Effects of Estrogen on Vascular Inflammation
A Matter of Timing

Susana Novella, Magda Heras, Carlos Hermenegildo, Ana Paula Dantas

Objective—Our study aims to determine the role of time of menopause on vascular inflammation biomarkers and how it affects their modulation by estrogen and raloxifene in postmenopausal women.

Methods and Results—Uterine arteries from 68 postmenopausal women were divided into 3 segments and cultured for 24 hours in tissue culture media containing 17β-estradiol (100 nmol/L), raloxifene (100 nmol/L), or vehicle. Assessment of arterial concentration of 13 inflammatory biomarkers was performed by multiplex immunobead-based assay. Aging per se has a positive correlation with the generation of several proinflammatory markers. Although short-term estradiol exposure correlates with lower expression of tumor necrosis factor-α, vascular endothelial growth factor, and interleukin-1β in all age groups, for most biomarkers aging was associated with a switch from a beneficial anti-inflammatory action by estrogen, at earlier stages of menopause, to a proinflammatory profile after 5 years past its onset. Raloxifene has no significant effect on the expression of all proinflammatory markers. Western blot analysis of estrogen receptor expression (estrogen receptor-α and estrogen receptor-β) showed that estrogen receptor-β increases with aging, and this increase has a positive correlation with the generation of several proinflammatory markers.

Conclusion—Aging alters estrogen-mediated effects on the modulation of inflammatory biomarkers in women. How aging affects estrogen responses on vascular inflammation is not clear, but our data show a positive association between increased estrogen receptor-β expression with aging and proinflammatory effects by estrogen. (***Arterioscler Thromb Vasc Biol.*** **2012;32:2035-2042.**)

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CLÍNIC BARCELONA Hospital Universitari

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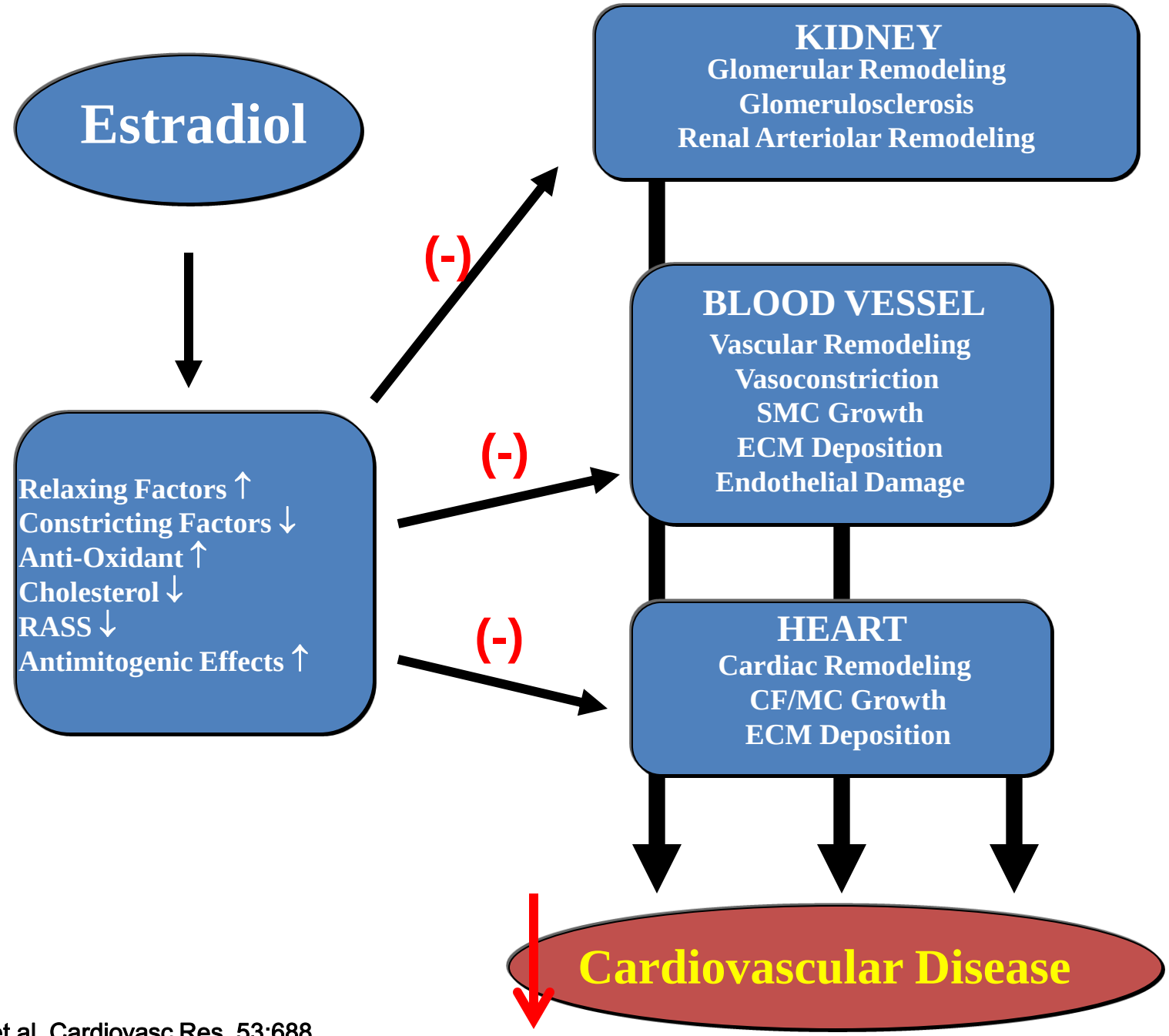
Fundación Investigación Clínico de Valencia

The Nurses' Health Study



Dubey RK et al. Cardiovasc Res, 53:688.

ORIGINAL CONTRIBUTION

JAMA - EXPRESS

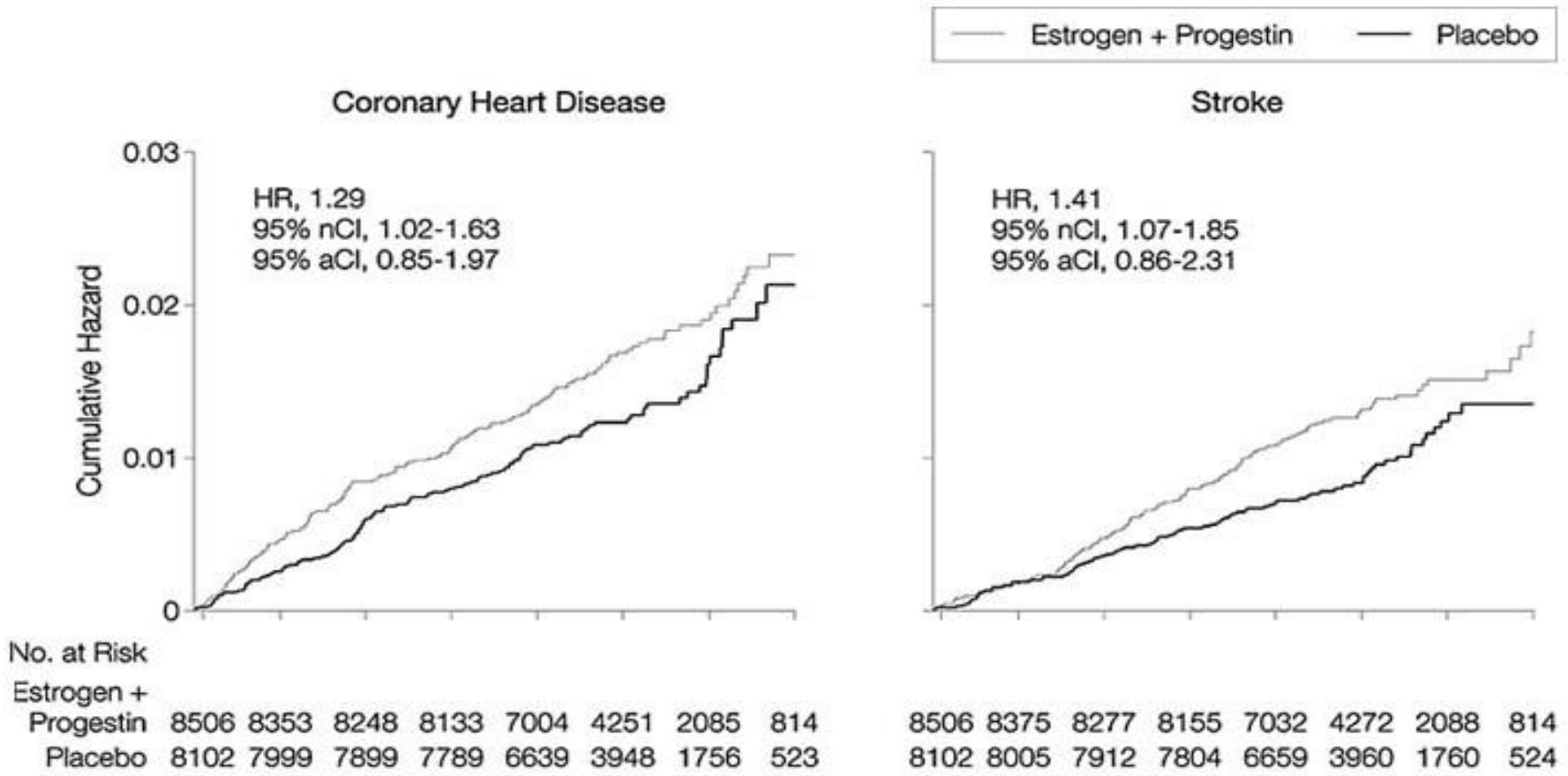
Risks and Benefits of Estrogen Plus Progestin in Healthy Postmenopausal Women

Principal Results From the Women's Health Initiative Randomized Controlled Trial

Writing Group for the
Women's Health Initiative
Investigators

JAMA, July 17, 2002 – Vol. 288, No. 3

Women's Health Initiative (WHI)

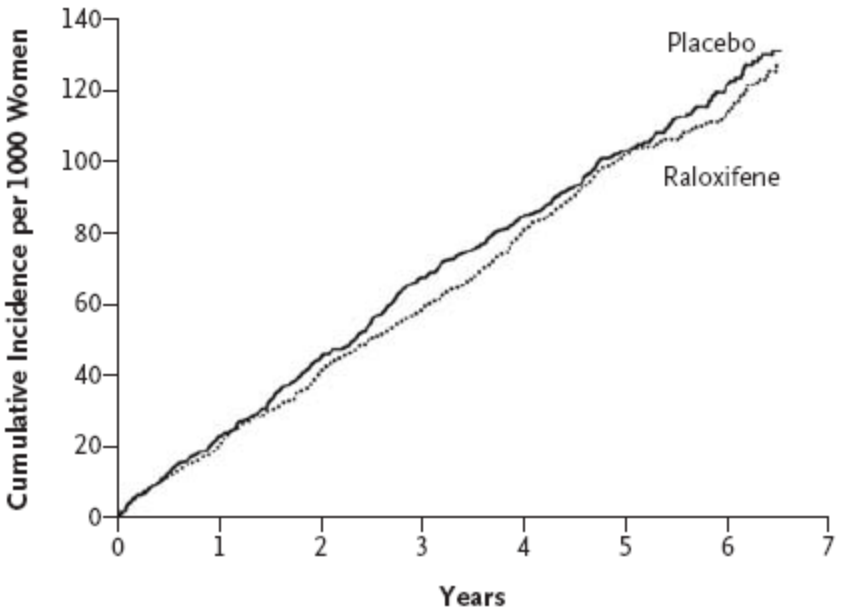


JAMA, July 17, 2002 – Vol. 288, No. 3

RUTH Trial

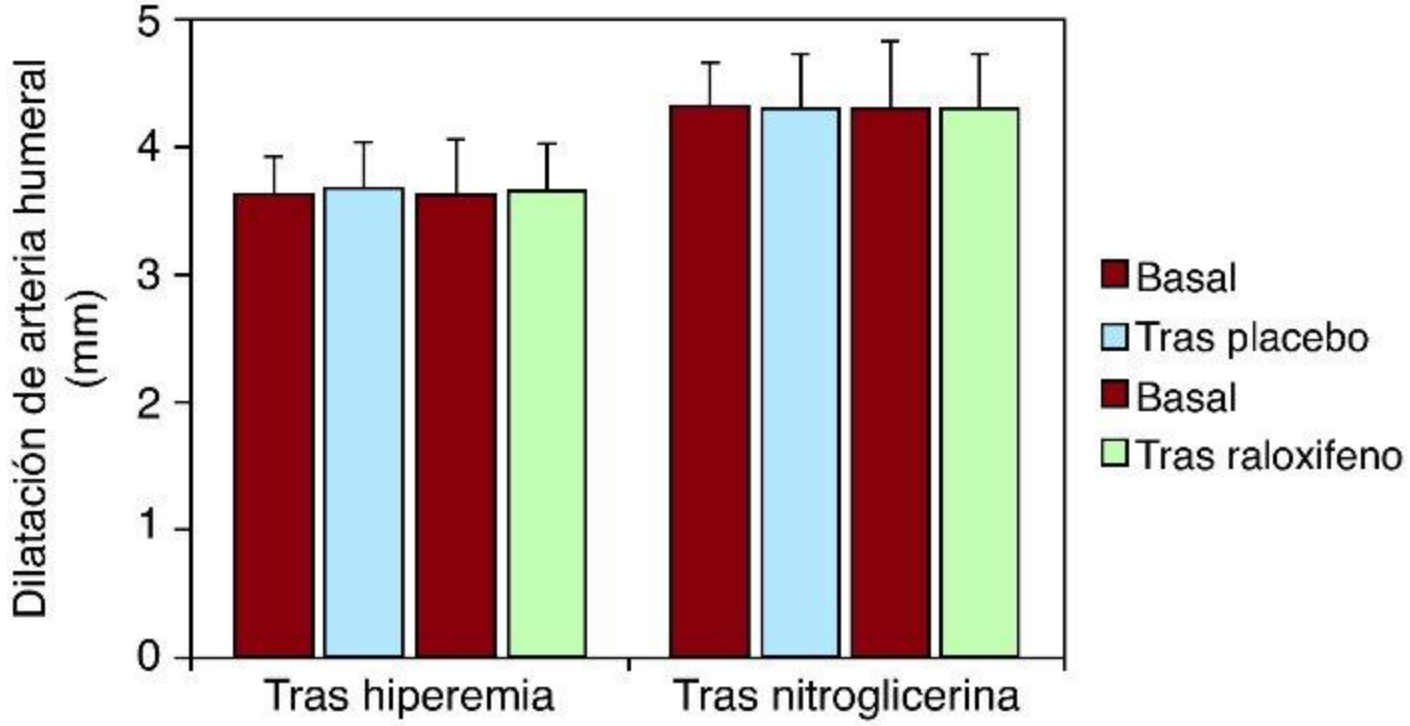
(Raloxifene Use for the Heart)

Coronary Events



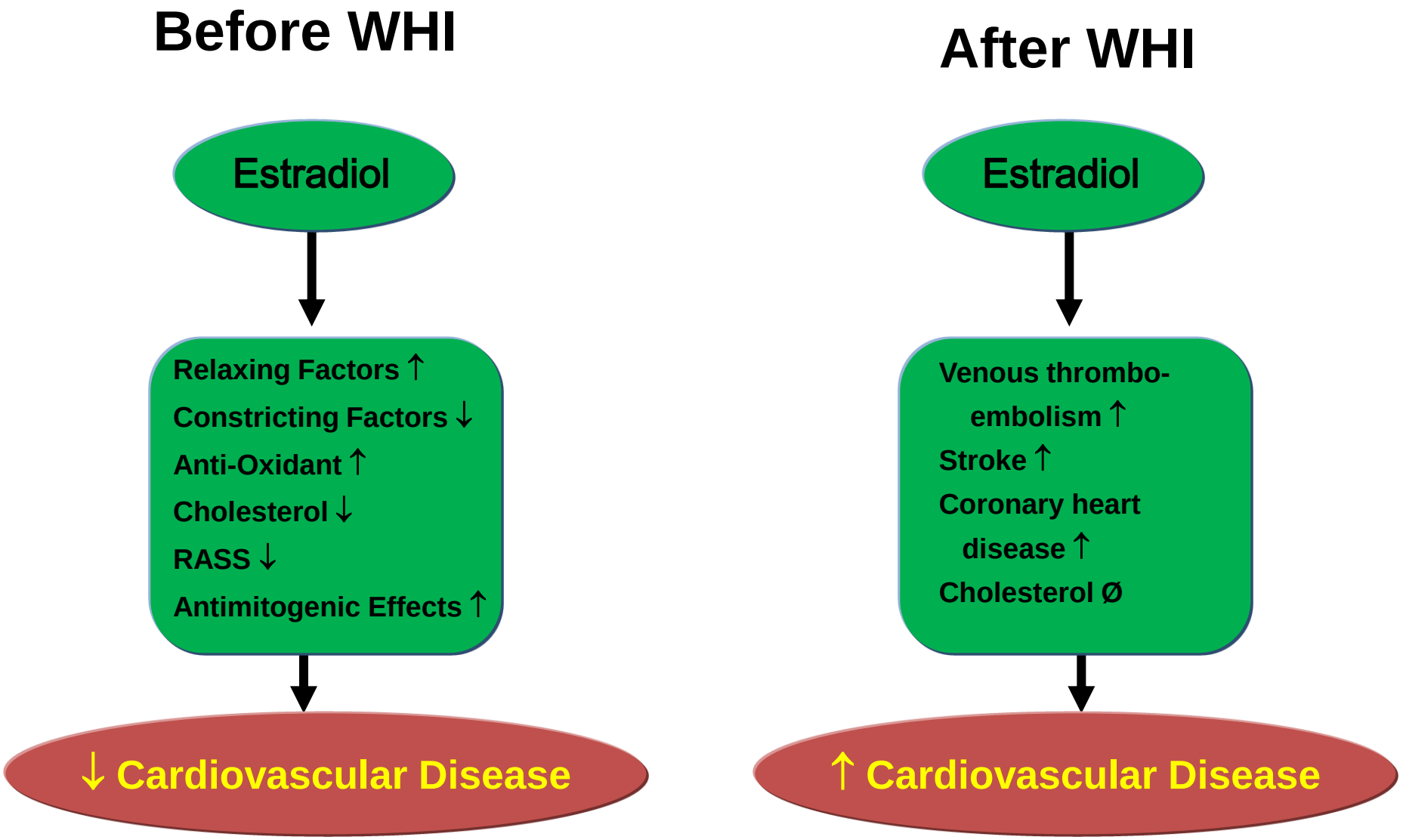
MERCED

(MEnopausia y Raloxifeno en la Cardiopatía isquémica: Efecto en la Disfunción endotelial)



Rev Esp Cardiol. 2011;64(7):572–578

Estrogen Replacement Therapy: before and after the Women's Health Initiative (WHI)

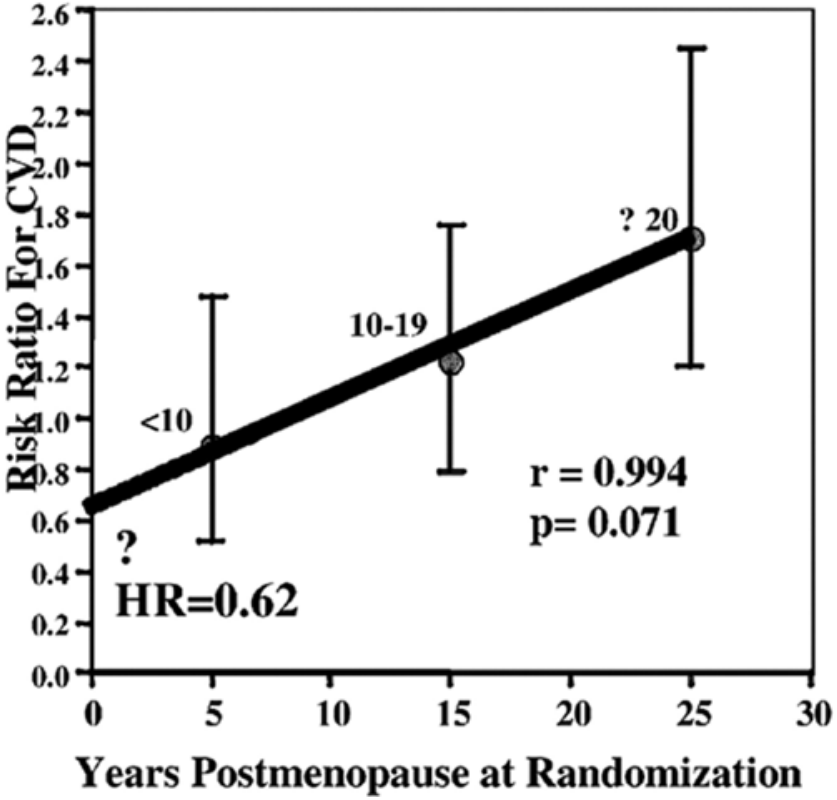


WHI?

- ✓ **Dose regimen**
- ✓ **Association of estrogen with progestins**
- ✓ **Administration route**
- ✓ **Type of Estrogen**
- ✓ **Average age of women beginning the trial**

THE "TIMING" HYPHOTESIS

Estrogen-mediated benefits to prevent cardiovascular disease may occur only when treatment is initiated before the detrimental effects of aging or cardiovascular disease are established in the vasculature.



Ann. N. Y. Acad. Sci.
1052: 43-56. 2005

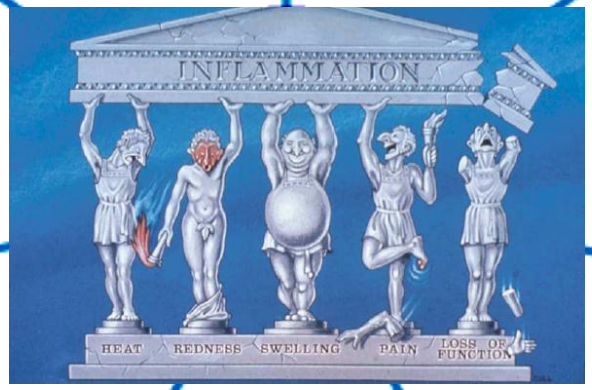
OBJECTIVES

The present study aims to determine the relationship of aging on vascular inflammation biomarkers and how time since menopause affects the modulation of inflammatory biomarkers by estrogen and Raloxifene in arteries from postmenopausal women.

Cardiovascular Diseases:
Atherosclerosis
Cerebrovascular dis.
Heart failure
Cardiomyopathy
Stroke

Neurological Disorders:
Alzheimer's
Parkinson's
AIS
Dementia

Diabetic Complications:
Cardiomyopathy
Atherosclerosis
Chronic renal failure
Retinopathy, Sepsis
Neuropathy



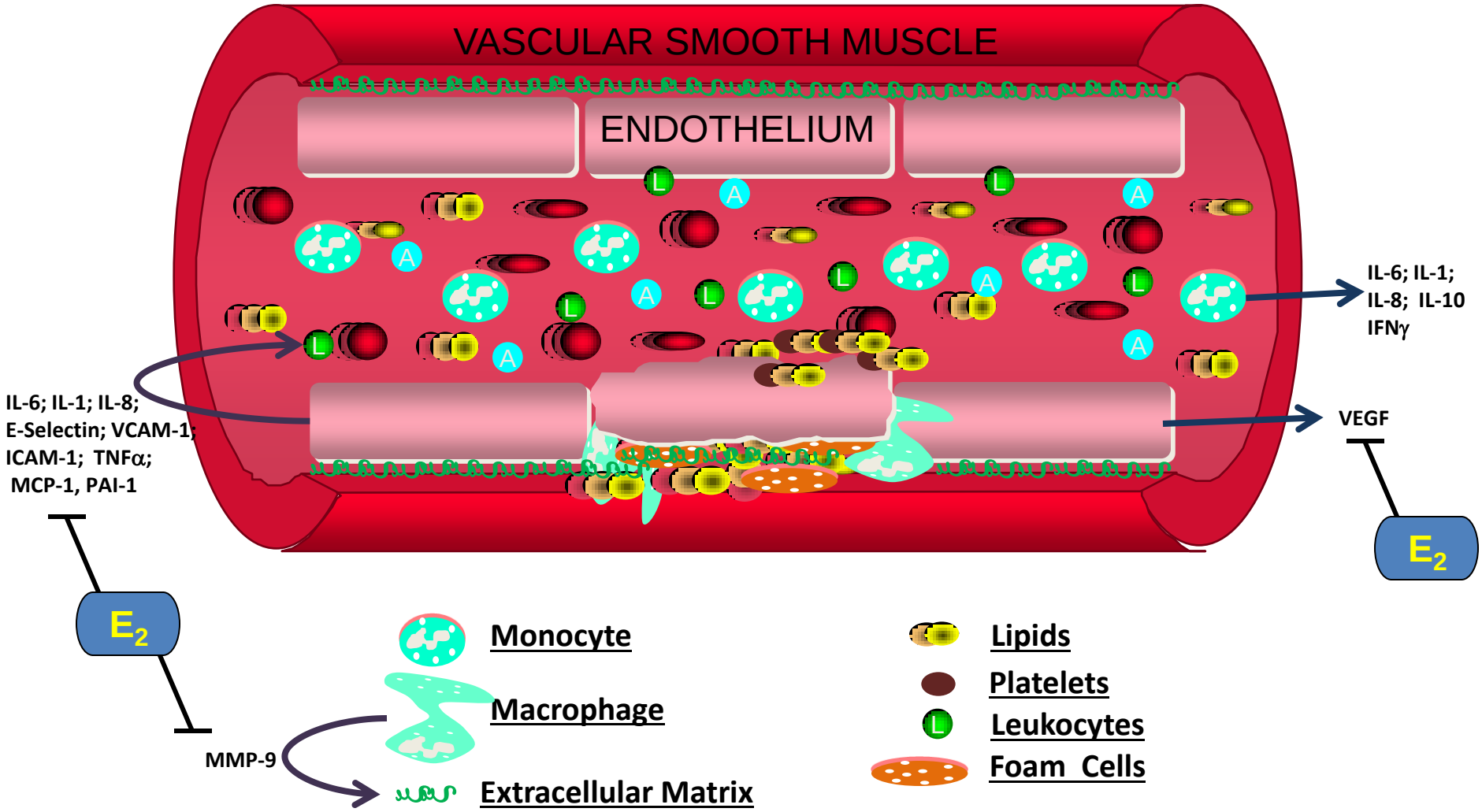
Metabolic Disorder Complications:
Fatty liver disease
Heart disease
Type 2 Diabetes
CKD
Sleep apnea

Chronic Inflammatory Dis:
IBD, COPD,
RA, Psoriasis
Chronic pancreatitis
CIDP, CICTD

Cancer:
Lung cancer
Kidney cancer
Gastric cancer
Colon cancer
Pancreatic cancer
Lymphoma

Bone, Muscular & Skeletal Disease:
Osteoporosis
Osteoarthritis
DDD
Muscular dystrophy

MOLECULAR MECHANISMS OF VASCULAR INFLAMMATION

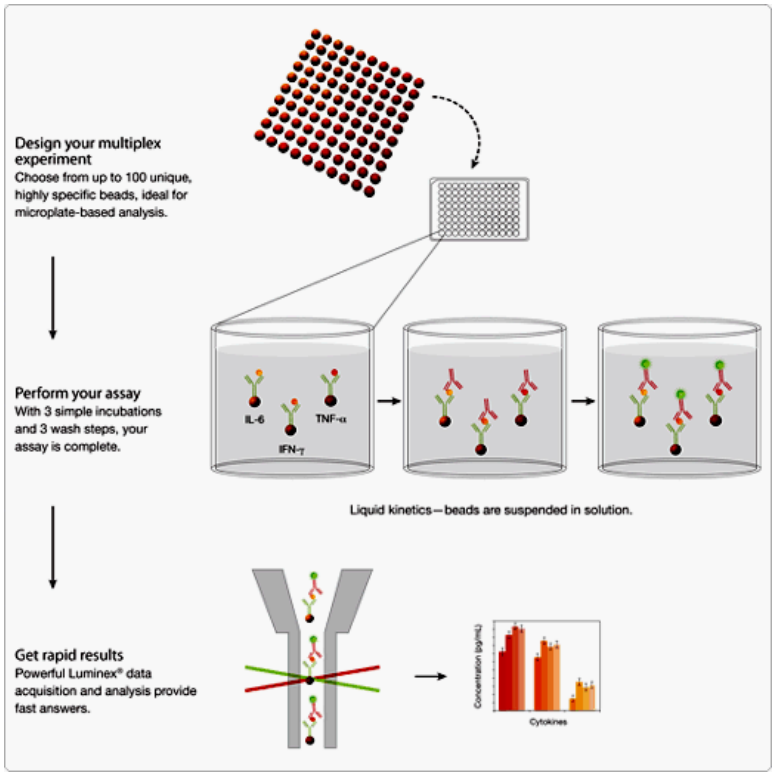


→ **Uterine arteries obtained from 68 women (age 41-86) - COLMAH-HERACLES.**

→ **At the moment of hysterectomy arteries were cleaned, divided into three segments and cultured for 24h in tissue culture media containing 17beta-estradiol (100nM), Raloxifen (100nM) or vehicle.**

→ **Exclusion criterion: use of hormone replacement therapy, SERMs (Raloxifen, Tamoxifen...) , chronic anti-inflammatory therapy, satins, RAS inhibitors, diabetes.**

→ **Multiplex, immunobead-based assay, was performed to measured 13 cardiovascular-related inflammatory biomarkers.**



CVD1: MMP-9; sE-Selectin; s-ECAM; s-VCAM; t-PAI

CVD3: IFNγ; IL-10; IL-1b; IL-6; IL-8; MCP-1; TNFα; VEGF

Table 1. Baseline Demographic and Clinical Characteristics

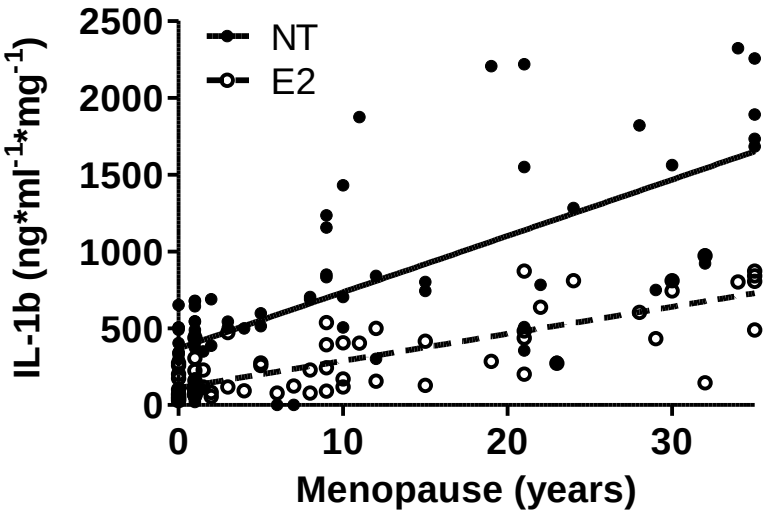
Characteristics	
Age, mean (SD)	58 (13)
Time since menopause, No (%) < 5 years > 5 – 10 years > > 10 years	 30 (44) 17 (25) 21 (31)
Reason for Hysterectomy. No (%): Myoma Uterine Prolapse	 39 (57) 29 (43)
BMI, mean (SD)	28 (4)
Blood Pressure (mmHg), mean (SD) Systolic Diastolic	 129 (19) 75 (10)
Total Cholesterol (mg/dL), mean (SD)	195 (28)
Triglycerides (mg/dL), mean (SD)	90 (19)
Smoking, No (%) Never, Past, Current,	 54 (80) 9 (13) 5 (7)

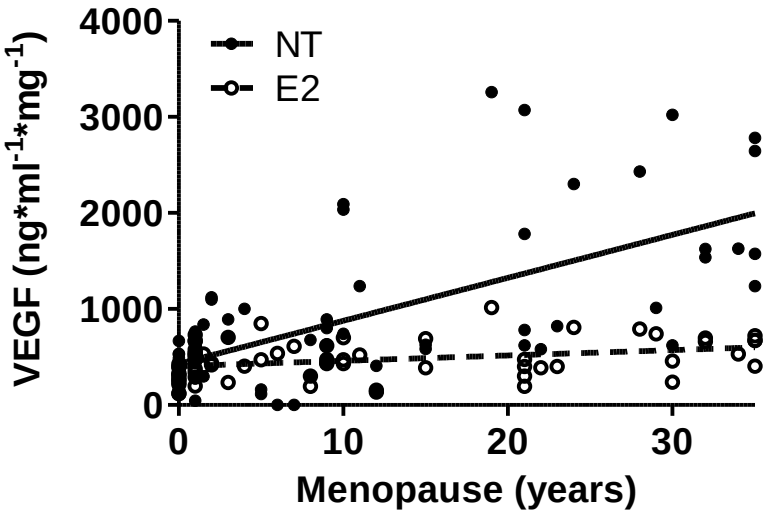
Table 2. *Pierson's Correlation Coefficients*

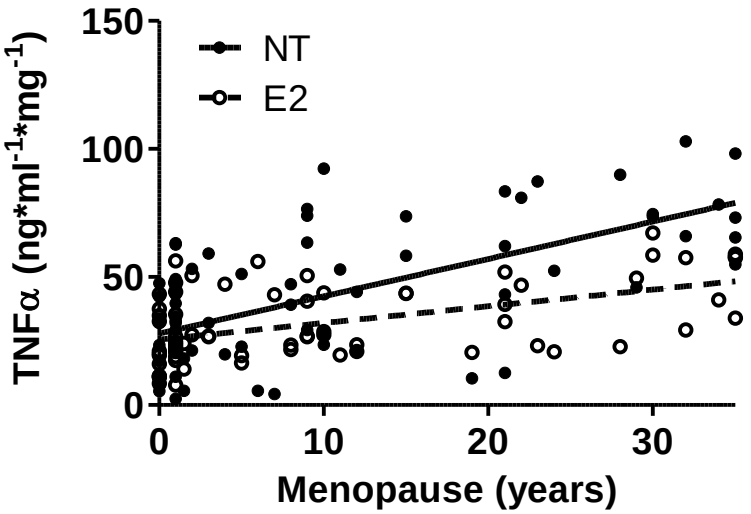
	Untreated				Estrogen				Raloxifene			
	Pierson r	r^2	P value	Summary	Pierson r	r^2	P value	Summary	Pierson r	r^2	P value	Summary
MMP-9	0.1522	0.023	0.5737	ns	0.1459	0.021	0.0987	ns	0.0592	0.004	0.8275	ns
E-Selectin	0.7205	0.519	<0.0001	***	0.7274	0.529	0.0003	***	0.6893	0.475	<0.0001	***
s-ICAM	0.4549	0.207	<0.0001	***	0.9057	0.820	<0.0001	*** (a)	0.5463	0.298	<0.0001	***
s-VCAM	0.6602	0.436	<0.0001	***	0.8463	0.716	<0.0001	*** (a)	0.5723	0.328	<0.0001	***
tPAI	0.6778	0.459	<0.0001	***	0.6221	0.387	<0.0001	***	0.5861	0.344	<0.0001	***
IFNγ	0.2048	0.042	0.4467	ns	0.2390	0.057	0.4105	ns	0.1428	0.020	0.8796	ns
IL-1β	0.7161	0.513	<0.0001	***	0.5302	0.281	<0.0001	*** (a)	0.6740	0.454	<0.0001	***
IL-6	0.1715	0.029	0.1587	ns	0.8357	0.699	<0.0001	*** (a)	0.2589	0.067	0.1265	ns
IL-8	0.1319	0.017	0.7479	ns	0.7193	0.518	<0.0001	*** (a)	0.4127	0.170	0.1121	ns
IL-10	0.4440	0.197	0.0849	ns	0.4192	0.176	0.1357	ns	0.5350	0.286	0.0611	ns
TNFα	0.6308	0.407	<0.0001	***	0.5161	0.266	<0.0001	*** (a)	0.7104	0.504	<0.0001	***
MCP1	0.6202	0.385	<0.0001	***	0.8311	0.691	<0.0001	*** (a)	0.6729	0.453	<0.0001	***
VEGF	0.6610	0.437	<0.0001	***	0.3362	0.113	0.0047	** (a)	0.5896	0.348	<0.0001	***

(a): Analysis of Covariance (ANCOVA) reveals significant difference ($p < 0.05$) in comparison to untreated group.

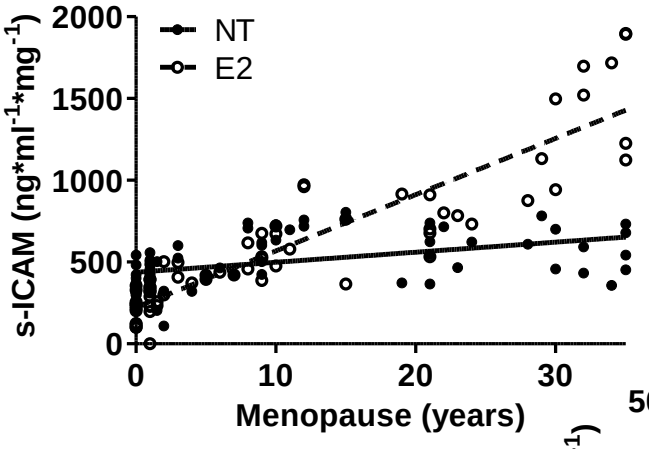
For some markers of inflammation a down-regulation by estrogen is observed in all stages of post-menopause.

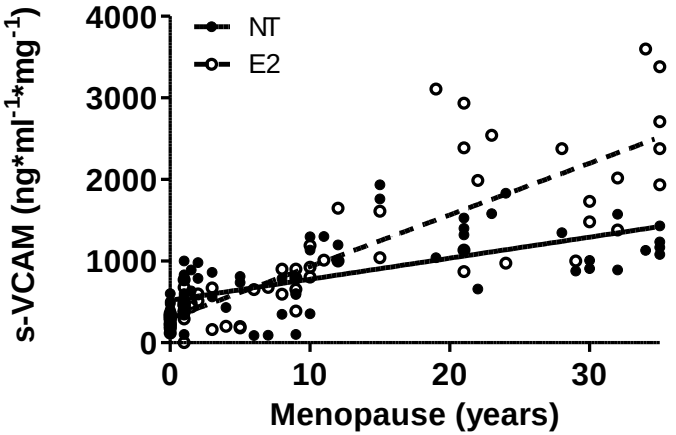


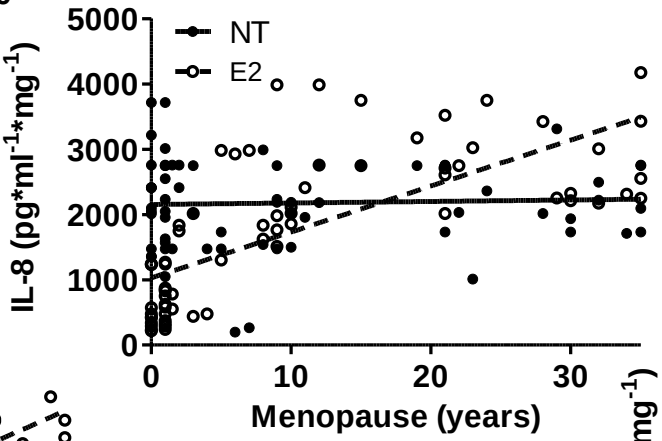


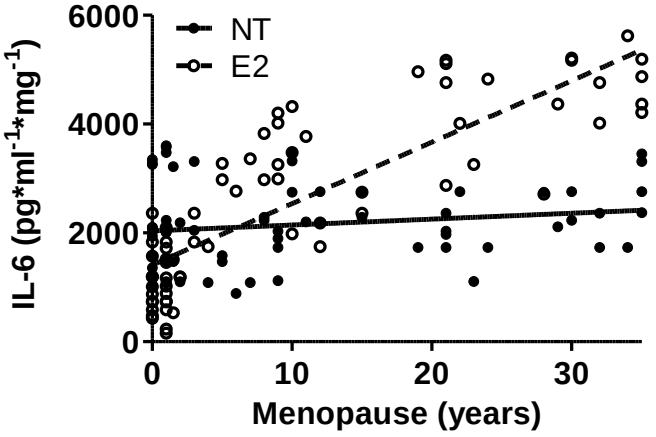


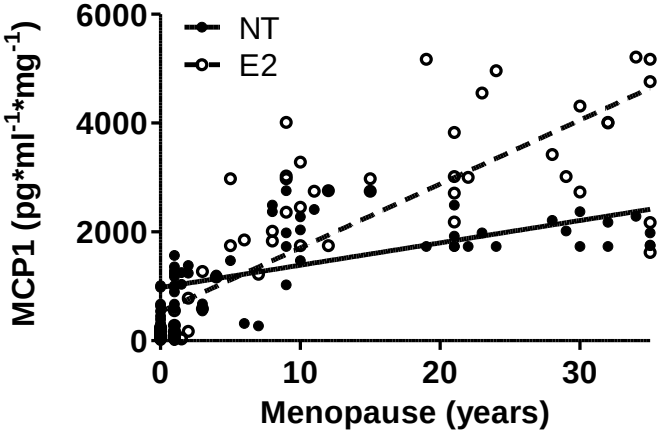
Aging can be associated to a switch from a beneficial anti-inflammatory action by estrogen, at earlier stages of menopause, to a pro-inflammatory profile after 5 year past its onset.











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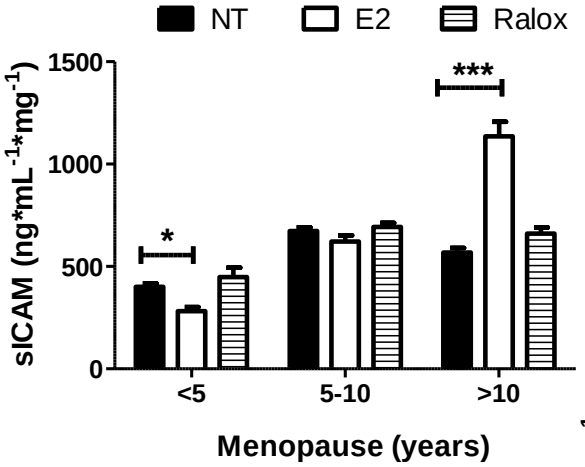
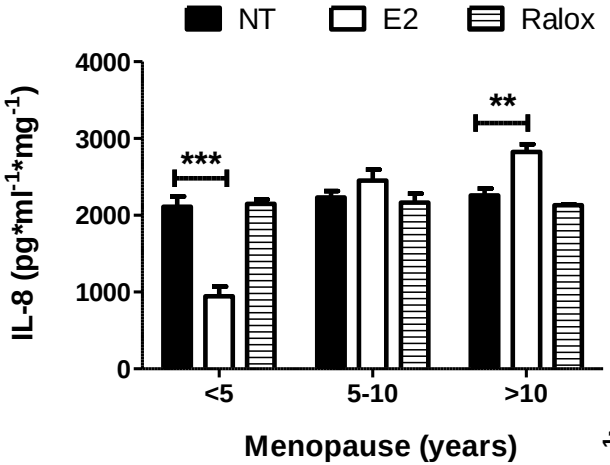
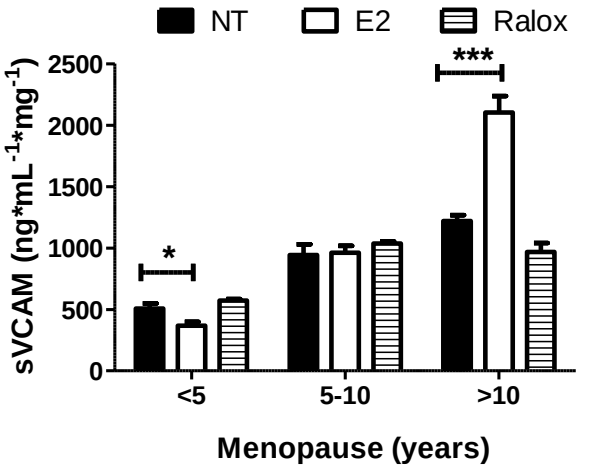
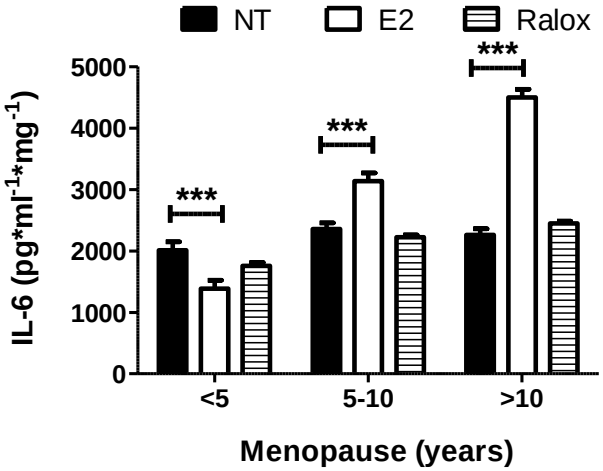
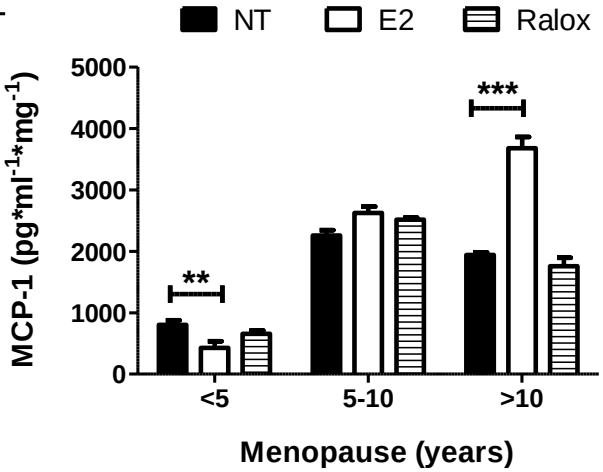


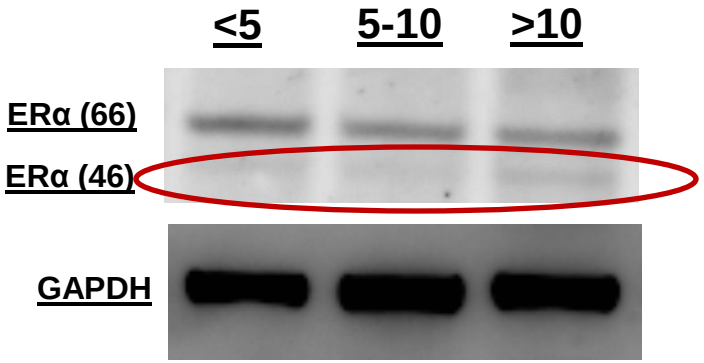


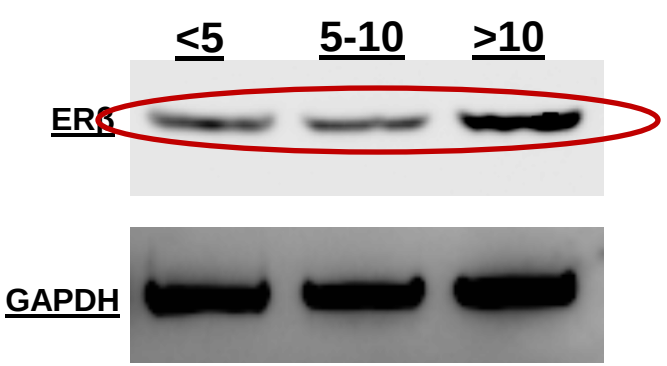


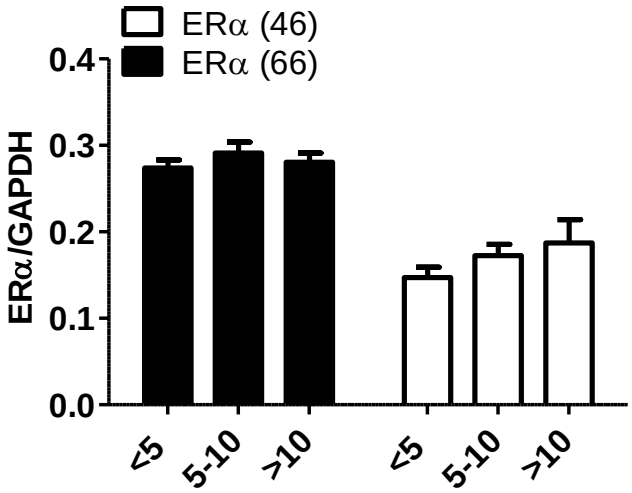


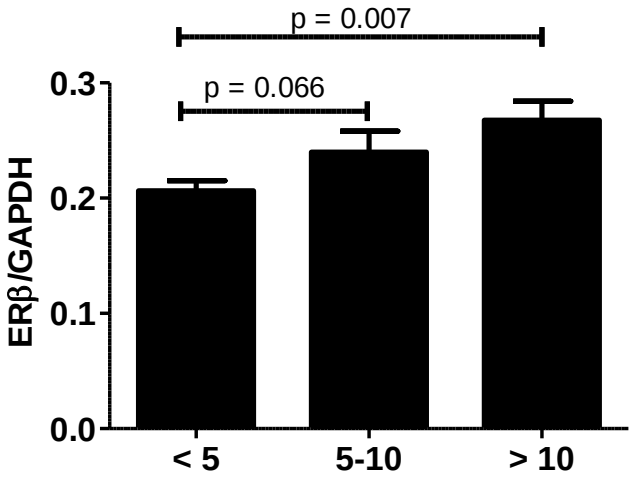


Aging modifies the pattern of Estrogen Receptors (ERs) expression.

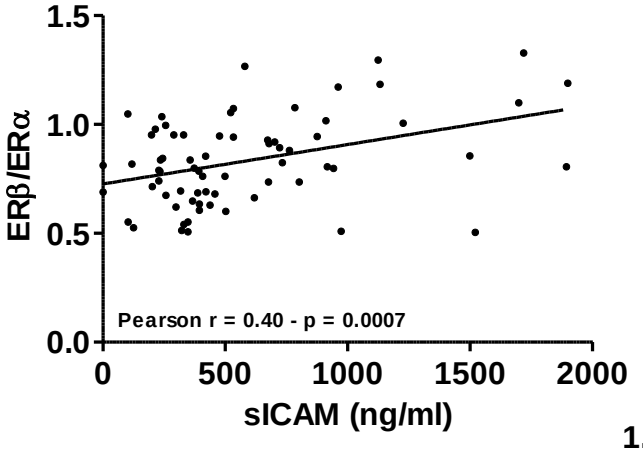


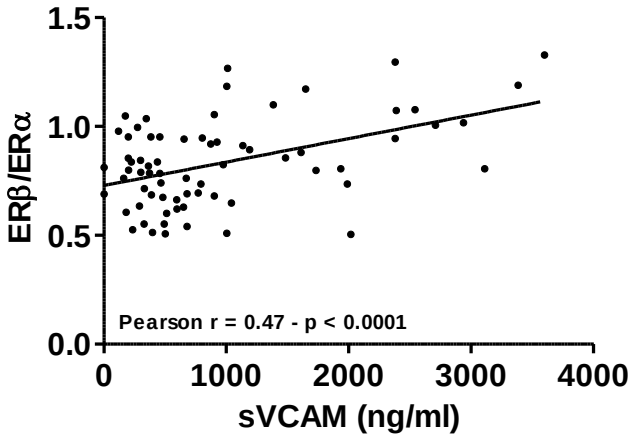


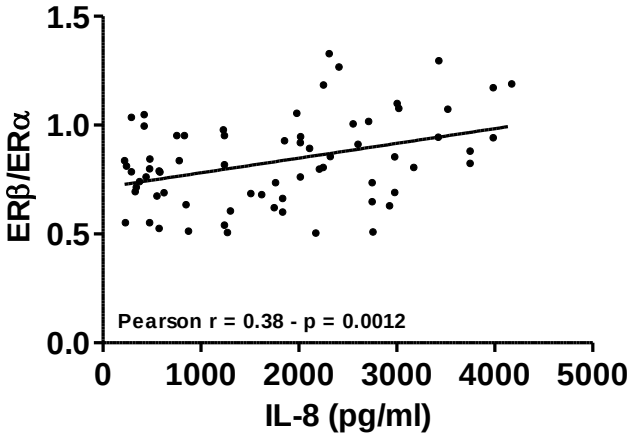


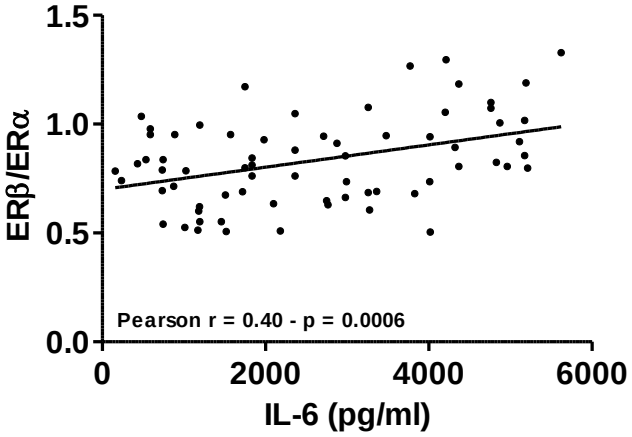


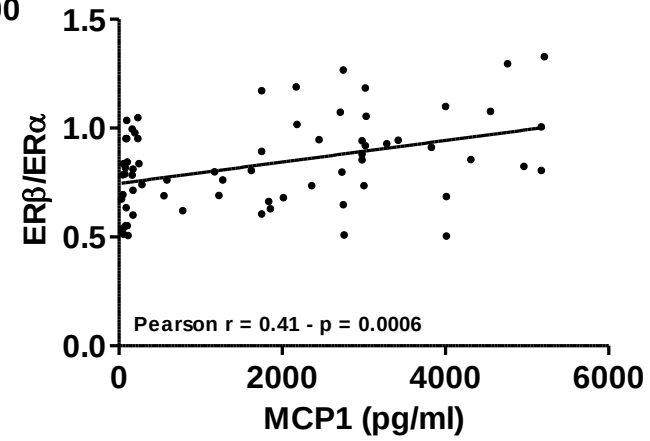
Aging-associated increase of ERβ/ERα ratio is correlated to estrogen-mediated increase of inflammatory biomarkers production.











CONCLUSIONS

✓Aging is associated with an increase of several markers of vascular inflammation. Although estrogen can display an "anti-inflammatory" action by down-regulating of some markers.; time since menopause alters estrogen-mediated effects on the modulation of most of inflammatory biomarkers.

✓The mechanisms whereby aging and long-term estrogen withdrawn affect estrogenic responses are largely unknown, but our data suggest a relationship between changes on balance of ERs (ER-α isoforms and ER-β) with increased proinflammatory effect by estrogen.

✓How aging affects estrogen responses and to what extent these changes can modify the risk for cardiovascular disease remains unknown, but our data strongly suggest that timing to start hormone replacement therapy should be taken into account when deciding the best therapy to treat postmenopausal women.

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