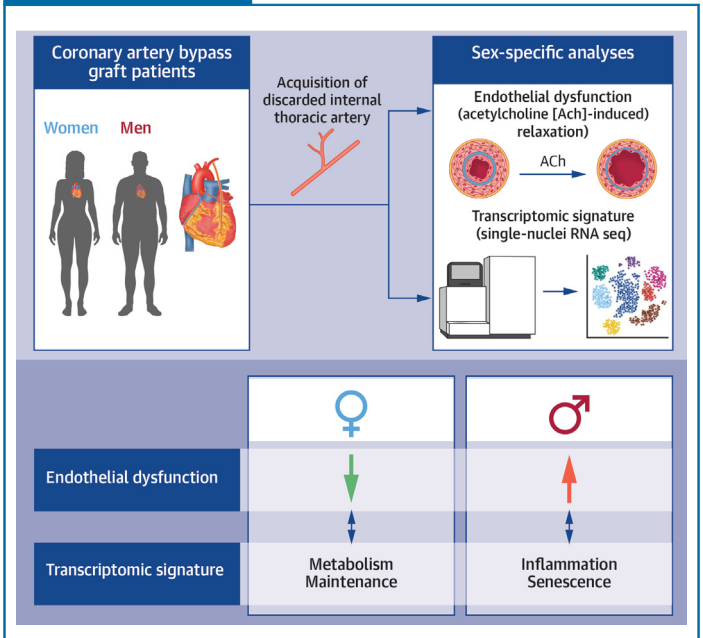
JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 9, NO. 10, 2024

ORIGINAL RESEARCH - CLINICAL

Senescence and Inflamm-Aging Are Associated With Endothelial Dysfunction in Men But Not Women With Atherosclerosis

Pauline Mury, PhD,[a,b] Gael Cagnone, PhD,[c] Olina Dagher, MD,[a,b,d] Florian Wünnemann, PhD,[a] Guillaume Voghel, MD, PhD,[e] Melissa Beaudoin, MSc,[a] Mélanie Lambert, PhD,[a,b] Géraldine Miquel, BSc,[a] Pierre-Emmanuel Noly, MD, PhD,[a,f] Louis P. Perrault, MD, PhD,[a,f] Michel Carrier, MD,[a,f,†] Nathalie Thorin-Trescases, PhD,[a] Jean-Sébastien Joyal, MD, PhD,[c,g,h] Guillaume Lettre, PhD,[a,i,*] Eric Thorin, PhD[a,f,*]

VISUAL ABSTRACT



Mury P, et al. JACC Basic Transl Sci. 2024;9(10):1163-1177.

HIGHLIGHTS

- **In patients undergoing coronary artery bypass graft surgeries, internal thoracic arteries from postmenopausal women display a better endothelial sensitivity to ACh ex vivo than age-matched men.**
- **At the molecular level, divergence in sensitivity to acetylcholine is observed particularly in ECs among all cell clusters, and mostly in male ECs, confirming the vascular reactivity results.**
- **Senescence- and inflammation-specific signaling pathways genes are overexpressed in male ECs, whereas extracellular matrix and metabolism-specific pathways dominate in female ECs.**
- **ECs of female patients were neither pro-senescent nor inflamed. The cause of atherogenesis may be less dependent on a dysfunctional endothelium in women.**
- **These results suggest that the use of senolytics have a great potential in men with coronary artery disease, but it may not be as effective in women.**

From the [a]Montreal Heart Institute Research Center, University of Montreal, Montreal, Quebec, Canada; [b]Department of Pharmacology and Physiology, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada; [c]University Hospital Sainte Justine Research Center, University of Montreal, Montreal, Quebec, Canada; [d]Department of Cardiac Sciences, Libin Cardiovascular Institute, Calgary, Alberta, Canada; [e]Department of Family Medicine and Emergency Medicine, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada; [f]Department of Surgery, Faculty of Medicine, University of Montreal, Montreal,

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2024.06.012

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 9, NO. 10, 2024
OCTOBER 2024:1163–1177

ABBREVIATIONS AND ACRONYMS

ACh = acetylcholine

BMI = body mass index

CABG = coronary artery bypass graft

CAD = coronary artery disease

CVD = cardiovascular disease

DEG = differentially expressed genes

EC = endothelial cell

EC_{50} = half maximal effective concentration

E_{max} = maximum relaxation

FIB = fibroblast

GSVA = gene set variation analysis

IMM = immune cell

ITA = internal thoracic artery

L2FC = log2 fold change

PER = pericyte

PGI_2 = prostacyclin I_2

SASP = senescence-associated secretory phenotype

SMC = smooth muscle cell

snRNA-seq = single nuclei RNA sequencing

UMAP = uniform manifold approximation and projection

SUMMARY

Coronary artery disease (CAD) is more prevalent in men than in women, with endothelial dysfunction, prodromal to CAD, developing a decade earlier in middle-aged men. We investigated the molecular basis of this dimorphism ex vivo in arterial segments discarded during surgery of CAD patients. The results reveal a lower endothelial relaxant sensitivity in men, and a senescence-associated inflammaging transcriptomic signature in endothelial cells. In women, cellular metabolism and endothelial maintenance pathways are conserved. This suggests that senolytic therapies to reduce risk of cardiovascular events in women with CAD may not be as effective as in men. (JACC Basic Transl Sci. 2024;9:1163–1177)

The prevalence of coronary artery disease (CAD) is higher in men than in women,[1,2] but the underlying molecular basis for this sexual dimorphism are poorly understood.[2,3] There is a consensus on the protective role of estrogens and CAD risk increasing following menopause.[4] Likewise, men develop lipid-rich plaques, whereas women are more likely to develop fibrous plaques[4] with a unique transcriptomic and proteomic signatures in the plaque.[5-8] Otherwise, presentation of oxidative stress and inflammation may differ between women and men but are inconsistent.[9-11]

In healthy humans, aging is associated with a progressive endothelium-dependent dilatory decline,[12,13] which appears 10 years earlier in men than in women[12] and is highly predictive of future cardiovascular events.[14-16] In recent years, research has established that age-related accumulation of senescent cells[17] could cause chronic low-grade cold inflammation, also known as inflammaging,[18] through the release of the senescence-associated secretory phenotype (SASP).[19] Because SASP involves a range of proinflammatory factors with important paracrine and autocrine effects on cell and tissue biology,[19] inflammaging could promote cardiovascular disease (CVD).[20] Senescent endothelial cells (ECs) have been identified lining human atherosclerotic lesions[21] and EC telomere attrition driven by both cellular damage and division is accelerated by exposure to risk factors for CVD,[22] suggesting that biological aging rather than chronological aging is the primary driver of CVD.[23] In older humans, senescent ECs accumulate[24] while the concomitant immuno-senescence slows their clearance.[25] The use of senolytics-inducing apoptosis of senescent cells has confirmed that vascular senescence causes endothelial dysfunction and atherosclerosis in mouse models.[26,27] Short telomere length is a strong index of the susceptibility of a cell to enter senescence,[22] especially for ECs that actively divide for repair while exposed to damaging high mechanical and oxidative stress.[28] Telomere length is similar at birth between sexes;[29] therefore, telomere length is an indicator of both replicative history and replicative potential of dividing cells.[30]

To investigate the molecular bases of the sexual dimorphism in CAD-associated endothelial dysfunction, we prospectively collected distal segments of lesion-free internal thoracic arteries (ITAs) during coronary artery bypass graft (CABG) surgeries from both men and women. Similar to plaque-free coronary arterial segments isolated from severely atherosclerotic patients,[31] ITA segments have a functional relaxant endothelium. Although this is not optimal,[32] it may be representative of the pre-plaque endothelial state in these CAD patients. Our data show that endothelial dysfunction is more pronounced in men compared to women. Importantly, using single-nuclei transcriptomics, senescent and inflammatory transcriptomic signatures suggestive of

Quebec, Canada; [g]Department of Pediatrics, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada; [h]Department of Ophthalmology, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada; and the [i]Department of Medicine, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada. *Drs Lettre and Thorin contributed equally to this work and are co-senior authors. †Dr Carrier is deceased.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

Manuscript received May 24, 2024; revised manuscript received June 26, 2024, accepted June 26, 2024.

the inflammaging were only identified in male EC, not in female EC. Therefore, EC senescence-associated endothelial dysfunction may contribute to atherogenesis in men.

METHODS

STUDY POPULATION. Sixty-five patients undergoing an in-patient CABG-only surgery at the Montreal Heart Institute (Canada) were prospectively recruited in the study between 2018 and 2019. All patients signed an informed consent. The study (ICM 13-1492) was approved by the Montreal Heart Institute ethics committee for human research (Comité d'éthique de la recherche et du développement des nouvelles technologies). All patients were treated according to the optimal standard of care. The main cardiovascular risk factors, including hypertension, dyslipidemia, obesity, diabetes mellitus, smoking status, and body mass index (BMI), as well as other comorbidities such as renal failure, previous myocardial infarct, and atrial fibrillation were recorded and found to be similar between the 2 sexes. None of the women received hormonal replacement therapy.

For all patients, a discarded distal segment of the ITA harvested as a graft conduit was provided by the surgeon in the operating room. The resulting tissue sample was placed in ice-cold sterile cell culture medium for immediate processing. The segment of ITA was cut in at least 3 rings that were 4 mm in length: 2 were used within 30 minutes to quantify ex vivo endothelium-dependent relaxation to acetylcholine (ACh); the third ring was snap frozen within 15 minutes of harvest for subsequent analysis of the transcriptomic signature of the arterial wall using single nucleus RNA sequencing (snRNA-seq).

STATISTICAL ANALYSIS. Data are presented as mean ± SEM in the text, tables, and figures. Comparison of CVD risk factors, ACh half maximal effective concentration (EC_{50}), maximal relaxation (E_{max}), and percent of ITA pre-contraction between sexes was performed using GraphPad Prism software (version 9). At first, normality of distribution was assessed using the D'Agostino-Pearson test between groups. Normally distributed data were compared using a parametric unpaired Student's *t*-test (age, E_{max}, and precontraction, telomere restriction fragment length); otherwise, a nonparametric Mann-Whitney test was used (BMI, troponin peak, cardiopulmonary bypass and aortic clamp duration, ACh-EC_{50}). Categorical variables are presented as count (percentage) and compared using Fisher exact or chi square test (patient presentation, number of diseased vessels, coronaries treated with ITA or saphenous vein. All the snRNA-seq analyses were performed using R software (v 4.1.2, R Core Team, 2021), as discussed in the methods section. The significance level was fixed to $P < 0.05$.

AVAILABILITY OF DATA. All single-cell data for the study have been deposited in the U.S. National Center for Biotechnology Information Gene Expression Omnibus database (GSE255895).

A detailed description of all methods is provided in the Supplemental Appendix.

RESULTS

POPULATION CHARACTERISTICS. We recruited 40 men and 25 women undergoing CABG surgery whose ages were 66.7 ± 1.4 years and 67.8 ± 2.1 years on average, respectively (Table 1). Neither age nor cardiovascular risk factors for CVD differed between men and women. Only the use of diuretics was more frequent in males compared to females (40.0% vs 8.0%, respectively; $P = 0.005$). No difference in presentation for surgical parameters were noted between male and female patients (ie, stable or instable angina, ST-segment elevation myocardial infarction or non-ST-segment elevated myocardial infarction, troponin peak, cardiopulmonary bypass and aortic cross clamp durations, and number of diseased vessels including treatment with ITA or saphenous vein grafts) (Table 1).

HIGHER ENDOTHELIAL SENSITIVITY TO ACh IN ARTERIES ISOLATED FROM WOMEN. We measured 2 indexes of vascular reactivity to assess endothelial function: ACh-EC_{50} for the endothelial sensitivity to ACh, and E_{max} for the maximal relaxation (Figure 1A). EC_{50} in female arteries was significantly lower than in male arteries (females = 18.9 nM [range: 6.55-47.7 nM] vs males = 63.9 nM [range: 18.6-121 nM]; $P = 0.004$), demonstrating a higher endothelial sensitivity to ACh (Figure 1B), and thus endothelial function in women. The maximal relaxation, however, was similar between sexes (E_{max} to ACh: females: 57% ± 5% vs males: 54% ± 4%; $P = 0.73$) (Figure 1B). Although these values are within the range of previous data reported for male patients,[32] this appears to be the first report showing that, in patients >65 years of age, endothelial function is better in women than in age-matched men.

We performed snRNA-seq on ITA segments from 6 male and 6 female patients to identify potentially different transcriptomic signatures between sexes. We randomly selected arterial segments of 2 patients per sex with high (E_{max} >71.5%), mild (E_{max} <71.5% to

TABLE 1 General Characteristics of the Cohort

	Men (n = 40)	Women (n = 25)	*P* Value
Cardiovascular risk factors			
Age, y	66.7 ± 1.4	67.8 ± 2.1	0.63
Hypertension	32 (80.0)	22 (88.0)	0.51
Dyslipidemia	32 (80.0)	22 (88.0)	0.51
Obesity	14 (35.0)	9 (36.0)	0.79
Diabetes mellitus	19 (47.5)	12 (48.0)	>0.99
Smoking			
Active	5 (12.5)	4 (16.0)	0.72
Ex-smoker	17 (42.5)	6 (24.0)	0.18
COPD	3 (7.5)	2 (8.0)	>0.99
Previous MI	9 (22.5)	3 (12.0)	0.34
Known AF	3 (7.5)	2 (8.0)	>0.99
BMI, kg/m^2	27.9 (24.7-31.8)	28.2 (25.7-35.6)	0.68
Medication			
Beta-blocker	30 (75.0)	13 (52.0)	0.067
ACEI	20 (50.0)	9 (36.0)	0.60
ARA II	5 (12.5)	1 (4.0)	0.39
Diuretic	16 (40.0)	2 (8.0)	0.005[a]
Hypoglycemic agent	17 (42.5)	11 (44.0)	>0.99
Anticoagulant	23 (57.5)	12 (48.0)	0.61
Aspirin	33 (82.5)	20 (80.0)	>0.99
Statin	35 (87.5)	19 (76.0)	0.31
CaCh blocker	8 (20.0)	3 (12.0)	0.51
Surgical characteristics			
Troponin peak, ng/L	12.0 (8.00-25.7)	13.5 (8.75-22.6)	0.96
Stable angina	13 (32.5)	6 (24.0)	0.33
Unstable angina	10 (25.0)	4 (16.0)	
STEMI	1 (2.5)	3 (12.0)	
NSTEMI	16 (40.0)	12 (48.0)	
Number of diseased vessels			
1	3 (7.5)	1 (4.0)	0.15
2	5 (12.5)	8 (32.0)	
3	32 (80.0)	16 (64.0)	
Coronaries treated with ITA			
1	35 (79.5)	21 (84.0)	0.89
2	7 (15.9)	3 (12.0)	
3	2 (4.5)	1 (4.0)	
Coronaries treated with SV			
1	16 (47.0)	10 (55.5)	0.32
2	17 (50.0)	6 (33.3)	
3	1 (2.9)	2 (11.1)	
CBP duration, min	60.0 (44.1-80.5)	68.0 (52.4-107)	0.11
Aortic clamp duration, min	34.3 (23.7-55.6)	43.6 (33.8-57.9)	0.27

Values are mean ± SEM, n (%) within the group, and median (Q1-Q3) when nonparametric test used. [a]$P < 0.05$.

ACEI = angiotensin-converting enzyme inhibitor; AF = atrial fibrillation; ARA = angiotensin II receptor antagonist; BMI = body mass index; CBP = cardiopulmonary bypass; CaCh = calcium channel; COPD = chronic obstructive pulmonary disease; ITA = internal thoracic artery; MI = myocardial infarction; NSTEMI = non-ST-segment elevation myocardial infarction; STEMI = ST-segment elevation myocardial infarction; SV = saphenous veins.

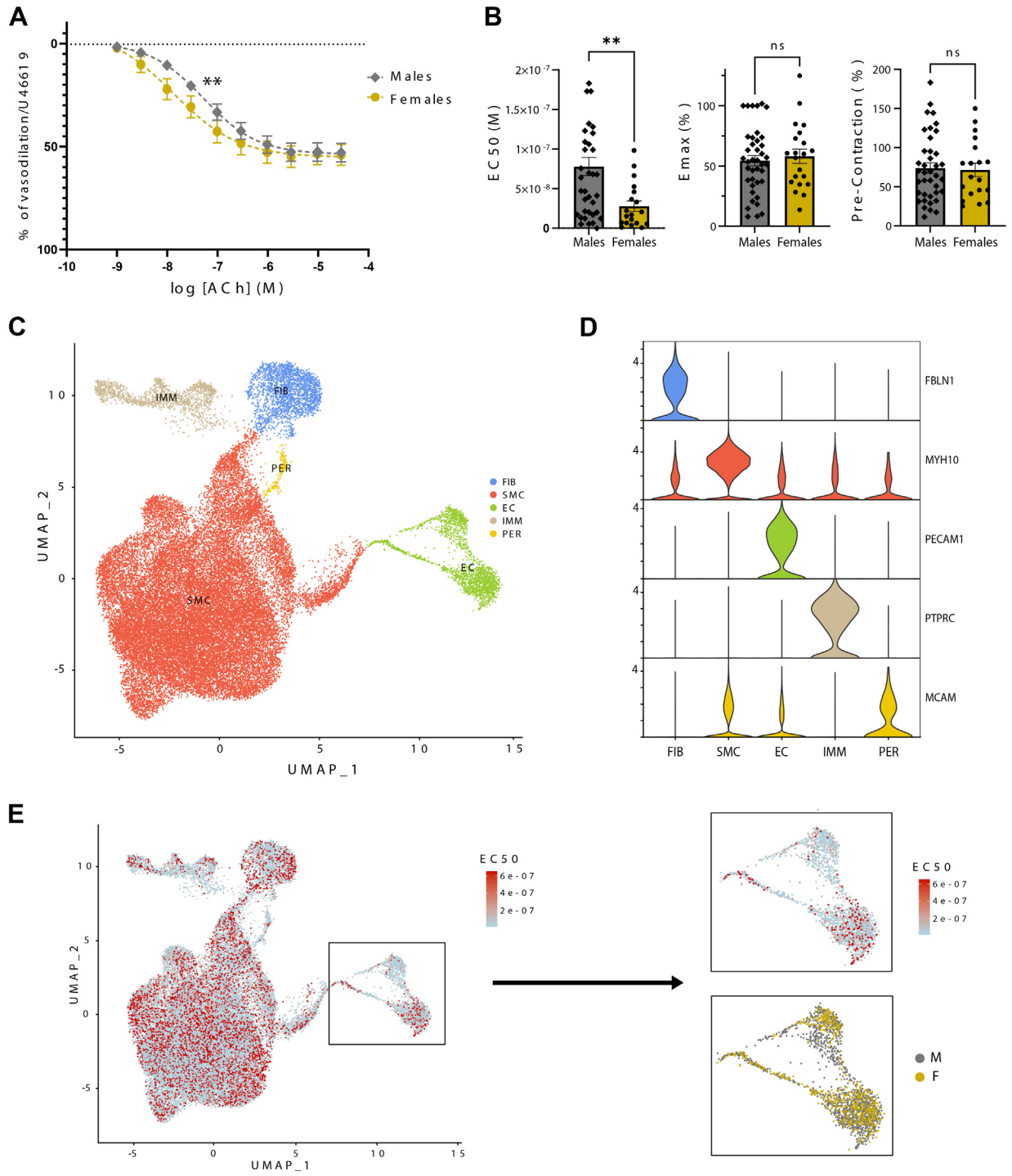
E_{max} >36.5%), and low endothelium-dependent maximal relaxation (E_{max} <36.5%) (Supplemental Figure 1A) to avoid bias of selection based on EC_{50}. In this small cohort, sensitivity to ACh in female arteries still showed a trend to be better than in male arteries (females: 27.6 nM [range: 17.2-144nM] vs males: 136 nM [range: 44.8-351 nM]; $P = 0.06$) (Supplemental Figure 1B), whereas E_{max} and preconstraction levels were similar between sexes (Supplemental Figures 1C and 1D). However, in this subgroup, the average age of the 6 women was 77 years, 14 years older than the average age of the 6 men ($P = 0.03$) (Supplemental Figure 1E). Nuclei were then isolated from an adjacent segment not used for the reactivity study.

TRANSCRIPTOMIC PROFILING OF ITA SEGMENT CELL TYPES. We clustered cells using unsupervised graph-based clustering and visualized them as a uniform manifold approximation and projection (UMAP) graph to identify the cells (**Figure 1C**) using canonical vasculature markers (**Figure 1D**, Supplemental Figure 2A, Supplemental Dataset 2). As expected,5 large cell types uniformly distributed between men and women were identified: smooth muscle cells (SMCs) (83.5%), fibroblasts (FIBs) (6.2%), ECs (5.6%), immune cells (IMMs) (4.2%) and pericytes (PERs: 0.6%) (**Figure 1C**, Supplemental Figure 2B).

The distribution of the cell types was similar between the 12 patients, except for male patient ITA3 exhibiting a significantly larger proportion of immune cells (Supplemental Figure 2C). This patient was excluded from downstream EC subclustering analysis to avoid unanticipated effect of high immune cell infiltration. Then, because endothelium-dependent function was characterized by the sensitivity to ACh (EC_{50}), we examined whether the distribution of the EC_{50} was uniform within the cell clusters (**Figure 1E**). Interestingly, higher (red dots) and lower values (blue dots) of EC_{50} were similarly distributed among the cells, except for ECs (**Figure 1E**, top zoom-in). Identification of the sex of the nuclei in this EC cluster showed that male sex was mostly concentrated in areas of high EC_{50} values (ie, of poor sensitivity to Ach) (**Figure 1E**, bottom zoom-in).

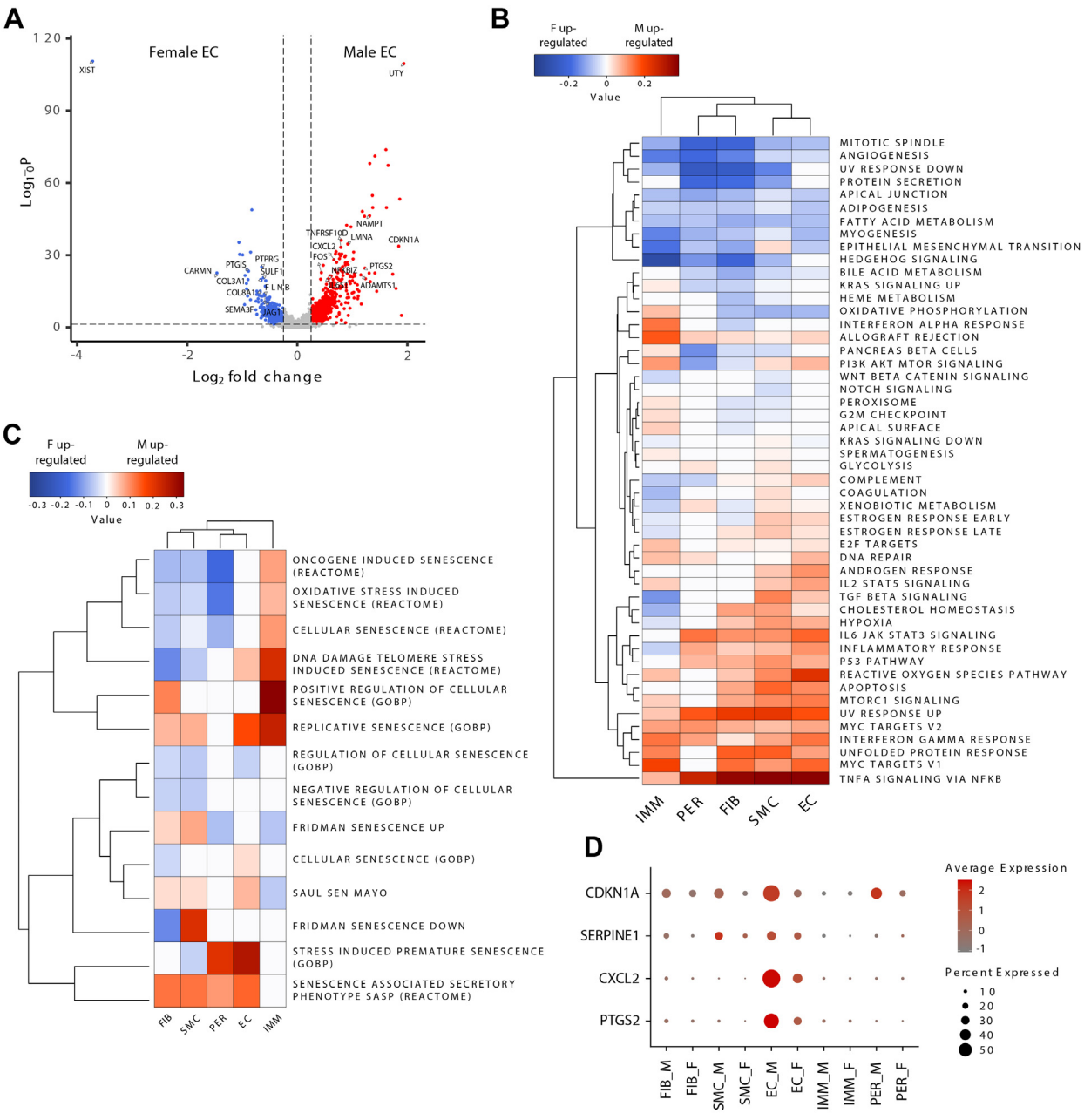
ECs FROM MALE PATIENTS ARE ASSOCIATED WITH CELLULAR SENESCENCE AND PROINFLAMMATORY GENE EXPRESSION. We then focused on the EC population for downstream analysis to explore the mechanisms underlying the sexual dimorphism linked to endothelial dysfunction and performed a differentially expressed gene (DEG) analysis. Comparison of gene expression levels identified 1,182 DEG (false discovery rate [FDR] <0.05 and log2 fold change [L2FC] >0.25) similarly distributed between women (581 upregulated genes) and men (601 upregulated genes) (**Figure 2A**, Supplemental Datasets 1 and 2). Excluding sex-specific genes (eg, *UTY*, *TTTY14*, *DDX3Y*, *USP9Y*, *PRKY* for males, and *TSIX*,

FIGURE 1 **Internal Thoracic Arteries From Female Donors Display a Better Endothelial Sensitivity to Acetylcholine**



(A) Endothelial-dependent relaxation to acetylcholine (ACh) was assessed ex vivo on pre-constricted arterial segments. (B) Half maximal effective concentration (EC_{50}), maximal efficacy (E_{max}), and pre-contraction expressed as % of the maximal contraction induced by 127 mM KCl-physiological solution. Values are expressed as mean ± SEM of 40 male and 25 female arterial rings. The Mann-Whitney test was used to compare groups: male (M) = 63.9 nM (range: 18.6-121 nM) vs female (F) = 18.9 nM (range: 6.55-47.7 nM); $P = 0.004$. (C) Uniform manifold approximation and projection (UMAP) plot of annotated cell-types present in arteries. (D) Violin plot representing expression of one canonical marker by cell-type (*FBLN1*: fibroblast, *MYH10*: smooth muscle cell, *PECAM1*: endothelial cell, *PTPRC*: immune cell, *MCAM*: pericytes). (E) Feature plot showing the distribution of EC_{50} in the entire dataset. Red dots represent higher values of EC_{50} (low sensitivity to ACh), and blue dots represent lower values of EC_{50} (high sensitivity to ACh). Black boxes in the right represent a zoom-in of the endothelia cell (EC) cluster of interest for both EC_{50} distribution (top) and sex distribution (bottom). **$P < 0.01$. FIB = fibroblast; IMM = immune cell; PER = pericyte; SMC = smooth muscle cell.

FIGURE 2 Male ECs Exhibit a More Prominent Pro-Senescent and Proinflammatory Signature



(A) Volcano plot showing differentially expressed genes between male and female ECs. Genes are considered significantly DE when FDR <0.05 and L2FC >±0.25. (B) Heatmap showing Gene set variation analysis (GSVA) of hallmark collection in the 5 cell types between males and females. Red and blue squares represent male and female overexpressed pathways, respectively. (C) Heatmap showing GSVA analysis of selected senescence-related gene sets between male and female cells. Red and blue squares represent male and female overexpressed pathways, respectively. (D) Dot plot showing expression of 4 selected genes associated to senescence and inflammation. AKT = protein kinase B; IL2 = interleukin 2; IL6 = interleukin 6; JAK = Janus kinase; KRAS = Kirsten rat sarcoma virus; MTOR = mammalian target of rapamycin; NFKB = nuclear factor kappa B; P13K = phosphatidylinositol 3-kinase; STAT3 = signal transducer and activator of transcription 3; STAT5 = signal transducer and activator of transcription 5; TGF = transforming growth factor; TNFA = tumor necrosis factor alpha; UV = ultraviolet; WNT = wingless-related integration site; all other abbreviations as in Figure 1.

XIST for females), we observed the presence of senescence and inflammation-specific genes (eg, *CDKN1A*, *NFKBIZ*, *TNFRSF10D, LMNA, PTGS2*) among upregulated genes in men, whereas the presence of extracellular matrix, defense and metabolism-specific genes (eg, *COL3A1*, *FLNB*, *PTGIS, PTPRG*) among the genes upregulated in women (**Figure 2A**).

To refine our analysis, we used the gene set variation analysis (GSVA) method to evaluate pathway enrichment scores for each individual cell (**Figure 2B**). GSVA of hallmark pathways (Molecular Signatures Database) between sexes first revealed that EC is the cell type with the least enriched pathways in female arteries (ie, blue squares). The overexpressed pathways in female ECs are mostly related to metabolism (**Figure 2B**). Inversely, overexpressed pathways in male ECs (ie, red squares) are mostly linked to inflammation, oxidative stress, and cell damage (**Figure 2B**), all favoring senescence and thus inflammaging.[20] Therefore, we selected several senescence-related pathways and ran a GSVA analysis revealing important differences between sexes in ECs (**Figure 2C**). Female ECs overexpressed one pathway involved in the "regulation of cellular senescence," whereas male ECs overexpressed pathways linked to oxidative stress, replicative- and stress-senescence, and the SASP (**Figure 2C**). These analyses reveal a strong dichotomy between sexes suggestive in men, unlike in women, of a prominent susceptibility to an EC inflammaging profile.

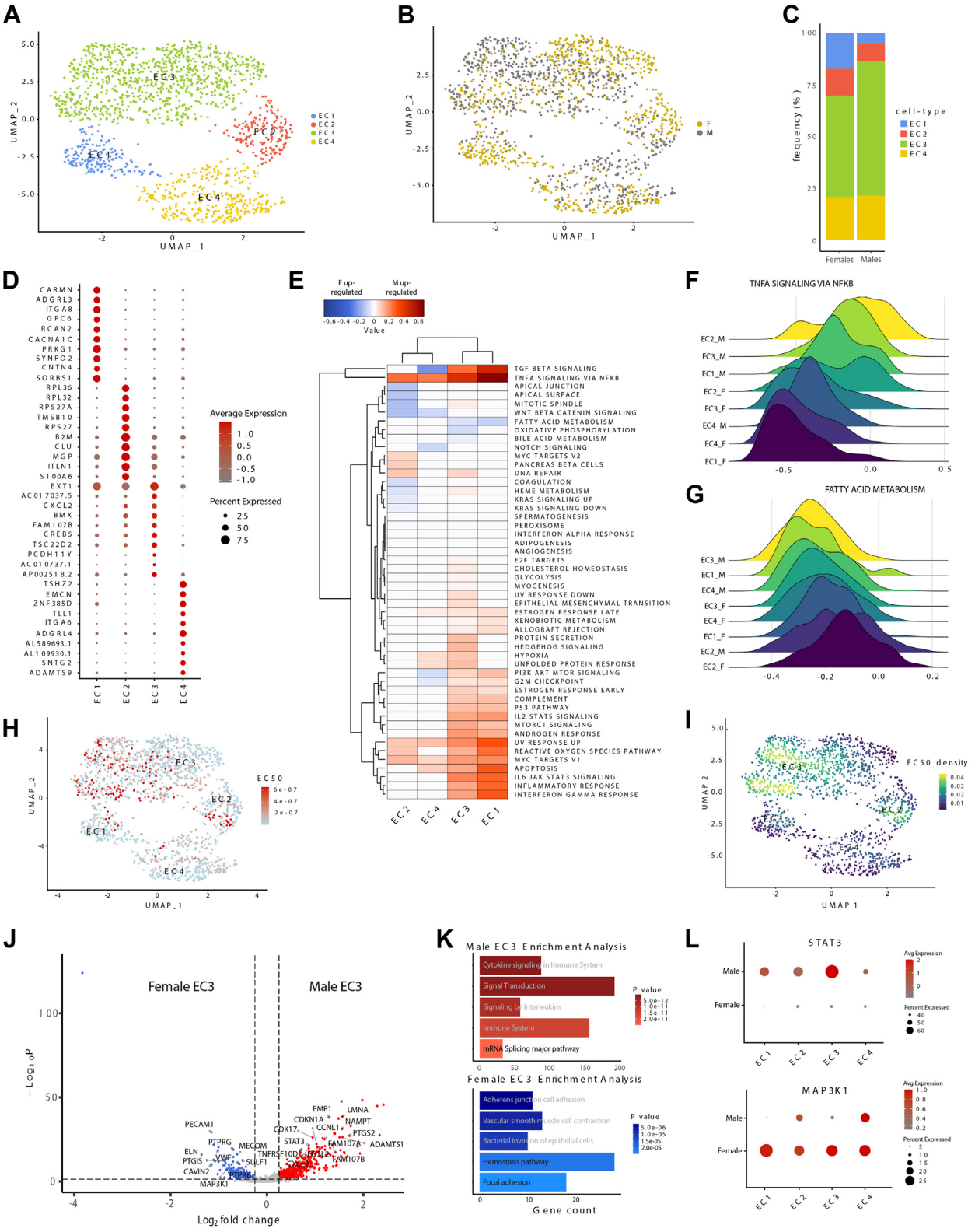
The prevalence of inflammation and senescence in male ECs was confirmed by a greater expression of 4 hallmark transcripts (**Figure 2D**): *CDKN1A* (p21; FDR = 9.08e-87, L2FC = 0.69) and to a lesser extent *SERPINE1* (FDR = 4.02e-12, L2FC = 0.82) were highly enriched in ECs compared to all other cell types. Likewise, *CXCL2* (FDR <1e-300, L2FC = 1.0) and *PTGS2* (*FDR* <1e-300, L2FC = 0.79), 2 contributors of the inflammatory response, were mainly expressed by male ECs compared to all other cell types.

Pathways analyses in other cell types of the wall (IMMs, FIBs, PERs, and SMCs) did not stand as robustly differentially expressed between male and female donors (Supplemental Figure 3). In hallmark pathways (Supplemental Figure 3A), "TNFα [tumor necrosis factor α] signaling via NFκB [nuclear factor kappa B]," "TGFβ [transforming growth factor β] signaling," "inflammatory response," "interferon γ response," "IL6 [interleukin 6] Janus kinase, signal transducer and activator of transcription signaling," and "IL2 STAT5 signaling" are upregulated in male ECs (all EC subclusters merged for this analysis) compared to female ECs and compared to other cell types. These 6 pathways are also upregulated in male SMCs, FIBs, and IMMs, although to a lesser extent than in male ECs. Three pathways are similarly upregulated in male ECs and SMCs: "ROS [reactive oxygen species] pathway," "apoptosis," and "MTORC1 signaling." Only the "UV response up" pathway is more upregulated in male FIBs and SMCs than in male ECs. In females, only 2 pathways are upregulated in PER: "TGFβ signaling" and "IL2 STAT5 signaling."

Senescence pathways are not as uniformly expressed in other cell types of the wall compared to ECs from male donors (Supplemental Figure 3B). "GOBP stress-induced premature senescence" and "GOBP replicative senescence" stand out in male ECs and are not discriminated by sex in the other cell families, suggestive of more damage in the male EC than in any other cell families of the cell wall. Only the "Fridman senescence down" pathway is upregulated in male IMMs, SMCs, and PERs, but not in ECs. Finally, some senescence pathways are upregulated in female cells, particularly female PERs, including "reactome-oncogene induced senescence," "reactome oxidative stress induced senescence," "reactome-cellular senescence," "GOBP replicative senescence," and "Fridman senescence up." Globally, unlike in ECs that either show no sex-related differential expression or a strong male-biased increased in senescence pathways, the other cell types are showing a more erratic pattern of differential expression. Altogether, these data show that senescence pathways are mostly upregulated in male ECs, whereas others are upregulated in female PERs.

EC SUBCLUSTERING REVEALS SEX-BASED HETEROGENEITY IN INFLAMMAGING MARKERS. To determine whether different EC subtypes may underly the sexual dimorphism of the endothelial dysfunction, we subclustered ECs to focus our analysis. UMAP analysis identified 4 subtypes of ECs (**Figure 3A**). We also mapped all ECs according to the sex of the patients (**Figure 3B**). Although the proportion of ECs in male and female arteries is similar in EC2 and EC4 subclusters (**Figures 3B and 3C**), the EC1 subcluster is enriched by female ECs, and the EC3 subcluster is mainly populated by male ECs ($P < 0.001$) (**Figure 3C**). To identify the potential biological functions of each EC subtype, we selected their top 10 specific genes (**Figure 3D**) and applied all the upregulated genes per cluster to the EnrichR pathway analysis (Supplemental Figure 4).[33] These combined approaches reveal that the 4 EC subclusters are likely varying in primary function, with a sex difference in expression profile (EC3) and in numbers (EC1 and EC3). Therefore, we performed GSVA to analyze the differences between male and female patients in each

FIGURE 3 Characterization of Sex-Specific Signatures of EC3 Subtype



Continued on the next page

EC by comparing the scores of the 50 hallmark gene sets (Figure 3E). Higher scores (red) represent overexpression in male ECs, and lower scores (blue) in female ECs. As previously identified (Figure 2B), we found the highest scores for gene sets linked to inflammation in the 4 EC subtypes. Notably, the "TNFα signaling via NFκB" pathway is the most divergent between sexes and is exacerbated in male ECs (Figures 3E and 3F). Twenty gene sets are homogeneously overexpressed in male ECs mostly in EC1 and EC3, including "IL6 JAK STAT3 signaling," "inflammatory response," "ROS pathway," and "interferon gamma response," confirming the large implication of inflammation in endothelial dysfunction observed in males (Figure 3E). In contrast, we observed that the "fatty acid metabolism" pathway is overexpressed in female ECs (Figures 3E to 3G), which is suggestive of a better use and metabolism of fatty acids by female ECs. Enrichment pathways in female ECs are not as homogeneous as in male ECs (Figure 3E), but the shift remains dependent on metabolism (ie, "fatty acid metabolism" and "oxidative phosphorylation" pathways), again suggestive of a better metabolic regulation. The EC3 subcluster almost exclusively overexpresses male EC pathways (Figure 3E); thus, the EC3 subtype might be of interest to understand sex-specific divergences in endothelial dysfunction.

SEX-SPECIFIC PATTERNS REVEAL ACTIVATION OF 2 DIFFERENT SIGNAL TRANSDUCERS IN THE EC3 SUBTYPE. We then plotted the distribution of the EC_{50} in all EC subtypes (Figure 3H). As expected, nuclei expressing highest values of EC_{50} are not uniformly distributed in the 4 subtypes. Red dots (low endothelial sensitivity to ACh) are mostly present in EC3, and to a lesser extent in EC2 as highlighted in the subsequent density plot (Figure 3I). Thus, as already hypothesized in Figure 3E, EC3 might be critical in determining the sex-specific divergence in endothelial function. Therefore, we focused our subsequent analyses on this EC3 subcluster.

Differential expression analysis (Figure 3J) revealed a large amount of DEGs, with a higher number of genes significantly upregulated in male (n = 695) than in female (n = 477) ECs (Supplemental Datasets 3 and 4). In males, at least 7 genes of the top 15 (*CDKN1A*, *NAMPT*, *LMNA*, *TNFRSF10D*, *CXCL2*, *ADAMTS1*, and *PTGS2,* respectively) are shared with the top 15 of the overall male ECs (Figure 2A), comforting that the inflammaging signature previously observed in ECs mainly stems from this EC3 subcluster.

The top 5 enriched pathways (Figure 3K) in male EC3 include inflammation-associated pathways ("cytokines and interleukins signaling") associated with the senescence phenotype.[19] In female EC3, overexpression of vascular wall interactions ("focal adhesion and adherens junction cell adhesion") and maintenance ("homeostasis pathway") pathways are supportive of a tighter cellular communication and regulatory function (Figure 3K). The differential expression analysis revealed *STAT3* gene as the top 15 transcript differentially overexpressed in male EC3 (Figure 3J). Dot plot reveals that the *STAT3* gene is specific to males and preferentially to EC3 (Figure 3L). In contrast, the *MAP3K1* gene transcripts are in the top 15 differentially expressed in female EC3 (Figure 3J). The *MAP3K1* gene is more expressed by female than male ECs of all subtypes (Figure 3L).

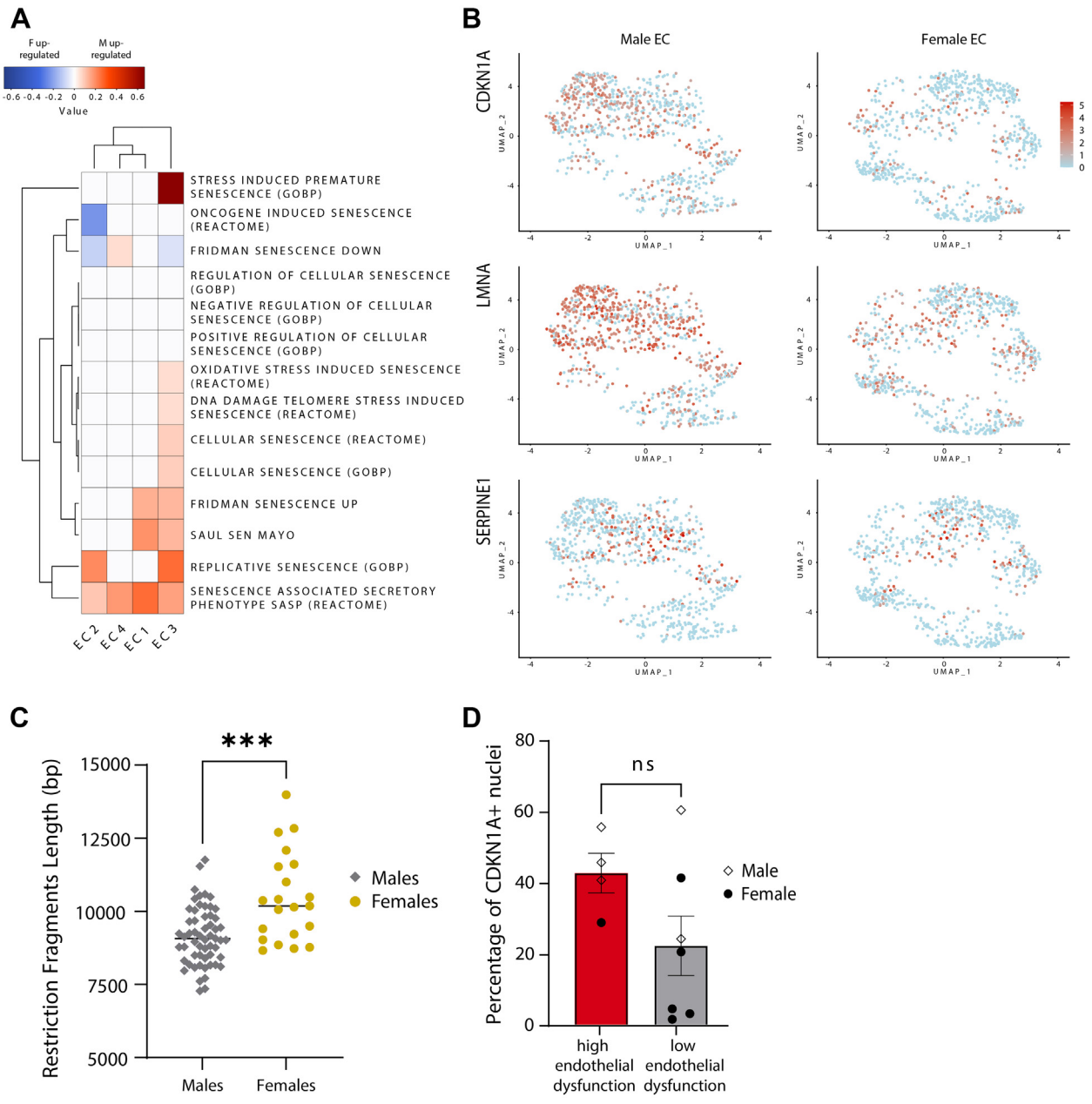
We next used the GSVA approach to measure sex-difference enrichment in gene sets associated with senescence (Figure 4A). Overall, we observed that upregulated pathways promoting senescence are only observed in male EC3 (Figure 4A); "stress induced premature senescence," "replicative senescence," "Fridman senescence up," "Saul sen Mayo," and "senescence associated secretory phenotype SASP" are among the most upregulated pathways, particularly in EC3 (Figure 4A).

To confirm this result, we plotted by sex the distribution of 3 selected genes associated with senescence, *CDKN1A*, *LMNA* and *SERPINE1* (*CDKN1A*: FDR = 7.89e-31, L2FC = 0.92; *LMNA*: FDR = 5.58e-46, L2FC = 1.37; *SERPINE1*: FDR = 1.74e-03, L2FC = 0.22

FIGURE 3 Continued

(A) UMAP plot showing EC subtypes clustered by similar transcriptomic patterns. (B) UMAP plot colored by sex. (C) Proportion of the 4 subclusters in male and female EC subtypes. (D) Dot plot showing the top 10 markers of our dataset for each EC subtype. (E) Heatmap showing GSVA analysis of hallmark collection in the 4 EC subtypes revealing the differential expression of biological pathways between male and female. Red and blue squares represent male and female overexpressed pathways, respectively. (F) Ridge plot showing the distribution of the "TNFA signaling via NFKB" gene set grouped by subtypes and sex. (G) Ridge plot showing the distribution of the "fatty acid metabolism" gene set grouped by subtypes and sex. (H) Feature plot showing the distribution of EC_{50} in the EC. Red dots represent higher values of EC_{50} and blue dots represent lower values of EC_{50} (higher endothelial sensitivity to ACh). (I) Density plot of EC_{50} distribution in EC subtypes. Values are scaled EC_{50}. (J) Volcano plot showing differentially expressed genes between male and female EC3. Genes are considered significantly DE when FDR <0.05 and L2FC $>\pm$ 0.25. (K) EnrichR pathway enrichment analysis from upregulated differentially expressed gen in male and female EC3. (L) Dot plots showing *STAT3* gene expression and *MAP3K1* gene expression in the 4 EC subtypes divided by sex. Abbreviations as in Figures 1 and 2.

FIGURE 4 Upregulation of Senescence-Associated Pathways in Male ECs and Implication of CDKN1A



(A) Heatmap showing GSVA analysis of selected senescence-related gene sets between male and female ECs. (B) Feature plot showing the distribution of *CDKN1A*, *LMNA*, and *SERPINE1* in the overall EC population. (C) Telomere restriction fragment length (in kilobase pair [kbp]) quantified in primary cultures of ECs isolated from internal thoracic arteries (ITAs) of male (n = 57) and female (n = 21) patients. Student's *t*-test was used to compare groups (M = 9.16 kbp ± 0.13 kbp vs F = 10.4 kbp ± 0.33 kbp; $P < 0.001$). (D) Percent of CDKN1A^{+} ECs (nuclei) present in ITA segments with either high or low endothelial dysfunction (ED) measured by high and low EC_{50} value, respectively. Threshold of endothelial dysfunction was selected using the mean EC_{50} of the 11 patients, that is, EC_{50} = 137 nM. Student's *t*-test was used to compare groups (high ED = 42.6% ± 5.6% vs low ED = 22.2% ± 8.3%; $P = 0.073$). ***$P < 0.001$. ns = not significant; other abbreviations as in Figures 1 and 2.

in M vs F) (Figure 4B). First, there is a higher number of cells expressing these genes in men than in women, and in EC3 more than in any other subtypes. Second, there is a strong homogeneity between the cells expressing these 3 genes. This homogeneity leads us to hypothesize that these cells are either in an active senescent state, or in the path toward senescence.

Given these important differences in senescence-associated signatures between sexes, we retrieved unpublished data of telomere restriction fragment length measured in human ECs from ITAs from 3 former studies from our laboratory.[22,34,35] These data show that ECs isolated from men had significantly shorter telomeres than those from women (**Figure 4C**). This supports the transcriptomic data (**Figures 2C, 3E, and 4A**) and shows that ECs isolated from male patients are more prone to enter senescence upon stress or division.

Finally, we tested whether there was a link between the phenotypic endothelium-dependent dysfunctional relaxation and the expression of *CDKN1A* (encoding for p21), a well-known senescence marker.[36] We assessed the percent of ECs expressing this gene (ie, *CDKN1A* >0.5) as a function of the severity of the endothelial dysfunction. Even in this small subgroup of patients, we found a positive, although not significant ($P = 0.073$), association between expression of *CDKN1A* and the severity of endothelial dysfunction (**Figure 4D**), with 5 of 6 women being in the better endothelial function group. In situ expression of p21 was also detectable in arteries, demonstrating protein expression in these ITA sections (Supplemental Figure 5), such as LMNA, PTGS2, and PTGIS protein expression.

DISCUSSION

CAD affects more men than women,[1,2] which translates to a lower proportion of CABG surgeries performed in women. Our understanding of the molecular bases of this sexual dimorphism remains limited. The aim of our study was to partly fill this gap of knowledge. Our data uniquely show that relaxant endothelial sensitivity remains significantly better for those older than 65 years of age in women than men. Remarkably, this difference was maintained in the subgroup of arterial segments subjected to snRNA-seq of women older than 75 years of age. Therefore, this protection extends beyond the estrogen umbrella that protects women up to their early 50s,[12,13] and supports the existence of a female sex-specific stress-resistant endothelial function[37] that may limit the impact of the traditional risk factors for CVD leading to atherosclerosis.

Unlike previous single-cell transcriptomic analyses performed in fibrotic and calcified carotid atherosclerotic plaques from patients[38-42] or from coronary arteries of explanted hearts,[43,44] the ITA used in the present study is free of lesion. Nonetheless, endothelial dysfunction is present because ACh did not induce a full relaxation; this reflects the damage to the arterial vasculature inherent to a lifelong exposure to risk factors for CVD.[22,28] Our data provide insights on the possible molecular mechanisms at the root of lesion genesis, which is different from the molecular heterogeneity[45] reported in carotid artery plaques[38-42] or in coronary arteries of ischemic hearts,[43,44] the final degenerative outcomes of risk factors for CVD. Importantly, none of these latter studies integrated sex as a variable, except in the study by Depuydt et al[39] where cluster composition of the carotid plaque did not differ between sexes; however, there was no mention of a sex-dependent transcriptomic signature. Only recently have data based on plaque transcriptomics[4,5,7,8] and proteomics[6] revealed sex differences and confirmed the morphological differences in plaque structure, fibrotic vs lipidic, in women vs men.[4]

Inflammation is a prominent signature of CAD,[20] and our transcriptomic analyses confirm the activation of inflammatory pathways, including NFκB/TNFα and IL6/JAK/STAT3 signaling involved in EC dysfunction and inflammation in atherosclerosis.[46] However, this proinflammatory transcriptome was consistently expressed in male ECs only. Female ECs did not upregulate inflammatory pathways; instead, they upregulated pathways involved in extracellular matrix regulation and fatty acid metabolism. The striking outcome of our analyses is the demonstration that, on top of a proinflammatory transcriptome, cellular senescence was also much more prominent in male ECs: stress-induced premature senescence, replicative senescence, SASP and DNA damage, and telomere stress-induced senescence pathways were upregulated in male ECs, and particularly in the EC3 subcluster. Senescence in male ECs was characterized by high numbers of CDKN1A^{+} ECs with the associated molecular signature (eg, LMNA, STAT3) including the SASP (eg, IL6, SERPINE1, TNFR), as well as by shorter telomeres in male ECs. Because cellular senescence is a significant cause of inflammation via the SASP,[19] it could be a key contributor of the higher endothelial dysfunction measured in men in the present study. The deleterious impact of cellular senescence on endothelial function is supported by recent studies showing that elimination of senescent ECs using senolytics (fesitin, dasatinib + quercetin) improves endothelium-dependent relaxation in older male[27,47] and atherosclerotic male[27] mice; male and female data were either combined or not analyzed in these latter studies. Therefore, our data support the current concept that inflammation and, more specifically, senescence-driven inflammaging in the endothelium stems from vascular diseases in men with atherosclerosis.[20]

We previously reported in ITA that senescence of ECs was independent of the chronological age of the patient, but was driven by the length of lifetime exposure to risk factors for CVD,[22] leading to a premature replicative- and stress-induced senescence.[34,35] In our study, although men and women exhibited similar risk factors for CVD, we observed shorter telomeres in ECs from men undergoing CABG compared to women; this, supported by the transcriptomic signature, shows that male ECs have a reduced stress resistance and a higher susceptibility to enter senescence. The stress of the endothelium in men is evidenced by an increase in PTGS2 transcripts coding for cyclooxygenase-2 (COX-2), the inducible enzyme responsible for production of the compensatory vasculoprotective factor prostacyclin I_2 (PGI_2). Accordingly, we previously reported that COX-2 expression was proportional to the EC senescence load in these patients.[22] This is also in agreement with the increased incidence of cardiovascular events and death in patients (85% men) treated with a coxib in-hospital to relieve pain post-CABG surgery.[48]

However, the female ITA endothelium is neither pro-senescent nor inflamed, a profile likely contributing to the better endothelium-dependent relaxant sensitivity in women with CAD. Telomeres are also longer in female ECs, demonstrating less replicative repair in vivo, as well as less oxidative stress-dependent DNA damage.[34,35] The milder endothelial dysfunction in females could be in part related to the maintained expression of PTGIS coding for prostacyclin synthase that is constitutively expressed in the endothelium[49] and produces cytoprotective PGI_2.[50] PTGIS expression is sensitive to oxidative stress[51] and thus to a proinflammatory and pro-oxidative environment such as that generated by senescent ECs[34,35] observed in male ECs. Better resilience and lower oxidative stress of the female endothelium is also supported by the higher expression of phosphatases (eg, PTPRG) that are also negatively regulated by oxidative stress.[52] Likewise, CAVIN2 coding for a caveolae-associated protein is maintained in female ECs: its absence induces sustained endothelial nitric oxide synthase hyperphosphorylation and pulmonary hypertension.[53] All these data indicate that oxidative stress is contained in female ECs; thus, they are better equipped to counteract stress.

The maintenance of the better endothelial function in women older than 75 years of age suggests the role of additional regulatory mechanisms independent of estrogens. The high expression of extracellular matrix components (FLNB, COL3, COL8, and TGFβ) may be a characteristic of the female sex that translates into the propensity to develop more fibrous plaque unlike the lipid-rich atheroma in males.[4-8] Although data interrogating sex dimorphism in the atherosclerotic plaque are available,[4-8] it is our understanding that the present dataset is the first to compare the transcriptome of the native endothelium of men and women in association with its endothelium-dependent relaxant function. The identification of subcluster analysis in ECs also provided evidence of the heterogeneity of the native endothelium, as suggested by others.[7,54] On the one hand, the EC3 subcluster was better associated with the endothelial relaxant function; on the other hand, all ECs differentially expressed STAT3 in males and MAP3K1 in females. STAT3 and MAP3K1 may therefore represent a hallmark trait of ECs in aging atherosclerotic male and female patients, respectively, whereas each subcluster has a specific role in the maintenance of the overall endothelial functions. More analyses will be needed to understand the contributions of these subclusters.

STUDY LIMITATIONS. Given the observational nature of this study, it cannot establish causation, but only association, between inflammaging and vascular endothelial dysfunction in these patients. Only the use of a senolytic could settle the issue as planed in our ongoing study.[55] In addition, telomere length was measured in explanted and cultured ECs from the ITAs, which may have driven replicative senescence prematurely in men; nonetheless, the analysis of the complete dataset collected by snRNA-seq strengthens these initial findings: PTGS2 (coxib-sensitive COX2), oxidative stress, and premature senescence were shown to be positively associated with the percent of EC senescence measured by both β-galactosidase activity and telomere length,[22,34,35] which reinforces the value of the current data. Yet, there is no definite cell senescent marker[56] and our combination of markers, used by others,[25,57] suggests that if ECs are not in a definitive cell cycle arrest, they express all required markers to be qualified as senescent, a signature that may be conducive to atherogenesis when exposed to disturb shear stress conditions as in coronary arteries. In a recent review, Ogrodnik[54] proposes that “there is a process of ‘cellular aging’ leading to the induction of cellular senescence and culminating in cell death.” This is possibly what the differential transcriptomic signature in the 4 EC subclusters reveal. Nonetheless, it is accepted that premature endothelial telomere shortening is a risk for vascular dysfunction,[58] and we observed accelerated shortening of the male endothelium compared to female. Another perceived limitation is the large age difference between the male and female subjects

whose ITAs underwent snRNA-seq: this could have had unknown confounding effects on the results. However, because it is the female samples that were the oldest by ~15 years, we would have expected an unfavorable bias toward females, which was not the case. The relevance of using the ITA for this study is also unknown as it is relatively protected against developing atherosclerosis,[59] with only up to 7% in patients with multivessel CAD.[60] Clearly, the ideal arteries would have been human plaque-free coronary arteries, similar to a muscular artery such as the ITA. We previously reported that lesion-free coronary artery segments of explanted ischemic hearts responded to ACh by a contraction ex vivo,[31,61] a response well described in these patients in vivo.[62] However, substance P induced an endothelium-dependent relaxation that was blocked by NOS inhibition,[31] similar to the ITA;[32] hence, the plaque-free coronary endothelium is not entirely dysfunctional, similar to the ITA. Therefore, the function of the ITA measured ex vivo may be well representative of a pre-plaque endothelium exposed lifelong to risk factors for CVD.[22]

CONCLUSIONS

We have shown that, in men, EC senescence and inflammation are closely associated with reduced endothelium-dependent relaxation known to be prodromal to atherogenesis.[14-16] In contrast, ECs of female patients undergoing CABG surgery were neither pro-senescent nor inflamed. Therefore, if the endothelial dysfunction and the associated inflammaging signature could likely contribute to CAD in men, there are, however, no indication that the endothelium and its dysfunction cannot participate to the pathology in women. Further studies are necessary to reconciliate the paradox of a more relaxant endothelium and upregulation of less senescent pathways in women with severe CAD requiring CABG. Thus, an attempt to explain the molecular etiology of the atherogenic trajectory in female arteries based solely on the "endothelial theory of atherosclerosis" would be speculative. More generally, "one unifying model cannot fully recapitulate the natural history of atherosclerosis".[45] The sexual dimorphism related to EC senescence strongly suggests that therapeutic targeting of this canonical pathway with senolytics may not improve clinical outcome in women with CAD. Large clinical trials in women are mandatory to settle this outstanding question.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

This work was supported by the Canadian Institutes of Health Research grant (PJT-162446 to Dr Thorin), and the Foundation of the Montreal Heart Institute (Dr Thorin). Drs Mury and Lambert have been supported by the postdoctoral program of the Fonds de la recherche du Québec. Dr Cagnone has received support from the Vision Health Research Network. Dr Dagher has received support from the Canadian Institutes of Health Research (grant FBD-183276). All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Dr Pauline Mury, Montreal Heart Institute, Research Center, 5000 rue Bélanger East, Montreal, Quebec H1T 1C8, Canada. E-mail: pauline.mury@umontreal.ca.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: In patients undergoing CABG surgeries, the endothelium of lesion-free thoracic arteries reveals a strong sexual dichotomy both functionally, with a worsened endothelial dysfunction in men, and molecularly, by the presence in male ECs of senescence-associated inflammaging. These data suggest that the use of senolytic and anti-inflammatory therapies to reduce the residual risk of cardiovascular events in women with CAD may not be as effective as in men.

TRANSLATIONAL OUTLOOK: Future studies should investigate the molecular mechanisms involved in CAD progression in women by focusing beyond the vascular endothelium.

REFERENCES

1. Roth GA, Mensah GA, Johnson CO, et al. Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 study. *J Am Coll Cardiol*. 2020;76:2982-3021.

2. Jebari-Benslaiman S, Galicia-García U, Larrea-Sebal A, et al. Pathophysiology of atherosclerosis. *Int J Mol Sci*. 2022;23(6):3346.

3. Man JJ, Beckman JA, Jaffe IZ. Sex as a biological variable in atherosclerosis. *Circ Res*. 2020;126:1297-1319.

4. Sakkers TR, Mokry M, Civelek M, et al. Sex differences in the genetic and molecular mechanisms of coronary artery disease. *Atherosclerosis*. 2023;384:117279.

5. Hartman RJG, Owsiany K, Ma L, et al. Sex-stratified gene regulatory networks reveal female key driver genes of atherosclerosis involved in smooth muscle cell phenotype switching. *Circulation*. 2021;143:713-726.

6. Theofilatos K, Stojkovic S, Hasman M, et al. Proteomic atlas of atherosclerosis: the

contribution of proteoglycans to sex differences, plaque phenotypes, and outcomes. *Circ Res*. 2023;133:542–558.

7. Diez Benavente E, Karnewar S, Buono M, et al. Female gene networks are expressed in myofibroblast-like smooth muscle cells in vulnerable atherosclerotic plaques. *Arterioscler Thromb Vasc Biol*. 2023;43:1836–1850.

8. Jin H, Mees BME, Biessen EAL, Sluimer JC. Transcriptional sex dimorphism in human atherosclerosis relates to plaque type. *Circ Res*. 2021;129:1175–1177.

9. Dela Justina V, Miguez JSG, Priviero F, Sullivan JC, Giachini FR, Webb RC. Sex differences in molecular mechanisms of cardiovascular aging. *Front Aging*. 2021;2:725884.

10. Shin J, Hong J, Edwards-Glenn J, et al. Unraveling the role of sex in endothelial cell dysfunction: evidence from lineage tracing mice and cultured cells. *Arterioscler Thromb Vasc Biol*. 2024;44:238–253.

11. Lorenz M, Koschate J, Kaufmann K, et al. Does cellular sex matter? Dimorphic transcriptional differences between female and male endothelial cells. *Atherosclerosis*. 2015;240:61–72.

12. Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol*. 1994;24:471–476.

13. Taddei S, Virdis A, Ghiadoni L, et al. Menopause is associated with endothelial dysfunction in women. *Hypertension*. 1996;28:576–582.

14. Shechter M, Issachar A, Marai I, et al. Long-term association of brachial artery flow-mediated vasodilation and cardiovascular events in middle-aged subjects with no apparent heart disease. *Int J Cardiol*. 2009;134:52–58.

15. Widlansky ME, Gokce N, Keaney JF Jr, Vita JA. The clinical implications of endothelial dysfunction. *J Am Coll Cardiol*. 2003;42:1149–1160.

16. Schächinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation*. 2000;101:1899–1906.

17. Childs BG, Gluscevic M, Baker DJ, et al. Senescent cells: an emerging target for diseases of ageing. *Nat Rev Drug Discov*. 2017;16:718–735.

18. Franceschi C, Bonafè M, Valensin S, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci*. 2000;908:244–254.

19. Coppé JP, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu Rev Pathol*. 2010;5:99–118.

20. Liberale L, Badimon L, Montecucco F, Lüscher TF, Libby P, Camici GG. Inflammation, aging, and cardiovascular disease: JACC review topic of the week. *J Am Coll Cardiol*. 2022;79:837–847.

21. Minamino T, Miyauchi H, Yoshida T, Ishida Y, Yoshida H, Komuro I. Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction. *Circulation*. 2002;105:1541–1544.

22. Voghel G, Thorin-Trescases N, Farhat N, et al. Cellular senescence in endothelial cells from atherosclerotic patients is accelerated by oxidative stress associated with cardiovascular risk factors. *Mech Ageing Dev*. 2007;128:662–671.

23. Donato AJ, Machin DR, Lesniewski LA. Mechanisms of dysfunction in the aging vasculature and role in age-related disease. *Circ Res*. 2018;123:825–848.

24. Rossman MJ, Kaplon RE, Hill SD, et al. Endothelial cell senescence with aging in healthy humans: prevention by habitual exercise and relation to vascular endothelial function. *Am J Physiol Heart Circ Physiol*. 2017;313:H890–H895.

25. Bloom SI, Islam MT, Lesniewski LA, Donato AJ. Mechanisms and consequences of endothelial cell senescence. *Nat Rev Cardiol*. 2023;20:38–51.

26. Caland L, Labbé P, Mamarbachi M, et al. Knockdown of angiopoietin-like 2 induces clearance of vascular endothelial senescent cells by apoptosis, promotes endothelial repair and slows atherogenesis in mice. *Aging (Albany NY)*. 2019;11:3832–3850.

27. Roos CM, Zhang B, Palmer AK, et al. Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. *Aging Cell*. 2016;15:973–977.

28. Thorin E, Thorin-Trescases N. Vascular endothelial ageing, heartbeat after heartbeat. *Cardiovasc Res*. 2009;84:24–32.

29. Barrett EL, Richardson DS. Sex differences in telomeres and lifespan. *Aging Cell*. 2011;10:913–921.

30. Aviv A, Aviv H. Reflections on telomeres, growth, aging, and essential hypertension. *Hypertension*. 1997;29:1067–1072.

31. Thorin E. Different contribution of endothelial nitric oxide in the relaxation of human coronary arteries of ischemic and dilated cardiomyopathic hearts. *J Cardiovasc Pharmacol*. 2001;37:227–232.

32. Hamilton CA, Williams R, Pathi V, et al. Pharmacological characterisation of endothelium-dependent relaxation in human radial artery: comparison with internal thoracic artery. *Cardiovasc Res*. 1999;42:214–223.

33. Kuleshov MV, Jones MR, Rouillard AD, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res*. 2016;44:W90–W97.

34. Voghel G, Thorin-Trescases N, Farhat N, et al. Chronic treatment with N-acetyl-cystein delays cellular senescence in endothelial cells isolated from a subgroup of atherosclerotic patients. *Mech Ageing Dev*. 2008;129:261–270.

35. Voghel G, Thorin-Trescases N, Mamarbachi AM, et al. Endogenous oxidative stress prevents telomerase-dependent immortalization of human endothelial cells. *Mech Ageing Dev*. 2010;131:354–363.

36. Yosef R, Pilpel N, Papismadov N, et al. p21 maintains senescent cell viability under persistent DNA damage response by restraining JNK and caspase signaling. *EMBO J*. 2017;36:2280–2295.

37. Tiberi J, Cesarini V, Stefanelli R, Canterini S, Fiorenza MT, La Rosa P. Sex differences in antioxidant defence and the regulation of redox homeostasis in physiology and pathology. *Mech Ageing Dev*. 2023;211:111802.

38. Alsaigh T, Evans D, Frankel D, Torkamani A. Decoding the transcriptome of calcified atherosclerotic plaque at single-cell resolution. *Commun Biol*. 2022;5:1084.

39. Depuydt MAC, Prange KHM, Slenders L, et al. Microanatomy of the human atherosclerotic plaque by single-cell transcriptomics. *Circ Res*. 2020;127:1437–1455.

40. Fernandez DM, Rahman AH, Fernandez NF, et al. Single-cell immune landscape of human atherosclerotic plaques. *Nat Med*. 2019;25:1576–1588.

41. Pan H, Xue C, Auerbach BJ, et al. Single-cell genomics reveals a novel cell state during smooth muscle cell phenotypic switching and potential therapeutic targets for atherosclerosis in mouse and human. *Circulation*. 2020;142:2060–2075.

42. Slenders L, Landsmeer LPL, Cui K, et al. Intersecting single-cell transcriptomics and genome-wide association studies identifies crucial cell populations and candidate genes for atherosclerosis. *Eur Heart J Open*. 2022;2:oeab043.

43. Hu Z, Liu W, Hua X, et al. Single-cell transcriptomic atlas of different human cardiac arteries identifies cell types associated with vascular physiology. *Arterioscler Thromb Vasc Biol*. 2021;41:1408–1427.

44. Wirka RC, Wagh D, Paik DT, et al. Atheroprotective roles of smooth muscle cell phenotypic modulation and the TCF21 disease gene as revealed by single-cell analysis. *Nat Med*. 2019;25:1280–1289.

45. Pasterkamp G, den Ruijter HM, Giannarelli C. False utopia of one unifying description of the vulnerable atherosclerotic plaque: a call for recalibration that appreciates the diversity of mechanisms leading to atherosclerotic disease. *Arterioscler Thromb Vasc Biol*. 2022;42:e86–e95.

46. Luo C, Ruan Y, Sun P, et al. The role of transcription factors in coronary artery disease and myocardial infarction. *Front Biosci (Landmark Ed)*. 2022;27:329.

47. Mahoney SA, Venkatasubramanian R, Darrah MA, et al. Intermittent supplementation with fisetin improves arterial function in old mice by decreasing cellular senescence. *Aging Cell*. 2023:e14060.

48. Nussmeier NA, Whelton AA, Brown MT, et al. Complications of the COX-2 inhibitors parecoxib and valdecoxib after cardiac surgery. *N Engl J Med*. 2005;352:1081–1091.

49. Spisni E, Bartolini G, Orlandi M, Belletti B, Santi S, Tomasi V. Prostacyclin (PGI2) synthase is a constitutively expressed enzyme in human endothelial cells. *Exp Cell Res*. 1995;219:507–513.

50. Mitchell JA, Kirkby NS, Ahmetaj-Shala B, et al. Cyclooxygenases and the cardiovascular system. *Pharmacol Ther*. 2021;217:107624.

51. Zou MH. Peroxynitrite and protein tyrosine nitration of prostacyclin synthase. *Prostaglandins Other Lipid Mediat*. 2007;82:119–127.

52. Madamanchi NR, Runge MS. Redox signaling in cardiovascular health and disease. *Free Radic Biol Med*. 2013;61:473–501.

53. Kasahara T, Ogata T, Nakanishi N, et al. Cavin-2 loss exacerbates hypoxia-induced pulmonary hypertension with excessive eNOS phosphorylation and protein nitration. *Heliyon*. 2023;9:e17193.

54. Ogrodnik M. Cellular aging beyond cellular senescence: markers of senescence prior to cell cycle arrest in vitro and in vivo. *Aging Cell*. 2021;20:e13338.

55. Dagher O, Mury P, Noly PE, et al. Design of a randomized placebo-controlled trial to evaluate the anti-inflammatory and senolytic effects of quercetin in patients undergoing coronary artery bypass graft surgery. *Front Cardiovasc Med*. 2021;8:741542.

56. Gorgoulis V, Adams PD, Alimonti A, et al. Cellular senescence: defining a path forward. *Cell*. 2019;179:813–827.

57. Childs BG, Baker DJ, Wijshake T, Conover CA, Campisi J, van Deursen JM. Senescent intimal foam cells are deleterious at all stages of atherosclerosis. *Science*. 2016;354:472–477.

58. Bloom SI, Liu Y, Tucker JR, et al. Endothelial cell telomere dysfunction induces senescence and results in vascular and metabolic impairments. *Aging Cell*. 2023;22:e13875.

59. Kraler S, Libby P, Evans PC, et al. Resilience of the internal mammary artery to atherogenesis: shifting from risk to resistance to address unmet needs. *Arterioscler Thromb Vasc Biol*. 2021;41:2237–2251.

60. Ozkan S, Akay TH, Gultekin B, et al. Atherosclerosis of radial and internal thoracic arteries used in coronary bypass: atherosclerosis in arterial grafts. *J Card Surg*. 2007;22:385–389.

61. Thorin E. Influence of nitric oxide synthase inhibition and endothelin-1 receptor blockade on acetylcholine-induced coronary artery contraction in vitro in dilated and ischemic cardiomyopathies. *J Cardiovasc Pharmacol*. 2001;38:90–98.

62. Ludmer PL, Selwyn AP, Shook TL, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med*. 1986;315:1046–1051.

KEY WORDS coronary artery disease, endothelium-dependent relaxation, inflammaging, sex-dimorphism

APPENDIX For supplemental Methods, tables, figures, and datasets, please see the online version of this paper.