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Relative differences in resting-state brain connectivity associated with long term intensive lifestyle intervention

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ABSTRACT

A number of studies have reported that type 2 diabetes mellitus (T2DM) is associated with alterations in resting-state activity and connectivity in the brain. There is also evidence that interventions involving physical activity and weight loss may affect brain functional connectivity. In this study, we examined the effects of nearly 10 years of an intensive lifestyle intervention (ILI), designed to induce and sustain weight loss through lower caloric intake and increased physical activity, on resting-state networks in adults with T2DM. We performed a cross-sectional comparison of global and local characteristics from functional brain networks between individuals who had been randomly assigned to ILI or a control condition of health education and support. Upon examining brain networks from 312 participants (average age: 68.8 for ILI and 67.9 for controls), we found that ILI participants (N = 160) had attenuated local efficiency at the network-level compared with controls (N = 152). Although there was no group difference in the network-level global efficiency, we found that, among ILI participants, nodal global efficiency was elevated in left fusiform gyrus, right middle frontal gyrus, and pars opercularis of right inferior frontal gyrus. These effects were age-dependent, with more pronounced effects for older participants. Overall these results indicate that the individuals assigned to the ILI had brain networks with less regional and more global connectivity, particularly involving frontal lobes. Such patterns would support greater distributed information processing. Future studies are needed to determine if these differences are associated with age-related compensatory function in the ILI group or worse pathology in the control group.

Functional magnetic resonance imaging (fMRI) studies evaluating brain activity at rest using the blood oxygen level dependent (BOLD) technique allows identification and evaluation of several brain networks defined by synchronous activity patterns. The network of brain areas known as the default mode network (DMN), so named due to higher activity in this network when the brain is at rest, contains the posterior cingulate cortex, precuneus, medial temporal lobe, inferior parietal lobe, and medial prefrontal cortex. Alterations in brain functional architecture have been reported among adults with type 2 diabetes mellitus (T2DM). Recent studies among individuals with T2DM have found patterns of decreased functional and structural connectivity in these individuals compared with healthy controls (Zhou et al., 2010; Musen et al., 2012; Chen et al., 2014; Hoogenboom et al., 2014), particularly in the DMN. A similar trend was also discovered among obese people

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compared with healthy controls (Kullmann et al., 2012). In addition to resting-state connectivity, resting-state activity is also reduced in some brain areas in T2DM patients (Xia et al., 2013; Cui et al., 2014).

Recent short-term studies with interventions in obese and/or older individuals have shown that increased physical activities have protective effects on the brain, including increases in the volume (Erickson et al., 2011) and blood flow (Burdette et al., 2010) to the hippocampus and improved functional connectivity in the DMN (Burdette et al., 2010; Voss et al., 2010a,b; Li et al., 2014). Alterations in resting-state brain activity are also reported. Increased resting-state activity has been observed in the middle and superior frontal gyri associated with an intervention involving Tai Chi, cognitive training, and counseling (Yin et al., 2014). On the other hand, reduced resting-state activity was observed in the DMN associated with a treadmill-walking exercise intervention (McFadden et al., 2013). Less is known about whether weight loss, separate from physical activity, alters resting state brain activity. The few published results are based on small studies (Frank et al., 2014; Prehn et al., 2016).

We report findings from resting-state functional brain MRI collected from overweight and obese adults with T2DM who enrolled in a randomized controlled clinical trial of 9.8 years of intensive lifestyle intervention designed to induce and sustain weight loss. We have previously reported that this intervention was associated with improvements in brain structure: lower ischemic lesion and ventricle volumes (Espeland et al., 2016). The hypothesis framing this current manuscript is that random assignment to this long term lifestyle intervention, compared with a control condition of diabetes support and education, is associated with differences in local and global network efficiency.

1. Materials and methods

1.1. Subjects

The design and methods of the parent study, the Action for Health in Diabetes (Look AHEAD) trial, have been published previously (Ryan et al., 2003). At baseline (2001–2004), participants had T2DM, age between 45 and 76 years, body mass index $\geq$25 kg/m^2 ($\geq$27 kg/m^2 if taking insulin), HbA1c < 11%, systolic blood pressure <160 mmHg, diastolic blood pressure <100 mmHg, and triglycerides <600 mg/dl. All subjects passed a maximal graded exercise test in order to ensure that exercise could be safely prescribed. Additional eligibility criteria included run-in and an interview with a behavioralist for judging the ability of the participant to adhere to lifestyle intervention, which may have culled some with overt cognitive impairment.

A subset of participants from three Look AHEAD sites (Philadelphia, PA; Pittsburgh, PA; Providence, RI) enrolled in the Look AHEAD Brain Magnetic Resonance Imaging (Look AHEAD Brain) study in 2012–2014, an ancillary study examining brain structure and function (Espeland et al., 2016), at their 10th, 11th, or 12th anniversary from their Look AHEAD enrollment. Eligibility was limited to active participants for whom MRI was safe (e.g. metal implants and claustrophobia excluded some participants) and could be obtained (some of the largest Look AHEAD participants could not be scanned). All participants signed a separate informed consent form for the Look AHEAD Brain study, approved by local Institutional Review Boards prior to their enrollment.

1.2. Interventions

At the time of Look AHEAD enrollment, participants were randomly assigned, with equal probability, to the Intensive Lifestyle Intervention (ILI), or to the control arm referred to as Diabetes Support and Education (DSE). The ILI, which included diet modification and physical activity, was designed to induce $\geq$7% weight loss during the first year and to maintain this weight loss for the following years (Look AHEAD, 2006). ILI individuals were provided frequent group and individual treatment sessions for the study's duration, as described previously (Look AHEAD, 2006). The DSE participants were offered three group sessions each year (for the first 4 years) that provided education (but not behavioral instruction) about diet, physical activity, or social support (Wesche-Thobaben et al., 2011). Medical care for participants was provided by their personal physicians, except for temporary changes in diabetes medication to treat hypoglycemia during the intensive weight loss periods in ILI (Ryan et al., 2003). The intervention phase of Look AHEAD ended September 2012.

Intervention adherence was assessed by centrally trained staff that was masked to intervention assignment (Ryan et al., 2003). Body mass index (weight in kilograms divided by the square of height in meters) was measured annually. A maximal graded exercise test was administered at baseline and a submaximal exercise test at years 1 and 4, and on a subset of participants at year 2 (Jakicic et al., 2009). Changes in fitness at years 1 and 4 were computed as the difference between estimated metabolic equivalents (METS) when the participants achieved or exceeded 80% of age-predicted maximal heart rate or Borg Rating of Perceived Exertion of >16 at baseline and at the subsequent assessment. The Paffenbarger Physical Activity Questionnaire was used to estimate weekly minutes of moderate-to-vigorous physical activity at years 1, 4, and 8 in a subset of participants.

1.3. Brain MRI data

Each participant's brain MRI scan included structural MRI and resting-state functional MRI data. The Look AHEAD T1-weighted structural MRI was acquired with a 1 mm volumetric MPRAGE sequence (Espeland et al., 2016). The structural image was spatially normalized to the Jakob template space after skull-stripping. The Jakob template is one of the MNI (Montréal Neurological Institute) templates. The resting-state fMRI data consisted of a series of 152 scans acquired with TR = 2 s while participants rested with eyes open, fixating on a centrally located crosshair inside the MRI scanner. The fMRI frames were aligned to correct for head motion during the scan, co-registered to the participant's structural image, and spatially normalized to the MNI space. The fMRI data were then band-pass filtered (0.009–0.08 Hz) to attenuate respiration and other physiological noises. In addition, six affine transformation parameters from the alignment process, as well as the mean time courses from the brain parenchyma including all gray and white matter tissues, deep white matter, and ventricles were regressed out in order to correct further motion and physiological noises. The use of global signal regression remains a topic of research as there are mixed views on the importance of this procedure. We have chosen to use global signal regression because there is evidence that it is important for assessing regional differences and helps reduce artifacts associated with large draining veins (Hayasaka, 2013). To reduce the effects from motion artifacts, time points with a large displacement were identified. In the process known as motion scrubbing, a time point with the frame displacement (FD) greater than 0.5 was considered excessive as suggested by Power et al., and that time point as well as the one prior and the two following were removed (Power et al., 2012).

The MRI Reading Center at the University of Pennsylvania oversaw quality control of the brain MRI data collected from the three imaging sites, utilizing Siemens Tim Trio scanners. Both ADNI (Alzheimer's Disease Neuroimaging Initiative) and fBIRN (functional Biomedical Informatics Research Network) phantoms were

scanned quarterly at each site in order to assure image quality and calibrate signal-to-noise ratio and spatial distortion; scanners from the Look AHEAD brain MRI study uniformly passed quality thresholds throughout the study. Because of this rigorous cross-site calibration process, and because of the robustness of resting-state fMRI network organization across different sites, scanners, and protocols (Moussa et al., 2012), we did not explicitly correct for sites as a covariate in our statistical analyses.

1.4. Functional connectivity network

A functional connectivity network was constructed for each participant's pre-processed fMRI data. First, fMRI data were parcellated into regions of interest (ROIs) defined by a multi-atlas segmentation method (Doshi et al., 2013). One hundred fourteen ROIs belonging to gray matter areas in the cerebrum were used as nodes in functional connectivity networks. A cross-correlation matrix of the average fMRI time courses from these 114 ROIs was then calculated. The resulting correlation matrix was thresholded to generate binary sparse matrices with a matrix value of one (1) indicating that two nodes are neighbors or are connected and a value of zero (0) indicating that two nodes are not connected. Thresholding procedures in use include statistical thresholds (Garrison et al., 2015), proportional or sparcity thresholds (Garrison et al., 2015), soft thresholds (Schwarz and McGonigle, 2011), or no thresholds (Rubinov and Sporns, 2011). Nevertheless, the ideal procedure for thresholding brain networks remains unknown. In the current work we used a thresholding procedure previously demonstrated to yield highly connected spare matrices with critical behavior (Ruan et al., 2010; Hayasaka, 2016). The threshold was applied to individual rows in the correlation matrix, rather than setting a single universal threshold, and resulted in networks with comparable sparcity across study participants. This thresholding approach can preserve connections in nodes with only weak links and limits network fragmentation (Ruan et al., 2010). This method has recently been applied to neuroimaging data, with resulting networks exhibiting limited fragmentation, critical behavior, and power law degree distributions (Hayasaka, 2016). We applied multiple thresholds to the correlation matrix to ensure that the results are robust with respect to the choice of the threshold. In particular, the threshold was adjusted so that the number of above-threshold elements per row, d, ranged from 3 to 10. A larger or smaller value of d corresponded to more or less abundant edges, respectively.

As opposed to methods such as independent components analysis (ICA) or seed-based connectivity, networks focus on the relationship between each node and every other node. Network analyses not only identify which nodes are directly connected but can help identify alternative paths that information could travel to be exchanged between nodes that are not directly connected. The highly multivariate data are typically summarized using network statistics that quantify certain properties of the networks. The current study used several nodal and network-level metrics that were calculated at each threshold. The first metric used is one of the most fundamental measures of network connectivity called degree. Degree (K) is the number of connections or edges at a given node. High degree nodes, or hubs, have the potential to spread information widely through the network. Degree was calculated for individual nodes and was averaged across all nodes to generate a network-level measure.

Global efficiency (Eglob) is a measure of network integration (distributed processing) and is defined at the individual node level as the average of the inverse of the shortest path length between node i and all other network nodes (Latora and Marchiori, 2001; Rubinov and Sporns, 2011). Eglob was calculated for every node (Eglob(i)) and averaged across all nodes to generate a network-level measure as in described by Eq. (1):

$$Eglob = \frac{1}{N}\sum_{i\in G} Eglob(i) = \frac{1}{N}\sum_{i\in G}\frac{\sum_{j\in G, j\neq i} d_{ij}^{-1}}{n-1} \quad (1)$$

where N is the set of all nodes in graph (G) and d_{ij} is the shortest path between nodes i and j in the network. Eglob is scaled from 0 to 1, with 0 indicating that a node is isolated and has no connections and a value of 1 indicating that a node is connected to all other nodes in the network. In functional brain networks, global efficiency provides a measure of the overall capacity for parallel information transfer and integrated processing among distributed components of the system (Bullmore and Sporns, 2012).

Local efficiency (Eloc) is a measure of network segregation (regional specificity) and is defined as the average of inverse of the shortest average path length between all neighbors of node i (Latora and Marchiori, 2001). Neighboring nodes are defined as any nodes directly connected to node i. Eloc was calculated for every node (Eloc(i)) and averaged across all nodes to generate a network-level measure as described by Eq. (2):

$$Eloc = \frac{1}{N}\sum_{i\in G} Eloc\,(i) = \frac{1}{N}\sum_{i\in G}\frac{\sum_{j,h\in G_i, j\neq i} a_{ij}a_{ih}\left[d_{jh}\,(Ni)\right]^{-1}}{K_i\,(K_i-1)} \quad (2)$$

where N is the set of all nodes in the original graph (G) and $Eloc\,(i)$ is the efficiency of the subgraph of node i (G_i) that contains all neighbors of node I, K_i is the degree of a given node a_{ij} the corresponding element of the thresholded binary matrix. The local efficiency of a node reveals how effectively information is transferred among its neighbors. In a social science analogy, Eloc measures how likely a person's friends are also friends to each other. Eloc is a scaled measure ranging from 0 to 1, with a value of 0 indicating that there are no connections between the neighbors of a node and a value of 1 indicating that all neighbors of a node are interconnected. In functional brain networks, high Eloc suggests a topological organization indicative of segregated neural processing (Rubinov and Sporns, 2010). Eloc was calculated for individual nodes and was averaged across all nodes to generate a network-level measure.

1.5. Statistical analysis

Both network-level and nodal metrics were compared by two-sample t-tests. Three network-level metrics, Eglob, Eloc, and K, were compared between the ILI and DSE groups. For each nodal metric, a two-sample t-test was performed at each node separately. Because the distribution of node degree was skewed, degree data were log-transformed in the analysis. Since the brain network organization may be influenced by aging (Mevel et al., 2013; Ferreira et al., 2015), we also adjusted our analysis by age and age-group interaction in a linear regression model. The age-group interaction enabled us to assess any differential aging effects on network characteristics between the two groups. For the network-wide metric analysis, a group difference with $p < 0.05$ was considered statistically significant. For the nodal metric analysis, the Benjamini-Hochberg method (Benjamini and Hochberg, 1995) was used to correct p-values for multiple comparisons among 114 ROIs, controlling the false-discovery rate (FDR). FDR-corrected $p < 0.05$ was considered a significant group difference. We report robust group differences consistently found in 2 or more d threshold levels.

Table 1
Characteristics at the time of enrollment into the Look AHEAD (Action for Health in Diabetes) trial of participants who had successful MRI scans in the Look AHEAD Brain Study by intervention assignment.

	Diabetes Support and Education (N = 152)	Intensive Lifestyle Intervention (N = 160)	P-value
Age (years), No. (%)			0.37
45–54	43 (28.3%)	40 (25.0%)	
55–64	90 (59.2%)	91 (56.9%)	
65–76	19 (12.5%)	29 (18.1%)	
Female sex, No. (%)	112 (73.7%)	108 (67.5%)	0.23
Race/Ethnicity, No. (%)			0.53
African-American	36 (23.7%)	32 (20.0%)	
Non-Hispanic White	108 (71.0%)	120 (75.0%)	
Other/Multiple	8 (5.3%)	8 (5.0%)	
Education, No. (%), Miss = 10			0.49
Not College Graduate	81 (55.5%)	82 (52.6%)	
College Graduate	24 (16.4%)	34 (21.8%)	
Post College	41 (28.1%)	40 (25.6%)	
Body Mass Index (kg/m^2), No. (%)			0.04
25–29	18 (11.8%)	32 (20.0%)	
30–39	97 (63.8%)	103 (64.4%)	
≥40	37 (24.3%)	25 (15.6%)	
HbA1c[a] (%), No. (%)			0.50
<7.0	65 (42.8%)	71 (44.4%)	
7.0–8.9	72 (47.4%)	79 (49.4%)	
≥9.0	15 (9.9%)	10 (6.2%)	
Diabetes Duration, No. (%), Miss = 6			0.92
<5 years	72 (48.0%)	74 (47.4%)	
≥5 years	78 (52.0%)	82 (52.6%)	
Insulin Use, No. (%)	20 (13.9%)	18 (11.8%)	0.58
Hypertension, No. (%)	127 (83.6%)	132 (82.5%)	0.80
Prior Cardiovascular Disease,[*] No. (%)	13 (8.6%)	13 (8.1%)	0.89
Depressive Symptoms, No. (%), Miss = 1			0.76
Beck < 11	132 (87.4%)	138 (86.2%)	
Beck ≥ 11	19 (12.6%)	22 (13.8%)	
Alcohol intake, No. (%), Miss = 1			0.77
None	95 (62.5%)	98 (61.2%)	
<1/day	45 (29.6%)	52 (32,5%)	
≥1/day	12 (7.9%)	10 (6.2%)	
Baseline Smoking Status, No. (%), Miss = 1			0.96
Never	75 (49.3%)	81 (50.9%)	
Past	71 (46.7%)	72 (45.3%)	
Present	6 (4.0%)	6 (3.8%)	
Fitness, METS, No. (%)			0.07
<7.1	84 (54.2%)	72 (43.9%)	
≥7.1	71 (45.8%)	92 (56.1%)	
Paffenbarger, No. (%)			0.72
<1060	73 (47.1%)	70 (42.7%)	
≥1060	35 (22.6%)	41 (25.0%)	
Not Collected	47 (30.3%)	53 (32.3%)	

[*] No participants had a history of stroke at Look AHEAD enrollment.
[a] Glycated hemoglobin.

2. Results

Of 1008 participants in the parent Look AHEAD study at the three clinics, 321 agreed to participate in the Look AHEAD Brain ancillary study. Among those 321 participants, 319 (99%) participants completed brain MRI scans from October 2011 through October 2014. Of these, useable resting-state fMRI scans were acquired from 312 (97.8%) participants: 152 in the DSE arm and 160 in the ILI arm. Scans were obtained an average (standard deviation) of 10.4 (0.5) years from the date of randomization in both groups. These dates ranged from 0.8 years prior to the end of the intervention to 2.0 years afterward. At this time, participants mean (standard deviation) age was 67.9 (6.2) for DSE participants compared with 68.8 (6.6) for ILI participants ($p = 0.19$).

The DSE participants averaged 9.9 (0.7) years of intervention; the ILI participants averaged 9.8 (0.7) years of intervention ($p = 0.13$). Table 1 lists demographic and clinical characteristics at enrollment in the Look AHEAD trial, in 2001–2004. No significant difference between the two groups was found for any of the factors listed in this table, except for a slight imbalance for baseline body mass index. The ILI intervention was successful in sustained differences in weight losses relative to DSE (Supplemental Exhibit 1). Changes in physical activity and fitness from baseline were markedly different between groups after year 1 of intervention, but tended to wane with time.

In the analysis of network-level statistics, a significant group difference in Eloc was found when corrected for age and age-group interaction. The group difference (DSE-ILI) as well as the age-group interaction were statistically significant for thresholds $d = 3–6$ (Fig. 1). These group differences were due to differential aging effects on Eloc between the groups. This age association can be seen from the scatterplot of Eloc versus age in Fig. 2 for networks formed at $d = 5$. In the ILI group, there is a trend for lower Eloc among older participants, while the trend is in the opposite direction in the DSE group. These results indicate that local brain connectivity was lower among older ILI participants. No significant group differences were found in Eglob or K.

Analysis at the nodal level found significant group differences in Eglob in several brain regions when corrected for age and age-group interaction. In particular, a significant age-group interaction (FDR-corrected $p < 0.05$) was found in the left fusiform gyrus, right middle frontal gyrus (MFG), and pars opercularis of the right infe-

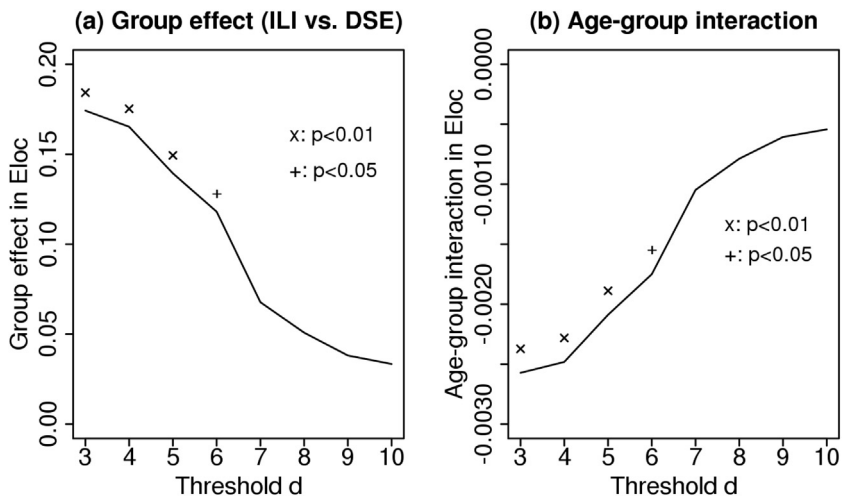

Fig. 1. The effects of age and age-group interaction from a linear regression model comparing Eloc between the DSE and ILI groups. The age effect (a) and the age-group interaction (b) are plotted over different network defining thresholds d. Statistically significant effects are indicated.

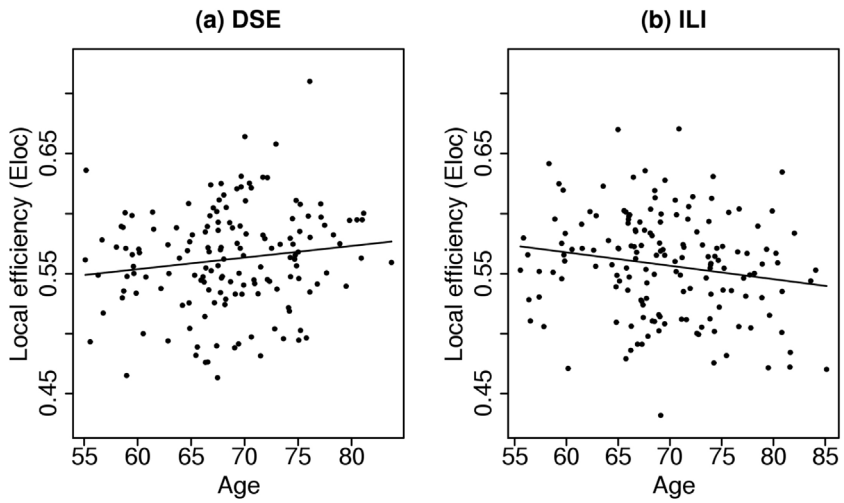

Fig. 2. Scatterplots of Eloc versus age for the DSE (a) and ILI (b) participants. The data points are based on networks with threshold d = 5. A fitted regression line is also shown in each group.

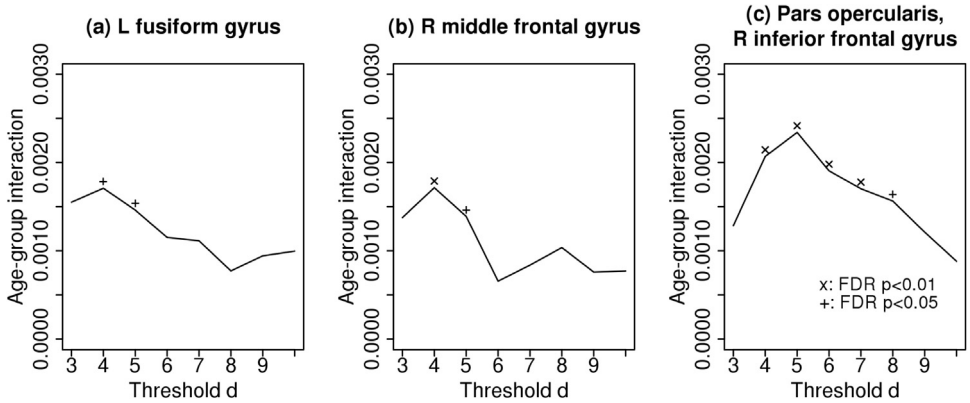

Fig. 3. The age-group interaction from a linear regression model of nodal Eglob. The age-group interaction (b) are plotted over different network defining thresholds d for the left fusiform gyrus (a), right middle frontal gyrus (b) and pars opercularis of the right inferior frontal gyrus (c). Statistically significant interactions, corrected for FDR over all ROIs, are indicated.

rior frontal gyrus (see Fig. 3). While the interaction was significant only for a few values of d (d = 4–5) for the left fusiform and right MFG, the interaction was more consistently significant in the right pars opercularis (d = 4–8). In all these brain areas, the age-group interaction was a manifestation of a differential aging effect on nodal Eglob between the groups. Fig. 4 shows scatterplots of nodal Eglob in the two groups and the three ROIs from networks formed at d = 5 threshold, along with fitted regression lines. These plots show

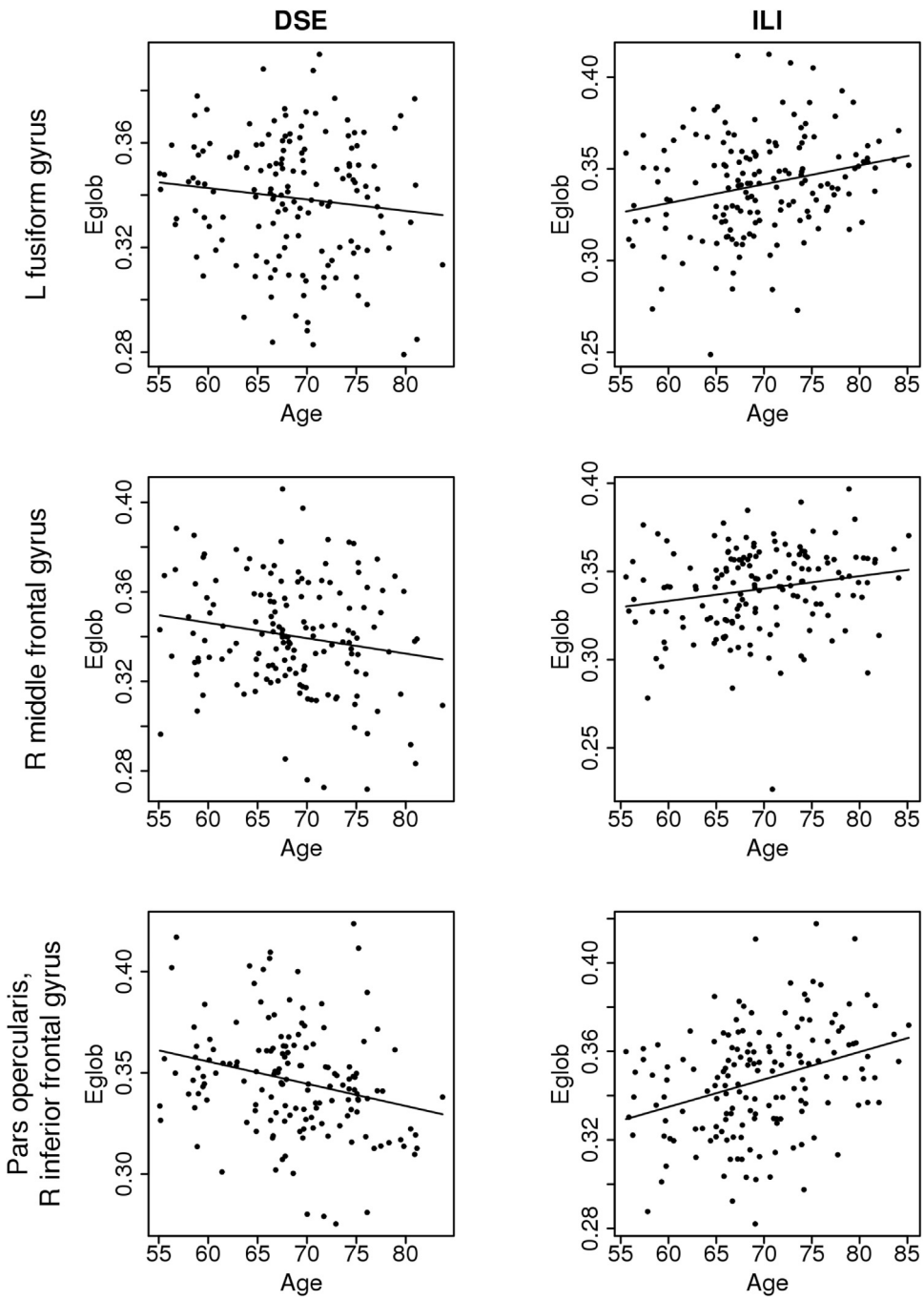

Fig. 4. Scatterplots of nodal Eglob versus age for the DSE (left) and ILI (right) participants in the left fusiform gyrus (top), right middle frontal gyrus (middle), and pars opercularis in the right inferior frontal gyrus (bottom). The data points are based on networks with threshold d = 5. A fitted regression line is also shown in each plot.

that Eglob is higher among older subjects in the ILI group, whereas the trend appears opposite in the DSE group. Elevated Eglob in these areas indicates a relative ease to reach to/from these ROIs from/to any other ROIs.

Among the three nodal ROIs with a significance group differences in Eglob, we also found a significant group difference in degree K in the pars opercularis of the right inferior frontal gyrus. In particular, the age-group interaction was significant for networks constructed at thresholds d = 5–6 (see Fig. 5a). The scatterplots in Fig. 5b and 5c show the age-related group difference between the two groups, based on the network generated at threshold d = 5. While log K tended to be higher among older participants in the ILI group, such trend was not observed in the DSE group. This trend indicates that older participants in the ILI group have more abundant connections in this region compared to the DSE counterparts.

3. Discussion

In this study, we have described the effects of ILI on brain functional connectivity networks. While some effects are significant at the whole brain level, such as the network-level Eloc, there are some region-specific effects in global efficiency (Eglob) and degree. The effects we found were age-dependent, and older participants tend to exhibit larger effects. Thus the intervention effect of ILI may be more pronounced among older individuals or may facilitate age-related compensation. We have recently demonstrated

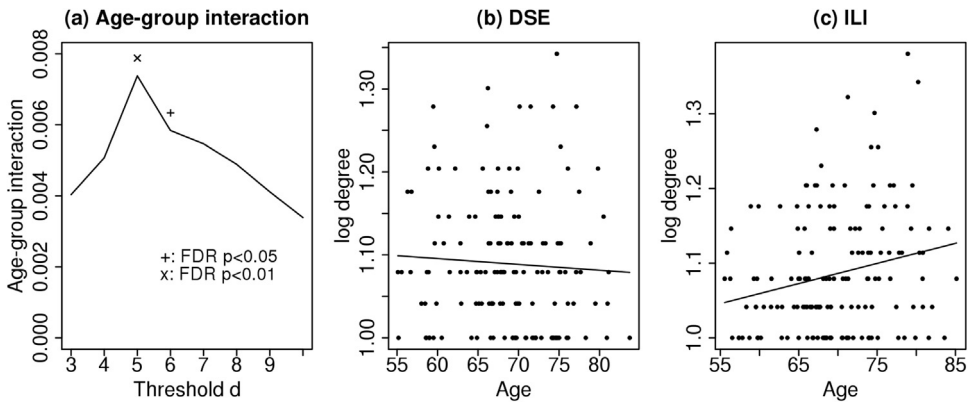

Fig. 5. The age-group interaction on node degree k at the pars opercularis of the right inferior frontal gyrus. The age-group interaction from a regression model on log degree is plotted over different network defining threshold d (a). Significant interactions, corrected for FDR, are indicated in the plot. Scatter plots of log degree k versus age for the DSE (b) and ILI (c) participants, based on networks with threshold d = 5, are also shown, along with a fitted regression line in each group.

that the ILI group had lower ischemic lesion volume and ventricular volume compared to the control population (Espeland et al., 2016). Given the well-established increase in both of these measures with normal aging, it is possible that the ILI modifies age-related and diabetes-related brain changes. In addition, given that brain pathology associated with diabetes accumulates over time, these changes are likely to be most pronounced in the older individuals that have longer disease duration. If the ILI reduces the detrimental effects of diabetes on the brain, it is likely that we will see the greatest benefit when comparing the oldest of the study populations.

Reduced network-level Eloc in older ILI participants can be interpreted in two different ways. First, older ILI participants' brain networks may have fewer local interconnections compared to that of the DSE participants. This means there are less redundant local connections in ILI participants' networks, potentially increasing the risk of network disruptions should some nodes or edges fail. Alternatively, reduced Eloc may also mean that connections in ILI participants' networks extend more globally to a wider extent rather than locally concentrated. This is likely accompanied by increased Eglob, either at the network-level or nodal-level. Since there were some regions with elevated Eglob in older ILI participants' networks, this explanation is plausible.

The regional differences found in this study are consistent with previous resting-state fMRI studies with T2DM and physical activity interventions. Xia et al. reported that spontaneous resting-state activity was reduced in left fusiform gyrus in T2DM subjects (Xia et al., 2013). It is plausible that such reduced activity may manifest as reduced global efficiency Eglob in T2DM individuals. In our study, we found that Eglob was elevated among ILI participants compared to DSE participants. Thus it is possible that ILI may preserve, or even counteract the deleterious effects of T2DM in the left fusiform gyrus.

Yin et al. reported that a physical activity-based intervention increased spontaneous resting-state activity in the right MFG (Yin et al., 2014). Moreover, Voss et al. reported an increase in resting-state connectivity resulting from a physical activity-based intervention in the frontal executive network, a collection of brain areas in the frontal cortex (Voss et al., 2010b). The frontal executive network includes two of the ROIs reported in this study: the right MFG and the pars opercularis of the right inferior frontal gyrus. Thus, the effects we observed in the right frontal lobe are consistent with these reports. It is interesting to note that our findings in the frontal lobe are located on the right hemisphere, rather than bilateral. It is possible that the right frontal cortex may be more susceptible to intervention effects than the left frontal cortex.

Two small studies have examined whether weight loss interventions alter brain function. Prehn et al. reported that hippocampal resting state activity was increased during a 12-week low-calorie diet in 19 postmenopausal obese women, however this effect was not sustained during a subsequent 4 week weight maintenance phase ( ). Frank et al. found the resting state network activity was increased among 11 women who had undergone gastric bypass an average of 3 years prior to the MRI compared to obese controls (Frank et al., 2014). No published studies of weight loss interventions have the duration or size to serve adequately as comparators to Look AHEAD.

There are several possible explanations for the significant regional differences in the frontal cortex we found in this study. Voss et al. hypothesize that aerobic exercise promotes integration of new neurons to the existing brain network (Voss et al., 2010a). Such reinforcement in brain connectivity appears prominent in fronto-hippocampal connections. Although our results do not identify any significant alterations in hippocampal connectivity, this hypothesis can explain the enhanced Eglob in the MFG and the pars opercularis. Moreover, opercular insular connectivity can be enhanced through active learning (Albert et al., 2009), which may be a result of the ILI. Yin et al. speculate that the increased activity and connectivity in the right MFG is associated with increased feeling of subjective well-being resulting from an intervention (Yin et al., 2014). This assessment is based on the right MFG's involvement in modulation of depressive states (Wang et al., 2008), as well as their prior study demonstrating the association between the functional connectivity in the right MFG and subjective well-being (Li et al., 2014). Considering these, we hypothesize the alterations in the frontal cortex among ILI participants may be the combination of these two factors. There may be new neuronal connections made in these areas due to improved physical fitness among ILI participants. Also, the improved sense of well-being due to weight loss may be associated with enhanced functional connectivity in these areas. Future studies are needed to further elucidate if these factors are contributing to alterations in functional connectivity in the frontal cortex.

Because weight loss may increase the risk for hypoglycemia, the Look AHEAD intervention protocol including provisions for reducing diabetes medications during the intensive phase of the intervention (Ryan et al., 2003). Overall, there was a slight increase in rate of hypoglycemia related to the intervention during its first year, but these cases were rare and there was no increased risk during the remaining years (Look AHEAD, 2016). Within the subset of Look AHEAD participants included in our MRI study, there were only two cases of severe hypoglycemia (e.g. involving loss of consciousness, seizure, or a glucose <70 mg/dl that prevented self-treatment) among ILI participants and no cases among DSE participants. Thus, the differences we report are unlikely to be

related to these rare events, however we cannot rule out whether less severe hypoglycemia may have influenced our findings. The ILI reduced the use of medications over time, including oral diabetes medications (Espeland et al., 2014a), thus it is possible the differences we report may be influenced by differential medication use over follow-up. They may also be related to improved diabetes control. At Year 1, mean levels of HbA1c in our MRI cohort were 6.52% for ILI participants and 7.20% for DSE participants ($p = 0.002$). At Year 8 these were 6.97% for ILI and 7.46% for DSE participants ($p = 0.006$). These parallel improvements seen in the full Look AHEAD cohort (Look AHEAD, 2013). The ILI intervention broadly affected many other factors that may influence brain function, including depression and hypertension (Rubin et al., 2013; Espeland et al., 2015). Thus, the mechanisms underlying the intervention effects that we describe may be complex and require further study.

Assignment to the ILI intervention was associated with lower overall mean ischemic lesions and ventricle volumes (Espeland et al., 2016), however there were no overall consistent differences in scores from a battery of cognitive function tests, both within the subset of participants with MRI and for larger segments of the cohort (Espeland et al., 2014b, 2016). Thus, why the improvements in markers of cerebrovascular disease and atrophy reported elsewhere, along with improvements in functional connectivity described here, have not translated into improved cognitive function also requires further study. Lack of difference in cognitive function in the setting of quantifiable structural and functional abnormalities in the brain may be due to resilience of these functions or insensitivity of the cognitive tests.

Although we were able to uncover possible intervention effects from ILI on brain functional connectivity, there are some limitations to this study. First, this is a cross-sectional study in which participants were scanned only once after the intervention. Consequently we are only able to identify group differences, as opposed to longitudinal changes before and after the intervention. Moreover, any group differences in functional connectivity before the intervention could not be examined, although such differences are highly unlikely due to the randomization process at the commencement of the Look AHEAD study. Another potential limitation of our study is that we have used a newly developed method to threshold the networks (Ruan et al., 2010). While this method has shown desirable properties such as reduced network fragmentation and scale-free degree distributions when applied to neuroimaging data (Hayasaka, 2016), further validation on a greater scale by the research community is needed. Despite the limitations, our study suggests possible differences in functional connectivity networks resulting from the lifestyle intervention, and our study may serve as a rationale for future intervention studies with longitudinal brain scans.

In summary, we demonstrated that a lifestyle intervention in T2DM may alter the connectivity in particular brain networks. Such alterations may result in improved access to/from a number of brain areas. We have demonstrated that the effects are more apparent in older adults.

Conflict of interests

No conflict to report.

Author contributions

HS and CR conceived and designed the study. HS and CR performed the statistical analyses and SH drafted the article. All authors contributed to data collection, analyses and interpretation of results. All authors revised the manuscript critically for important intellectual content. All authors provided final approval of the manuscript.

Role of the funding sources

The funders had no role in the study design, analysis of data, interpretation of findings, or the writing of the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2016.09.016.

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