Peripheral insulin resistance moderates cerebral glucose metabolism and impairs working memory in healthy younger adults.

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Abstract

People with insulin resistance are at increased risk for cognitive decline. Historically, insulin resistance was considered a condition of ageing but it is increasingly seen in younger adults. It is possible that changes in insulin function in early adulthood have both proximal effects, and moderate or even accelerate cerebral metabolism changes in ageing. Thirty-six younger (mean 27.8 years) and 43 older (mean 75.5) participants completed a battery of tests, including blood sampling, cognitive assessment and a simultaneous PET/MR scan. Cortical thickness and cerebral metabolic rate of glucose were derived for 100 regions, 17 networks and subcortical structures. Insulin resistance was measured with the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). Older adults had lower rates of cerebral glucose metabolism than younger adults across the brain. In younger adults, higher insulin resistance was associated with attenuated rates of cerebral glucose metabolism, but this was not seen in older adults. The largest effects of insulin resistance in younger adults were in prefrontal, parietal and temporal regions; and in the control, salience ventral attention, default and somatomotor networks. Higher rates of network glucose metabolism were associated with lower reaction time and psychomotor speed. Higher levels of insulin resistance were associated with lower working memory. Our results underscore the importance of insulin sensitivity to brain health and cognitive function across the adult lifespan, even in early adulthood.

Significance Statement

Glucose is the primary source of energy for the brain. Decreased glucose metabolism in the brain due to clinically significant levels of insulin resistance is associated with cognitive impairment and neurodegenerative disease. Although sub-clinical levels of insulin resistance have also been associated with grey matter atrophy, white matter integrity and cognitive decline, their impact on cerebral metabolism in healthy individuals is unclear. We showed for the first time that – while older adults have lower rates of cerebral metabolism - peripheral insulin resistance attenuates cerebral metabolism in healthy younger but not older adults. Lower rates of glucose metabolism were also associated with reduced psychomotor speed, and higher insulin resistance with impaired working memory performance. Preventing insulin resistance in early adulthood is important for ensuring efficient fuel supply for the brain and the maintenance of cognitive health across the adult lifespan.

1. Introduction

Insulin is a central player in many physiological processes, including the uptake of glucose into cells and the regulation of systemic blood glucose levels (1). Insulin enters the brain via insulin-independent mechanisms, where it used for a range of functions, such as cerebral glucose metabolism, the production of neurotransmitters, growth and regeneration of axons and neuron, circuit development, as well as the regulation of mood, behaviour and cognition (1, 2).

Insulin resistance describes how sensitive cells are to the effects of insulin. A person who is more insulin resistant (or has lower insulin sensitivity) requires larger amounts of insulin to lower systemic blood glucose levels than a person who has less insulin resistance. In insulin resistant people, tissues lose the ability to respond to normal insulin levels. The underlying aetiology of insulin resistance has been well described (see (3) for a review).

There has been a rapid rise in recent years in the number of people with insulin resistance (4, 5), with an associate increase in research on the pathophysiology of insulin in the brain. Much of this research has studied people with conditions in which insulin resistance is a hallmark characteristic, including ageing, type 2 diabetes and Alzheimer's disease (see (6-8) for a reviews). This research suggests that peripheral and central insulin resistance are linked (9, 10) and that clinical levels of insulin resistance are an important contributor to age-related diseases and cognitive decline (11-13). Moreover, the risk of cognitive decline often starts in midlife and increases as the duration and severity of insulin resistance increases (14). Although the causes of cognitive decline in insulin resistance are multifaceted, altered cerebral glucose metabolism is considered a major factor (2, 8).

Sub-clinical levels of insulin resistance, such as those seen in pre-diabetes, are also associated with grey matter atrophy and cognitive decline, even in the absence of comorbidities (15, 16). Longitudinal studies have linked peripheral insulin resistance in healthy subjects with subsequent declines in cognitive performance (17, 18). In healthy older adults, the impact of insulin resistance one whole-brain glucose metabolism is equivocal (6, 19-21). However, when regional glucose metabolism has been measured in healthy older adults, higher insulin resistance has been associated with lower glucose uptake in the thalamus and caudate (20), regions of the prefrontal and temporal cortices, cingulate and insula (19) and the medial orbital frontal cortex (20). Age-related reductions in cerebral metabolism occur independently from cortical atrophy (22), indicating that metabolic reductions are not simply a result of loss of tissue or cell bodies in ageing but also occur from a loss of metabolic efficiency or function.

Deleterious brain changes from insulin resistance are not limited to older adults, also being observed in younger adults. For example, variations in glycaemia in the absence of a clinical diagnoses of prediabetes or insulin resistance can cause grey matter atrophy (23, 24), reduced white matter integrity and impaired cognition in people in their late 20s, 30s and 40s (23-25). There is also a substantive range of insulin levels that occurs even before insulin resistance become clinically significant (26, 27). Elevated insulin levels precede other pathophysiological changes, such as weight gain, hyperglycaemia, hypertension, and cardiac function loss, by as much as 10 to 15 years (28-31), although it is unclear if the similar effects occur in the brains of younger and middle aged people.

The limited research on insulin and cerebral glucose in younger adults is surprising. It is possible that changes in insulin function in early adulthood have both proximal effects, and moderate or even accelerate cerebral metabolic changes in ageing. On the other hand, the maintenance of insulin sensitivity in early adulthood may act to preserve cerebral glucose metabolism and cognition through middle and later life, supporting the cognitive function and wellbeing of older people. These questions are especially important to answer as rates of insulin resistance are growing in younger people (32).

Here we investigated regional and network cerebral glucose metabolism, insulin resistance and cognition in healthy younger and older adults. In this pre-registered report, we first hypothesised that greater cortical thickness would be associated with higher cerebral metabolic rate of glucose. Our second hypothesis was that, after adjusting for cortical thickness differences in ageing, older adults would have lower cerebral metabolic rate of glucose than younger adults. Our third hypothesis was that older adults would have greater insulin resistance than younger adults, and that the reduction in glucose from insulin resistance would be moderated by age, with the effect being stronger in older adults. Our final hypothesis was that higher regional glucose would be associated with better cognitive test performance.

2. Results

We examined the relationship between insulin resistance, cerebral glucose metabolism and cognition in n=36 younger and n=43 older adults using simultaneous positron emission tomography and magnetic resonance imaging (PET/MR). Insulin resistance was measured using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). T1-weighted MRI was used to derive cortical thickness measures from 100 regions and 17 networks from the Schaeffer atlas; this parcellation was applied to the simultaneously acquired FDG-PET images to quantify cerebral metabolic rate of glucose (CMR_{GLC}) in each region and network. The relationship between CMR_{GLC}, HOMA-IR, cortical atrophy, and cognition was examined using general linear models (GLM) and their effect sizes indexed with partial eta squared (η^2_p) .

2.1 Demographic Factors

There was no significant difference in the proportion of females and males, years of education or BMI between the older and younger adult groups (Table 1). However, older adults had lower whole brain cortical thickness, systolic blood pressure (all $p < .001$) and resting heart rate ($p < .05$) compared to younger adults. Although participants did not have a previous diagnosis of hypertension upon study entry, nine younger and 29 older participants would meet the definition for hypertension from the measurements taken, i.e., a systolic blood pressure of 140 mmHg or higher, a diastolic blood pressure 90 mmHg or higher, or both (33). Because of the known impact of blood pressure and the other demographic factors on cortical thickness and other aspects of brain function (34) we ran additional GLMs with them as covariates, which are reported in the Supplement (Tables S7).

Older adults had higher fasting blood glucose levels, but no significant difference was found between groups for HOMA-IR. For younger adults, fasting blood glucose ranged from 4.0 to 5.4 mmol and HOMA-IR from 0.6 to 3.25. For older adults, fasting blood glucose ranged from 3.7 to 6.6 mmol and HOMA-IR from 0.1 to 3.14. A clinical diagnose of insulin resistance is not usually made from HOMA-IR, however, thresholds have been proposed based on correlates to cardiometabolic risk factors or other measures of insulin resistance. The recommended thresholds vary based on the age, sex and ethnicity, ranging from approximately 1.8 to 2.7 (35). In our sample, five younger and five older participants would meet a HOMA-IR threshold of 1.8 for insulin resistance; and two younger and four older participants would meet a threshold of 2.7.

2.2 Cortical Thickness and CMR_{GLC} (Hypothesis 1)

Higher regional CMR_{GLC} was positively associated with higher cortical thickness (see Figure 1, and Table S2) in 83 of the 100 regions. The largest effect sizes were in regions of the superior, middle and medial frontoparietal cortex.

2.3. Age, Insulin Resistance and CMR_{GLC} (Hypothesis 2)

2.3.1. Regional Level

The GLMs of the effect of age, HOMA-IR, age group X HOMA-IR and cortical thickness on regional CMR_{GLC} were significant in 99 of the 100 cortical regions and all eight subcortical structures (p-FRD < .05; Table S3). Older adults had lower CMR_{GLC} than younger adults in all regions (Figure 2 and Table S3). The effect sizes ranged from .20 to .24 in regions in the somatomotor, salience ventral attention, control and default networks, to less than .10 mostly in regions in the visual network and sub-cortical structures. The largest effects were in the medial, ventral and dorsal prefrontal cortices in the default network; the lateral prefrontal cortex, insula and the parietal operculum and lobule in the salience ventral attention network; the lateral prefrontal and temporal cortices in the control network; and regions of the somatomotor network.

Higher HOMA-IR was associated with lower CMR_{GLC} across all regions (Figure 2 and Table S3). The effect sizes ranged from .14 to .16 in regions in the salience ventral attention, somatomotor, default and control networks, to less than .08 in regions in the visual and control networks and the sub-cortical structures. The largest effects of HOMA-IR were in the lateral prefrontal, medial parietal and parietal operculum in the salience ventral attention network; the ventral and dorsal prefrontal cortices, parietal lobule and posterior cingulate in the default network; the lateral prefrontal cortex, precuneus and cingulate in the control network; and several somatomotor regions.

The interaction of age and HOMA-IR was significant in 41 regions (Figure 2; Table S3). Post-hoc tests showed that higher insulin resistance was associated with significantly lower CMR_{GLC} for younger adults but not older adults in regions in each of the networks (Table S4). We also calculated the percentage change in regional CMR_{GCL} from a 10% change in HOMA-IR from the slope of the regression lines for younger and older adults separately. A 10% increase in HOMA-IR was associated with 3.3% to 7.3% lower CMR_{GLC} across regions for younger adults; and for older adults, smaller and non-significant negative associations corresponding to zero to -2.2% decreases in CMR_{GLC} for each 10% increase in HOMA-IR. The largest reductions for younger adults were in the dorsal prefrontal cortex and parietal medial regions of the default network; the lateral prefrontal cortices in the control and salience ventral attention networks; the medial frontal cortex in the default network; the superior parietal and post central regions of the dorsal attention network; the temporal parietal cortex and insula; and regions of the somatomotor network.

2.3.2. Network Level

The GLMs at the network level were significant in all Schaefer 17 networks and the overall subcortical network (Table S5). The age group x HOMA-IR interaction was significant for all networks. Post-hoc tests showed that higher insulin resistance was associated with significantly lower CMR_{GLC} for younger adults but not older adults in all networks. Large effect sizes (η^2_p >.20) of HOMA-IR were found for younger adults in the control, salience ventral attention, somatomotor, default and visual networks.

2.4 CMR_{GLC} and Cognition (Hypothesis 3)

Five principal components (PCs) of cognition with eigenvalues greater than one were identified across the six cognitive tests and eleven measures, explaining 81% of the variance. The varimax rotation converged in five iterations (see Table S5). Cognitive control (task-switching and WASI FSIQ) loaded most strongly on the first principal component. Visuospatial processing (digit substitution) loaded most strongly on the second principal component; response inhibition (stop-signal) on the third component; working memory (digit span) on the fourth component; and verbal learning and memory (HVLT) on and the fifth component.

Higher CMR_{GLC} in the limbic, default and subcortical networks was associated with better performance on the first principal component of cognitive control (Table S6). In particular, higher network CMR_{GLC} was associated with a faster reaction time in task-switching (see Figure 3). Higher CMR_{GLC} in the control and default networks was associated with faster visuospatial processing speed (principal component 2, digit substitution). Higher CMR_{GLC} in the dorsal attention and control networks was associated with better response inhibition (principal component 3, stop signal). Interestingly, lower CRM_{GLC} was associated with higher WASI FSIQ2 and number of correct responses in the digit substitution task. Higher HOMA-IR had a direct effect on worse working memory (principal component 4, digit span). However, none of the interaction terms of HOMA-IR and network CMR_{GLC} were significant in the stepwise regression models, indicating that levels of HOMA-IR did not moderate the relationship between CMR_{GLC} and cognition.

3 Discussion

3.1 The Association Between CMR_{GLC} and Age, HOMA-IR and Cortical Thickness

Older and more insulin resistant people had lower rates of regional cerebral metabolism after controlling for cortical thickness, and higher regional cortical thickness was associated with higher metabolism. The largest age differences were in regions in the prefrontal and temporal cortices and the insula. These results are consistent with our recent meta-analyses findings of cerebral glucose metabolism differences in ageing (22). They suggest that older adults have less metabolic active cortical tissue than younger adults but they also have less efficient metabolism of glucose. Our results are also consistent with research in older adults in which higher HOMA-IR was associated with lower glucose metabolism in regions of the medial orbital, prefrontal and temporal cortices and the cingulate and insula (19, 36).

While we hypothesised that insulin resistance would attenuate glucose metabolism more in older than younger adults, we found that higher insulin resistance was associated with lower regional and network cerebral glucose metabolism in younger adults only. Age moderated the relationship between HOMA-IR and CMR_{GLC} in 41 regions, particularly in regions in the prefrontal, parietal, temporal and somatomotor cortices. Rates of cerebral metabolism in insulin resistant younger

adults were also lower in all networks, particularly in the control, salience ventral attention, somatomotor and default mode networks. Previous research has also shown a negative effect from metabolic dysfunction on grey matter volume, white matter integrity and cognition in people in their late 20s, 30s and 40s (23-25). Our results extend this research by showing that deleterious metabolic changes in the brain from insulin resistance also occur in younger, healthy adults.

Our results are compatible with a growing body of research suggesting that the brain is sensitive to the levels of peripheral insulin and uses insulin in a range of functions (37-39). Research also suggesting shared pathways or mechanisms driving changes to the brain in age and insulin resistance (8). The mechanism include metabolic disturbances from an increase in neuronal insulin resistance, decreased brain insulin receptor number and function, impaired insulin signalling, a pro-inflammatory state and mitochondrial dysfunction (40, 41). Prolonged peripheral hyperinsulinemia can also decrease insulin receptors at the blood-brain barrier, thereby reducing insulin transport into the brain (40). Neurons in the hypothalamus and brainstem that are responsible for energy homoestatis and feeding are also impaired in insulin resistance (2). Much of the research in this area has been with participants with a diagnosis of insulin resistance or Type 2 diabetes (7) or been limited to older participants (6, 19, 21). However, our results indicate that variability in peripheral insulin resistance within a healthy range is also associated with changes in cerebral metabolism, even in younger adults.

It is striking that higher insulin resistance was associated with greater CMR_{GLC} reductions in younger than older adults. This is somewhat counterintuitive, as we had expected that insulin resistance would be related to CMR_{GLC} reductions more so in older adults. Our results are also different to the one other study by Nugent et al. (36) that also included younger adults, in which HOMA-IR did not correlate with CMR_{GLC} in any brain region. One possible reason for these different results is that our younger sample had a broader age by 12 years and slightly higher HOMA-IR2 than Nugent et al. (20-42 years vs 18-30 years; HOMA-IR2 0.6 vs 0.5). We also note that our older adult group had slightly lower mean and standard deviation fasting insulin and HOMA-IR levels than our younger adult group (see Table S1). This is in contrast to the association between ageing and increasing insulin resistance shown in studies of the general population (42, 43). As such, our older cohort seems to be insulin sensitive compared to the general older adult population. Replicating and extending our findings with other younger adult populations and with a wider range of insulin resistance levels in older adults levels is warranted.

3.2 CMR_{GLC} Primarily in "Higher Order" Brain Networks is Associated with Cognition

While associations between cognition and CMR_{GLC} were obtained, these were not moderated by insulin resistance. Higher CMR_{GLC} was associated with faster psychomotor speed or reaction time in the digit substitution, task switching and stop signal tasks; but lower CMR_{GLC} was associated with higher accuracy in the digit substation task and higher WASI score. WASI, which is an estimate of full scale IQ (FSIQ), was acquired in this study as a more robust estimate of cognitive reserve, which is often measured using reading tests or educational attainment (44). Our sample had a relatively high mean WASI FSIQ2 of 116 and seven participants scored above 140, all in the older age group, indicating high levels of cognitive reserve. Two younger participants scored above 130. Together these results suggests that reduced rates of cerebral glucose in the brain of adults primarily attenuate processing speed, even in adults who retain relatively high cognitive reserve.

Regions of the "higher order" networks (e.g., control and attention) as opposed to the primary sensory networks (e.g., visual) were particularly noteworthy in our results for two reasons. First, these were the regions and networks where older age and higher insulin resistance were associated with the lowest glucose metabolism. Second, higher glucose metabolism in those networks predicted processing speed during response inhibition and visuospatial tasks. This likely reflects the fact that the cognitive battery primarily indexed memory, spatiotemporal processing and attentional control.

We did not find significant network CMR_{GLC} associations with episodic and working memory (PC4 and PC5). However, we did find a direct effect of HOMA-IR on working memory, an effect also found in previous research (45, 46). Higher HOMA-IR was associated with lower working memory, suggesting that insulin resistance is an independent risk factor for working memory impairment. Unexpectedly, we did not find age group differences for episodic and working memory. A large body of research has shown that older adults typically show a decline in episodic and working memory compared with younger adults (see (47) (48), further highlighting that our sample of older

adults were particularly cognitively-healthy with high cognitive reserve. Our results suggest that maintaining higher CMR_{GLC} in ageing may help retain better and faster task execution in attention, executive function and response inhibition; and that maintaining insulin sensitivity may help to preserve working memory.

3.3 Limitations

The current study used a cross-sectional design, limiting conclusions about any causal relationships. The differences we found between groups may also reflect underlying cohort differences rather than age-related changes. As noted above, our older adult population appears to be particularly healthy and relatively homogenous, at least in terms of insulin resistance and cognitive reserve. Longitudinal research could test whether changes in insulin function in early adulthood not only have a proximal effect, such as those reported here, but also moderate or even accelerate cerebral metabolic changes in ageing.

Our study is also limited by the absence of middle age adults. Research on structural and functional brain networks have reported quadratic trajectories of age differences, with an inflection point somewhere in the third to fifth decade of life (see (49) for review). However, the lack of middle aged adults precludes the identification and quantification of ageing trajectories across the full adult lifespan. Additional research is needed to elucidate these patterns.

HOMA-IR is considered a reliable clinical and research tool for the assessment of levels of insulin resistance. Nevertheless, a limitation of HOMA-IR is that it represents a single snapshot of the complex glucose-insulin system (50). Research using dynamic measures (e.g., hyperinsulinemic-euglycemic clamp or 2-hour glucose tolerance tests) could improve our understanding of the complexity of insulin signalling and metabolism in the periphery and the brain in ageing and across the spectrum of health and metabolic-related diseases.

3.4 Implications for the Maintenance of Brain Health Across the Adult Lifespan

The results of the current study underscore the importance of insulin sensitivity to brain health across the adult lifespan – especially at younger ages. The maintenance of insulin sensitivity in early adulthood may act to preserve cerebral glucose metabolism through middle and later life, supporting cognition and the quality of life of older people. Diet, lifestyle (e.g., sleep, exercise, stress), and genetics are risk factors for an increase in insulin resistance and would appear to be targets for public health interventions and clinical application to optimise both peripheral and central glucose metabolism (2, 51). Pharmaceutical treatments for diabetes that target peripheral glucose and bodyweight reductions may reduce the risk for cognitive decline (52, 53). Future research is recommended to test whether these pharmaceuticals support glucose metabolism in the brain, reducing or even negating the reductions seen in ageing and insulin resistance.

4. Method

Full details of the methods are provided in the Supplement. This study design, hypotheses and analyses were preregistered at OSF registrations (https://osf.io/93mnd).

4.1 Participants

Participants were recruited from the general community via local advertising. The final sample included 79 individuals, 36 younger (mean 27.8; SD 6.2;) and 43 older (mean 75.5; SD 5.8; range 66-86 years) adults (see Table S1). Exclusion criteria included a known diagnosis or history of hypertension, diabetes, or both, reported by participants at the time of recruitment to the study.

4.2 Data Acquisitions

Participants completed an online demographic and lifestyle questionnaire and a cognitive test battery. Briefly, the following cognitive measure were used (see Supplement for details): a combined and age-normalised index of verbal comprehension and perceptual reasoning from the Wechsler Abbreviated Scale of Intelligence; delayed recall and a recognition discrimination index from the Hopkins Verbal Learning Test; length of longest correct series for forward and backward recall from a digit span test to index working memory; switching cost in a task switching test to index cognitive flexibility; reaction time in a stop signal task to measure response inhibition; and number and seconds per correct response in a digit substitution task to measure visuospatial and processing speed.

Participants underwent a 90-minute simultaneous MR-PET scan in a Siemens (Erlangen) Biograph 3-Tesla molecular MR scanner (for scan parameters see Supplementary Information).

At the beginning of the scan, half of the 260 MBq FDG tracer was administered via the left forearm as a bolus, the remaining 130 MBq of the FDG tracer dose was infused at a rate of 36ml/hour over 50 minutes (54). Non-functional MRI scans were acquired during the first 12 minutes, including a T1 3DMPRAGE and T2 FLAIR. Thirteen minutes into the scan, list-mode PET and T2* EPI BOLD-EPI sequences were initiated. A 40-minute resting-state scan was undertaken in naturalistic viewing conditions. Plasma radioactivity levels were measured every 10 minutes throughout the scan.

4.3 MRI Pre-Processing, Cortical Thickness and Grey matter Volume

For the T1 images, the brain was extracted in Freesurfer; quality of the pial/white matter surface was manually checked, corrected and registered to MNI152 space using Advanced Normalization Tools (ANTs). Cortical thickness for the Schaefer 100 regions was obtained from the Freesurfer reconstruction statistics for each participant.

4.4 PET Image Reconstruction and Pre-Processing

The list-mode PET data for each subject were binned into 344 3D sinogram frames of 16s intervals. Attenuation was corrected via the pseudo-CT method for hybrid PET-MR scanners (55). Ordinary Poisson-Ordered Subset Expectation Maximization algorithm (3 iterations, 21 subsets) with point spread function correction was used to reconstruct 3D volumes from the sinogram frames. The reconstructed DICOM slices were converted to NIFTI format with size 344 × 344 × 127 (d size: 1.39 × 1.39 × 2.03 mm^3) for each volume. All 3D volumes were temporally concatenated to form a single 4D NIFTI volume. After concatenation, the PET volumes were motion corrected using FSL MCFLIRT (56), with the mean PET image used to mask the 4D data. PET images were corrected for partial volume effects using the modified Müller-Gartner method (57) implemented in Petsurf.

4.5 Cerebral Metabolic Rates of Glucose

Calculations of regional CMR_{GLC} were undertaken in PMOD 4.4 (http://www.pmod.com) using the FDG time activity curves for the Schaefer 100 atlas parcellation and AAL subcortical structures. The FDG in the plasma samples was decay-corrected for the time between sampling and counting, and used as the input function to Patlak models. A lumped constant of 0.89 was used (58), and equilibrium (t) set at 10 mins, the time corresponding to the peak of the bolus and onset of a stable signal (54). The fractional blood space (vB) was set at 0.05 (59). Participant's plasma glucose (mmol) was entered in the model from their baseline blood sample.

CRM_{GLC} in the 17 networks was calculated from the regional CMR_{GLC} values for each participant. Because the regions within a network differ in cortical volume, the regional CRM_{GLC} values could not simply be averaged. Rather, each regional CRM_{GLC} value was weighted by the percentage its volume represented within the total network cortical volume. An overall subcortical CMR_{GLC} value was calculated by weighting each structure by the percentage its volume represented from the total volume of the subcortical structures.

4.6 HOMA-IR

A blood sample taken prior to FD infusion was used to collect 2ml of plasma for insulin and glucose measurement, which was undertaken by a commercial laboratory. HOMA-IR was calculated as fasting glucose (mmol/L) x fasting insulin (μU/ml) / 22.5 (60). The constant of 22.5 is a normalising factor for normal fasting plasma insulin and glucose (i.e., 4.5 mmol/L x 5 μU/ml = 22.5). Higher HOMA-IR values indicate greater insulin resistance. We also calculated HOMA-IR2 (https://www.rdm.ox.ac.uk/). We compared the relationship between HOMA-IR and HOMA-IR2 with CMR_{GLC} and found minimal to no differences. This was expected as HOMA-IR2 models increases in the insulin secretion curve for plasma glucose concentrations above 10 mmol/L (61); a threshold that than none of our participants reached. Hence, the results reported here are based on HOMA-IR.

4.7 Data Analysis

The CMR_{GLC} and HOMA-IR data was inspected for and found to be satisfactory for assumptions of normality and potential impact of any outliers (see Supplement Figure S2).

4.7.1 Association, Moderating and Mediating Effects of Age, HOMA-IR, Grey Matter Volume, Cortical Thickness and CMR_{GLC}

Hypothesis 1: To test hypothesis 1 – that there is a positive relationship between cortical thickness and CMR_{GLC} – a series of general linear models (GLM) was run testing whether cortical thickness predicts regional CMR_{GLC}.

Hypothesis 2: To test hypothesis 2 - that after controlling for cortical thickness, there is a negative association between age group and insulin resistance and CMR_{GLC} – a series of GLMs was used. Regional CMR_{GLC} was the dependent variable in each model, with age group, HOMA-IR and age group x HOMA-IR as the predictors. Cortical thickness in the same region was included as a covariate. For the subcortical structures, whole brain average cortical thickness was used as the covariate. For significant age group x HOMA-IR effects, post-hoc GLMs were run separately for younger and older adults. A series of GLMs were also run at the 17 network level with the same design.

Partial eta squared (η^2_p) was used to quantify the effect sizes in the general linear models. Each series of analyses was also FDR-corrected at $p < .05$ for the overall GLM.

4.7.2 CMR_{GLC} and Cognition

Hypothesis 3: We applied data reduction techniques to reduce the dimensions in the cognitive test data. The cognitive scores (see Supplement) were converted to Z-scores and entered in a Principal Component Analysis (PCA). Principal components (PCs) with eigenvalues greater than one were retained and subject to varimax rotation to optimally reduce dimensionality (62). Participant component scores were saved for further analyses.

To test hypothesis 3 - that cognitive function negatively correlates with CMR_{GLC} after controlling for cortical thickness and age, and the relationships are moderated by the insulin resistance - a series of GLMs was run. The five cognition PCs were entered as the dependent variable, with CMR_{GLC} in 17 networks entered as independent variables, together with age group, whole brain cortical thickness and HOMA-IR. To test for a moderating effect of HOMA-IR, where a network CMR_{GLC} predicted a principal component of cognition, a product term was created between CMR_{GLC} and HOMA-IR. Stepwise regression was used with the significant CMR_{GLC} network(s) entered in block 1, and the product term(s) with HOMA-IR in block 2. A significant increase in variance explained from block 1 to block 2 was indicative of moderation.

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Titles and legends to figures

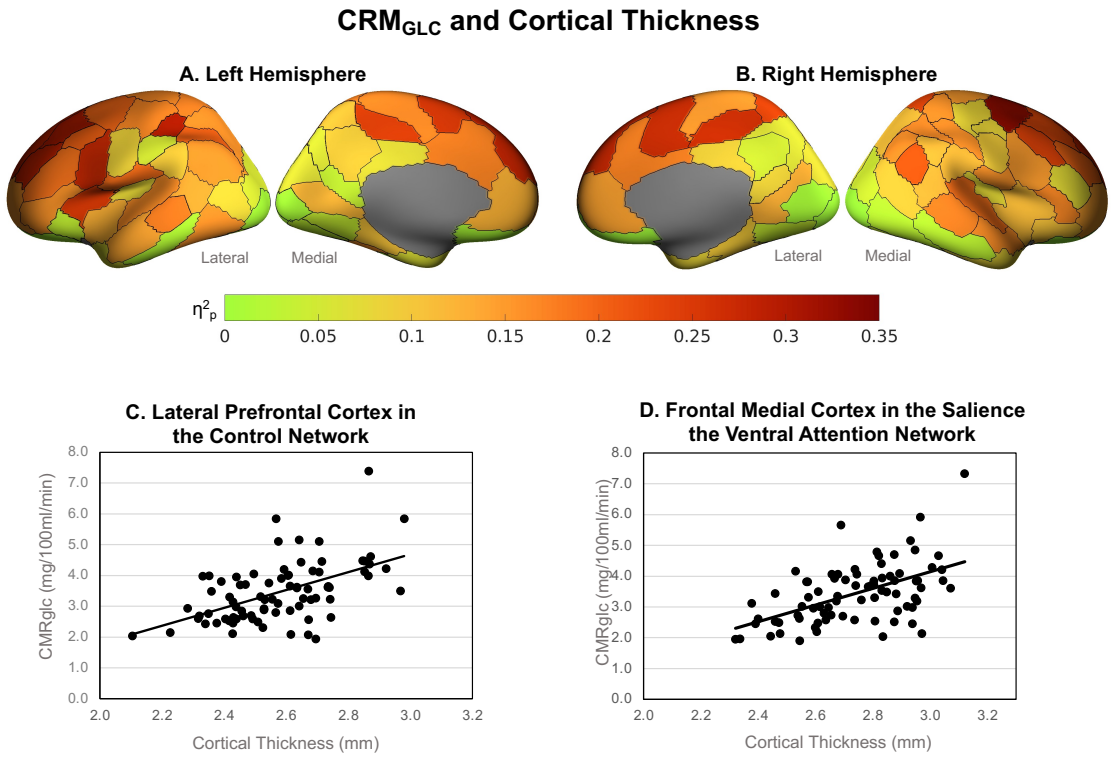

Figure 1. Effect sizes (η^2_p) of higher regional CMR_{GLC} associations with higher cortical thickness (data is from Table S2). Example associations in (A) the lateral prefrontal cortex in the control network, and (B) the frontal medial cortex in the salience ventral attention network. Figures were produced using the toolbox at: https://github.com/StuartJO/plotSurfaceROIBoundary

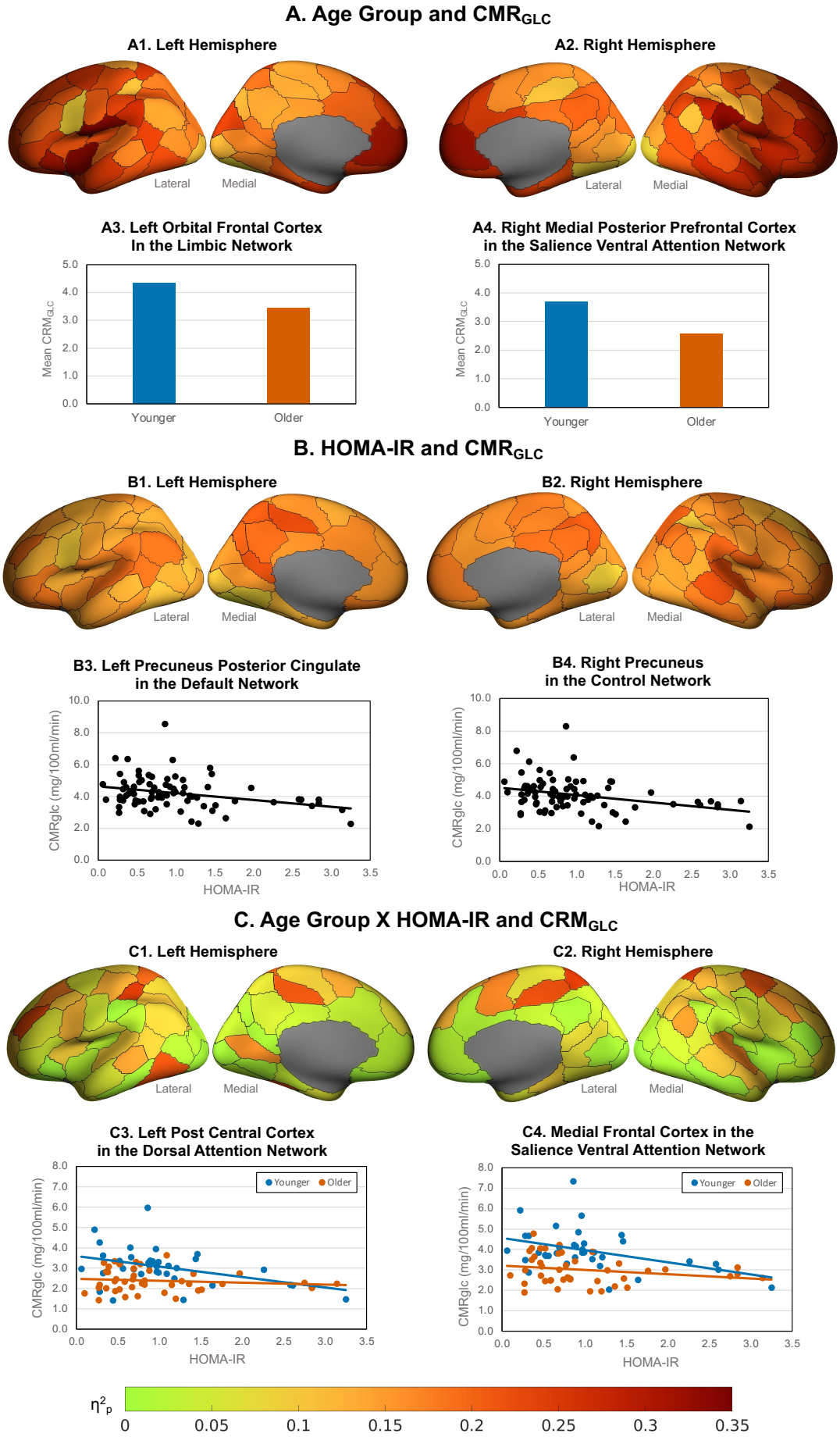
A. Age Group and CMRGLC
A1. Left Hemisphere
A2. Right Hemisphere
Lateral
Medial
A3. Left Orbital Frontal Cortex
In the Limbic Network
A4. Right Medial Posterior Prefrontal Cortex
in the Salience Ventral Attention Network
Mean CRMGLC
Younger
Older
B. HOMA-IR and CMRGLC
B1. Left Hemisphere
B2. Right Hemisphere
B3. Left Precuneus Posterior Cingulate
in the Default Network
B4. Right Precuneus
in the Control Network
CMRglc (mg/100ml/min)
HOMA-IR
C. Age Group X HOMA-IR and CRMGLC
C1. Left Hemisphere
C2. Right Hemisphere
C3. Left Post Central Cortex
in the Dorsal Attention Network
C4. Medial Frontal Cortex in the
Salience Ventral Attention Network
Younger
Older
η2p
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35

Figure 2. Effect sizes (η^2_p) of regional CMR_{GLC} associations with (A) age group, (B) HOMA-IR and (C) age group x HOMA-IR, controlling for cortical thickness (data in Table S3). Example age group and CMR_{GLC} relationships in (A3) the left orbital frontal cortex in the limbic network, and (A4) the right medial posterior prefrontal cortex in the salience ventral attention network. Example HOMA-IR effects in (B3) the left precuneus posterior cingulate in the default network and (B4) right precuneus In the control network . Example age group X HOMA-IR interactions in (C3) left post central cortex in the dorsal attention network and (C4) right medial frontal cortex in the salience ventral attention network.

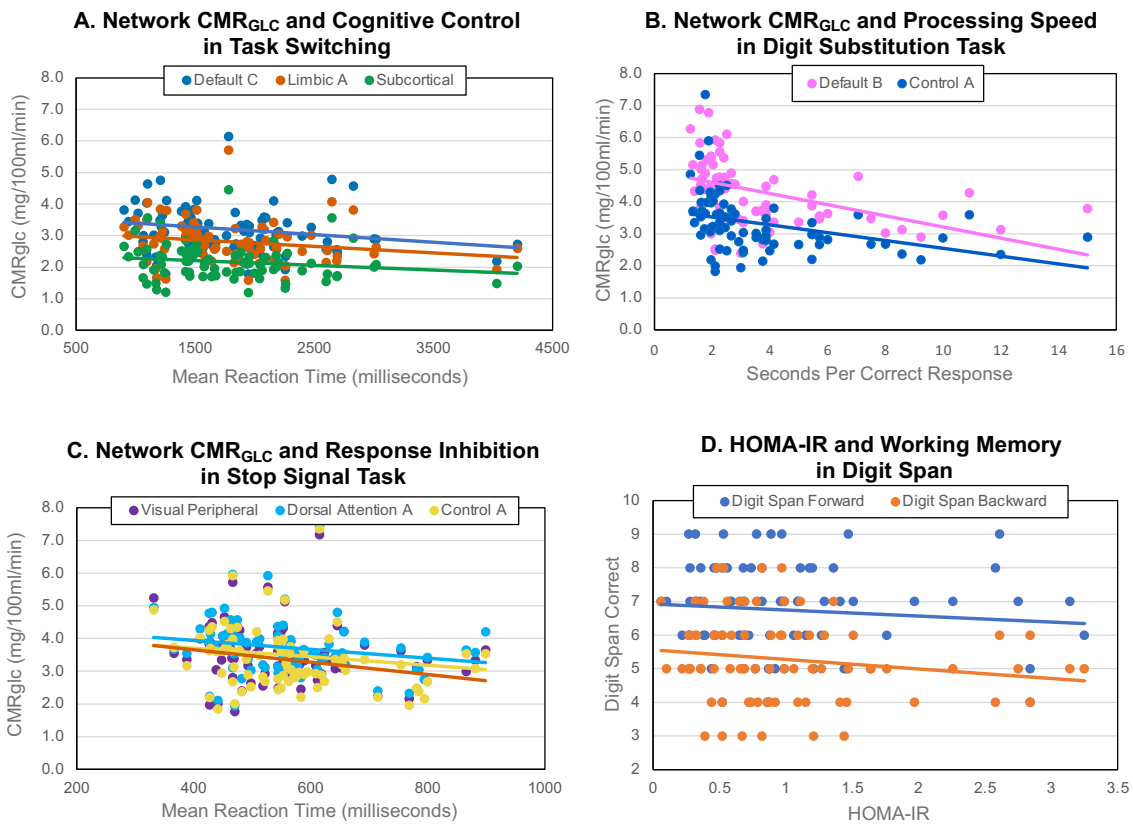

Figure 3. Relationship between network CMR_{GLC} and cognitive measures to illustrate significant effects from the general linear models (Tables S6). Significant negative associations of higher network CMR_{GLC} and: (A) shorter mean reaction time in task switching trials (PC1), (B) lower processing speed in the digit substitution task (loading on PC2) and (c) shorter mean reaction time in stop signal trials measuring response inhibition (PC3). (D) Significant negative association of higher HOMA-IR and lower longest forward and backward digit span performance.

Tables

Table 1. Demographics comparison of older and younger groups. Continuous variables are mean (standard deviation); categorical variables are %.

	Younger (N = 36)	Older (N = 43)	p-value[1]
Age (years)	27.8 (6.2)	75.5 (5.8)	<.001
Sex (% female)	58%	47%	.295
Education (years)	18 (2.8)	16.9 (3.6)	.167
Fasting blood glucose (mmol/L)	4.7 (0.3)	5.1 (0.5)	<.001
Fasting insulin (mIU/L)	4.7 (3.1)	4.0 (2.7)	.268
HOMA-IR	1.0 (0.7)	0.9 (0.7)	.783
HOMA-IR2	0.61 (0.40)	0.53 (0.38)	.410
Cortical Thickness (cm)	2.52 (0.07)	2.36 (0.11)	<.001
Systolic Blood Pressure	119.5 (17.6)	148.7 (24.3)	<.001
Diastolic Blood Pressure	79.4 (13.4)	83.6 (11.9)	.144
Resting Heart Rate	82.6 (17.3)	73.9 (13)	.013
BMI	24.1 (4.6)	25.6 (3.5)	.133

[1]P-values are based on T-test for continuous and Ch-square for categorical variables.