 – 300]	271 [248 – 290] (0.74)	263 [237 – 302] (0.22)
Iron saturation, %	18 [14 – 24]	18 [14 – 22] (0.82)	22 [15 – 29] (<0.01)
FSH, mIU/mL	35.6 [15.6 – 61.9]	58.1 [34.4 – 78.0] (<0.01)	n/a
SHBG, nmol/L	42.6 [24.5 – 59.6]	39.5 [24.8 – 60.4] (0.52)	31.8 [22.9 – 47.5] (0.03)
Free estradiol, pg/mL	0.6 [0.4 – 2.4]	0.3 [0.2 – 0.5] (<0.01)	0.8 [0.6 – 0.9] (0.91)
Wall volume/Height, mL/cm	4.1 [3.5 – 4.5]	4.3 [3.8 – 4.7] (0.81)	4.7 [4.4 – 5.5] (<0.01)

¹ Median [inter-quartile range]. N=101 women and 47 men.

² Compared to women at baseline based on Wilcoxon signed rank tests.

³ Compared to women at baseline based on Wilcoxon rank-sum tests.