◆ Human Brain Mapping 38:3249–3261 (2017) ◆

Higher Serum Cholesterol Is Associated With Intensified Age-Related Neural Network Decoupling and Cognitive Decline in Early- to Mid-Life

Jeffrey M. Spielberg,[1,2,3]* **Naomi Sadeh**,[1,4] **Elizabeth C. Leritz**,[5,6] **Regina E. McGlinchey**,[5,6] **William P. Milberg**,[5,6] **Jasmeet P. Hayes**,[3,4] and **David H. Salat**[2,5,7]

[1]*Department of Psychological and Brain Sciences, University of Delaware, Newark, Delaware 19716*
[2]*Neuroimaging Research for Veterans Center, VA Boston Healthcare System, Boston, Massachusetts 02130*
[3]*Department of Psychiatry, Boston University School of Medicine, Boston, Massachusetts 02130*
[4]*National Center for Posttraumatic Stress Disorder, Behavioral Sciences Division, VA Boston Healthcare System, Boston, Massachusetts 02130*
[5]*Geriatric Research, Education & Clinical Center & Translational Research Center for TBI and Stress Disorders, VA Boston Healthcare System, Boston, Massachusetts 02130*
[6]*Department of Psychiatry, Harvard Medical School, Boston, Massachusetts 02215*
[7]*Department of Radiology, Harvard Medical School, Boston, Massachusetts 02129*

Abstract: Mounting evidence indicates that serum cholesterol and other risk factors for cardiovascular disease intensify normative trajectories of age-related cognitive decline. However, the neural mechanisms by which this occurs remain largely unknown. To understand the impact of cholesterol on brain networks, we applied graph theory to resting-state fMRI in a large sample of early- to mid-life Veterans ($N = 206$, $\text{Mean}_{age} = 32$). A network emerged (centered on the banks of the superior temporal sulcus) that evidenced age-related decoupling (i.e., decreased network connectivity with age), but *only* in participants with clinically-elevated total cholesterol (≥180 mg/dL). Crucially, decoupling in this network corresponded to greater day-to-day disability and mediated age-related declines in psychomotor speed. Finally, examination of network organization revealed a pattern of age-related dedifferentiation for the banks of the superior temporal sulcus, again present only with higher cholesterol. More specifically, age was related to decreasing within-module communication (indexed by Within-Module Degree *Z*-Score) and increasing between-module communication (indexed by Participation Coefficient), but only in participants with clinically-elevated cholesterol. Follow-up analyses indicated that all findings were driven by low-density lipoprotein (LDL) levels, rather than high-density

Contract grant sponsors: Translational Research Center for TBI and Stress Disorders (TRACTS), a VA Rehabilitation Research and Development (RR&D) Traumatic Brain Injury Center of Excellence (B9254-C), and the Spivack Center for Clinical and Translational Neuroscience at Boston University School of Medicine

*Correspondence to: Jeffrey M. Spielberg, Department of Psychological and Brain Sciences, University of Delaware, Newark, DE 19716. E-mail: jmsp@udel.edu

Received for publication 22 August 2016; Revised 13 March 2017; Accepted 14 March 2017.

DOI: 10.1002/hbm.23587

Published online 31 March 2017 in Wiley Online Library (wileyonlinelibrary.com).

lipoprotein (HDL) or triglycerides, which is interesting as LDL levels have been linked to increased risk for cardiovascular disease, whereas HDL levels appear inversely related to such disease. These findings provide novel insight into the deleterious effects of cholesterol on brain health and suggest that cholesterol accelerates the impact of age on neural trajectories by disrupting connectivity in circuits implicated in integrative processes and behavioral control. *Hum Brain Mapp 38:3249–3261, 2017*.

Key words: aging; caudate nucleus; cognition; graph theory; lipids; magnetic resonance imaging; risk factors; temporal lobe; veterans

INTRODUCTION

Aging across the lifespan is associated with well-described trajectories of cognitive and neural alterations [Park and Reuter-Lorenz, 2009]. Cognitive abilities such as psychomotor speed, working memory, and inhibitory control tend to increase steadily during childhood, plateau during early adulthood, then decline at a relatively steady rate [Craik and Bialystok, 2006]. These cognitive changes are accompanied by significant shifts in both brain structure and functional organization. For example, late life is associated with reductions in both gray and white matter volume [Raz et al., 2005], along with weaker functional connectivity within certain networks (e.g., default-mode network, [Damoiseaux et al., 2008]). Evidence also points toward a pattern of late-life dedifferentiation in neural networks, whereby weaker coupling is observed *within* certain network modules (sets of highly interconnected brain regions; e.g., default mode, salience) and stronger coupling is found *between* such modules [Ferreira and Busatto 2013; Geerligs et al., 2014; Goh, 2011]. Combined with evidence of greater inter-correlation among cognitive abilities in older adults, this pattern of findings suggests that late-life individuals experience greater difficulty selectively recruiting specific neural circuits and related cognitive abilities [Cabeza et al., 2002].

Mounting evidence indicates that metabolic factors, particularly those related to cardiovascular disease, intensify this normative pattern of age-related cognitive decline. For example, higher total serum (blood) cholesterol (TC) in *mid-life* predicts worse *late-life* cognitive capacity [Solomon et al., 2009]. Interestingly, late-life cognitive function is not related to *concurrent* TC levels (only mid-life TC), suggesting that the impact of TC on age-related neural trajectories occurs during middle age [van Vliet et al., 2009]. Other key risk factors for cardiovascular disease, including hypertension, obesity, and diabetes mellitus, have also been linked to age-related cognitive decline [see Morra et al., 2013, for review]. In addition, growing evidence suggests that extreme, chronic stress exposure is an environmental risk factor that potentiates accelerated aging [Wolf et al., 2016] and links cardio-metabolic pathologies with neurocognitive decline [Wolf et al., 2016]. Thus, stress-related psychiatric conditions, such as posttraumatic stress disorder (PTSD) may accelerate age-related changes in neural functioning, particularly in highly traumatized groups. Military Veterans are at particularly high risk for both cardiovascular disease [Hoerster et al., 2012] and extreme stress exposure and chronic PTSD from warzone trauma, and consequently may be particularly vulnerable to premature age-related neural decline related to cardiovascular disease [Miller and Sadeh, 2014].

Despite accumulating evidence that cardiovascular disease risk factors impact age-related cognitive decline, the specific neural mechanisms by which these processes occur remains largely unknown. Emerging structural research in mid- to late-life individuals indicates that TC has a widespread influence on both gray and white matter integrity. For example, TC has been linked to reduced integrity of several white matter tracts, including longitudinal frontotemporal fibers [Williams et al., 2013]. Although these findings provide insight into the structural correlates of TC, there remains a dearth of research on the impact of TC on functional brain *networks* (i.e., communication between regions). Given that networks are a fundamental unit of brain organization [Bressler, 1995; McIntosh, 1999], an in-depth characterization of connectivity patterns is a crucially important step in elucidating the precise neural mechanisms by which TC may intensify age-related cognitive decline. Thus, the focus of the present investigation is to characterize how the interplay between TC and age impacts functional brain networks.

Recent advances in graph theory and related methods allow for brain networks to be assayed at a level of complexity not previously possible [Atluri et al., 2013; Turk-Browne, 2013]. Graph theory tools can identify disturbances in clusters of network connections and elucidate key organizational properties of the global network, sub-networks, and the function of nodes (brain regions) within local and global networks [Rubinov and Sporns, 2010]. Categories of graph properties include: *Centrality*—the extent to which a particular node facilitates network intercommunication, and *Functional Integration*—how well a network combines information across distributed regions [Rubinov and Sporns, 2010]. Thus, examining graph properties can help to delineate the complex mechanisms by which TC may alter network structure and, in turn, intensify age-related cognitive decline.

To address these critical questions, we applied graph theory to resting-state functional magnetic resonance imaging (fMRI) in a large sample ($N = 206$) of early- to mid-life

Veterans with trauma exposure (age range = 19–62, mean = 32). Although any of the cardiovascular disease risk factors mentioned above may prove informative for understanding the impact of cardiovascular disease on age-related neural changes, the present work focused specifically on TC for several reasons. First, hypertension is potentially problematic for the study of functional networks, because blood pressure directly impacts the blood-oxygenation-level dependent response [Kalisch et al., 2001], and thus hypertension-related network differences are more likely to be due to uninteresting methodological variance. Second, the pathway by which obesity (and related constructs such as body-mass index) impacts cardiovascular disease is thought to involve factors such as elevated cholesterol and hypertension [Van Gaal et al., 2006]. As such, cholesterol appears closer in the causal pathway to cardiovascular disease than obesity. Third, the incidence of diabetes in this sample is 1%, as expected given the age range of our sample. We focused on total cholesterol (TC) rather than constituent factors (i.e., low-density lipoprotein [LDL], high-density lipoprotein [HDL], triglycerides), because it remains unknown which, if any, of these constituent factors are particularly impactful on neural networks. Thus, our primary analyses examined TC, and we conducted subsidiary analyses to identify which of constituent factors were driving the observed effects.

We focused on the early- to mid-life (as opposed to late-life) age range, given that higher TC and other forms of vascular risk in this period are associated with adverse events in later life, such as increased vulnerability for cognitive decline [van Vliet et al., 2009]. Thus, neural evidence of premature aging may be evident earlier than the cognitive declines observed in later life. We also performed exploratory analyses testing whether PTSD exacerbates the impact of TC and age on brain networks and cognitive health, which was possible given that our sample consisted of Veterans with large variation in stress-related pathology. Finally, to further characterize observed differences in network properties, we examined the relationship between network findings and an index of day-to-day functional disability, along with several measures of neuropsychological function.

MATERIALS AND METHODS

Participants

Veterans of Operations Enduring Freedom, Iraqi Freedom, and New Dawn were recruited via outreach at military-associated events. Study exclusion criteria were: history of neurologic illness or seizure disorders unrelated to head injury, current diagnosis of schizophrenia, bipolar, or other psychotic disorder, severe depression or anxiety, current active homicidal and/or suicidal ideation with intent, cognitive disorder due to a general medical condition other than traumatic brain injury (TBI), and psychological diagnosis that would interfere with accurate data collection. Data was collected from 221 participants, and data for 15 participants were excluded from analyses due to: missing TC ($n = 3$), susceptibility artifact ($n = 4$), motion > 3 mm ($n = 8$). The final sample size was 206 (9.7% female, mean age = 32.3, SD = 8.7; 69% White, 10% Black/African American, 16% Hispanic/Latino, 1% American Indian, 3% Asian). Ninety-five percent of the sample had one or more deployments (1 deployment = 64%, 2 = 26%, >2 = 5%) and the remainder were pre-deployed. All research was conducted with the understanding and written consent of each participant.

Assessment

Cholesterol

TC (mean = 182.2, SD = 36.3), HDL (mean = 47.8, SD = 12.4), LDL (mean = 112.7, SD = 32.3), and triglycerides (mean = 133.0, SD = 98.3) were assessed via a fasting blood panel (Quest Diagnostics). Triglyceride level for one participant was found to be an extreme outlier and was reigned in to the 2.5 standard deviation level. Reliability information for cholesterol measures was not available for the present data, in particular. However, Quest Diagnostics participates in the College of American Pathologists proficiency testing program, and the results of this testing indicates very high reliability. For example, coefficients of variation (lower values indicate higher reliability) for January 2016 for the laboratory where the present testing was completed were: TC = 1.4%, HDL = 2.5%, and triglycerides = 1.9%. Thus, these data indicate that cholesterol measurement via the methods used herein are highly stable and reliable.

Figure 1 displays a histogram of age, plotted separately for low and high TC (as determined by a median split). The TC median was 180 mg/dL, and values above this point are indicative of clinically elevated TC levels [Stamler et al., 1986].

Posttraumatic stress disorder

Current PTSD diagnosis was assessed via the Clinician Administered PTSD Scale [Blake et al., 1995], a semi-structured clinical interview (51.5% of the sample met criteria).

Functional disability

Functional disability within the past 30 days was assessed via the World Health Organization Disability Assessment Schedule II (WHODAS) [World Health Organization, 2001]. We summed two subscales to create one measure of disability. The first measured the number of days in which the participant was "totally unable to carry out…usual activities or work," and the second measured the number of days in which the participant had to "cut back or reduce…usual activities" (excluding days in which participants were "totally unable").

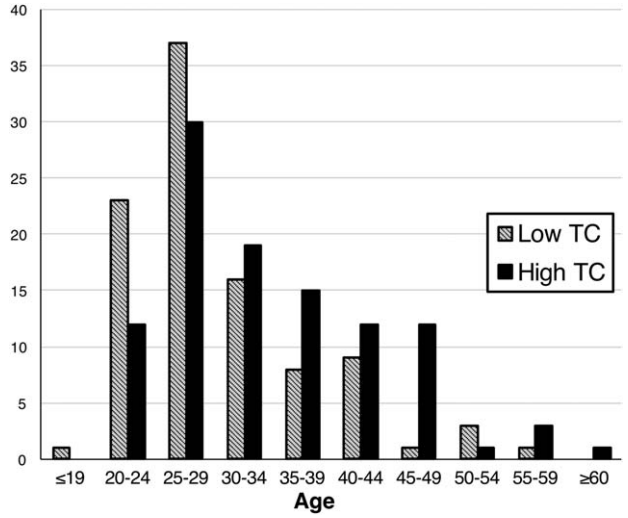


Figure 1.

Histogram of age separated by low and high total cholesterol. The height of each column reflects the number of participants whose age falls between in range denoted by the corresponding label on the *x*-axis, plotted separately for low and high total cholesterol (TC). Low TC participants had TC < 180 mg/dL and are represented by striped bars, whereas high TC participants had TC > 180 mg/dL and are represented by solid black bars.

Traumatic brain injury

TBI was examined as a covariate in analyses and was assessed via the Boston Assessment of TBI-Lifetime [Fortier et al., 2013], a semi-structured clinical interview.

MRI Acquisition and First Level Processing

Acquisition

Participants were instructed to remain still with eyes open while 2 EPI runs (voxel size = 3 × 3 × 3.75 mm, TR = 3,000 ms, TE = 30 ms, scan time per run = 360 s, grid = 64 × 64, # of slices = 38) were acquired on a Siemens 3T TIM Trio. Full brain coverage was achieved with the exception of cerebellum, which was thus not examined. Two MPRAGEs (voxel size = 1 × 1 × 1.2 mm, T1 = 1,000 ms, TR = 2,530 s, TE = 3.32 ms, grid = 240 × 256, # of slices = 160) were acquired and averaged to create a single high contrast-to-noise image.

FreeSurfer processing

Individualized cortical parcellations and subcortical segmentations were created via FreeSurfer [Fischl et al., 2002]. Cortical surface models were manually checked slice-by-slice and edited for accuracy. The Desikan/Killany parcellation was used (34 regions per hemisphere), along with subcortical segmentation (7 regions per hemisphere). Frontal pole was excluded due to frequent susceptibility artifact. Node maps were transformed from each participant's anatomical space to functional space via a 6 dof (i.e., rigid body) affine registration implemented by FSL's FLIRT.

Preprocessing of resting state fMRI

Data were preprocessed using the Graph Theory GLM (GTG) toolbox version 0.42 [Spielberg et al., 2015] (www.nitrc.org/projects/metalab_gtg; RRID: SCR_014075). Data were motion corrected, detrended (linear and quadratic), bandpass filtered (retaining 0.1–0.10 Hz), and the mean global, ventricular, and white matter signals were partialled out, along with estimated motion parameters. Timeseries for FreeSurfer nodes were extracted by calculating mean signal across node for each time point, for each EPI run. Timeseries for the EPI runs were concatenated after mean-centering each timeseries within run, and an 80 × 80 Pearson correlation matrix created for each participant. The top panel of Figure 2 displays the mean connectivity matrix across all participants, organized by functional module (see below for description of modules and the procedures used to derive these modules). The bottom panel of Figure 2 displays a histogram of connectivity values across all nodes and participants, along with the distributions for each participant divided by older/younger participants with higher/lower TC.

To insure that our choice of particular preprocessing stream did not drive findings, preprocessing was repeated with other options: (i) slice-timing correction, (ii) partialling of the square, autocorrelation, and first derivative of motion parameters, (iii) partialling the first derivative of global, ventricular, and white matter signals, (iv) determination on a participant-specific basis whether partialling of global signal was necessary (via the Global Negative Index), (v) motion scrubbing. Connectivity matrices remained extremely similar (correlations > 0.97), indicating that present findings were not specific to our choice of preprocessing stream.

Identification of Age/Cholesterol/PTSD-Related Network Connections

To identify network connections that varied with age, TC, and/or PTSD, connectivity matrices were entered as dependent variables into the Network Based Statistic (NBS) toolbox [Zalesky et al., 2010]. The first model contained age, TC, and PTSD diagnosis as predictors, the 2-way interactions were added in a second model, and the 3-way interaction was added in a third model. In NBS, the regression model is first tested for each individual link, following which a *t*-threshold is applied ($t < 3.25$ in the present study) to remove unassociated links. Next, clusters of supra-threshold links (i.e., sets of links that share a node with at least one of the other links in the cluster) are located, and the *corrected* significance of each cluster is computed based on the number of links in that cluster. In particular, a corrected *P*-value is calculated by comparing

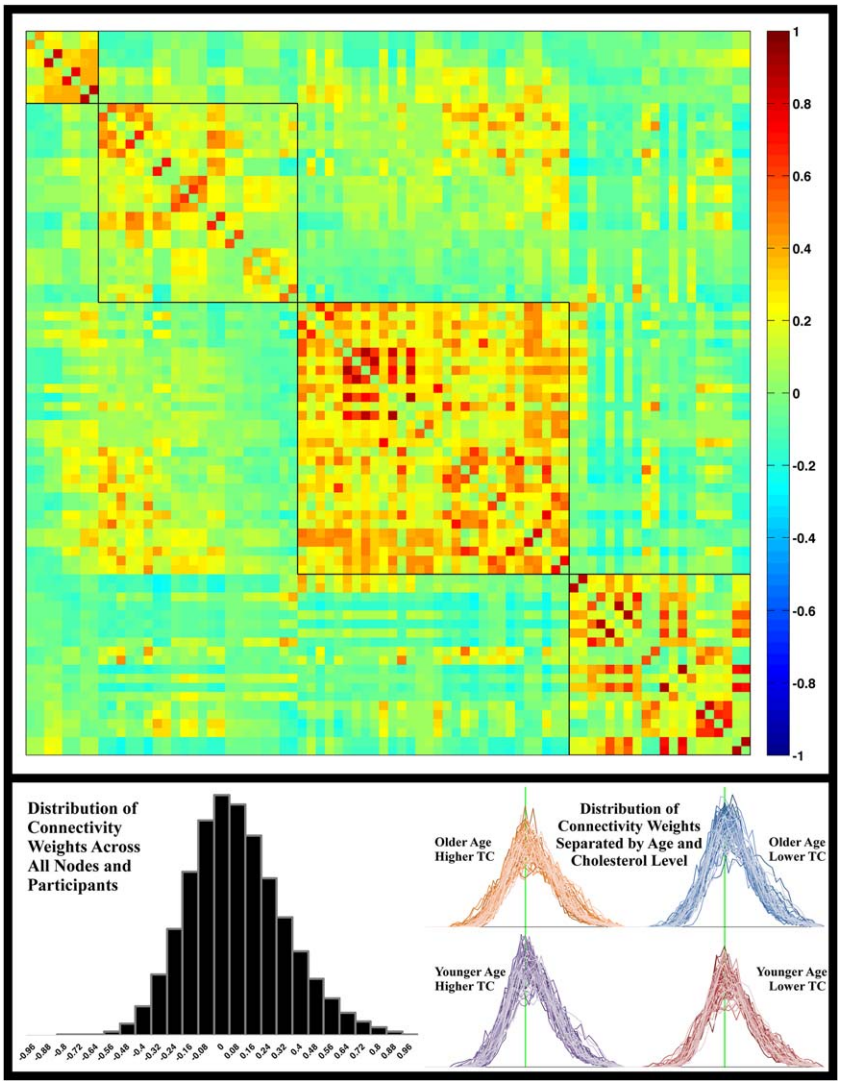


Figure 2.

Mean connectivity matrix and histograms of connectivity values across all participants. Top panel: Each row/column reflects one node, and each cell reflects the mean connectivity (Pearson correlation) across all participants between the row and column nodes. The black lines delineate the four modules. Bottom panel: The black histogram reflects connectivity values across all nodes and participants. The height of each column reflects the number of correlations whose value falls between ± 0.04 of the corresponding label on the *x*-axis (e.g., the height of the column above 0.08 reflects the number of correlations between 0.04 and 0.12). The four small images on the right reflect the distributions of connectivity values for older participants with higher cholesterol (image with orange lines), older participants with lower cholesterol (blue lines), younger participants with higher cholesterol (purple lines), and younger participants with lower cholesterol (red lines) (older/younger determined via median split, higher/lower cholesterol split at 180 mg/dL). For each of the four image, each line reflects the distribution (across nodes) for a single participant, and the vertical green line marks the 0 bin. [Color figure can be viewed at wileyonlinelibrary.com]

the observed size against a null distribution of maximal supra-threshold cluster sizes created via a non-parametric permutation approach (5,000 randomizations in the present study). This leaves an overall corrected $\alpha < 0.05$.

All models contained TBI and ethnicity nuisance covariates. To decompose significant interactions, the sample was divided at the median of the moderator, and regressions computed in each group.

Relationship to Functional Disability and Cognitive Function

To determine the impact of connectivity differences on day-to-day functioning and cognitive processes, mean connectivity in significant networks was correlated with a measure of functional disability in the last 30 days (WHODAS) and neuropsychological indices assaying four domains of cognitive functioning: psychomotor speed, inhibition/switching, working memory, and episodic memory. The relationship between neuropsychological indices/WHODAS and age/TC/PTSD were also examined.

We examined four domains of cognitive functioning: psychomotor speed, inhibition/switching, working memory, and episodic memory. Psychomotor speed was assessed using average reaction time for correct responses on the Simple Reaction Time (SRT) task of the Cambridge Neuropsychological Test Automated Battery [Sahakian and Owen, 1992]. Inhibition/switching were measured using the Inhibition and Inhibition/Switching subtests from the Delis-Kaplan Executive Function System Color-Word Interference Test [Delis et al., 2001]. Specifically, performance on each subtest was measured as the sum of the total time and error standard scores. Working memory was assessed using the total standard score of the Digit Span subtest of the Wechsler Adult Intelligence Scale, Fourth Edition [Wechsler, 2008], and episodic memory was measured using delayed free recall on the California Verbal Learning Test – second Edition [Delis et al., 2000]. False discovery rate [Benjamini and Yekutieli, 2010] was used to correct (across tests) for multiple comparisons, and corrected *P*-values are in brackets.

Mediation Analyses

For all neuropsychological indices significantly related to both age/TC/PTSD and an extracted network, we tested whether network connectivity mediated the relationship between age/TC/PTSD and neuropsychological performance. Analyses were computed using the PROCESS macro (PROCESS model 4) [Hayes, 2013] in SPSS v20 with bias-corrected bootstrapped confidence intervals (CI). For networks related to interactions, moderated mediation analyses were carried out (PROCESS model 7, see top of Fig. 5 for an example of this type of model).

Identification of Age/Cholesterol/ PTSD-Related Graph Theory Properties

To identify graph theory properties that varied with age, TC, PTSD, and/or their interactions, graph properties were computed and tested as dependent variables in robust regressions in the GTG toolbox [Spielberg et al., 2015] (www.nitrc.org/projects/metalab_gtg). The GTG toolbox computes properties for each participant using the Brain Connectivity Toolbox [Rubinov and Sporns, 2010]. Before graph properties were computed, each participant's connectivity matrix was thresholded to include only positive weights and normalized via division by the median positive (excluding zeros) weight for each matrix. Normalization was performed to remove bias due to individual differences in overall network weight.

Three graph properties were examined, one indexing *Functional Integration* (Global Efficiency) and two indexing *Centrality* (Participation Coefficient, Within-Module Degree *Z*-Score). Global Efficiency assays the efficiency of global network communication. Specifically, Global Efficiency is the mean of the (inverse) shortest paths between all nodes (i.e., the extent to which nodes are connected to each other through the fewest possible number of nodes). Participation Coefficient assays the diversity of between-module communication (i.e., the extent to which a node is connected to other modules, relative to its overall connectivity). Within-Module Degree *Z*-Score assays within-module communication (i.e., the extent to which a node is connected to other nodes within its own module). Formally defined, modules are sets of regions having more within- than between-module coupling. Modularity was computed on the mean (across sample) network using the Louvain algorithm followed by the Kernighan-Lin fine-tuning algorithm. The Louvain algorithm is a greedy optimization method that attempts to maximize *Q* modularity, which is the sum of the within-module connectivities minus what would be expected by chance. In the Louvain algorithm, each node is first assigned to its own module, after which a node is randomly selected and the change in modularity due to moving that node to each neighboring node's community is calculated. The move with the maximum change is made, and this is repeated for all nodes until no increase can be achieved. Next, a new network is created wherein each module (i.e., set of nodes) becomes a node and the links between these new nodes are the sum of the links for each of the component nodes, and the process begins anew using the new network. This loop is applied until no further increase can be made, and the final "nodes" reflect the modules. Thus, the number of modules is derived from the data rather than defined a priori. This module assignment is then passed to a variant of the Kernighan-Lin algorithm, also a greedy optimization method, which performs fine-tuning using a similar procedure. Given that the selection of nodes is random, this method is not determinant. Thus, this entire process is (independently) repeated 10,000 times, and the partition with the highest ultimate modularity is chosen.

Module membership: module 1 (red nodes in Figs. 3 and 4) = caudate, putamen, pallidum, thalamus. Module 2 (green nodes) = amygdala, hippocampus, nucleus accumbens, medial orbitofrontal cortex, lateral orbitofrontal cortex, inferior frontal gyrus pars orbitalis, rostral anterior cingulate cortex, entorhinal cortex, middle temporal gyrus, inferior temporal gyrus (ITG), temporal pole. Module 3 (yellow nodes) = insula, superior frontal gyrus, rostral

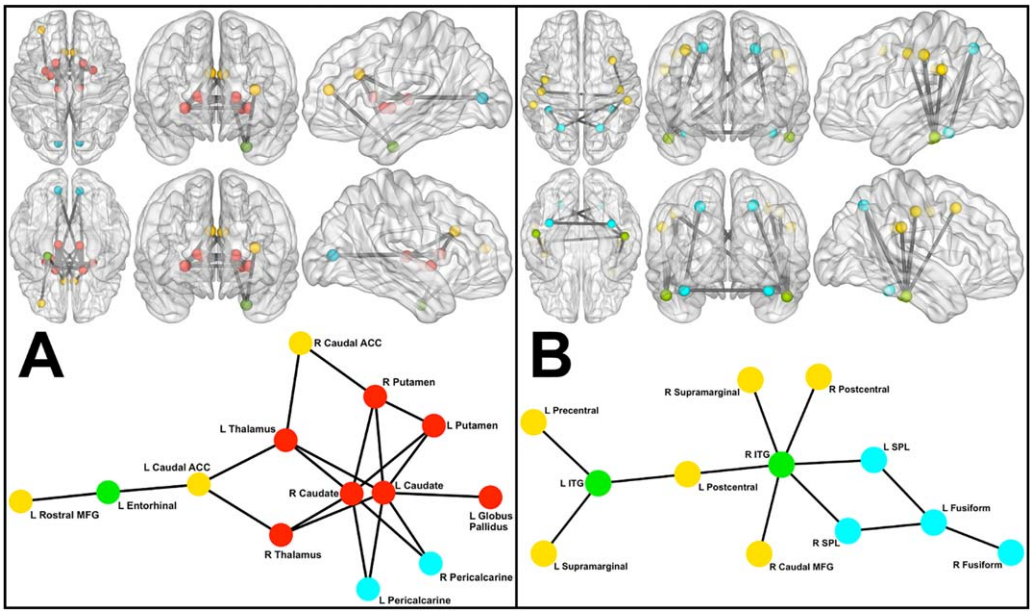


Figure 3.

Networks in which connectivity strength is negatively related to age. Representation of the two significant networks in which connectivity strength was negatively related to age. Correction for multiple comparison (across connections) was carried out via permutation-based cluster correction. Circle/sphere color reflects module. The module represented by red nodes consists of striatal and thalamic nodes; the module represented by green nodes consists of subcortical and inferior frontal and temporal nodes, along with subcortical nodes; the module represented by yellow nodes consists largely of superior frontal, temporal, and parietal nodes; the modules represented by the blue nodes consists of occipital and posterior parietal and temporal nodes (see text for specific membership). Stick/ball figures created using Kamada-Kawai spring embedder algorithm. R = right; L = left; ACC = anterior cingulate cortex; MFG = middle frontal gyrus; ITG = inferior temporal gyrus; SPL = superior parietal lobule. For both **A** and **B**, the six 3d brain images at the top of each panel show (clockwise from top left) an axial view from superior to the brain, a coronal view from anterior to the brain, a sagittal view from left of the brain, a sagittal view from right of the brain, a coronal view from posterior to the brain, and an axial view from inferior to the brain. [Color figure can be viewed at wileyonlinelibrary.com]

middle frontal gyrus, caudal middle frontal gyrus, inferior frontal gyrus pars opercularis, inferior frontal gyrus pars triangularis, caudal anterior cingulate cortex, posterior cingulate cortex, banks of the superior temporal sulcus, superior temporal gyrus, transverse temporal gyrus, precentral gyrus, postcentral gyrus, paracentral lobule, supramarginal gyrus. Module 4 (blue nodes) = isthmus of the cingulate, fusiform gyrus, parahippocampus, precuneus, superior parietal lobule, inferior parietal lobule, cuneus, lateral occipital cortex, lingual gyrus, pericalcarine cortex.

Importantly for the present work, Participation Coefficient and Within-Module Degree Z-Score can be used to assess (age-related) network dedifferentiation (i.e., weaker Within-Module Degree *Z*-score and stronger Participation Coefficient). Node-specific properties were examined only for the node with the highest number of differential connections in the equivalent NBS analysis. Properties were entered as dependent variables in robust regressions in the GTG toolbox. Predictor models were the same as NBS analyses. However, relationships were tested only for the predictor that was significant in the equivalent NBS analysis. Significance was determined via permutation tests (5,000 repetitions). False discovery rate was used to correct (across properties) for multiple comparisons, and adjusted *P*-values are in brackets. Significant interactions were decomposed as described above.

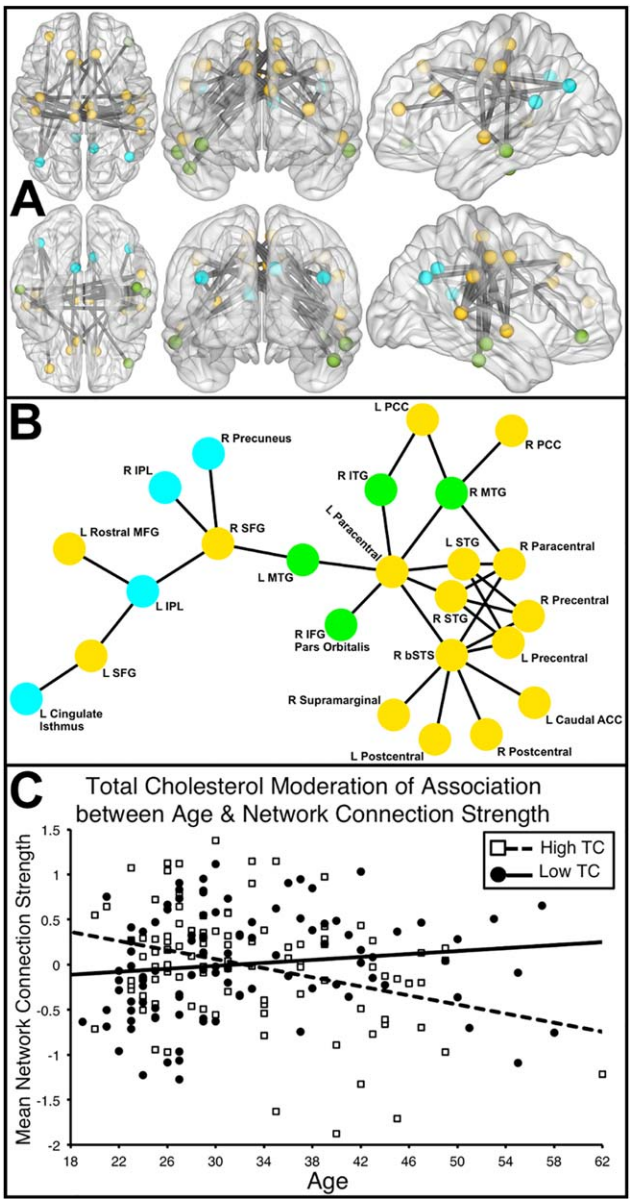
Analyses to Rule out Confounds

Although TBI and ethnicity were included as nuisance covariates in all NBS and GTG analyses, it remains possible that findings were driven by other potential confounding factors. To rule this out, analyses were rerun with additional nuisance covariates: gender, education, current/past DSM-IV Axis I diagnoses (assessed via the Structured Clinical Interview for the DSM-IV), alcohol use (assessed via Lifetime Drinking History [Skinner and Sheu, 1982] and the Short Michigan Alcohol Screening Test [Selzer et al., 1975]), body mass index, weight, blood pressure, cardiometabolic syndrome, diabetes mellitus, cigarette use,

current prescription medication use, and average and maximum estimated motion. Medication categories tested were: antihypertensive (used by 8% of the sample), cholesterol (4%), diabetes (0.4%), antidepressant (19%), antiepileptic (6%), sedative/hypnotic (5%), and pain (23%). Importantly, all analyses remained significant when partialling out variance associated with these measures.

RESULTS

For all analyses, only significant findings are reported. At the bivariate level, PTSD was not correlated with TC ($r = -0.01$, $P = 0.90$) or age ($r = -0.13$, $P = 0.06$). TC was correlated with age, albeit relatively weakly ($r = 0.28$, $P < 0.01$).



Identification of Age/TC/PTSD-Related Networks

Increased age was associated with weaker connectivity in two networks, one of which was largely subcortical (13 nodes, 20 links, corrected $P < 0.01$, $\Delta R^2 = 0.24$, Fig. 3A) and the other temporoparietal (12 nodes, 12 links, corrected $P = 0.01$, $\Delta R^2 = 0.13$, Fig. 3B). Mean connectivity in the subcortical network evidenced a significant negative relationship with psychomotor speed (SRT; $r = -0.22$, $P < 0.01$ [corrected $P = 0.01$]), indicating that weaker network connectivity is associated with slower psychomotor speed.

When the age × TC interaction was examined, we discovered a network (24 nodes, 31 links, corrected $P < 0.01$, $\Delta R^2 = 0.18$, Fig. 4) centered on the banks of the right superior temporal sulcus in which participants with relatively higher TC evidenced age-related decoupling. To decompose this significant interaction, the sample was divided at the TC median, and regressions computed in each group. These analyses revealed that age was a significant predictor of decoupling in the high ($P < 0.01$) but not the low TC group. Across all participants, mean connectivity in this network evidenced a significant negative relationship with disability (WHODAS; $r = -0.19$, $P < 0.01$ [corrected $P = 0.01$]), with decoupling in this network predicting greater real-world functional impairment. Mean connectivity also evidenced a negative relationship with psychomotor speed (SRT; $r = -0.20$, $P < 0.01$ [corrected $P = 0.01$]) and a positive relationship with inhibition/switching ($r = 0.20$,

Figure 4.

Total cholesterol moderating the association between age and network coupling. Representation of the significant network associated with the interactive effect of age and total cholesterol. Specifically, connectivity strength in this network was negatively associated with age in participants with higher levels of cholesterol, whereas a positive relationship was found for participants with lower cholesterol levels. Correction for multiple comparisons (across connections) was carried out via permutation-based cluster correction. Circle/sphere color reflects module. The module represented by green nodes consists of subcortical and inferior frontal and temporal nodes; the module represented by yellow nodes consists largely of superior frontal, temporal, and parietal nodes; the modules represented by the blue nodes consists of occipital and posterior parietal and temporal nodes (see text for specific membership). TC = total cholesterol; R = right; L = left; ACC/PCC = anterior/posterior cingulate cortex; SFG/MFG/IFG = superior/middle/inferior frontal gyrus; STG/MTG/ITG = superior/middle/inferior temporal gyrus; bSTS = banks of the superior temporal sulcus; IPL = inferior parietal lobule. The six 3d brain images in panel **A** show (clockwise from top left) an axial view from superior to the brain, a coronal view from anterior to the brain, a sagittal view from left of the brain, a sagittal view from right of the brain, a coronal view from posterior to the brain, and an axial view from inferior to the brain. Panel **C** visualizes the relationship between age and connection strength for high and low total cholesterol based on median split (180 mg/dL). [Color figure can be viewed at wileyonlinelibrary.com]

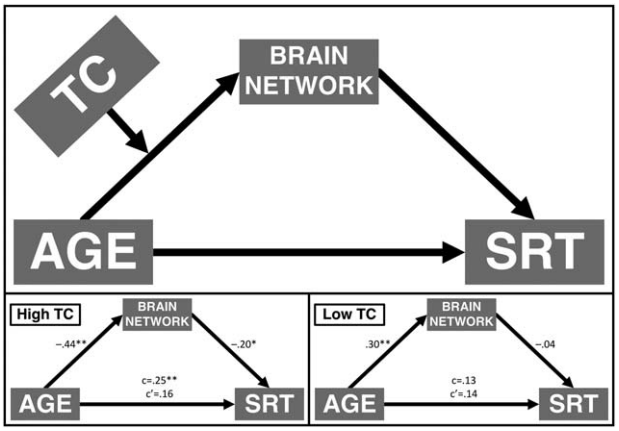


Figure 5.

Network connectivity mediating relationship between age and psychomotor speed differentially by cholesterol level. The top panel depicts the overall (moderated mediation) model. The bottom left and right panels decompose the age × total cholesterol interaction by depicting the mediation aspect of the model at high and low levels of total cholesterol, respectively (as determined via a total cholesterol median split at 180 mg/dL). Thus, the bottom two panels reflect splitting of the top model by total cholesterol levels. The indirect effect was significant for high (bottom left) but not low (bottom right) TC. Numbers represent regression beta values. BRAIN NETWORK = mean connection strength for the network associated with the age × TC interaction; SRT = simple reaction time (indexing psychomotor speed); c = direct path of age to SRT *without* the brain network variable in the model; c' = direct path of age to SRT *with* the network variable in the model; * = $P < 0.05$; ** = $P < 0.01$.

$P < 0.01$ [corrected $P = 0.01$]), both of which indicate that weaker network connectivity is associated with worse task performance.

To determine which components of TC accounted for the age × TC interaction, regressions were computed substituting HDL, LDL, or triglycerides for TC. LDL significantly interacted with TC to predict mean connectivity ($P < 0.01$), whereas HDL and triglycerides did not. To determine whether findings were being driven solely by relatively older participants, all analyses were repeated after excluding participants older than 40 ($n = 39$, leaving $n = 167$ for analysis). Importantly, all analyses remained significant in this subsample.

No significant main or interactive effects emerged for PTSD diagnosis, and no main effects emerged for TC.

Network Connectivity as a Mediator of Age-Related Decline in Psychomotor Speed

Given that increasing age was also directly related to slower psychomotor speed in our sample ($r = 0.25$, $P < 0.01$ [corrected $P < 0.01$]), we assessed whether mean connectivity in the largely subcortical age-related network mediated this relationship (for parameter scaling reasons, all variables were *z*-scored for these analyses). Mean connectivity in this network was found to be a significant mediator of the relationship between age and psychomotor speed (indirect effect $\beta = 0.06$, 95% CI = [0.01, 0.13]).

For the network related to the age × TC interaction, we conducted moderated mediation (see top panel of Fig. 5), with age as the independent variable, mean network connectivity as the mediator, TC as the moderator of the age↔network connectivity relationship, and psychomotor speed as the dependent variable. Moderated mediation was significant (MM index = 0.06, 95% CI = [0.01, 0.12]). To decompose this effect, we examined mediation separately in high and low TC groups, based on median split (≥180 mg/dL). The indirect effect of age on slower psychomotor speed via network decoupling was significant in the high (indirect effect $\beta = 0.09$, 95% CI = [0.02, 0.21], bottom left of Fig. 5) but not low ($\beta = -0.01$, 95% CI = [–0.07, 0.02], bottom right of Fig. 5) TC group.

To determine whether findings were being driven solely by relatively older participants, all analyses were repeated after excluding participants older than 40. Importantly, all analyses remained significant in this subsample.

Age/TC/PTSD Associations With Graph Theory Properties

Age was negatively related to Global Efficiency ($P < 0.01$ [corrected $P = 0.02$], $\Delta R^2 = 0.03$), indicating that relatively older (i.e., middle age) participants had less efficient overall communication in the global network. Age was also negatively related to Participation Coefficient for both left caudate ($P = 0.03$ [corrected $P = 0.03$], $\Delta R^2 = 0.02$) and right ITG ($P = 0.01$ [corrected $P = 0.02$], $\Delta R^2 = 0.04$), indicating that these regions interact with fewer modules in middle age participants. Finally, age was negatively related to Within-Module Degree *Z*-Score for left caudate ($P = 0.01$ [corrected $P = 0.02$], $\Delta R^2 = 0.03$), indicating that this region had weaker connections with nodes within its module in middle age participants.

The interaction between age and TC predicted Participation Coefficient ($P < 0.01$ [corrected $P = 0.01$], $\Delta R^2 = 0.06$) and Within-Module Degree *Z*-Score ($P < 0.01$ [corrected $P = 0.01$], $\Delta R^2 = 0.05$) for the banks of the right superior temporal sulcus. Follow-up analyses revealed that age was positively related to Participation Coefficient in the high TC group ($P = 0.03$) and was not a significant predictor at lower TC. Follow-up analyses also indicated that age related negatively to Within-Module Degree *Z*-Score in high TC and positively in low TC, although this was not significant in either group, indicating that the interaction was driven by the relative differences in slope across TC levels. Thus, age-related network dedifferentiation (i.e., higher Participation Coefficient and lower Within-Module Degree *Z*-Score with age) was found for participants with higher TC.

To determine which components of TC accounted the age × TC interaction, regressions were computed substituting HDL, LDL, or triglycerides for TC. Only LDL significantly interacted with age to predict graph properties for the banks of the right superior temporal sulcus (Participation Coefficient $P = 0.01$, Within-Module Degree *Z*-Score $P < 0.01$). To determine whether findings were being driven solely by relatively older participants, all analyses were repeated after excluding participants older than 40. The relationships between age and Global Efficiency and caudate Within-Module Degree *Z*-Score, along with the interaction of age and TC predicting Participation Coefficient for the banks of the right superior temporal sulcus, remained significant in this subsample. Additionally, the age × TC interaction predicting Within-Module Degree *Z*-Score for the banks of the right superior temporal sulcus was marginally significant ($P = 0.08$), suggesting that the failure to find significance in this subsample is simply due to the reduction in power (i.e., removal of 19% of the sample). The relationships between age and Participation Coefficient for caudate and ITG were not significant in this subsample, although the direction of effects remained consistent. These failures to remain significant may be due to the reduction in power and/or that these effects are more applicable to older participants.

DISCUSSION

Does TC intensify age-related declines in functional brain networks and related cognitive function? In the present study, we provide preliminary evidence that this does occur. First, we found a network centered on the banks of the right superior temporal sulcus that evidenced age-related decoupling, but *only* in participants with clinically elevated (≥180 mg/dL) TC levels (Fig. 4). Decoupling in this network was also associated with slower psychomotor speed and worse inhibition/switching (but not working or episodic memory), indicating that weaker connectivity in this network is associated with worse cognitive efficiency and control. Furthermore, network decoupling mediated age-related declines in psychomotor speed, but again only in high TC participants (Fig. 5). Consistent with these findings, network decoupling also corresponded to greater day-to-day disability, demonstrating the translational implications of this network for real-world functioning.

Second, we found that TC modulated the impact of age on two graph theory properties of the banks of the right superior temporal sulcus, each of which assays a different aspect of network organization. In particular, age was related to *higher* Participation Coefficient and *lower* Within-Module Degree *Z*-Score, but only in high TC participants.

Participation Coefficient indexes the extent to which a brain region interacts with different network modules (i.e., the diversity of between-module communication). In other words, a high Participation Coefficient indicates that a node functions as a hub for communicating with other modules. Within-Module Degree *Z*-Score assays the extent to which a region is connected to nodes within its own module. In other words, a high Within-Module Degree *Z*-Score indicates that a node functions as a hub for facilitating communication between nodes in a module. This pattern of findings is consistent with age-related dedifferentiation in higher TC participants in the network surrounding the banks of the right superior temporal sulcus. Specifically, for participants with high TC, age was related to *de*creasing *within*-module and *in*creasing *between*-module communication for the banks of the right superior temporal sulcus, whereas this was not the case for low TC participants.

The banks of the right superior temporal sulcus encompass the posterior portion of superior temporal sulcus and makes up part of temporoparietal junction (along with inferior parietal lobule). Temporoparietal junction is reciprocally connected with prefrontal and temporal cortices and receives input from an array of regions, including thalamus and perceptual regions [Decety and Lamm, 2007]. As such, temporoparietal junction is thought to play a key role in the integration of low-level information into more complex representations [Decety and Lamm, 2007]. Right temporoparietal junction, in particular, is also reliably recruited during bottom-up (stimulus-driven) attention [Corbetta et al., 2008]. Thus, the age-related dedifferentiation (in the banks of the right superior temporal sulcus) we observed in high TC may lead to inappropriate interruption of ongoing processing by stimulus-level information. More broadly, the network in which TC and age interacted to predict connectivity (Fig. 4) includes (temporal and parietal) association and (frontal) executive regions that show strong anatomical connectivity to the banks of the right superior temporal sulcus/temporoparietal junction. Thus, in the presence of higher TC, age may be associated with disruption in the integration of lower-level stimulus information into goal-directed behavior (instantiated in executive and motor regions). This hypothesis is consistent with our finding that decoupling in this network mediated the relationship between age and psychomotor speed (for high TC participants). Interestingly, although this is a relatively young cohort, the banks of the right superior temporal sulcus are highly vulnerable to neuronal changes in Alzheimer's disease [Gómez-Isla et al., 1997; Killiany et al., 2000] and the nature of the link between cholesterol and the development of Alzheimer's disease remains unclear [Ricciarelli et al., 2012]. Although highly speculative, present findings may reflect a piece of the mechanism by which higher TC levels may increase risk for Alzheimer's disease, at least in some individuals.

Follow-up analyses indicated that our findings were driven by LDL levels, rather than by HDL or triglyceride levels. LDL transports lipids (fats) extracellularly and is associated with increased risk for cardiovascular disease (e.g., atherosclerosis), whereas HDL is inversely associated with cardiovascular disease [Manninen et al., 1992].

Importantly, brain and serum (blood) cholesterol are independently regulated, and serum cholesterol does not appear to cross the blood-brain barrier (although, brain cholesterol is excreted into the blood) [Dietschy, 2009]. Thus, serum TC likely has an indirect impact on neural function, potentially via neurovascular changes. Alternatively, serum TC may serve as an indirect proxy for brain cholesterol levels linked by metabolic processes general to central and peripheral regulation. Further research using measures more closely related to brain cholesterol is needed to determine potential mechanisms of action.

Age also evidenced several independent effects on brain networks in this sample. Specifically, age was related to decoupling in two networks, one largely subcortical (Fig. 3A) and one temporoparietal (Fig. 3B). Decoupling in the subcortical network predicted slower psychomotor speed, and network decoupling mediated the relationship between age and psychomotor speed (across all participants), highlighting the behavioral importance of this network. The subcortical regions central to this network (e.g., caudate, putamen) are crucial for direct regulation of motor cortex, and thus behavior. These regions integrate an array of neural signals via dopaminergic inputs from substantia nigra and glutamatergic inputs from cingulate and dorsal regions of prefrontal cortex [Haber, 2014]. Therefore, our finding that network decoupling mediated the relationship between age and psychomotor speed may reflect a disruption in this control circuit. Age was also associated with less efficient communication across the entire brain (Global Efficiency), along with lower left caudate between- and within-module communication (Participation Coefficient, Within-Module Degree Z-Score, although these findings did not remain significant when excluding participants over 40). Thus, overall connectivity for this region appears to be reduced as age increases, rather than evidencing age-related dedifferentiation as found for the banks of the right superior temporal sulcus.

Interestingly, no effects (main or interaction with age and/or TC) were found for PTSD diagnosis. This may be due to the complex relationship between stress, TC, and cognitive health in the aging process, which may not be reflected in differential brain network topology. Regardless, research examining the effects of stress exposure on physical health and neural network functioning over the life course will be important for explaining the elevated rates of neurodegenerative disorders in samples with chronic stress exposure [Yaffe et al., 2010].

Strengths and Limitations

The present work benefited from a number of strengths, including a large sample size, extensive characterization of medical and clinical phenomenon, and the use of analysis methods that assay network function at a high level of complexity. As with any study, some limitations must be considered. For example, as mentioned above, it is possible that serum TC influenced brain circuitry via its impact on neurovascular function (e.g., atherosclerosis). Given that the fMRI signal is dependent on blood flow, present findings may reflect overall vascular differences (i.e., reduced blood flow) rather than connectivity per se. However, vascular reduction would likely have a more widespread impact than the relatively small network observed herein, which makes up less than 1% of the potential connections. Thus, it is unlikely that our findings are primarily driven by overall vascular reduction. However, future research that corrects for overall vascular response (e.g., via measurement of hypercapnia) is needed before firm conclusions can be drawn. In addition, it is possible that the use of a Veteran sample influenced present findings. Future studies in civilian cohorts are needed to assess the generalizability of the present work.

Although not a limitation, per se, it is important to note that the present work focused on *functional* networks, which do not map directly onto anatomical (white matter) connections. Thus, observed differences in connectivity may not be directly anatomically mediated (via white matter). Future work should conduct similar network analyses on white matter networks (via diffusion-weighted imaging) to determine what aspects of present findings are reflected anatomically.

Despite these limitations, present work provides novel preliminary evidence that TC intensifies age-related brain network decoupling that, in turn, impacts cognitive function. Given the relatively young age of the sample, it is noteworthy that we observed age-related decline in network connectivity that was associated with real-world functional impairment. Present findings provide novel insight into the deleterious effects of cholesterol on brain health in early to midlife and suggest that cholesterol accelerates the impact of age on neural trajectories by disrupting network connectivity in regions implicated in integrative processes and behavioral control.

ACKNOWLEDGMENTS

We thank Wally Musto for his tireless recruitment efforts on our behalf, as well as the entire TRACTS team for their assistance with data collection and management, and in particular Drs. Corbo and Robinson for MRI data collection/processing. All authors report no conflicts of interest. Authors declare no competing financial interests.

REFERENCES

Atluri G, Padmanabhan K, Fang G, Steinbach M, Petrella JR, Lim K, MacDonald A, Samatova NF, Doraiswamy PM, Kumar V (2013): Complex biomarker discovery in neuroimaging data: Finding a needle in a haystack. Neuroimage Clin 3:123–131.

Benjamini Y, Yekutieli D (2010): The control of the false discovery rate in multiple testing under dependency. Ann Stat 29: 1165–1188.

Blake DD, Weathers FW, Nagy LM, Kaloupek DG, Gusman FD, Charney DS, Keane TM (1995): The development of a Clinician-Administered PTSD Scale. J Trauma Stress 8:75–90.

Bressler SL (1995): Large-scale cortical networks and cognition. Brain Res Rev 20:288–304.

Cabeza R, Anderson ND, Locantore JK, McIntosh AR (2002): Aging gracefully: Compensatory brain activity in high-performing older adults. Neuroimage 17:1394–1402.

Corbetta M, Patel G, Shulman GL (2008): The reorienting system of the human brain: From environment to theory of mind. Neuron 58:306–324.

Craik FI, Bialystok E (2006): Brain changes in development and aging. Trends Cogn Sci 3:131–138.

Damoiseaux JS, Beckmann CF, Arigita ES, Barkhof F, Scheltens P, Stam CJ, Smith SM, Rombouts SARB (2008): Reduced resting-state brain activity in the "default network" in normal aging. Cereb Cortex 18:1856–1864.

Decety J, Lamm C (2007): The role of the right temporoparietal junction in social interaction: How low-level computational processes contribute to meta-cognition. Neuroscientist 13: 580–593.

Delis DC, Kaplan E, Kramer JH. (2001): Delis-Kaplan Executive Function System (D-KEFS). New York: Psychological Corporation.

Delis DC, Kramer JH, Kaplan E, Ober BA. (2000): CVLT-II. New York: Psychological Corporation.

Dietschy JM (2009): Central nervous system: Cholesterol turnover, brain development and neurodegeneration. Biol Chem 390: 287–293.

Ferreira LK, Busatto GF (2013): Resting-state functional connectivity in normal brain aging. Neurosci Biobehav Rev 37:384–400.

Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, Van Der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montillo A, Makris N, Rosen B, Dale AM (2002): Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. Neuron 33:341–355.

Fortier CB, Amick MM, Grande L, McGlynn S, Kenna A, Morra L, Clark A, Milberg WP, McGlinchey RE (2013): The Boston Assessment of Traumatic Brain Injury-Lifetime (BAT-L) semi-structured interview: Evidence of research utility and validity. J Head Trauma Rehabil 29:89–98.

Geerligs L, Maurits NM, Renken RJ, Lorist MM (2014): Reduced specificity of functional connectivity in the aging brain during task performance. Hum Brain Mapp 35:319–330.

Goh JO (2011): Functional dedifferentiation and altered connectivity in older adults: Neural accounts of cognitive aging. Aging Dis 2:30–48.

Gómez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, Parisi JE, Hyman BT (1997): Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann Neurol 41:17–24.

Haber SN (2014): The place of dopamine in the cortico-basal ganglia circuit. Neuroscience 282:248–257.

Hayes AF. (2013): Introduction to Mediation, Moderation, and Conditional Process Analysis. New York: Guilford Press.

Hoerster KD, Lehavot K, Simpson T, McFall M, Reiber G, Nelson KM (2012): Health and health behavior differences: US Military, veteran, and civilian men. Am J Prev Med 43:483–489.

Kalisch R, Elbel GK, Gössl C, Czisch M, Auer DP (2001): Blood pressure changes induced by arterial blood withdrawal influence bold signal in anesthetized rats at 7 Tesla: Implications for pharmacologic MRI. Neuroimage 14:891–898.

Killiany RJ, Gomez-Isla T, Moss M, Kikinis R, Sandor T, Jolesz F, Tanzi R, Jones K, Hyman BT, Albert MS (2000): Use of structural magnetic resonance imaging to predict who will get Alzheimer's disease. Ann Neurol 47:430–439.

Manninen V, Tenkanen L, Koskinen P, Huttunen JK, Mänttäri M, Heinonen OP, Frick MH (1992): Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study. Circulation 85:37–45.

McIntosh AR (1999): Mapping cognition to the brain through neural interactions. Memory 7:523–548.

Miller MW, Sadeh N (2014): Traumatic stress, oxidative stress and posttraumatic stress disorder: Neurodegeneration and the accelerated-aging hypothesis. Mol Psychiatry 19:1156–1162.

Morra L, Zade D, McGlinchey RE, Milberg WP (2013): Normal aging and cognition: The unacknowledged contribution of cerebrovascular risk factors. Aging Neuropsychol C 20: 271–297.

Park DC, Reuter-Lorenz P (2009): The adaptive brain: Aging and neurocognitive scaffolding. Annu Rev Psychol 60:173–196.

Raz N, Lindenberger U, Rodrigue KM, Kennedy KM, Head D, Williamson A, Dahle C, Gerstorf D, Acker JD (2005): Regional brain changes in aging healthy adults: General trends, individual differences and modifiers. Cereb Cortex 15: 1676–1689.

Ricciarelli R, Canepa E, Marengo B, Marinari UM, Poli G, Pronzato MA, Domenicotti C (2012): Cholesterol and Alzheimer's disease: A still poorly understood correlation. IUBMB Life 64:931–935.

Rubinov M, Sporns O (2010): Complex network measures of brain connectivity: Uses and interpretations. Neuroimage 52: 1059–1069.

Sahakian BJ, Owen AM (1992): Computerized assessment in neuropsychiatry using CANTAB: Discussion paper. J R Soc Med 85:399–402.

Selzer ML, Vinokur A, Rooijen LV (1975): A self-administered short Michigan alcoholism screening test (SMAST). J Stud Alcohol Drugs 36:117–126.

Skinner HA, Sheu WJ (1982): Reliability of alcohol use indices; the Lifetime Drinking History and the MAST. J Stud Alcohol Drugs 43:1157–1170.

Solomon A, Kivipelto M, Wolozin B, Zhou J, Whitmer RA (2009): Midlife serum cholesterol and increased risk of Alzheimer's and vascular dementia three decades later. Dement Geriatr Cogn Disord 28:75–80.

Spielberg JM, McGlinchey RE, Milberg WP, Salat DH (2015): Brain network disturbance related to posttraumatic stress and traumatic brain injury in veterans. Biol Psychiatry 78:210–216.

Stamler J, Wentworth D, Neaton JD (1986): Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded?: Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). JAMA 256:2823–2828.

Turk-Browne NB (2013): Functional interactions as big data in the human brain. Science 342:580–584.

Van Gaal LF, Mertens IL, Christophe E (2006): Mechanisms linking obesity with cardiovascular disease. Nature 444:875–880.

van Vliet P, van de Water W, de Craen AJ, Westendorp RG (2009): The influence of age on the association between cholesterol and cognitive function. Exp Gerontol 44:112–122.

Wechsler D. (2008): Wechsler Adult Intelligence Scale–Fourth Edition (WAIS–IV). San Antonio: NCS Pearson.

Williams VJ, Leritz EC, Shepel J, McGlinchey RE, Milberg WP, Rudolph JL, Lipsitz LA, Salat DH (2013): Interindividual variation in serum cholesterol is associated with regional white matter tissue integrity in older adults. Hum Brain Mapp 34: 1826–1841.

Wolf EJ, Sadeh N, Leritz EC, Logue MW, Stoop T, Milberg W, Miller MW (2016): Posttraumatic Stress Disorder as a catalyst for the association between metabolic syndrome and reduced cortical thickness. Biol Psychiatry 80:363–371.

Wolf EJ, Logue MW, Hayes JP, Sadeh N, Schichman SA, Stone A, Salat DH, Milberg W, McGlinchey R, Miller MW (2016): Accelerated DNA methylation age: Associations with PTSD and neural integrity. Psychoneuroendocrinology 63:155–162.

World Health Organization. (2001): WHODAS II Disability Assessment Schedule. Geneva: World Health Organization.

Yaffe K, Vittinghoff E, Lindquist K, Barnes D, Covinsky KE, Neylan T, Kluse M, Marmar C (2010): Posttraumatic stress disorder and risk of dementia among US veterans. Arch Gen Psychiatry 67:608–613.

Zalesky A, Fornito A, Bullmore ET (2010): Network-based statistic: Identifying differences in brain networks. Neuroimage 53: 1197–1207.