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The KEEPS-Cognitive and Affective Study: Baseline Associations between Vascular Risk Factors and Cognition

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Abstract

Background—Midlife vascular risk factors influence later cognitive decline and Alzheimer's disease (AD). The decrease in serum estradiol levels during menopause has been associated with cognitive impairment and increased vascular risk, such as high blood pressure (BP), which independently contribute to cognitive dysfunction and AD.

Methods—We describe the extent to which vascular risk factors relate to cognition in healthy, middle–aged, recently postmenopausal women enrolled in the Kronos Early Estrogen Prevention Cognitive and Affective Study (KEEPS-Cog) at baseline. KEEPS-Cog is a double-blind, randomized, placebo-controlled, parallel group design, clinical trial, investigating the efficacy of low-dose, transdermal 17β-estradiol and oral conjugated equine estrogen on cognition.

Results—The KEEPS-Cog cohort (N=662) is healthy and free of cognitive dysfunction. Higher systolic BP was related to poorer performance in auditory working memory and attention (unadjusted p=0.004; adjusted p=0.10). This relationship was not associated with endogenous hormone levels.

Conclusions—Lower BP early in menopause may positively affect cognitive domains known to be associated with AD.

Keywords

Clinical Trial; Estrogen; Blood Pressure; Vascular Risk; Hormone Therapy; Estradiol; Cognition; Attention; Memory

Introduction

Declining serum estrogen levels during the menopausal transition have been linked to increased vascular risk factors and subtle cognitive decline (1, 2). Results from basic science, observational studies and clinical trials suggest that hormone therapy (HT) administered soon after menopause may reduce these deleterious vascular and cognitive effects (3, 4). Specifically, HT's salutary vascular effects have been linked to protection of arterial wall function and lowering blood pressure (BP) (5, 6). HT administration may also have beneficial cognitive effects, both via direct actions on estrogen receptors in the brain, and indirectly, through HT's beneficial effects on the vasculature.

Increases in vascular risk factors, including hypertension and insulin resistance during midlife are associated with an increased risk of Alzheimer's disease (AD) in later life (for a comprehensive review see (7); (8, 9)). Similarly, controlled vascular risk factors such as reductions in BP are associated with protection against AD (10, 11). This indicates that long-standing uncontrolled BP and other vascular risk factors may contribute to AD pathology, possibly through decreased cerebral blood flow (CBF) and accumulation of β-amyloid (Aβ), a key pathological feature of preclinical AD (12, 13). Some studies show that precipitous decrease in serum estradiol levels during the menopausal transition is associated with some cognitive impairment, particularly in the domains of attention and memory (14). Moreover, the decline in serum estradiol levels increases the risk of hypertension and hypercholesterolemia, factors that independently contribute to cognitive dysfunction and AD in later life (15).

While there is a clear relationship between cognition and clinically diagnosable hypertension and hypercholesterolemia, the point at which vascular risk factors influence cognitive task performance is less understood. Moreover, while the menopausal decline in serum estradiol levels has been linked to subtle reductions in cognition and increased vascular risk factors, the extent to which serum estradiol levels influence vascular risk factors and cognition independently or in tandem is unclear.

The majority of research linking vascular factors and cognition has been conducted in older populations and in samples with established vascular disease (16). Recently, studies have shown that vascular risk factors can influence cognitive task performance in younger

populations, whose vascular disease risk factors are within clinically ‘normal’ limits (17). For instance, our group (18) and others (19) found a relationship between cognitive task performance and blood pressure (BP) extending into the normotensive range (i.e., around 120/80 mmHg) in younger samples of healthy men and women (i.e., 18–25 years). These changes in cognition are similar to changes observed in older populations (20).

The Kronos Early Estrogen Prevention Cognitive and Affective Study (KEEPS-Cog) is a 4-year, randomized, double-blind, placebo-controlled, parallel group, clinical trial, designed to investigate the differential efficacy of low-dose estrogen formulations on cognition in recently postmenopausal women. Women enrolled in the KEEPS-Cog sub-study serve as an excellent cohort to investigate the relationship among vascular risk factors, cognitive task performance and endogenous sex hormone levels in recently postmenopausal women, prior to randomization to study medication. Continued follow-up of this cohort will clarify the relationship between midlife HT use, cardiovascular function and cognitive performance.

The purpose of this study was to define the relationships among vascular risk factors, cognitive task performance and endogenous sex hormone levels (e.g. estradiol, estrone, progesterone and testosterone) at baseline in healthy, middle–aged, recently postmenopausal women enrolled in the KEEPS-Cog sub-study at baseline. Additionally, we sought to explore the extent to which this relationship might be associated with serum endogenous sex hormone levels.

Methods

The KEEPS-cog is a sub-study of the Kronos Early Estrogen Prevention Study (KEEPS) funded by the National Institute on Aging (NIA). Details of the trial design, inclusion and exclusion criteria, treatment assignment, and participant characteristics have been published previously (21, 22). The University of Wisconsin in Madison was the coordinating site for the KEEPS-Cog trial. The trial consisted of four visits over four years (baseline and months 18, 36 and 48). KEEPS-Cog cognitive and affective testing sessions coincided with visits for the parent KEEPS trial. The current manuscript includes measures ascertained at the baseline study visit only, prior to randomization to treatment and thus hormone levels reflect only endogenous levels.

Participants

KEEPS-Cog participants consisted of 662 recently menopausal women (42–59 years old), between 6 months and 3 years of their last menses. Participants included women without known or suspected cognitive or cardiovascular disease, and meeting the inclusion criteria for KEEPS (KEEPS-Cog NCT000154180) enrolled at one of nine clinical testing sites. KEEPS is a multicenter, randomized, double-blinded, placebo-controlled trial, designed to test the hypothesis that low-dose HT initiated in recently postmenopausal women will reduce the progression of subclinical atherosclerosis as measured by carotid artery intima-media thickness (CIMT) and coronary artery calcification (CAC) over four years (21, 22).

Potential participants in the KEEPS Cog study underwent a depression and cognitive dysfunction screening, a medical examination, blood tests and an electrocardiogram at

baseline. Women scoring 18 or greater on the Beck Depression Inventory (BDI), or reporting suicidal ideation as assessed by the Beck Depression Inventory, or scoring 22/30 or lower on the Mini Mental State Exam (MMSE) were excluded from the KEEPS-Cog study. All women underwent a high-resolution B-mode ultrasound examination for the assessment of CIMT (23) and computed tomography for the assessment of CAC (24). Women were also excluded if they had a history of clinically defined cardiovascular disease; were current heavy smokers (more than ten cigarettes/day by self-report); their CAC score was ≥50 Agatston units (AU); body mass index was >35 kg/m^2; or if they had dyslipidemia (low-density lipoprotein (LDL) cholesterol >190 mg/dL), hypertriglyceridemia (>400 mg/dL), serum 17β-estradiol >40 mg/dL, uncontrolled hypertension (systolic BP >150 mmHg or diastolic BP >95 mmHg), or fasting blood glucose (FBG) >126 mg/dL (21). Women using lipid-lowering medications at baseline were excluded. KEEPS-Cog was approved by Institutional Review Boards at each of the nine clinical testing sites and the University of Wisconsin (UW) in Madison. All participants provided written informed consent.

1.1. Cognitive task measures—Cognitive tasks included the Modified Mini-Mental State Exam (25), Prospective Memory Test (26), NYU Paragraph Recall (27), Stroop Color Word Interference Test (28), Letter-Number Sequencing (29), Digit Symbol (30), Trail Making Test (Trails A & B) (31), the California Verbal Learning Test (CVLT-II) (32), the Benton Visual Retention Test (33), Digit Span (29) and Verbal Fluency (31). We combined components of the individual cognitive tests into a four-factor structure for analyses (described below).

Laboratory values and vascular risk factors—Blood pressure was recorded in the morning by a registered nurse familiar with BP measurement methodology. Participants were seated for five minutes prior to when BP was taken. A conventional mercury sphygmomanometer, appropriate sized BP cuff and a stethoscope with a bell were used. Two BP determinations were obtained from the same arm, ten minutes apart. The average of the two BP readings was used for analyses. Venous blood samples were obtained from the arm opposite of and after measurements of BP. Low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C) triglycerides and blood glucose (FBG) were measured by Kronos Science Laboratories (Phoenix AZ).

Hormone analyses—Serum estradiol, estrone, testosterone and progesterone were measured at the University of Wisconsins Clinical and Translational Science Award (CTSA) - funded Institute for Clinical and Translational Research (ICTR) laboratory in the Assay Services Core. Baseline samples were batched and assays were conducted at one time. Ultrapure water (500 µl) with 40 µl of internal standards (deuterated steroids: testosterone, estradiol, estrone, progesterone; CDN Isotopes in Pointe-Claire, Quebec, Canada) were added to 400 µl of serum and extracted with 2 ml of methyl ether. The ether layer was dried and re-suspended in ethanol and 500 µl water and 1 ml of dichloromethane. The dichloromethane portion was dried and re-suspended in 25 µl acetonitrile/water (50:50). To derivatize the estrogens, 25 µl dansyl chloride was added, heated for 3 minutes and samples were prepared for injection of 30 µl into the LC/MS. A 150×2.10 mm column (2.6 µ, C18, Kinetex, Phenomenx, Torrance, California) was used for the HPLC separation on our

Agilent 1100 series system. Positive ion identification was used for testosterone (*m/z* 289), progesterone (*m/z* 315), estradiol (*m/z* 506) and estrone (*m/z* 504) measurements. Separation was performed by using a gradient in mobile phase B = 95% acetonitrile (ACN)/5% water (H2O) and A 95% H20/5% ACN where %B begins at 44%, increases to 60% at 10 minutes, to 90% at 16 minutes and back to 70% at 20 minutes, to 44% at 23 minutes. Flow rate was 100 μl/minutes. Coefficients of variation for sex hormones assayed in the present study are as follows: testosterone (18.8%); progesterone (25.3%); estradiol (12.9%); and estrone (22.4%).

Statistical Analyses—All analyses were controlled for age, education, race, study site and apolipoprotein E ε4 (*APOE* ε4) status based on *APOE's* reported independent effects on cardiovascular risk and cognitive task performance (34, 35).

A confirmatory factor analysis (CFA) was used to examine cognitive function at baseline. Compilation of factor scores was comprised of components of the 12 cognitive tests utilized in the KEEPS-Cog battery. Using standard criteria for model fit (36) a four-factor solution provided an acceptable fit (χ^2=360.58 with a p-value<0.001; comparative fit index - CFI=0.92, root mean squared error of approximation-RMSEA=0.05). The four cognitive domains used in the analyses included 1) Verbal Learning 2) Auditory Attention & Working Memory, 3) Verbal Attention & Executive Function and 4) Speeded Language & Flexibility. The factor scores served as outcome measures in the mixed regression models. The model was estimated using the R package.

To explore the relationship between vascular risk factors and cognitive factor scores, we employed a linear mixed effect modeling approach. Vascular risk factors entered into the model included systolic BP, LDL-C, HDL-C, FBG and body mass index (BMI) as well as CIMT and CAC. We controlled for gender, age, race, education level and *APOE* 4 status. All p values were set at 0.05 and were adjusted for multiple comparisons using Benhamini-Hochberg's procedures (37)

In order to investigate a potential impact of sex endogenous hormone levels on the systolic BP - cognition relationship, we next employed another linear effects model. In this model, we included endogenous sex hormone levels including estradiol, estrone, testosterone and progesterone. As was the case with the first model, we controlled for gender, age race, education level and *APOE* 4 status, p values were set at 0.05 and we adjusted for multiple comparisons (37).

Procedures—Administration of the cognitive battery was conducted by trained psychometricians at each of the nine testing sites. Before the KEEPS-cog sub-study initiated, all psychometricians took part in a two-day group training covering KEEPS-Cog rationale, methodology, test administration and scoring and a full day of interactive, mock cognitive and affective testing. Each site was provided with a cognitive testing manual and instructions for test administration and scoring and a DVD of a mock testing session. In the instance of psychometrician turnover, the KEEPS-Cog coordinator, a trained cognitive neuroscientist at the UW Madison, traveled to the testing site and trained the new psychometrician on testing administration and scoring techniques. Additionally, we

conducted training sessions every two years, and quarterly conference calls were held between the UW Madison and all sites to ensure continuity of the project. To further ensure the quality of the data, 10% of participant data at each testing site was audited to ensure correct test scoring and data entry. Quality assurance checks exceeded 98% accuracy for all sites for all cognitive variables.

The KEEPS-Cog testing session lasted approximately 1.5 hours. The cognitive testing area was free of excessive noise and approved by the KEEPS-Cog study personnel at the UW Madison. Because the KEEPS-Cog visit coincided with the parent KEEPS visits that required a fasting blood draw, all participants were tested in the morning and were given a light breakfast before cognitive testing began. All participants were tested by the same psychometrician at their respective site. Order of neuropsychological test administration was consistent across sites and tests were grouped to minimize between test interference. Tests were scored and entered into a centralized database within 3 days of the visit.

Results

Participants

Of the 662 women meeting inclusion criteria for KEEPS and consenting to participate in the KEEPS-Cog, 91 were excluded from the present analyses due to missingness of cognitive data or unwillingness to take part in genetic testing for *APOE* genotyping.

Demographic information for participants enrolled in the KEEPS-Cog sub-study is listed in Tale 1. Participants (N = 571) were middle aged (mean 52.7 years) and well-educated, with 73.8% reporting at least some college education. The majority of participants identified themselves as White (78.1%). Those identifying as Black or Hispanic were 7.1% and 6.2%, respectively. Percentage of Non-White, non-Hispanic, participants from each of the nine clinical testing sites ranged from 5.6% to 17.7% of the total sample.

Table 2 shows KEEPS-Cog participants' vascular risk factors. By design, the middle-aged sample is very healthy and at low risk for vascular disease based on BP measures, BMI, and HDL-C, LDL-C and FBG levels. Percent of women enrolled in KEEPS-Cog with an ApoE 4 allele is 14.3%. There is no significant difference on any measure between KEEPS-Cog participants and participants enrolled in the parent KEEPS study.

Results of the fixed effects models are shown in Table 3. Results reveal a significant association between systolic BP and the auditory attention and working memory factor score (unadjusted, $p = .004$) after controlling for age, education and *APOE* ε4 status. This relationship, however, fades after adjusting for multiple comparisons (adjusted, $p = 0.10$). Additional analyses showed no relationship between BP and the other three factor scores.

To ensure the relationship between systolic BP and cognition was not attributed to endogenous sex hormone levels, we entered estradiol, estrone, progesterone and testosterone into a fixed effects model. Analyses revealed that the relationship between the auditory attention and working memory factor score and systolic BP was not altered after including baseline sex hormones (all p values > 0.28) (See Table 4).

Discussion

Our results show that the KEEPS-Cog cohort was healthy and free of major medical conditions and comorbidities at baseline. As such, our results are less prone to confounding related to concomitant vascular and cognitive interference. Our main result was a significant relationship between auditory attention/working memory and systolic BP such that participants with higher systolic BP performed worse on tests within this domain.

Present results are consistent with other studies showing an inverse relation between BP and cognition (i.e., higher BP is associated with poorer cognitive performance) (38). A number of studies have demonstrated that midlife hypertension has a negative impact on cognitive performance (39, 40), is an established risk factor for AD (9, 41), and has been linked to increased cognitive impairment among AD patients (42). Hypertension has been linked to deficits in attention (43), and memory (44), the factor score found to be related to BP in the present study. The auditory attention/working memory factor score is comprised of digit span forward, digit span backward and the letter number sequencing task. Prior research has shown that the individual components of the attention/working memory factor score, particularly digit span, have been linked to BP dysregulation during midlife (45, 46).

Our data revealed that the relationship between BP and cognition was not influenced by endogenous sex hormone levels (p values > .28) for estradiol, estrone, testosterone and progesterone. This result is consistent with prior findings linking hypertension to cognitive impairment and mild cognitive impairment (MCI), independent of the effects of endogenous estrogen levels or HT administration (47, 48). These results suggest that subtle increases in vascular risk factors influence cognition, independent of estrogen's effects on cognition and disease incidence. It is likely that both increased midlife vascular risk factors and the loss of estradiol during the menopausal transition serve as independent risk factors that work synergistically to increase the risk for cognitive decline and incident AD in later life.

Midlife hypertension has been associated with decreasing estradiol levels during menopause and cognitive impairment. Hypertension during midlife has also been associated with an increased risk of AD in later life, while reductions in BP are associated with protection against the disease (7, 13). This indicates that long-standing, uncontrolled BP may contribute to AD neuropathology, possibly through decreased cerebral blood flow (CBF) and accumulation of β-amyloid (Aβ), a key pathological feature of preclinical AD (13). For instance, plasma Aβ42 has been shown to be significantly and positively correlated with systolic and diastolic BP and pulse pressure (49), and the Honolulu Heart Program/ Honolulu-Asia Aging Study reported that midlife systolic BP variation is associated with increased Aβ in the hippocampus (50). Moreover, studies involving BP medications suggest that some antihypertensives reduce the risk for AD and improve cognition in patients with AD via improved CBF (51–53). Although collectively, these studies suggest that cognition is impaired in the presence of prolonged uncontrolled hypertension and the mechanism driving this relationship may be directly related to AD neuropathology. Future studies investigating the relationship between midlife cognition and BP would likely benefit from the inclusion of neuroimaging and measures of potential soluble or cellular biomarkers in order to assess the potential mechanisms driving this relationship.

It should be noted that our prior work (18) as well as other studies (19) have shown that even subclinical vascular dysfunction, (e.g., slightly high or low BP), may pose a significant additional risk factor for cognitive decline and AD, compounded by genetics and family history. While our participants were normotensive, we observed a relationship between higher systolic BP and poorer cognitive task performance in a healthy, middle-aged cohort. The healthy KEEPS-Cog cohort is ideally positioned to address this important issue- as the participants do not have clinical evidence of vascular disease at baseline.

A potential limitation of the current data is that the KEEPS-Cog study cohort primarily identifies as White, though the study as a whole is more representative than the Women's Health Initiative (WHI) or the Women's Health Initiative Memory Study (WHIMS) (22, 54). Black and Hispanic populations are more likely to be afflicted with increased risk for hypertension across the lifespan than White participants.

An important distinction between the KEEPS-Cog sub-study and the WHIMS, is that our participants were younger, more educated, and arguably most important for the current project, have healthy cardiovascular profiles. Compared to WHIMS participants, women in the KEEPS-Cog have lower systolic BP levels, are more likely to be never smokers, have lower lipid levels and lower BMI. While the WHI and WHIMS studies have contributed knowledge surrounding standard-dose HT, vascular risk factors and AD, the KEEPS and KEEPS-Cog sub-studies are ideally positioned to examine the effects of low-dose HT when initiated soon after menopause. Additionally, the KEEPS-Cog sub-study provides the opportunity to determine early postmenopausal initiation of low-dose HT in conjunction with subclinical vascular risk factors during midlife, and their contribution to cognitive task performance and subsequent AD in later life. Thus, KEEPS is positioned to answer questions regarding the long term use of differential low-dose estrogen formulations administered soon after menopause, the impact on vascular risk and how these factors may work independently and synergistically to affect cognition over time.

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Abbreviations

AD	Alzheimer's disease
BP	blood pressure

KEEPS	Kronos Early Estrogen Prevention Study
KEEPS-Cog	Kronos Early Estrogen Prevention Cognitive and Affective Study
HT	hormone therapy
CBF	cerebral blood flow
CIMT	carotid artery intima-media thickness
AU	Agatston units
HDL-C	Agatston units
LDL-C	low-density lipoprotein-cholesterol
UW	University of Wisconsin
CVLT-II	California Verbal Learning Test
FBG	Fasting blood glucose
ICTR	Institute for Clinical and Translational Research
ACN	acetonitrile
BMI	body mass index
APOE	apolipoprotein E
CFA	confirmatory factor analysis
Aβ	β-amyloid
WHI	Women's Health Initiative
WHIMS	Women's Health Initiative Memory Study

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Table 1

Demographic Information of KEEPS-Cog Participants.

Education	N	%
Some High School	3	.5
High School Diploma or GED	46	7.0
Some College/Vocational School	122	18.7
College Graduate	263	40.3
Some Graduate or professional school	30	4.6
Graduate or Professional degree	189	28.9

Site	N	%
Brigham and Women	81	12.3
Columbia	87	13.2
Mayo	117	17.7
Albert Einstein	69	10.4
University of California	51	7.7
Utah	90	13.6
Washington	37	5.6
Yale	67	10.1
Kronos	62	9.4

Race	N	%
No Answer	36	5.4
Asian Indian	4	.6
Black	47	7.1
White	516	78.1
Chinese	6	.9
Philipino	2	.3
Hispanic	41	6.2
Japanese	1	.2
Korean	1	.2
Other	7	1.1

Table 2

Description of vascular risk factors among women in the KEEPS-Cog study.

Vascular Risk Factor	Mean	SD
Age (years)	52.66	2.59
Height (ft)	5.45	2.40
Weight (lbs)	155.60	26.60
Waist Circumference (in)	33.32	6.82
Average systolic (mm/Hg)	118.58	15.25
Average diastolic (mm/Hg)	74.61	9.24
BMI (kg/m^2)	26.32	4.31
FBG (mg/dL)	89.17	9.77
Trig (mg/dL)	91.75	51.41
LDL-C (mg/dL)	128.99	29.73
HDL-C (mg/dL)	64.66	17.13
Current Tobacco Use (%users)	6.4%	
APOE ε4 (% E4 positive women)	14.3%	

BMI = body mass index, FBG = fasting blood glucose, Trig = triglycerides LDL-C = low density lipoprotein-cholesterol, HDL-C = high density lipoprotein-cholesterol

Table 3

Results of the mixed effects model describing the relationship between the auditory attention and working memory factor score and vascular risk factors and subclinical atherosclerosis measures of CIMT and CAC. Age, education, race and ApoE 4 status were entered as covariates.

Attention/Working Memory	t-value	p-value
Systolic Blood Pressure	−2.93	0.003
CIMT	0.533	0.594
CAC	−0.778	0.436
LDL-C (mg/dL)	−.0.225	0.821
HDL-C (mg/dL)	−0.345	0.730
FBG (mg/dL)	0.807	0.420
BMI	0.737	0.460

CIMT = carotid artery intima–media thickness, CAC = coronary arterial calcification, LDL-C = low density lipoprotein-cholesterol, HDL-C = high density lipoprotein-cholesterol, FBG = fasting blood glucose, BMI = body mass index.

Table 4

Results of the mixed effects model describing the relationship between the auditory attention and working memory factor score and sex hormone levels.

Serum Hormone Levels	Mean (SD)	t-value	p-value
Estradiol, pg/mL	21.7 (30.9)	0.81	0.42
Estrone, pg/mL	23.8 (16.7)	−1.03	0.29
Testosterone, pg/mL	217.8 (128.3)	−0.60	0.54
Progesterone, pg/mL	355.7 (288.8)	1.00	0.31