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Identifying postmenopausal women at risk for cognitive decline within a healthy cohort using a panel of clinical metabolic indicators: potential for detecting an at-Alzheimer's risk metabolic phenotype

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ABSTRACT

Detecting at-risk individuals within a healthy population is critical for preventing or delaying Alzheimer's disease. Systems biology integration of brain and body metabolism enables peripheral metabolic biomarkers to serve as reporters of brain bioenergetic status. Using clinical metabolic data derived from healthy postmenopausal women in the Early versus Late Intervention Trial with Estradiol (ELITE), we conducted principal components and k-means clustering analyses of 9 biomarkers to define metabolic phenotypes. Metabolic clusters were correlated with cognitive performance and analyzed for change over 5 years. Metabolic biomarkers at baseline generated 3 clusters, representing women with healthy, high blood pressure, and poor metabolic phenotypes. Compared with healthy women, poor metabolic women had significantly lower executive, global and memory cognitive performance. Hormone therapy provided metabolic benefit to women in high blood pressure and poor metabolic phenotypes. This panel of well-established clinical peripheral biomarkers represents an initial step toward developing an affordable, rapidly deployable, and clinically relevant strategy to detect an at-risk phenotype of late-onset Alzheimer's disease.

1. Introduction

Effective prevention and delay of Alzheimer's disease (AD) will require intervention during the preclinical phase (Carrillo et al., 2013; Jack et al., 2011; Sperling et al., 2011; Yao et al., 2011). Achievement of this goal entails accurate identification of at-risk individuals before clinically symptomatic disease. Successful screening of at-risk populations requires an accurate, rapidly deployable, clinically accessible, and economically feasible biomarker strategy. To achieve these criteria, biomarkers based on peripheral indicators that accurately predict early risk status of the brain would be advantageous. Interrogating the metabolic system through peripheral indicators provides one such strategy, as substantial evidence supports the hypothesis that midlife metabolism affects cognitive health in older age (Cheng et al., 2012; Gottesman et al., 2014; Kenna et al., 2013; Kivipelto et al., 2001; Norton et al., 2014; Rawlings et al., 2014; Roberts et al., 2014; Wharton et al., 2014; Whitmer et al., 2005).

One strategy to enrich an at-risk population for biomarker development is to focus on individuals with a greater lifetime risk of AD. Women have a 2-fold greater lifetime risk of developing AD, and thus, constitute a target population for which biomarkers for early detection of risk could have substantial public health impact (Alzheimer's Association, 2014). Although the biological basis for gender differences in AD remains to be established, basic and clinical science indicate that the menopausal transition and decline in estrogen can adversely affect brain and whole-body metabolism

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(Brinton et al, 2015; Henderson and Brinton, 2010; Rettberg et al., 2014; Yin et al., 2015).

Based on basic and clinical science, we hypothesized that metabolically based biomarkers would identify individuals at the tipping point for developing an at-risk for Alzheimer's phenotype in a population of healthy postmenopausal women. To test this hypothesis, we conducted a clustering analysis using baseline data from the Early versus Late Intervention Trial with Estradiol (ELITE; Hodis et al., 2015) to identify metabolic phenotypes. We subsequently investigated the association of these phenotypes with cognitive performance and the longitudinal change in both metabolic phenotypes and cognitive performance over 5 years. We further hypothesized that the administration of hormone therapy (HT) would differentially impact both overall metabolism and cognitive performance within women of different metabolic phenotypes.

2. Methods

2.1. The ELITE clinical trial

ELITE was a double-blinded, placebo–controlled clinical trial randomizing 643 postmenopausal women. It was designed to test the timing hypothesis of postmenopausal HT, such that HT benefits and risks depend on the temporal initiation of HT relative to time-since-menopause, which is in turn related to underlying tissue health (Henderson et al., 2013; Karim et al., 2015). Women were recruited into 2 cohorts: early menopause (n = 271), defined as within 6 years of menopause, and late menopause (n = 372), defined as 10 or more years postmenopause.

Eligible women were postmenopausal, defined as absence of menses for ≥6 months or surgical menopause and serum estradiol below 25 pg/mL. Of the women included, 14 were between 6 months and 1 year postmenopausal, and the remainder were all >1 year postmenopausal. Women were excluded if they had clinical signs, symptoms, or personal history of cardiovascular disease; diabetes mellitus (fasting serum glucose ≥140 mg/dL); uncontrolled hypertension (diastolic blood pressure ≥110 mmHg); untreated thyroid disease; plasma triglyceride levels >500 mg/dL; serum creatinine >2.0 mg/dL; cirrhosis or liver disease; a life threatening disease with prognosis <5 years; or inability to determine time-since-menopause. Women with a history of deep vein thrombosis, pulmonary embolism, or breast cancer were excluded. Within each postmenopause cohort, women were randomized to receive either HT (17β-estradiol, 1 mg daily) or placebo. Women who had not undergone a hysterectomy also used vaginal 4% progesterone (or placebo) gel for the last 10 days of each month.

The primary trial outcome was rate of change of distal common carotid artery far wall intima-media thickness (Hodis et al., 2015). A secondary outcome was change in cognitive function (Henderson et al., 2013). A comprehensive battery of neuropsychological tests was administered before randomization, at about 2.5 years, and at each participant's final study visit, approximately 5 years after randomization. The battery included 14 neuropsychological tests that emphasized standardized tests sensitive to age-associated change in middle-aged and older adults (Henderson et al., 2013). ELITE was approved by the Institutional Review Board of the University of Southern California. All participants provided written informed consent.

For the longitudinal analysis, the full sample of 643 women was restricted to those completing cognitive testing at baseline and again at either 2.5 years, 5 years, or both (n = 502). Of the 502 women, 216 were in the early menopause and 286 in the late menopause groups.

2.2. Clinical and laboratory measurements

At each 6-month clinic visit, 8-hour fasting blood was drawn, and blood pressure was measured. Current medication use was recorded. Samples were prepared and stored at −70 °C.

Fasting glucose, β-hydroxybutyrate, and insulin were measured in stored plasma using kits (glucose and β-hydroxybutyrate: Cayman Chemical, Ann Arbor, MI, USA; insulin: Alpco Diagnostics, Salem, NH, USA), according to each manufacturer's protocol. Fasting total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol levels were measured in fresh plasma using an enzymatic method of the Standardization Program of the National Centers for Disease Control and Prevention as described previously (Hodis et al., 2015). Low-density lipoprotein (LDL) cholesterol was computed using the Friedewald equation (Friedewald et al., 1972). Fasting HbA1c was measured in fresh whole blood using Bio-Rad Hemoglobin A1c high performance liquid chromatography.

2.3. Statistical analysis

The analysis included 9 metabolic variables: glucose, the homeostatic model assessment score (HOMA; a measure of insulin resistance: [glucose mmol/L × insulin]/22.5), ketones (β-hydroxybutyrate), HDL cholesterol, LDL cholesterol, triglycerides, HbA1c, and systolic and diastolic blood pressure (SBP, DBP). These biomarkers were selected on the basis of their contribution to metabolic, cardiovascular, and neurologic health. Insulin and total cholesterol were excluded as these were respectively highly correlated with the HOMA score ($R^2 = 0.98$, $p < 0.0001$) and LDL cholesterol ($R^2 = 0.89$, $p < 0.0001$). All variables were standardized using baseline averages and standard deviations from the entire ELITE sample. A principal components analysis on the 9 standardized variables identified the number of potential clusters that best explained the variance in the dataset. Specifying 3 clusters, a nonhierarchical K-means clustering algorithm was performed; the resulting 3 clusters were descriptively identified based on their means profile. The 3 clusters were compared on demographic factors and metabolic variables using analysis of variance and covariance for continuous variables and χ^2 tests for categorical variables.

Three cognitive composite scores (global cognition, executive functions, and verbal memory) were generated from the 14-item test battery. Composite scores were a linear sum of the standardized test scores within each domain, with each standard test score inversely weighted by its correlation with other contributing cognitive tests (Henderson et al., 2013). The verbal memory composite score was defined a priori by Word List Free Recall (a short version of the California Verbal Learning Test II) immediate and delayed recall, and Paragraph Recall (East Boston Memory Test) immediate and delayed recall (Henderson et al., 2013). Tests included in the executive functions composite score were Symbol Digit Modalities Test, Trail Making Test part B, Shipley Abstraction Scale, and category fluency (Animal Naming). These tests were determined by a principal components analysis of baseline scores (Henderson et al., 2013). The composite score for global cognition was similarly calculated as a weighted average, including all tests in the battery. Analysis of covariance was used to test for overall cross-sectional differences among metabolic clusters on each cognitive composite and test; covariates included postmenopause cohort (early/late), random intervention assignment, and education.

Measurements of longitudinal change in metabolic biomarkers used measures at 3 time points (baseline, 2.5 years, and end of study at approximately 5 years) to match with cognitive assessment times. Modeling each metabolic biomarker or cognitive composite separately as the longitudinal dependent variable, data were analyzed using mixed effects linear models, testing the effects of baseline