Published in final edited form as:

Schizophr Res. 2019 June ; 208: 324–330. doi:10.1016/j.schres.2019.01.031.

Brain Insulin Resistance and Altered Brain Glucose are Related to Memory Impairments in Schizophrenia

S. Andrea Wijtenburg^{*,a}, Dimitrios Kapogiannis^b, Stephanie Korenic^a, Roger J. Mullins^b, Joyce Tran^b, Frank Gaston^a, Shuo Chen^a, Maja Mustapic^b, L. Elliot Hong^a, Laura M. Rowland^a

^aMaryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD, USA

^bLaboratory of Neurosciences, Intramural Research Program, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA

Abstract

Memory is robustly impaired in schizophrenia (SZ) and related to functional outcome. Memory dysfunction has been shown to be related to altered brain glucose metabolism and brain insulin resistance in animal models and human studies of Alzheimer's disease. In this study, differences in brain glucose using magnetic resonance spectroscopy (MRS) and blood Extracellular Vesicle (EV) biomarkers of neuronal insulin resistance (i.e. Akt and signaling effectors) between SZ and controls were investigated, as well as whether these measures were related to memory impairments. Neuronal insulin resistance biomarkers showed a trend for being lower in SZ compared to controls, and memory measures were lower in SZ compared to controls. Occipital cortex glucose was higher in SZ compared to controls indicating lower brain glucose utilization. Linear regression analyses revealed significant relationships between neuronal insulin resistance biomarkers, memory measures, and brain glucose. More specifically, p70S6K, an insulin signaling effector, was related to verbal learning and brain MRS glucose in the SZ group. For the first time, we show that memory impairments in SZ may be related to brain glucose and brain insulin resistance. These data suggest that brain insulin resistance may play a role in the pathophysiology of learning and memory dysfunction in SZ.

*Corresponding Author: S. Andrea Wijtenburg, Ph.D., Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, P.O. Box 21247, Baltimore, MD 21228, Phone: 410-402-6886, Fax: 410-402-6077, awijtenburg@som.umaryland.edu.

Author Contributions

SAW, LMR, and DK wrote the manuscript. SK, RJM, JT, FG, MM recruited participants and analyzed data. SAW and SC performed statistical analyses. All authors reviewed, edited, and approved the manuscript.

Author Conflicts of Interest

The authors have no conflicts to report.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Brain insulin resistance; schizophrenia; glucose; magnetic resonance spectroscopy; memory; EVs; Extracellular Vesicles

1. Introduction

Memory dysfunction has been linked to poorer functional outcomes and quality of life in schizophrenia (SZ) (Al-Uzri et al., 2006). Adults with SZ are at a greater risk for developing dementia than those without SZ, especially before the age of 65 (Ribe et al., 2015). Altered glucose (Glc) metabolism impairs memory function (Hoyer, 2003; Mayer et al., 1990), and studies in SZ show ingestion of Glc improves learning and memory (Fucetola et al., 1999; Newcomer et al., 1999; Stone et al., 2003). Studies of SZ also show lower cerebral Glc metabolism using FDG-PET in frontal areas (Buchsbaum and Hazlett, 1998) and in the visual cortex (Desco et al., 2003). Cerebral Glc hypometabolism on FDG-PET is one of the most consistently replicated findings in Alzheimer's disease (AD) and mild cognitive impairment (Daulatzai, 2017; Langbaum et al., 2009), in which it has been associated with insulin resistance (IR), operationally defined via a model that incorporates fasting Glc and insulin concentrations (Baker et al., 2011; Willette et al., 2015b). In a study of cognitively normal adults at risk for AD, higher IR was associated with lower global and regional Glc metabolism in the frontal and medial temporal lobes (Willette et al., 2015a). Moreover, IR has been shown to negatively relate to verbal episodic memory in cognitively normal adults (Laws et al., 2017). In another study of older adults, homeostatic model assessment of insulin resistance (HOMA-IR) and fasting insulin levels were negatively related to performance on a visuospatial memory task (Tan et al., 2011). In an animal model of induced IR by high-fat diet, learning and memory were impaired (Ma et al., 2015), suggesting a causal role of IR in memory impairment. Collectively, this evidence suggests that Glc hypometabolism and brain IR could be partial underlying causes for memory impairment in both AD and SZ. However, to our knowledge, brain IR has not been studied in SZ.

Recent work has identified biomarkers of neuronal-specific IR in AD by isolating blood Extracellular Vesicles (EVs) of neuronal origin and measuring levels of phosphorylated insulin receptor substrate 1 (IRS-1) (Kapogiannis et al., 2015). These initial neuronal IR biomarkers were shown to differentiate between AD, frontotemporal dementia, diabetes, and age-matched control groups and were associated with brain atrophy in AD (Kapogiannis et al., 2015; Mullins et al., 2017b). Plasma EVs of neuronal origin were also found to contain insulin signaling molecules downstream from IRS-1 (Mustapic et al., 2017), such as Akt, GSK3 β , and p70S6K. Since these protein kinases regulate the efficiency of the IR pathway by phosphorylation and are a site of convergence for multiple intracellular pathways, we hypothesized that they may better represent the state of neuronal IR compared to IRS-1. Recently, it was shown that IRS-1 and these downstream mediators change in synchronization in neuronal EVs in response to a drug engaging the insulin cascade (Althauda et al., 2019; Althauda et al., 2018).

Complementary to the development EV neuronal IR biomarkers, in vivo measures of brain Glc levels may provide insight into Glc hypometabolism in AD and SZ. Currently, MRS is the only non-invasive technique that does not use ionizing radiation to study cerebral Glc. MRS has been used to demonstrate that after ingestion of Glc, adults with AD had higher Glc levels in the hippocampus compared to baseline, which was not observed in healthy elderly or younger adults (Haley et al., 2006). This suggests higher hippocampal Glc levels may be the physiologic counterpart of reduced hippocampal Glc metabolism, which is known to occur in AD. In another study, precuneal/posterior cingulate Glc levels were significantly higher in AD compared to healthy older and younger controls and performed well in discriminating between groups (Mullins et al., 2017a). Due to its low concentration and spectral overlap with other metabolites (Govindaraju et al., 2000), Glc has been historically difficult to detect with MRS at 3 Tesla field strength. Newer MRS methods to measure Glc use shorter echo times to take advantage of decreased signal loss due to T₂-relaxation and phase dispersion from J-coupling (Bednarik et al., 2015; Haley et al., 2006; Seaquist et al., 2005) or two dimensional methods like 2D JPRESS that have improved fitting as a result of better separation of the metabolites and thus greater information content (Mullins et al., 2017a). Here, we employed a very short echo time sequence to quantify Glc from the occipital cortex.

In this study, we measured EV biomarkers of neuronal IR and brain Glc levels using MRS and examined whether these two measures were altered, related to each other, and to memory dysfunction in SZ. Based on previous studies showing altered Glc metabolism and IR in AD, memory impairments in SZ, and altered Glc metabolism in SZ, we hypothesized that there would be altered EV biomarkers of neuronal IR in SZ and that these would be related to impaired memory function and MRS Glc.

2. Experimental/Materials and methods

2.1 Participant Characteristics

This study was approved by the University of Maryland School of Medicine Institutional Review Board, and all participants provided informed consent prior to study initiation. Twenty-two adults with SZ and twenty-four healthy controls (HC) participated in this study. Participant demographics and characteristics are summarized in Table 1. Adults with SZ were evaluated for capacity to consent to ensure that each participant understood the study and its procedures. Participants with SZ were characterized and evaluated with the Structured Clinical Interview for DSM-IV (SCID), a general psychiatric interview for past illness characteristics and family history, the Brief Psychiatric Rating Scale (BPRS) and Brief Negative Symptom Scale (BNSS). Functional capacity and quality of life was assessed with the UCSD Performance-Based Skills Assessment (UPSA). Verbal and visuospatial learning and memory were assessed using the Hopkins Verbal Learning Task Revised (HVLTR) (Benedict et al., 1998) and Brief Visuospatial Memory Test (BVMTR) (Benedict et al., 1996), respectively. For the HVLTR, participants are asked to listen to a list of 12 words and asked to recall as many as possible. There were three trials. For the BVMTR, participants view geometric figures and are asked to draw as many figures as possible in their correct location on a sheet of paper. There were three trials. All participants were clinically stable

with no changes in symptoms or medication status for at least 4 weeks prior to study participation. To be included in the study, participants with SZ and healthy controls had: 1) No contraindication for MRI scanning (i.e. cardiac pacemaker, prosthesis, other metal in body), 2) No major medical illness that affects brain structure or diabetes determined via self-report, and 3) No current substance dependence/abuse excluding nicotine.

2.2 EV Analysis

Blood samples for measuring IR biomarkers were available from 19 adults with SZ and 16 HC. Blood draws were conducted between 8 – 10 am after a 12h fast. Pre-analytical factors for blood collection and storage comply with guidelines for EV biomarkers (Coumans et al., 2017; Witwer et al., 2013): Blood samples were collected by venipuncture into vacutainer EDTA tubes, centrifuged for 20 min at 2500 g at 18°C, and then the resulting plasma supernatant layer was pipetted in 0.5-ml aliquots and stored at –80°C. Samples were thawed to room temperature once before processing. For isolation of EVs enriched for neuronal origin, we used the protocol used in multiple previous studies (Fiandaca et al., 2015; Goetzl et al., 2018; Goetzl et al., 2015a; Goetzl et al., 2015b; Goetzl et al., 2016) and presented in detail in Mustapic et al. (Mustapic et al., 2017). Briefly, we defibrinated 0.5 ml plasma samples using Thrombin, precipitated total EVs using a high-throughput and high efficiency (Saenz-Cuesta et al., 2015) particle precipitation method (Exoquick®) and immunoprecipitated EVs expressing L1 Cell Adhesion Molecule (L1CAM) using mouse anti-human CD171 (or else L1CAM) biotinylated antibody (clone 5G3, Thermo Scientific, Inc.). To calculate EV concentration and average diameter, we used Nanoparticle Tracking Analysis (NTA) by Nanosight® NS500 (NanoSight, Amesbury, UK). Five exposures of 20 seconds each were recorded from fields chosen randomly by NanoSight software (NanoSight NTA 3.2), which was also used to calculate average EV concentration and diameter. After NTA, L1CAM+ EVs were lysed and stored at –80°C until prior to assays. Insulin signal transduction proteins were measured in lysed EVs by electrochemiluminescence assays in duplicate. We quantified Akt, GSK3β, and p70S6K (K15133); and pAkt (phosphorylated at the Ser473 residue), pGSK3β (phosphorylated at the Ser9 residue), and pp70S6K (phosphorylated at the Thr421/Ser424 residues) (K15177). All EV biomarker levels were referenced to the EV concentration for normalization and then log transformed to avoid skewness.

2.3 Magnetic Resonance Spectroscopy

All participants were scanned in the morning between the hours of 8 and 10am on a Siemens Trio 3T MR system (Erlangen, Germany) using a 32-channel phased array head coil housed at the Center for Brain Imaging Research at the University of Maryland School of Medicine. 3D MP-RAGE images were acquired for voxel placement. Voxels were placed in the midsagittal slice in the occipital cortex (Figure 1). Data presented here are part of a study of visual plasticity (data not presented but HC published elsewhere (Wijtenburg et al., 2017)) and memory function, thus the rationale for assessing the occipital cortex. Participants were asked to keep their eyes open and remain awake during the study. There was a fixation point on the screen for them to fixate their eyes. A very short TE phase rotation (PR)-STEAM sequence (Bustillo et al., 2016; Henning, 1992; Wijtenburg et al., 2014; Wijtenburg and Knight-Scott, 2011a, b; Wijtenburg et al., 2018) (TR/TE=2000/6.5ms, 128 excitations, VOI

~ 24 cm³, 2.5kHz spectral width, 2048 complex points, and RF phases of: $\phi_1=135^\circ$, $\phi_2=22.5^\circ$, $\phi_{13}=112.5^\circ$, $\phi_{ADC}=0^\circ$) was used to acquire Glc levels. A water reference (NEX=16) was also acquired for phase and eddy current correction as well as quantification. A basis set of 19 metabolites was simulated using the GAVA software package (Soher et al., 2007): alanine (Ala), aspartate (Asp), creatine (Cr), γ -aminobutyric acid (GABA), Glc, glutamate (Glu), glutamine (Gln), glutathione (GSH), glycine (Gly), glycerophosphocholine (GPC), lactate (Lac), myo-Inositol (ml), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), phosphocholine (PCh), phosphocreatine (PCr), phosphoethanolamine (PE), scyllo-Inositol (si), and taurine (Tau). The basis set was imported into LCModel and used for quantification (Provencher, 1993). Metabolite levels were corrected for the proportion of the gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) within each spectroscopic voxel using in-house Matlab code based directly on the work of Gasparovic et al (Gasparovic et al., 2006). Metabolite levels were reported in institutional units, and Glc levels with percent standard deviation (%SD) Cramer Rao Lower Bounds (CRLBs) 50% were included in statistical analyses (Kreis, 2016). SNR, as reported in Table 2, measured by LCModel is the ratio of maximum in the spectrum with the baseline removed to twice the root-mean-square of the residual (Provencher, 2018). For the MRS data quality measures, there were no group differences between linewidth ($p=0.96$), SNR ($p=0.29$), or Glc CRLB ($p=0.23$). A representative spectrum with corresponding LCModel fit is shown in Figure 2.

2.4 Statistical Analysis

R was used for all statistical analyses (R Core Team, 2018). To examine group differences for univariates, a Pearson Chi-square test was performed on categorical variables with $\alpha=0.05$, and Wilcoxon rank-sum tests or Wilcoxon-Mann-Whitney tests, which are the non-parametric alternative to the two sample t-test, were performed on continuous variables with significance set at $\alpha=0.05$ since the data were not normally distributed. In this study, the neuronal IR biomarkers were highly inter-related, and principle component analysis (PCA) showed that the first principle component accounted for 99% of the total variance. Therefore, a principal component score was used to represent the six neuronal IR biomarkers. This neuronal IR biomarker score was used in linear regression analyses with significance set at $\alpha=0.05$ to examine the relationships between Glc levels and memory function (HVLT and BVMT), neuronal IR biomarker score and memory function (HVLT and BVMT), and finally Glc levels and neuronal IR biomarker score. If the regression model was not significant, then each term was evaluated for its significance and the model was adjusted accordingly. Further, exploratory correlation analyses that were not corrected for multiple comparisons were conducted to determine which individual neuronal IR biomarkers were most strongly related to measures of Glc and memory function and to examine whether these relationships differed between in SZ and HC. Analyses were first conducted across diagnostic groups, and correlations that were significant ($\alpha<0.05$) or trend level ($0.5<\alpha<0.1$) were repeated in both HC and SZ groups separately.

3. Results

3.1 Group Differences in memory, neuronal IR biomarkers, and brain glucose

There were no significant differences in demographic variables including gender ($p=0.49$), age ($p=0.38$), smoking ($p=0.78$), education ($p=0.67$), and BMI ($p=0.22$). Data were available for all participants for BVMT and HVLT. For MRS, two participants did not have the MRS scan (2 SZ), two datasets were excluded due to poor data quality ($LW>0.1$, 1 HC and 1 SZ), two datasets were excluded due to $CRLB \%SD > 50\%$ (1 HC and 1 SZ), and Glc was not detected in five datasets (3 HC and 2 SZ). For EV measurements, blood samples were available from 19 adults with SZ and 16 HC. As expected, adults with SZ performed poorer on tests of spatial memory (BVMT, $p=0.006$), verbal learning (HVLT, $p=0.001$), and functional capacity (UPSA, $p=0.015$) compared to HC. Brain Glc levels were significantly higher in SZ compared to HC in SZ ($p=0.002$). In terms of neuronal IR biomarkers, only Akt levels were lower in adults with SZ compared to HC at trend level ($p=0.109$). Significance between groups for the other five EV biomarkers ranged from $p=0.16$ – 0.37 . From the principal component analysis, the neuronal IR biomarker score did not differ between groups ($p=0.20$).

3.2 Linear Regressions between neuronal IR biomarker score, brain Glc, and memory

Neuronal IR biomarker score and memory.—For HVLT, there was a trend level linear relationship with diagnosis ($\beta_{DX} = 75.3 \pm 38.8$, $t=1.94$, $p=0.061$) and a significant interaction term ($\beta_{int} = 5.06 \pm 2.4$, $t=2.08$, $p=0.046$) (Figure 3a). In SZ, lower neuronal IR biomarker scores were associated with poorer performance on the HVLT; whereas, in HC, neuronal IR biomarker score was not associated with HVLT performance. For BVMT, there was a significant relationship with diagnosis ($\beta_{DX} = -8.2 \pm 2.7$, $t=-3.07$, $p=0.004$), but not with neuronal IR biomarker score ($p=0.37$).

Brain Glc and Memory.—A regression with HVLT revealed a trend level relationship with diagnosis ($\beta_{DX} = -4.15 \pm 2.2$, $t=-1.91$, $p=0.065$), but not brain Glc levels ($p=0.19$). Thus, adults with SZ had lower HVLT scores compared to HC. Both brain Glc levels ($\beta_{Glc} = -4.94 \pm 2.2$, $t=-2.21$, $p=0.034$) and diagnosis ($\beta_{DX} = -5.30 \pm 2.6$, $t=-2.05$, $p=0.049$) were significantly related to BVMT (Figure 3b). Adults with SZ had higher Glc levels than HC, and higher Glc levels were associated with lower BVMT scores in both groups.

Neuronal IR biomarker score and brain Glc.—A regression with brain Glc levels revealed trend level relationships with the interaction term ($\beta_{int} = -0.45 \pm 0.26$, $t=-1.76$, $p=0.09$) (Figure 3c). In SZ, lower neuronal IR biomarker scores were associated with higher brain Glc levels; whereas, in HC, neuronal IR biomarker scores were not associated with Glc levels in HC.

3.3 Exploratory Correlation Analyses between neuronal IR biomarkers, brain Glc, and memory

Neuronal IR biomarkers and memory.—Across groups, HVLT scores were significantly related to pGSK3 β ($r=0.344$, $p=0.043$) and trend level related to Akt ($r=0.306$, $p=0.074$), p70S6K ($r=0.330$, $p=0.053$), pAkt ($r=0.306$, $p=0.093$), and pp70S6K ($r=0.298$,

$p=0.082$). When broken down by diagnostic group, there were no significant correlations between memory scores and IR biomarkers (p 's=0.752-0.937) in the HC group. In SZ, HVLT was significantly associated with p70S6K ($r=0.541$, $p=0.017$), pGSK3 β ($r=0.512$, $p=0.025$), and pp70S6K ($r=0.461$, $p=0.047$) and at trend level with Akt ($r=0.449$, $p=0.054$), GSK3 β ($r=0.425$, $p=0.070$), and pAkt ($r=0.437$, $p=0.062$). Z-scores computed on the three significant correlations in SZ (HVLT with p70S6K, pGSK3 β , and pp70S6K) showed a trend level significant difference between SZ and HC for p70S6K ($p=0.07$); whereas, the other correlations were not significantly different between groups ($p=0.14$).

Across groups, BVMT scores were trend level related to p70S6K ($r=0.306$, $p=0.074$) and pGSK3 β ($r=0.311$, $p=0.069$) across both groups. When broken down by diagnostic group, there were no significant correlations in the HC group (p 's=0.259-0.417) or the SZ group (p 's=0.317-0.752). Thus, lower IR biomarker levels were associated with poorer memory performance.

Brain Glc and Memory.—Brain Glc was significantly correlated with BVMT and HVLT with $r = -0.530$, $p=0.001$ and $r = -0.406$, $p=0.016$, respectively; thus, poorer performance on learning and memory tasks was associated with higher Glc levels across groups. When separated by diagnostic groups, Glc and HVLT were not significantly correlated in both groups. Glc and BVMT were also trend level correlated in SZ only ($r=-0.425$, $p=0.101$) compared to HC ($r=-0.169$, $p=0.490$). The Z-score between correlations was not significant ($p=0.45$).

Neuronal IR biomarkers and Glc.—There was a trend level association between pGSK3 β and brain Glc across groups. When broken down by diagnostic group, there were no significant correlations in the HC group (p 's = 0.473-0.888). Brain Glc was negatively correlated at trend level with p70S6K ($r=-0.454$, $p=0.090$) and pGSK3 β ($r=-0.504$, $p=0.055$) in the SZ group. The Z-scores for these two correlations were not significant ($p=0.16$). Correlations between brain Glc and the other neuronal IR biomarkers ranged from $p=0.120$ -0.214.

4. Discussion

These results suggest that poor glucose utilization, reflected by higher levels of brain Glc, perhaps due to brain IR, is evident in SZ. Furthermore, brain Glc and neuronal IR biomarkers are related to each other, and importantly to memory impairments in SZ. These relationships were specific to SZ and therefore, brain IR may play a role in the pathogenesis of memory dysfunction in SZ.

While impaired memory function is well-established in the SZ literature as well as altered Glc metabolism via PET (Aleman et al., 1999; Buchsbaum and Hazlett, 1998; Grimes et al., 2017; McDermid Vaz and Heinrichs, 2002), here we show that the brain canonical insulin/Akt signaling cascade as reflected in circulating neuronal EVs taken as a whole (via PCA score) and diagnosis predict memory function and MRS brain Glc levels. In terms of individual neuronal IR biomarkers, Akt, pGSK3 β , and p70S6K revealed important group differences and associations with memory and MRS Glc. Akt plays a central role in IR by

mediating insulin actions via regulation of expression and/or activity of a wide range of enzymes, transporters, and transcription factors, which comprise the canonical insulin signaling pathway (Boucher et al., 2014). In this relatively small study, we show trend differences in Akt levels between diagnostic groups. This data further supports postmortem studies in SZ showing altered Akt levels (McGuire et al., 2017) as well as genetics studies that report an association between Akt1 (a genetic variant of Akt) and SZ that has been replicated in diverse populations (Emamian, 2012), suggesting that impaired Akt signaling may be involved in the pathophysiology of SZ.

Another study finding is the association of pGSK3 β and p70S6K with memory function and trend-level negative association with brain Glc only in SZ. GSK3 β is an immediate downstream molecule from Akt (Emamian, 2012) and can positively regulate p70S6K activity (Shin et al., 2011). In turn, both Akt and p70S6K can phosphorylate GSK3 β and thereby homeostatically inactivate the protein (McCubrey et al., 2014). Impaired insulin signaling reduces phosphorylation of GSK3 β , and thus activates the molecule, which also homeostatically inhibits the cascade at the level of IRS-1 (Kim and Feldman, 2012; Talbot et al., 2012). Previous postmortem studies showed altered GSK3 β mRNA and GSK3 β levels in cortical regions in adults with SZ (Emamian et al., 2004; Kozlovsky et al., 2004). p70S6K, a member of the S6K kinase family and part of the mTOR pathway, directly affects IRS-1, rendering it unresponsive to insulin (Manning, 2004). A previous study found that p70S6K was associated with spatial memory formation in the hippocampus of rodents (Dash et al., 2006); whereas, we observed a relationship between p70S6K and verbal learning (HVLT), which was stronger in SZ. In this study, the relationship between pGSK3 β and p70S6K with HVLT was positive, suggesting that altered insulin cascade activation, as evidenced by more negative EV levels, is associated with memory impairment. Similarly, the trend-level association between pGSK3 β and p70S6K with MRS Glc was negative, reaffirming the notion that the poor glucose utilization is associated with impaired insulin signaling.

Adults with SZ who participated in this study were predominantly taking antipsychotic medications, which can be a contributing factor to peripheral IR. While peripheral and brain IR do overlap (de la Monte, 2009), it is important to note that animal model and AD postmortem studies show brain IR does occur without peripheral IR (Mullins et al., 2017b; Talbot et al., 2012). While it is unknown how antipsychotic medications impact brain IR, studies suggest that adults with SZ have an increased risk for diabetes or impaired glucose metabolism regardless of medication status (Rajkumar et al., 2017), glucose homeostasis is altered from illness onset of SZ (Pillinger et al., 2017), and glycemic abnormalities are likely intrinsic to the illness beyond the known effects of medication, lifestyle, and health care access (Perry et al., 2016). Finally, a study in patients with first episode psychosis, unaffected patient siblings, and HC found that patients and their siblings had lower sensitivity to insulin than HC (Chouinard et al., 2018). Abnormal peripheral glucose metabolism or insulin signaling may be an increased risk factor for psychosis and independent of treatment effects. Taken together this evidence suggests that peripheral IR is present prior to antipsychotic treatment at first-episode and during treatment with antipsychotics, and therefore there is a high likelihood that brain IR in SZ is present regardless of antipsychotic medication status. Thus, we believe that the study results are not

a direct result of antipsychotic medication status, but acknowledge that larger studies are needed to further probe this area.

The lack of a relationship between IR biomarkers and brain Glc in HC in conjunction with significant group differences in Glc and trend level differences in IR biomarkers, suggest that brain IR and changes in Glc metabolism emerge in SZ in synchronization, potentially as part of the same process. These findings specifically in SZ motivated our exploratory correlation analyses to be performed in each group separately.

Glc is a metabolite with a relatively low concentration in the normal brain of approximately 1 mM (Govindaraju et al., 2000). Historically, Glc has been challenging to detect because its resonances overlap with other metabolites of higher concentration such as glutamate, glutathione, myo-Inositol, etc. Previous MRS studies used very short echo times (TE)s (Bednarik et al., 2015; Haley et al., 2006) or 2D localization techniques to measure Glc (Mullins et al., 2018). The advantage of very short TEs as employed in this study is that there is less signal loss due to phase dispersion from J-coupling and T_2 relaxation. Using the suggested CRLB %SD threshold of 50% for low concentration metabolites (Kreis, 2016), Glc was not identified in five datasets (%SD CRLB of 999%) and excluded due to %SD CRLB > 50% in two datasets. While extensive phantom work has yet to be conducted to investigate the reproducibility of this metabolite at various concentrations with the PR-STEAM technique, test-retest reproducibility metrics from human data extracted from our previous paper (Wijtenburg et al., 2018) reported mean CV and mean ADs of 16% and 19%, respectively with %SD CRLBs all below 40%. These reproducibility metrics are similar to another study using semi-LASER at 3T to measure Glc that reported reproducibility metrics of 20% or less for Glc and CRLB less than approximately 30% (Bednarik et al., 2015). Thus, these data suggest that very short TE acquisitions may be a viable method for measuring Glc.

There are several limitations to this study. First, the sample size is relatively small and results need to be replicated in a larger study. Second, when acquiring very short TE MRS data, the macromolecule background is very prominent in the spectrum. While an individual macromolecule suppressed spectrum was not acquired for each participant, the contributions of the macromolecule background were still accounted for by LCModel using a macromolecule basis set. Two studies have shown that a simulated basis set is adequate for quantification (Cudalbu et al., 2009; Schaller et al., 2013). Although participants with diabetes were excluded in this study via self-report, this was not verified via clinical research standards such as the HOMA-IR or QUICKI metrics (Katz et al., 2000). While participants were asked to relax and keep their eyes open and look at the fixation during the study, a camera was not used to verify compliance with instructions. Another limitation is that the spectroscopic sequence used in this study was not specifically tailored for glucose detection and therefore, other sequences such as semi-Laser (Bednarik et al., 2015), 2D JPRESS (Prescot and Renshaw, 2013), or 4D EP-JRESI (Sarma et al., 2014) may be better suited for glucose detection. Another limitation is that we did not measure peripheral metabolic markers, specifically plasma glucose levels and HbA1c. Participants were asked to fast for 12 hours prior to the scan and to report whether they were diabetic or not, which would have excluded them from the study. However, without the blood measures, we were not able to

verify these statements. Further, by not measuring plasma glucose, we were unable to examine the relationship between peripheral plasma glucose levels and brain glucose levels. In future studies, we plan to measure both plasma glucose and HbA1c.

Thus, this is the first study to measure IR biomarkers from EVs of neuronal origin and brain Glc using MRS in SZ and show that these measures are related to one another as well as to memory dysfunction. Even though this is a small study and data may be considered preliminary, the results of this study suggest that brain IR may be an underlying cause of memory impairments in SZ and that treatments targeting brain IR may be useful in alleviating memory deficits in SZ.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank the patients with schizophrenia and healthy controls for participating in this study.

Funding Body Agreement and Policies

The authors would like to acknowledge support from the National Institutes of Health to R01MH094520 (LMR).

References

- Al-Uzri MM, Reveley MA, Owen L, Bruce J, Frost S, Mackintosh D, Moran PM, 2006 Measuring memory impairment in community-based patients with schizophrenia. Case-control study. *Br J Psychiatry* 189, 132–136. [PubMed: 16880482]
- Aleman A, Hijman R, de Haan EH, Kahn RS, 1999 Memory impairment in schizophrenia: a meta-analysis. *Am J Psychiatry* 156, 1358–1366. [PubMed: 10484945]
- Althauda D, Gulyani S, Karnati H, Li Y, Tweedie D, Mustapic M, Chawla S, Chowdhury K, Skene SS, Greig NH, Kapogiannis D, Foltynie T, 2019 Utility of Neuronal-Derived Exosomes to Examine Molecular Mechanisms that Affect Motor Function in Patients with Parkinson Disease: A Secondary Analysis of the Exenatide-PD Trial. *JAMA Neurol.*
- Althauda D, Gulyani S, Karnati HK, Li Y, Tweedie D, Mustapic M, Chawla S, Chowdhury K, Skene SS, Greig NH, Kapogiannis D, Foltynie T, 2018 Neuronal-1 derived exosomes reveal drug mechanisms in clinical trials: Exenatide in Parkinson's disease *JAMA Neurol* In press.
- Baker LD, Cross DJ, Minoshima S, Belongia D, Watson GS, Craft S, 2011 Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. *Arch Neurol* 68(1), 51–57. [PubMed: 20837822]
- Bednarik P, Moheet A, Deelchand DK, Emir UE, Eberly LE, Bares M, Seaquist ER, Oz G, 2015 Feasibility and reproducibility of neurochemical profile quantification in the human hippocampus at 3 T. *NMR Biomed* 28(6), 685–693. [PubMed: 25904240]
- Benedict RHB, Schretlen D, Groninger L, Brandt J, 1998 Hopkins Verbal Learning Test–Revised: Normative Data and Analysis of Inter-Form and Test-Retest Reliability. *The Clinical Neuropsychologist* 12(1), 43–55.
- Benedict RHB, Schretlen D, Groninger L, Dobraski M, Shpritz B, 1996 Revision of the Brief Visuospatial Memory Test: Studies of Normal Performance, Reliability, and Validity. *Psychological Assessment* 8(2), 145–153.
- Boucher J, Kleinridders A, Kahn CR, 2014 Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harb Perspect Biol* 6(1), a009191. [PubMed: 24384568]
- Buchsbaum MS, Hazlett EA, 1998 Positron emission tomography studies of abnormal glucose metabolism in schizophrenia. *Schizophr Bull* 24(3), 343–364. [PubMed: 9718628]

- Bustillo JR, Rediske N, Jones T, Rowland LM, Abbott C, Wijtenburg SA, 2016 Reproducibility of phase rotation stimulated echo acquisition mode at 3T in schizophrenia: Emphasis on glutamine. *Magnetic resonance in medicine* 75(2), 498–502. [PubMed: 25762462]
- Chouinard VA, Henderson DC, Dalla Man C, Valeri L, Gray BE, Ryan KP, Cypress AM, Cobelli C, Cohen BM, Ongur D, 2018 Impaired insulin signaling in unaffected siblings and patients with first-episode psychosis. *Mol Psychiatry*.
- Coumans FAW, Brisson AR, Buzas EI, Dignat-George F, Drees EEE, El-Andaloussi S, Emanuelli C, Gasecka A, Hendrix A, Hill AF, Lacroix R, Lee Y, van Leeuwen TG, Mackman N, Mager I, Nolan JP, van der Pol E, Pegtel DM, Sahoo S, Siljander PRM, Sturk G, de Wever O, Nieuwland R, 2017 Methodological Guidelines to Study Extracellular Vesicles. *Circ Res* 120(10), 1632–1648. [PubMed: 28495994]
- Cudalbu C, Mlynarik V, Xin L, Gruetter R, 2009 Quantification of in vivo short echo-time proton magnetic resonance spectra at 14.1 T using two different approaches of modeling the macromolecule spectrum. *Meas Sci Technol* 20, 104034.
- Dash PK, Orsi SA, Moore AN, 2006 Spatial memory formation and memory-enhancing effect of glucose involves activation of the tuberous sclerosis complex-Mammalian target of rapamycin pathway. *J Neurosci* 26(31), 8048–8056. [PubMed: 16885218]
- Daulatzai MA, 2017 Cerebral hypoperfusion and glucose hypometabolism: Key pathophysiological modulators promote neurodegeneration, cognitive impairment, and Alzheimer's disease. *J Neurosci Res* 95(4), 943–972. [PubMed: 27350397]
- de la Monte SM, 2009 Insulin resistance and Alzheimer's disease. *BMB Rep* 42(8), 475–481. [PubMed: 19712582]
- Desco M, Gisbert JD, Reig S, Sanz J, Pascual J, Sarraimea F, Benito C, Santos A, Palomo T, Molina V, 2003 Cerebral metabolic patterns in chronic and recent-onset schizophrenia. *Psychiatry Res* 122(2), 125–135. [PubMed: 12714176]
- Emamian ES, 2012 AKT/GSK3 signaling pathway and schizophrenia. *Front Mol Neurosci* 5, 33. [PubMed: 22435049]
- Emamian ES, Hall D, Birnbaum MJ, Karayiorgou M, Gogos JA, 2004 Convergent evidence for impaired AKT1-GSK3 β signaling in schizophrenia. *Nat Genet* 36(2), 131–137. [PubMed: 14745448]
- Fiandaca MS, Kapogiannis D, Mapstone M, Boxer A, Eitan E, Schwartz JB, Abner EL, Petersen RC, Federoff HJ, Miller BL, Goetzl EJ, 2015 Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: A case-control study. *Alzheimers Dement* 11(6), 600–607.e601. [PubMed: 25130657]
- Fucetola R, Newcomer JW, Craft S, Melson AK, 1999 Age- and dose-dependent glucose-induced increases in memory and attention in schizophrenia. *Psychiatry research* 88(1), 1–13. [PubMed: 10641582]
- Gasparovic C, Song T, Devier D, Bockholt HJ, Caprihan A, Mullins PG, Posse S, Jung RE, Morrison LA, 2006 Use of tissue water as a concentration reference for proton spectroscopic imaging. *Magn Reson Med* 55(6), 1219–1226. [PubMed: 16688703]
- Goetzl EJ, Abner EL, Jicha GA, Kapogiannis D, Schwartz JB, 2018 Declining levels of functionally specialized synaptic proteins in plasma neuronal exosomes with progression of Alzheimer's disease. *Faseb J* 32(2), 888–893. [PubMed: 29025866]
- Goetzl EJ, Boxer A, Schwartz JB, Abner EL, Petersen RC, Miller BL, Carlson OD, Mustapic M, Kapogiannis D, 2015a Low neural exosomal levels of cellular survival factors in Alzheimer's disease. *Ann Clin Transl Neurol* 2(7), 769–773. [PubMed: 26273689]
- Goetzl EJ, Boxer A, Schwartz JB, Abner EL, Petersen RC, Miller BL, Kapogiannis D, 2015b Altered lysosomal proteins in neural-derived plasma exosomes in preclinical Alzheimer disease. *Neurology* 85(1), 40–47. [PubMed: 26062630]
- Goetzl EJ, Kapogiannis D, Schwartz JB, Lobach IV, Goetzl L, Abner EL, Jicha GA, Karydas AM, Boxer A, Miller BL, 2016 Decreased synaptic proteins in neuronal exosomes of frontotemporal dementia and Alzheimer's disease. *Faseb J* 30(12), 4141–4148. [PubMed: 27601437]
- Govindaraju V, Young K, Maudsley AA, 2000 Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR Biomed* 13(3), 129–153. [PubMed: 10861994]

- Grimes KM, Zanjani A, Zakzanis KK, 2017 Memory impairment and the mediating role of task difficulty in patients with schizophrenia. *Psychiatry Clin Neurosci* 71(9), 600–611. [PubMed: 28294468]
- Haley AP, Knight-Scott J, Simnad VI, Manning CA, 2006 Increased glucose concentration in the hippocampus in early Alzheimer's disease following oral glucose ingestion. *Magn Reson Imaging* 24(6), 715–720. [PubMed: 16824966]
- Henning J, 1992 The application of phase rotation for localized in vivo proton spectroscopy with short echo times. *J Magn Reson* 96(1), 40–49.
- Hoyer S, 2003 Memory function and brain glucose metabolism. *Pharmacopsychiatry* 36 Suppl 1, S62–67. [PubMed: 13130391]
- Kapogiannis D, Boxer A, Schwartz JB, Abner EL, Biragyn A, Masharani U, Frassetto L, Petersen RC, Miller BL, Goetzl EJ, 2015 Dysfunctionally phosphorylated type 1 insulin receptor substrate in neural-derived blood exosomes of preclinical Alzheimer's disease. *Faseb J* 29(2), 589–596. [PubMed: 25342129]
- Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ, 2000 Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 85(7), 2402–2410. [PubMed: 10902785]
- Kim B, Feldman EL, 2012 Insulin resistance in the nervous system. *Trends Endocrinol Metab* 23(3), 133–141. [PubMed: 22245457]
- Kozlovsky N, Shanon-Weickert C, Tomaskovic-Crook E, Kleinman JE, Belmaker RH, Agam G, 2004 Reduced GSK-3 β mRNA levels in postmortem dorsolateral prefrontal cortex of schizophrenic patients. *J Neural Transm (Vienna)* 111(12), 1583–1592. [PubMed: 15565492]
- Kreis R, 2016 The trouble with quality filtering based on relative Cramer-Rao lower bounds. *Magnetic resonance in medicine* 75(1), 15–18. [PubMed: 25753153]
- Langbaum JB, Chen K, Lee W, Reschke C, Bandy D, Fleisher AS, Alexander GE, Foster NL, Weiner MW, Koeppe RA, Jagust WJ, Reiman EM, 2009 Categorical and correlational analyses of baseline fluorodeoxyglucose positron emission tomography images from the Alzheimer's Disease Neuroimaging Initiative (ADNI). *Neuroimage* 45(4), 1107–1116. [PubMed: 19349228]
- Laws SM, Gaskin S, Woodfield A, Srikanth V, Bruce D, Fraser PE, Porter T, Newsholme P, Wijesekara N, Burnham S, Dore V, Li QX, Maruff P, Masters CL, Rainey-Smith S, Rowe CC, Salvado O, Villemagne VL, Martins RN, Verdile G, 2017 Insulin resistance is associated with reductions in specific cognitive domains and increases in CSF tau in cognitively normal adults. *Sci Rep* 7(1), 9766. [PubMed: 28852028]
- Ma X, Fang F, Song M, Ma S, 2015 The effect of isoliquiritigenin on learning and memory impairments induced by high-fat diet via inhibiting TNF- α /JNK/IRS signaling. *Biochem Biophys Res Commun* 464(4), 1090–1095. [PubMed: 26188513]
- Manning BD, 2004 Balancing Akt with S6K: implications for both metabolic diseases and tumorigenesis. *J Cell Biol* 167(3), 399–403. [PubMed: 15533996]
- Mayer G, Nitsch R, Hoyer S, 1990 Effects of changes in peripheral and cerebral glucose metabolism on locomotor activity, learning and memory in adult male rats. *Brain Res* 532(1–2), 95–100. [PubMed: 2149302]
- McCubrey JA, Steelman LS, Bertrand FE, Davis NM, Sokolosky M, Abrams SL, Montalto G, D'Assoro AB, Libra M, Nicoletti F, Maestro R, Basecke J, Rakus D, Gizak A, Demidenko ZN, Cocco L, Martelli AM, Cervello M, 2014 GSK-3 as potential target for therapeutic intervention in cancer. *Oncotarget* 5(10), 2881–2911. [PubMed: 24931005]
- McDermid Vaz SA, Heinrichs RW, 2002 Schizophrenia and memory impairment: evidence for a neurocognitive subtype. *Psychiatry research* 113(1–2), 93–105. [PubMed: 12467949]
- McGuire JL, Depasquale EA, Funk AJ, O'Donnovan SM, Hasselfeld K, Marwaha S, Hammond JH, Hartounian V, Meador-Woodruff JH, Meller J, McCullumsmith RE, 2017 Abnormalities of signal transduction networks in chronic schizophrenia. *NPJ Schizophr* 3(1), 30. [PubMed: 28900113]
- Mullins R, Reiter D, Kapogiannis D, 2018 Magnetic resonance spectroscopy reveals abnormalities of glucose metabolism in the Alzheimer's brain. *Annals of clinical and translational neurology* 5(3), 262–272. [PubMed: 29560372]

- Mullins RJ, Diehl TC, Chia CW, Kapogiannis D, 2017a Insulin Resistance as a Link between Amyloid-Beta and Tau Pathologies in Alzheimer's Disease. *Front Aging Neurosci* 9, 118. [PubMed: 28515688]
- Mullins RJ, Mustapic M, Goetzl EJ, Kapogiannis D, 2017b Exosomal biomarkers of brain insulin resistance associated with regional atrophy in Alzheimer's disease. *Hum Brain Mapp* 38(4), 1933–1940. [PubMed: 28105773]
- Mustapic M, Eitan E, Werner JK Jr., Berkowitz ST, Lazaropoulos MP, Tran J, Goetzl EJ, Kapogiannis D, 2017 Plasma Extracellular Vesicles Enriched for Neuronal Origin: A Potential Window into Brain Pathologic Processes. *Front Neurosci* 11, 278. [PubMed: 28588440]
- Newcomer JW, Craft S, Fucetola R, Moldin SO, Selke G, Paras L, Miller R, 1999 Glucose-induced increase in memory performance in patients with schizophrenia. *Schizophr Bull* 25(2), 321–335. [PubMed: 10416734]
- Perry BI, McIntosh G, Weich S, Singh S, Rees K, 2016 The association between first-episode psychosis and abnormal glycaemic control: systematic review and meta-analysis. *Lancet Psychiatry* 3(11), 1049–1058.
- Pillinger T, Beck K, Gobjila C, Donocik JG, Jauhar D, Howes OD, 2017 Impaired glucose homeostasis in first-episode schizophrenia: a systematic review and meta-analysis. *JAMA psychiatry* 74(3), 261–269. [PubMed: 28097367]
- Prescot AP, Renshaw PF, 2013 Two-dimensional J-resolved proton MR spectroscopy and prior knowledge fitting (ProFit) in the frontal and parietal lobes of healthy volunteers: assessment of metabolite discrimination and general reproducibility. *J Magn Reson Imaging* 37(3), 642–651. [PubMed: 23055387]
- Provencher SW, 1993 Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magnetic resonance in medicine* 30(6), 672–679. [PubMed: 8139448]
- Provencher SW, 2018 LCMModel & LCMgui User's Manual.
- Rajkumar AP, Horsdal HT, Wimberley T, Cohen D, Mors O, Borglum AD, Gasse C, 2017 Endogenous and antipsychotic-related risks for diabetes mellitus in young people with schizophrenia: a danish population-based cohort study. *Am J Psychiatry* 174(7), 686–694. [PubMed: 28103712]
- Ribe AR, Laursen TM, Charles M, Katon W, Fenger-Gron M, Davydow D, Chwastiak L, Cerimele JM, Vestergaard M, 2015 Long-term Risk of Dementia in Persons With Schizophrenia: A Danish Population-Based Cohort Study. *JAMA psychiatry*, 1–7.
- Saenz-Cuesta M, Arbelaiz A, Oregi A, Irizar H, Osorio-Querejeta I, Munoz-Culla M, Banales JM, Falcon-Perez JM, Olascoaga J, Otaegui D, 2015 Methods for extracellular vesicles isolation in a hospital setting. *Front Immunol* 6, 50. [PubMed: 25762995]
- Sarma MK, Nagarajan R, Macey PM, Kumar R, Villablanca JP, Furuyama J, Thomas MA, 2014 Accelerated echo-planar J-resolved spectroscopic imaging in the human brain using compressed sensing: a pilot validation in obstructive sleep apnea. *AJNR Am J Neuroradiol* 35(6 Suppl), S81–89. [PubMed: 24503554]
- Schaller B, Xin L, Cudalbu C, Gruetter R, 2013 Quantification of the neurochemical profile using simulated macromolecule resonances at 3 T. *NMR Biomed* 26(5), 593–599. [PubMed: 23413241]
- Sequist ER, Tkac I, Damberg G, Thomas W, Gruetter R, 2005 Brain glucose concentrations in poorly controlled diabetes mellitus as measured by high-field magnetic resonance spectroscopy. *Metabolism* 54(8), 1008–1013. [PubMed: 16092049]
- Shin S, Wolgamott L, Yu Y, Blenis J, Yoon SO, 2011 Glycogen synthase kinase (GSK)-3 promotes p70 ribosomal protein S6 kinase (p70S6K) activity and cell proliferation. *Proc Natl Acad Sci U S A* 108(47), E1204–1213. [PubMed: 22065737]
- Soher BJ, Young K, Bernstein A, Aygula Z, Maudsley AA, 2007 GAVA: spectral simulation for in vivo MRS applications. *J Magn Reson* 185(2), 291–299. [PubMed: 17257868]
- Stone WS, Seidman LJ, Wojcik JD, Green AI, 2003 Glucose effects on cognition in schizophrenia. *Schizophrenia research* 62(1–2), 93–103. [PubMed: 12765749]
- Talbot K, Wang HY, Kazi H, Han LY, Bakshi KP, Stucky A, Fuino RL, Kawaguchi KR, Samoyedny AJ, Wilson RS, Arvanitakis Z, Schneider JA, Wolf BA, Bennett DA, Trojanowski JQ, Arnold SE, 2012 Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with

IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J Clin Invest* 122(4), 1316–1338. [PubMed: 22476197]

Tan ZS, Beiser AS, Fox CS, Au R, Himali JJ, Debette S, Decarli C, Vasan RS, Wolf PA, Seshadri S, 2011 Association of metabolic dysregulation with volumetric brain magnetic resonance imaging and cognitive markers of subclinical brain aging in middle-aged adults: the Framingham Offspring Study. *Diabetes Care* 34(8), 1766–1770. [PubMed: 21680719]

Wijtenburg SA, Gaston FE, Spieker EA, Korenic SA, Kochunov P, Hong LE, Rowland LM, 2014 Reproducibility of phase rotation STEAM at 3T: focus on glutathione. *Magnetic resonance in medicine* 72(3), 603–609. [PubMed: 24151202]

Wijtenburg SA, Knight-Scott J, 2011a Reconstructing very short TE phase rotation spectral data collected with multichannel phased-array coils at 3 T. *Magn Reson Imaging* 29(7), 937–942. [PubMed: 21550744]

Wijtenburg SA, Knight-Scott J, 2011b Very short echo time improves the precision of glutamate detection at 3T in 1H magnetic resonance spectroscopy. *J Magn Reson Imaging* 34(3), 645–652. [PubMed: 21761460]

Wijtenburg SA, Near J, Korenic SA, Gaston FE, Chen H, Mikkelsen M, Chen S, Kochunov P, Hong LE, Rowland LM, 2018 Comparing the reproducibility of commonly used magnetic resonance spectroscopy techniques to quantify cerebral glutathione. *J Magn Reson Imaging*.

Wijtenburg SA, West J, Korenic SA, Kuhney F, Gaston FE, Chen H, Roberts M, Kochunov P, Hong LE, Rowland LM, 2017 Glutamatergic metabolites are associated with visual plasticity in humans. *Neurosci Lett* 644, 30–36. [PubMed: 28189743]

Willette AA, Bendlin BB, Starks EJ, Birdsill AC, Johnson SC, Christian BT, Okonkwo OC, La Rue A, Hermann BP, Kosciak RL, Jonaitis EM, Sager MA, Asthana S, 2015a Association of Insulin Resistance With Cerebral Glucose Uptake in Late Middle-Aged Adults at Risk for Alzheimer Disease. *JAMA Neurol* 72(9), 1013–1020. [PubMed: 26214150]

Willette AA, Modanlo N, Kapogiannis D, Alzheimer's Disease Neuroimaging I, 2015b Insulin resistance predicts medial temporal hypermetabolism in mild cognitive impairment conversion to Alzheimer disease. *Diabetes* 64(6), 1933–1940. [PubMed: 25576061]

Witwer KW, Buzas EI, Bemis LT, Bora A, Lasser C, Lotvall J, Nolte-t Hoen EN, Piper MG, Sivaraman S, Skog J, Thery C, Wauben MH, Hochberg F, 2013 Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. *J Extracell Vesicles* 2, 20360.

Figure 1.
Three T₁-weighted voxel images showing placement along the midline in the occipital cortex.

Figure 2. Representative very short TE PR-STEAM spectrum acquired from the occipital cortex. LCMoel fit (in red) is shown overlaid the actual data (in gray) with the fit residual show above (in gray). The LCMoel fit to Glc is shown below, also in gray.

Figure 3.

(a) Plots of HVLt score as a function of IR biomarker score with data from HC shown in black and adults with SZ in gray. For HVLt, a linear regression model revealed a trend level relationship with diagnosis ($p=0.061$) and a significant interaction term ($p=0.046$) such that lower neuronal IR biomarker scores were associated with poor performance on HVLt in SZ, but not in HC. (b) Plot of BVMT score as a function of MRS brain Glc levels with data from HC shown in black and adults with SZ in gray. A linear regression model for BVMT score revealed significant effects of brain Glc levels ($p=0.034$) and diagnosis ($p=0.049$). Adults with SZ had higher Glc levels than HC, and higher Glc levels were associated with lower BVMT scores in both groups. (c) Plot of brain MRS Glc levels as a function of neuronal IR biomarker score with data from HC shown in black and adults with SZ in gray. A linear regression revealed trend level relationships with an interaction ($p=0.09$) such that lower neuronal IR biomarkers scores were associated with higher Glc levels in SZ, and not in HC.

Table 1.**Participant Demographics**

	SZ	HC
Male/Female	15/7	14/10
Age (years)	39.5 ± 14.5	36.7 ± 16.0
Education (years)	13.8 ± 2.1	14.3 ± 1.5
Duration of Illness (years)	19.0 ± 14.7	
Smoker (yes/no)	3/19	4/20
Psychiatric Ratings		
BPRS (total)	39.8 ± 13.5	
BPRS (positive)	8.7 ± 5.6	
BPRS (negative)	6.9 ± 2.0	
BNSS	16.2 ± 9.9	
Antipsychotic Medications		
1st generation	1	
2nd generation	17	
Both (1st+2 nd generation)	2	
None	2	
Other Psychotropic Medications		
Benzodiazepine	4	
Antidepressant	9	
Mood Stabilizer	2	
Cognitive Measures		
¹ Visual spatial Memory (BVMT)	17.7 ± 6.5	25.4 ± 5.6
² Verbal Memory (HVLIT)	22.9 ± 6.2	28.1 ± 4.3
³ UPSA	93.1 ± 10.9	101.7 ± 9.6

¹: p=0.006,²: p=0.001,³: p=0.015

Table 2.

EV and MRS Summary

	SZ	SZ N	HC	HC N
EV				
I_{Akt}^*	-6.65 ± 0.29	19	-6.45 ± 0.39	16
GSK3 β *	-6.47 ± 0.27	19	-6.32 ± 0.42	16
p70S6K *	-6.42 ± 0.25	19	-6.25 ± 0.37	16
pAkt *	-6.60 ± 0.28	19	-6.44 ± 0.40	16
pGSK3 β *	-6.61 ± 0.25	19	6.45 ± 0.38	16
pp70S6K *	-6.59 ± 0.28	19	-6.43 ± 0.40	16
PCA Score	-16.06 ± 0.65	19	-15.65 ± 0.95	16
MRS				
LW (ppm)	0.046 ± 0.02	16	0.046 ± 0.02	19
SNR	72.2 ± 16.7	16	75.6 ± 20.3	19
$^2_{Glc}$ (IU)	1.46 ± 0.66	16	0.88 ± 0.34	19
Glc CRLB (%SD)	21.2 ± 13.7	16	23.1 ± 10.2	19
Voxel Gray Matter (%)	49.4 ± 0.04	16	52.1 ± 0.04	19
$^3_{Voxel}$ White Matter (%)	44.9 ± 0.04	16	41.2 ± 0.05	19
Voxel CSF (%)	5.4 ± 0.02	16	6.1 ± 0.02	19

* Normalized to their EV concentration and log transformed;

1 : p=0.109,

2 : p=0.006,

3 : p=0.081