Contribution of Estrogen Deficiency to Age-Associated Increases in Large Artery Stiffness in Women

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Abstract.

Background: Stiffening of the large central (e.g., aorta and common carotid) arteries contributes to the pathogenesis of cardiovascular disease, the leading cause of death in postmenopausal women. Large artery stiffness increases across the stages of menopause and is accelerated around the final menstrual period, presumably due to the loss of vascular-protective estrogen (E2). Short-term ovarian hormone suppression (via gonadotropin releasing hormone antagonist [GnRHa]) with randomization to receive E2 or placebo (PL) added back is a research model that can be used to evaluate the effects of E2-deficiency on large artery stiffness, independent of confounding changes in CVD risk factors (e.g., aging, adiposity, etc) that occur with menopause. **Objective:** Evaluate the role of E2-deficiency on large artery stiffness in women across the stages of menopause. **Methods**: Large artery stiffness (carotid artery β-stiffness via ultrasonography) was measured in premenopausal (PreM; n=28; 33±7 yrs), perimenopausal (PeriM; n=43; 49±1 yrs) and postmenopausal (PostM; n=28; 58±1 yrs) women before and after 6 days of GnRHa (0.25 mg/day ganirelix) with randomization to either transdermal E2 (0.075 mg/day) or PL. **Results**: Carotid stiffness increased in PreM women randomized to GnRHa+PL (5.08±0.24 vs. 6.34±0.41U, p<0.001) and was prevented by E2 add-back (5.50±0.24 vs. 5.40±0.41U, p=0.75). Carotid stiffness tended to increase from baseline in PeriM women randomized to GnRHa+PL (6.83±0.36 vs. 7.45±0.41U, p=0.09) but not GnRHa+E2 (7.59±0.38 vs. 6.99±0.43U, p=0.11). Carotid stiffness did not change in PostM women regardless of randomization (p>0.74). **Conclusions:** E2-deficiency contributes to increased large artery stiffness, however, there may be a critical period for intervention in which the large arteries are acutely modifiable.

Introduction.

- Stiffening of the large central elastic (i.e., aorta and common carotids) arteries independently predicts cardiovascular disease (CVD) events.[1,2]
- Large artery stiffness increases across the stages of menopause and accelerates around the final menstrual period which is hypothesized to be related to the loss of estradiol (E2) during menopause.[3,4]
- Vascular aging is unique in women because adverse changes in CVD risk factors (i.e., adiposity, aging) coincide with changes in gonadal hormones with the menopause transition, making it difficult to isolate the effects of estrogen deficiency.[5]
- Short-term ovarian hormone suppression (via gonadotropin releasing hormone antagonist [$GnRH_{ant}$]) can be used to isolate the effects of ovarian hormones, independent of changes in CVD risk factors.[6]
- Therefore, this study evaluated the effects of short-term estrogen deficiency on in large artery stiffness in women across the stages of menopause.

Hypotheses.

1) Large artery stiffness will be increased in premenopausal and perimenopausal women but not estrogen-deficient postmenopausal women following the $GnRH_{ant}$ intervention alone.
2) Estradiol add-back will reverse the effect of the $GnRH_{ant}$ intervention in premenopausal and perimenopausal women and improve large artery stiffness in postmenopausal women.

Methods.

- Healthy women (18-75 years) not taking vascular-altering medications were recruited from the Denver, CO metropolitan area.
- All women were characterized according to menopausal status using the Stages of Reproductive Aging Workshop (STRAW) criteria.
- All women went through natural (i.e., non-surgical) menopause and were not using any form of hormone therapy.
- Study procedures were approved by the Institutional Review Board and registered to ClinicalTrials.gov (NCT00608062).

Vascular testing.

- Fasted vascular testing was performed in the supine position following 10 minutes of quiet rest.
- Common carotid β-stiffness was measured using carotid ultrasonography (Vivid I, GE Healthcare) and analyzed (Vascular Analysis Tools, MIA LLC)[3] with higher values indicative of greater large artery stiffness.

Methods Cont'd.

Ovarian Hormone Suppression Model.

- Pharmacologic ovarian suppression is an appropriate strategy for investigating regulatory actions of ovarian hormones in women.[3,4]
- Ganirelix acetate (Antagon, Organon Pharmaceuticals) is a $GnRH_{ant}$ that competes with natural GnRH receptors on the anterior pituitary, and induces a rapid, reversible suppression of luteinizing hormone (LH) and follicle stimulating hormone (FSH).
- Add-back of individual ovarian hormones (e.g., estradiol) allows for the isolation of the physiological actions of individual hormones.

Study Design.

- ***Phase 1.*** Carotid stiffness was measured at baseline and after 3 days of $GnRH_{ant}$ (initial dose: 0.5 mg followed by daily 0.25 mg/day of Ganirelix acetate)
- ***Phase 2.*** Carotid stiffness was measured after an additional 3 days of $GnRH_{ant}$ with randomization to either placebo (PL) or transdermal estradiol (E2, 0.075 mg/day) add-back (Figure 1).

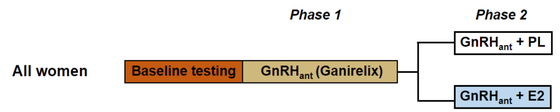


Figure 1. Study design. Carotid stiffness was measured at baseline and after 3 days of a gonadotropin-releasing hormone antagonist ($GnRH_{ant}$) intervention (Phase 1); 3 additional days of $GnRH_{ant}$ intervention with randomization to either placebo (PL) or estradiol (E2) add-back (Phase 2).

Results.

n	Premenopausal 28	Perimenopausal 43	Postmenopausal 28	*p-value* -
Race				
White	20 (71.4)	34 (79.1)	23 (83.1)	
Black	1 (3.6)	2 (4.7)	-	
Asian	3 (10.7)	2 (4.7)	1 (3.6)	
Hawaiian	-	-	1 (3.6)	
More than one	4 (14.3)	3 (7.0)	3 (10.7)	0.52
Ethnicity, n (%)				
Non-Hispanic	22 (78.6)	36 (83.7)	22 (78.6)	
Hispanic	5 (17.9)	3 (9.3)	5 (17.9)	
Unknown	1 (3.6)	0 (0)	1 (3.6)	0.71
Age (years)	33 ± 7	49 ± 1	58 ± 1	**<0.001**
Body mass index (kg/m^2)	24.5 ± 1.1	24.9 ± 0.6	26.8 ± 0.9	0.17
Body mass (kg)	66.7 ± 2.8	68.0 ± 1.8	70.8 ± 2.4	0.49
Total body fat (%)	30.7 ± 1.4	35.1 ± 0.9	39.4 ± 1.0	**<0.001**
Trunk body fat (%)	28.6 ± 8.9	34.0 ± 7.3	38.3 ± 6.3	**<0.001**
Seated Systolic BP (mmHg)	110 ± 2	114 ± 2	119 ± 3	**0.02**
Seated Diastolic BP (mmHg)	70 ± 1	72 ± 1	74 ± 2	0.26
Glucose (mg/L)	85 ± 2	83 ± 1	87 ± 2	0.20
Insulin (mIU/dL)	9.9 ± 2.5	6.3 ± 0.6	7.8 ± 1.7	0.26
Total cholesterol (mg/dL)	148 ± 5	165 ± 4	180 ± 6	**0.001**
LDL (mg/dL)	86 ± 4	97 ± 4	108 ± 6	**0.02**
HDL (mg/dL)	45 ± 2	50 ± 2	50 ± 2	0.15
Triglycerides (mg/dL)	69.5 [55.0, 90.5]	83.0 [68.0, 105.0]	84.0 [68.3, 116.0]	0.09

Table 1. Participant characteristics. All data are expressed as mean±SD. Non-normally distributed data are express as median [95% confidence intervals]. BP, blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein

Results Cont'd.

n		Premenopausal $GnRH_{ant}$ + PL 12	Premenopausal $GnRH_{ant}$ + E2 12	Perimenopausal $GnRH_{ant}$ + PL 21	Perimenopausal $GnRH_{ant}$ + E2 19	Postmenopausal $GnRH_{ant}$ + PL 15	Postmenopausal $GnRH_{ant}$ + E2 13
FSH (mIU/mL)	Baseline	7.2 [5.4, 8.7]	5.6 [5.2, 6.7]	26.5 [13.9, 64.4]	19.7 [8.0, 77.6]	89.5 [53.8, 97.9]	71.8 [52.5, 91.4]
	$GnRH_{ant}$	6.9 [5.3, 8.6]	6.2 [4.0, 8.4]	**22.0 [4.9, 38.8] †**	21.4 [6.1, 38.8]	**53.2 [39.7, 62.7] †**	**49.0 [32.0, 60.9] †**
	$GnRH_{ant}$ + Txt	6.7 [4.9, 8.3]	5.1 [3.5, 6.5]	**25.2 [6.4, 43.6] ***	18.5 [6.4, 43.6]	**50.3 [41.4, 70.1] †**	**35.6[20.7, 47.7]†*§**
Estradiol (pg/mL)	Baseline	74 [35, 132]	63 [32, 84]	40 [17, 86]	92 [15, 138]	10 [10, 12]	10 [10, 13]
	$GnRH_{ant}$	**35 [26, 66] †**	42 [24, 55]	27 [10, 88]	26 [10, 175]	10 [10, 12]	10 [10, 12]
	$GnRH_{ant}$ + Txt	**37 [32, 50] †**	53 [38, 123]	29 [10, 63]	**80 [22, 178]* §**	10 [10, 11]	**45 [35, 61] *†§**
Progesterone (ng/dL)	Baseline	0.7 [0.3, 1.0]	0.6 [0.2, 0.8]	0.4 [0.3, 0.6]	0.4 [0.2, 0.8]	0.3 [0.2, 0.4]	0.3 [0.2, 0.4]
	$GnRH_{ant}$	0.6 [0.2, 1.1]	0.4 [0.1, 0.9]	0.3 [0.2, 0.8]	0.3 [0.2, 0.6]	**0.2 [0.1, 0.4] †**	0.3 [0.1, 0.4]
	$GnRH_{ant}$ + Txt	0.3 [0.2, 0.7]	0.3 [0.2, 0.7]	0.5 [0.3, 1.8]	0.3 [0.2, 1.1]	0.2 [0.2, 0.4]	0.4 [0.2, 0.3]
Testosterone (ng/dL)	Baseline	29 [20, 43]	32 [21, 42]	19 [17, 26]	20 [17, 35]	17 [17, 25]	20 [17, 26]
	$GnRH_{ant}$	30 [17, 46]	**23 [19, 34] †**	17 [17, 22]	19 [17, 30]	17 [17, 20]	17 [17, 20]
	$GnRH_{ant}$ + Txt	**27 [17, 40]***	25 [18, 34]	17 [17, 20]	18 [17, 28]	**17 [17, 18] †**	17 [17, 18]

Table 2. Sex hormones. Change in sex hormones following gonadotropin releasing hormone antagonist ($GnRH_{ant}$) alone (Phase 1) and with estradiol (E2) or placebo (PL) add-back (Phase 2). All data are expressed as median [95% confidence intervals]. *p<0.05 compared with premenopausal; †p<0.05 versus baseline of same menopause stage; ɪ˙ versus GnRHant alone of same menopause stage; § versus placebo within same menopause stage

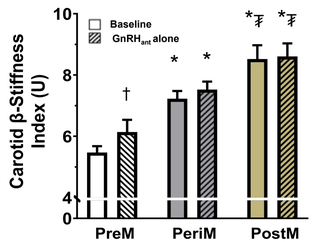
Phase 1.

Figure 2. Changes in carotid β-stiffness index at baseline (solid bars) and following $GnRH_{ant}$ treatment alone (hatched bars) in premenopausal (PreM), perimenopausal (PeriM) and postmenopausal (PostM) women.

* p<0.05 compared with premenopausal women.

Ŧ p<0.05 compared with perimenopausal women.

† p<0.05 compared with baseline of same menopausal group.



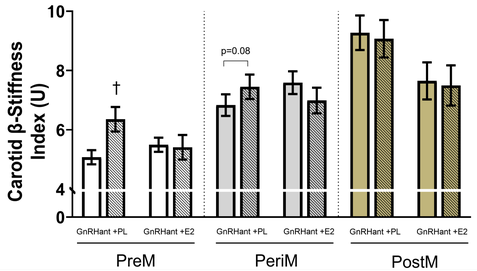
Phase 1 Findings:

1. Carotid β-stiffness index is progressively greater across the stages of menopause at baseline.
2. $GnRH_{ant}$ alone reduced estradiol (Table 2) and increased carotid β-stiffness index in premenopausal women only.

Phase 2.

Figure 3. Changes in carotid β-stiffness index at baseline (solid bars) and following $GnRH_{ant}$ with randomization to either placebo (PL) or estradiol (E2) add-back (hatched bars) in premenopausal (PreM), perimenopausal (PeriM) and postmenopausal (PostM) women.

† p<0.05 compared with baseline of same menopausal group.



Phase 2 Findings:

1. Reductions in estradiol increased carotid β-stiffness index in premenopausal and perimenopausal women that is prevented by the administration of estradiol added-back.
2. Carotid β-stiffness index is not altered in postmenopausal women receiving $GnRH_{ant}$ treatment regardless of randomization to estradiol.

Conclusions.

- Estrogen-deficiency increases carotid β-stiffness in premenopausal and perimenopausal women that is prevented by the administration of estradiol.
- In postmenopausal women, carotid β-stiffness is not altered by the $GnRH_{ant}$ treatment regardless of randomization to estradiol add-back.
- These data suggest that estrogen-deficiency contributes to increased large artery stiffness; however, there may be a critical period for intervention in which the large arteries may be acutely modifiable to therapies and interventions.

Citations. 1. Mitchell, Gary F., et al. "Arterial stiffness and cardiovascular events: the Framingham Heart Study." *Circulation* 121.4 (2010): 505-511.
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