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Glucose levels during life and neuropathologic findings at autopsy among people never treated for diabetes

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Abstract

We evaluated associations between glucose and dementia-related neuropathologic findings among people without diabetes treatment history to elucidate mechanisms of glucose's potential effect on dementia. We used glucose and hemoglobin A1c values to characterize glucose exposures over five years prior to death (primary) and age bands from 55–59 through 80–84 (secondary). Autopsy evaluations included Braak stage for neurofibrillary tangles, CERAD grade for neuritic plaques, macroscopic infarcts including lacunar infarcts, Lewy bodies, cerebral microinfarcts, and hippocampal sclerosis. Of 529 who came to autopsy, we included 430 with no history of diabetes treatment. We found no associations between glucose levels and Braak stage or CERAD grade. There was a suggestion of a relationship between glucose and hippocampal sclerosis, though this was inconsistent across analyses. There was higher risk of Lewy bodies in substantia nigra and locus ceruleus with higher glucose levels in age band analyses. We did not find interactions

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between glucose levels, neuropathologic findings, and dementia. The mechanism by which glucose may impact dementia risk is still unknown.

Keywords

glucose; neuropathology; neuritic plaques; hippocampal sclerosis; Lewy bodies; neurofibrillary tangles

1. Introduction

Diabetes has been found to be a risk factor for Alzheimer's disease and dementia (Gorelick, et al., 2011,Kloppenborg, et al., 2008). Recently we reported an association between glucose levels and dementia risk, even among people with no history of diabetes treatment (Crane, et al., 2013). The mechanisms by which elevated glucose levels may lead to increased dementia risk are not known.

Given this scientific uncertainty we sought to determine whether there were associations between glucose levels and neuropathologic outcomes evaluated at the time of autopsy among people with no history of treatment for diabetes. We specifically focused on neuropathologic findings that are in turn associated with dementia. Our *a priori* hypothesis was that higher glucose levels would be associated with higher levels of Alzheimer's disease-related pathology of neuritic plaques and neurofibrillary tangles. These elements are captured by Braak stage (Braak and Braak, 1991) for neurofibrillary tangles and Consortium to Establish a Registry for Alzheimer's Disease (CERAD) grade for neuritic plaques (Mirra, et al., 1991). Previous work has also found that Lewy bodies, microinfarcts, and macroscopic infarcts including lacunar infarcts are associated with dementia (Sonnen, et al., 2007), so we also evaluated associations between glucose levels and those neuropathologic outcomes, as well as amyloid angiopathy.

Glucose levels may directly lead to higher levels of any particular neuropathologic finding and thus to increased dementia risk. Alternatively, elevated glucose levels may impact dementia risk by altering cognitive resilience such that the burden of neurodegenerative changes one could tolerate before manifesting dementia is reduced. To address this, we used interaction models to explore whether glucose levels may alter thresholds of dementia risk for each neuropathologic finding. We therefore evaluated both whether there were associations between glucose levels and risk of dementia-related neuropathologic outcomes evaluated at death, and whether glucose levels modified the relationship between these neuropathologic outcomes and risk of expressing the clinical manifestation of dementia.

In our prior analysis of the association between glucose levels and dementia risk, we considered glucose levels over the five years prior to dementia onset. For the current analyses, we identified two time periods of interest. Our *a priori* primary approach was based on biological considerations, and considered the time period prior to death. These models in essence considered average glucose levels over the last several years of life as the exposure of interest.

Our *a priori* biological models may provide insight into disease etiology, but would be difficult to use to make clinical decisions, because we would have no way of knowing if a particular person was within the last few years of life. These considerations led us to a second, exploratory approach, which was to consider age bands late in life as our exposure-time axis. Thus, models for these secondary analyses considered average glucose levels over specific ages of life as the exposure of interest.

The present report thus follows up our finding of an association between higher glucose levels and increased dementia risk among people with no history of treatment for diabetes (Crane, et al., 2013) by exploring associations between glucose levels over several different time-windows and neuropathologic findings at autopsy. Our overarching goal was to help elucidate mechanisms by which higher glucose levels in late life could contribute to dementia risk.

2. Methods

2.1 Study description

Our analyses used autopsied participants with no history of diabetes treatment from Adult Changes in Thought (ACT), a population-based prospective cohort study examining risk factors for dementia. The study is described in detail elsewhere (Crane, et al., 2013,Kukull, et al., 2002,Larson, et al., 2006). Briefly, ACT participants are community-dwelling members of Group Health (GH), an integrated health care delivery system in the Pacific Northwest of the United States. Participants were required to be age 65 or older and not demented at study enrollment; then they were followed with biennial interviews for continued demographic and risk factor ascertainment and cognitive screening evaluations with the Cognitive Abilities Screening Instrument (CASI), for which scores range from 0 to 100 and higher scores indicate better functioning (Teng, et al., 1994). The CASI assesses attention, concentration, orientation, memory, language, visual construction, verbal fluency, and judgment. Participants with scores of 85 or less underwent further clinical and psychometric evaluation, including a battery of neuropsychological tests. The dementia psychometric battery includes clock drawing (Spreen and Strauss, 1991), verbal fluency (Morris, et al., 1989), Mattis Dementia Rating Scale (Mattis, 1988), Boston naming (Morris, et al., 1989), verbal paired associations and recall, logical memory and recall (Wechsler, 1987), Word List Memory (Morris, et al., 1989), Constructional Praxis and recall (Morris, et al., 1989), Trails A and B (Reitan and Wolfson, 1985), and Information and Comprehension subtest items (Wechsler, 1987). The results of these evaluations and laboratory testing and imaging records were then reviewed in a consensus conference. Diagnoses of dementia (American Psychiatric Association. Task Force on DSM-IV, 1994) and of probable or possible Alzheimer's disease (McKhann, 1984) were made on the basis of research criteria. Dementia-free participants continued with scheduled follow-up visits.

As participants were members of GH, information from electronic administrative databases – including lab measures, pharmacy dispensings, and diagnosis codes resulting from clinical encounters – could be linked to participants to augment data collected at ACT study visits. Additionally, participants who died and had consented to brain autopsy underwent a complete historical medical record review to determine even more extensive comorbid

history. All procedures were approved by the institutional review boards of GH and the University of Washington, and participants gave written informed consent.

2.2 Glucose characterization and modeling

GH pharmacy records were used to identify autopsied participants from ACT who never appeared to have been using medications for treatment of diabetes. Among these participants we ascertained measures of random and fasting glucose and glycated hemoglobin (HbA_{1c} or total glycated hemoglobin), taken as part of regular clinical care, through linkage with the GH computerized laboratory databases, as well from a complete historical medical record review. As in our prior glucose work within ACT (Crane, et al., 2013), total glycated hemoglobin was converted to HbA1c using the formula (HbA1c = 0.6*(glycated hemoglobin) + 1.7), and HbA1c was transformed to daily average glucose using the formula (avg = 28.7*(HbA1c) – 46.7) (Nathan, et al., 2008). Then, as in the prior analysis of glucose levels and dementia risk, we combined individual random and fasting glucose measures and daily average glucose using a hierarchical Bayesian framework (Carlin and Louis, 2000) to compute an estimated average glucose level for each 5-year period of each autopsied participant's life, covering time as recently as the 5 years prior to death and all the way back to the first availability of glucose/HbA1c measures for the individual. Computerized clinical laboratory data were available from 1988 onward; clinical laboratory data from medical records extended back as far as the 1940s for some individuals. Our prior report on clinical dementia outcomes was limited to the period from 1988 onwards (Crane, et al., 2013).

2.3 Neuropathology evaluation

Neuropathology workup for ACT has been reported elsewhere; we quote here from two studies (Li, et al., 2007,Sonnen, et al., 2007). Neuropathologic examinations were performed in the UW Division of Neuropathology and the University of Washington Alzheimer's Disease Research Center Neuropathology Core. All neuropathologic assessments were performed blind to clinical diagnosis and status of risk factors. Brains were immersion-fixed in formalin for at least 2 weeks prior to dissection. Following fixation, all brains were evaluated (wholly and after coronal sectioning) for any gross lesions, including the extent of atherosclerosis ("mild" when restricted to branch points in the circle of Willis, "moderate" when also in other regions at the base of the brain, and "severe" when present on the convexity of cerebrum) and the number of gross (macroscopic) infarcts including lacunar infarcts. We limited our evaluation to remote (estimated >1 year old) macroscopic infarcts, as acute and subacute infarcts were thought unlikely to have contributed to longstanding cognitive decline. Tissue sections were dissected from middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobule, anterior cingulate gyrus, primary visual cortex, basal ganglia at the level of the anterior commissure, thalamus, hippocampus at the level of the uncus, amygdala, midbrain including substantia nigra, pons at the level of the locus ceruleus, medulla, cerebellar hemisphere, and pituitary gland. These tissue sections were processed and embedded in paraffin prior to sectioning and staining. Neuritic plaques were scored according to the criteria of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (Mirra, 1997,Mirra, et al., 1991). Neurofibrillary tangles were staged according to the methods of Braak and Braak (Braak, et al., 1999,Braak and Braak, 1991). Amyloid angiopathy was scored according to the method of Vonsattel

(Vonsattel, et al., 1991). Microvascular lesions were evaluated in bilateral sections of frontal lobe, temporal lobe, parietal lobe, occipital lobe, caudate nucleus, putamen, internal capsule, and thalamus similarly to the protocol published by the Honolulu Asian Aging Study (White, et al., 2002). A microvascular lesion microinfarct was defined as an encephalomalacic lesion, 2 mm or smaller in its greatest dimension, which was not visible on gross inspection of the brain.

Immunohistochemistry for α-synuclein is performed in sections from frontal cortex and amygdala and evaluated in brainstem (substantia nigra and locus ceruleus) on H&E / luxol fast blue stained sections. A classification of neocortical Lewy body disease was made if immunohistochemically confirmed Lewy bodies were identified in mid-frontal cortex, and was typically associated with brainstem and amygdala Lewy bodies (Leverenz, et al., 2008,Leverenz and McKeith, 2002).

2.4 Other covariates

Ages at study baseline and at death were calculated based on birthdate. Sex, level of formal schooling, smoking history, and exercise levels were ascertained by ACT interview data. ACT study data also provided information on participants' dementia status as of their last ACT follow-up visit. Body mass index was assessed from medical record review for each autopsied participant, with their value closest to age 65 presented in our descriptive tables. Diagnoses of hypertension were ascertained from ACT interview data and medical record review, and blood pressure measures were obtained from medical record reviews. Up to three blood pressure values are recorded for each calendar year from medical record abstraction, with the first recorded value for 4-month time blocks included in the database. All values for each five-year block were included, and we used the average of these values in analyses. Coronary artery disease was summarized from four self-reported data elements at ACT study visits or analogous chart review data, based on angina, history of coronary artery bypass grafting, history of myocardial infarction, and history of coronary artery angioplasty. Cerebrovascular disease was defined on the basis of a clinical stroke or TIA, or carotid endarterectomy, measured from self-report at ACT study visits or analogous information found on medical record review. Atrial fibrillation was based on medical record reviews and ICD-9 codes. *APOE* genotype was obtained for participants who consented to genotyping.

2.5 Inclusion criteria

Inclusion criteria for the autopsy sample in the primary and secondary models are summarized in Table 1. For all models, we included only those ACT participants who never received a prescription treatment for diabetes during life (based on automated GH pharmacy dispensing data). For the primary models, we required continuous GH enrollment for the five years prior to death, and required adequate laboratory data to estimate glucose exposure. As shown in Table 1, this meant either at least one HbA1c value, or at least three different random glucose values spread across 90+ days. For the secondary models, we only included people in age-bands for which they had available glucose data (see Table 1)

2.6 Statistical analyses

For the primary analyses, we estimated the association between average glucose level in the five years prior to death (categorized as <100, 100–110, and >110 mg/dL) and risk of the dichotomized neuropathologic outcomes using modified Poisson regression models for binary data estimated via generalized estimating equations (GEE) (Zou, 2004). We chose these categories for glucose exposure for our primary analyses based on the distribution of glucose values observed in our sample (see Table 2). We chose to use this categorical exposure approach so as not to assume a strictly linear relationship between glucose levels and outcomes. A spline based approach could have been employed to avoid this linearity assumption while granting more flexibility than simple categorizations, but we still decided that the categorical approach in this instance would provide some simplicity in facilitating presentation, interpretation, and comparisons of results across all outcome models. We modeled each neuropathologic outcome separately. We adjusted all models for a core set of variables: ACT study cohort (original cohort enrolled 1994–1996; expansion cohort enrolled 2000–2003; expansion cohort enrolled 2005-onwards), age at death, sex, and level of formal education. As shown in Table 1, we performed additional analyses for each neuropathologic outcome in which we controlled for a broader set of covariates including average blood pressure levels, and histories of smoking, coronary artery disease, cerebrovascular disease, and atrial fibrillation.

To account for possible selection bias due to factors influencing continued ACT enrollment, consent to autopsy, and death, we incorporated inverse probability weights in the estimation of the aforementioned analytic models. We derived these weights from a logistic regression model of the probability of selection into the autopsy sample from the broader eligible ACT cohort (Haneuse, et al., 2009). The logistic regression model included variables thought to influence selection and potentially to be associated with the glucose exposure and neuropathologic outcomes. These variables included the adjustment variables that were included in outcome models, as well as dementia status as of last ACT visit. Finally, because estimation of the selection weights involves uncertainty, we computed standard errors and bias-corrected and accelerated bootstrap 95% confidence intervals (CIs) for primary relative risk (RR) estimates from our neuropathology outcome models using a bootstrap approach, as in prior publications (Dublin, et al., 2014,Haneuse, et al., 2009).

For our secondary analyses, we considered each 5-year age band from age 55–59, 56–60, 57–61, etc. on through 80–84. For each of these 26 age bands, we performed a separate analysis of the association between each neuropathologic outcome and the average glucose level measured in that age band using an analytic framework similar to that for our primary analyses (see Table 1). For any given age band model, we only included individuals with at least one glucose value in the specified age range. We used different categories of glucose exposure: <95 mg/dL, 95–105 mg/dL, and >105 mg/dL; see Figure 1 and its note for a discussion of the rationale for this categorization. We accounted for selection using inverse probability weighting as described above for the primary analyses, generating new weights for each of these models given the changing eligible sample, but we did not perform bootstrapping for each of the 26 age-bands x 7 outcomes = 182 regression models. We summarized results from these exploratory analyses graphically, showing estimated RRs and

their 95% CIs along with a lowess smooth fit to illustrate trends. We did not perform bootstrapping for these confidence intervals.

To explore the hypothesis that glucose might be associated with increased dementia risk through a possible alteration of cognitive resilience, we performed a series of additional analyses to investigate whether there were interactions between glucose levels and the dementia risk associated with neuropathologic findings. The outcome for these models was dementia status at the time of death. As such, we excluded 23 subjects whose last ACT visit was dementia-free but had occurred more than 2.25 years prior to death, as we thought the dementia status at death would not be reliably known for this handful of individuals. The exposure summary measures for these analyses were average glucose levels in the five years prior to death, categorized as <100 mg/dL, 100–110 mg/dL, and >110 mg/dL. As in the primary analyses, each neuropathologic finding was dichotomized (e.g. Braak stage 0-IV vs. Braak stage V-VI). For each neuropathologic measure, we used logistic regression with main effects for glucose level and the neuropathologic finding and the interaction terms between them. We computed an omnibus p-value to test for the overall significance of the interaction terms (a two degrees of freedom test) between glucose levels and neuropathologic findings on dementia risk. We present these results in a table stratified by glucose levels in the five years preceding death, showing the relative risks of dementia for higher levels of neuropathologic findings within each of the glucose groups. As in the primary analyses, we accounted for selection using inverse probability weighting and accounted for uncertainty in the weights using a bootstrapping procedure. For many of the more rare neuropathologic findings, bootstrapping often resulted in samples with too sparse data to estimate the interaction terms. For those analyses we present results that account for selection using inverse probability weights but that do not have bootstrapping; therefore, the presented confidence intervals in those instances are likely too narrow and not an accurate representation of the uncertainty.

Analyses were performed using SAS software, version 9.2 (SAS Institute, Inc., Cary, NC) and R, version 2.15.3 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1 Participants

At the time of this analysis, among 3,964 potentially eligible ACT participants with no known history of treatment for diabetes, 430 had died, consented to- and undergone autopsy and medical record review, and had at least some glucose or HbA1c measures recorded in the medical record or GH laboratory database. Of these 430, 318 had sufficient measures and GH enrollment to be included in the primary analysis examining glucose levels in the five years prior to death. Demographic characteristics, vascular risk factors, *APOE* genotype, clinical conditions, and average glucose level stratified by glucose exposure category for this group are summarized in Table 2. Cerebrovascular disease, and to a lesser extent *APOE* ε4 alleles, tended to be more common in the lowest glucose exposure category, while hypertension was more common among those in the highest glucose category. Many other characteristics were similar across groups.

3.2 Neuropathology findings

Neuropathology findings for the 318 participants included in the primary analyses, stratified by glucose exposure category, are shown in the left columns of Table 3, and for the 430 participants who had data included in at least one secondary analysis are shown in the right hand column of Table 3. In this sample of people never treated with diabetes medications during life, 50% had moderate or frequent neuritic plaques by CERAD criteria, 31% had widespread distribution of neurofibrillary tangles as indicated by Braak stage V or VI, and 30% had amyloid angiopathy. Atherosclerosis was common, being present in 61% of the sample. Microinfarcts (14%) and macriscopic infarcts including lacunar infarcts (30%) were less common. Lewy bodies and hippocampal sclerosis were less common still (see Table 3).

3.3 Primary analyses: Associations between glucose levels and neuropathology findings in the 5 years prior to death

Associations between glucose levels in the five years prior to death and neuropathologic findings at autopsy are shown in Table 4. The first set of RR estimates (with 95% CIs) shown are from models that include the core adjustment variables of ACT study cohort, age at death, sex, and education, and used inverse probability weighting to account for selection bias; these were our pre-specified primary models. Our *a priori* hypothesis was that higher glucose levels would increase risk for dementia-levels of neuritic plaques as measured by CERAD grade or of neurofibrillary tangles as measured by Braak stage. While the adjusted RR point estimates comparing the middle (100–110 mg/dL) and highest (>110 mg/dL) glucose level groups to the lowest level group (<100 mg/dL was the reference group) were slightly greater than 1, these findings were not statistically significant (p-values were 0.53 for association with CERAD grade and 0.85 for Braak stage). Models for Lewy bodies and the vascular neuropathologic measures also did not suggest associations between higher glucose levels and greater risk for these outcomes (all p-values > 0.50). The only association estimated to be significant at our pre-specified alpha level was between glucose and risk of hippocampal sclerosis (p-value=0.01). These results showed a J-shaped relationship, with lowest risk in the intermediate glucose exposure group of 100–110 mg/dL (RR 0.26, CI 0.05–1.60) and greatest risk in the highest glucose exposure group of >110 mg/dL (RR 1.89, CI 0.58–5.36). The uncertainty around these estimates was quite large, though, as evidenced by the wide confidence intervals. This reflects the small sample size for analyses of this outcome; as shown in Table 3 there were only 25 people identified with hippocampal sclerosis in these models, which may be too few for reliable regression modeling.

The final set of results shown in Table 4 are from models that adjusted for a more extensive set of covariates while still incorporating weighting to account for selection. The pattern of findings was very similar to the primary adjusted models. In particular, there was still no indication of significant associations between higher glucose exposures and CERAD grade or Braak stage. For hippocampal sclerosis, the point estimates for the intermediate and high exposure groups were somewhat further away from the null but still reflected high uncertainty.

Additional models incorporating *APOE* genotype were very similar. We did not find strong evidence of a relationship between glucose levels and CERAD grade or Braak stage (or any

of the other neuropathologic outcomes) when additionally adjusting for this genotype (see Supplemental Table 2).

3.4 Secondary analyses: Associations between glucose levels in five year age bands and neuropathology findings at autopsy

Figure 2 shows regression model results for estimated associations between average glucose levels in five-year age bands and CERAD findings at autopsy. Specifically, it plots adjusted RR point estimates (with 95% CIs) comparing the risk of intermediate or frequent plaques in the middle exposure group (95–105 mg/dL, in blue) and highest exposure group (>105 mg/dL, in red) to the lowest exposure group (<95 mg/dL, the reference group) for each of the age-band analyses, along with fitted lowess curves to each of the point estimates to provide a descriptive summary of trends in estimates across the many age analyses. We had hypothesized that there would be a dose-response relationship between higher glucose levels and higher levels of neuritic plaques. Such a relationship would have tended to produce RR point estimates above 1 across age groups for the intermediate exposure group (blue) and even higher point estimates for the higher exposure group (red). Instead, we find no associations, with the blue and red curves remaining very close to 1 and no evidence of a dose-response relationship.

Figure 3 shows similar results for Braak stage at autopsy. As was the case for CERAD, these results do not support a dose-response relationship between higher glucose exposures and higher probability of Braak stage V or VI at autopsy.

Figure 4 shows somewhat different results for risk of Lewy bodies in the substantia nigra or locus ceruleus and its association with glucose levels at various ages. The figure suggests a dose-response relationship, where the red lower curve summarizing the point estimates of relative risks for the highest glucose exposure group is consistently higher than the blue curve for the intermediate exposure group across multiple five-year age bands. However, the confidence intervals around the point estimates are large, as shown by the vertical red and blue bars. The large confidence intervals reflect the small numbers of people who had this neuropathologic finding at autopsy. Also, in our primary analyses covering five years preceding death, we found no relationship between Lewy body findings in the substantia nigra or locus ceruleus and glucose levels (Table 4).

Online Supplemental Figure 2 shows age-band analysis results for hippocampal sclerosis. In our primary analyses we found a J-shaped relationship between glucose levels in the five years preceding death and risk of hippocampal sclerosis at autopsy. We did not find supporting evidence for this relationship when we considered five year age-bands. Instead, we see inconsistent relationships, and wide confidence intervals reflecting the small numbers of people with hippocampal sclerosis.

Findings for other neuropathologic outcomes showed no consistent or conclusive relationships (see online Supplementary Figures 3–8).

3.5 Interactions between glucose levels and neuropathologic findings on dementia risk

Online Supplemental Table 3 shows the results from models of dementia risk and its relationship with glucose exposures in the five years prior to death and neuropathologic findings, allowing for interactions between these factors. These models do not explain the increased risk of dementia associated with higher glucose levels that were observed in the prior study. For some relationships the point estimates appear to follow a dose-response relationship. For example, the relative risks for dementia associated with moderate or frequent plaques by CERAD criteria are 2.30 for those with glucose levels 100 mg/dL, 2.22 in those with glucose levels 100–110 mg/dL, and 3.95 in those with glucose levels >110 mg/dL. However, the variability around these estimates was large, leading to a p value of 0.48. Furthermore, as also shown in Online Supplemental Table 3, when we adjust those models further for *APOE* genotype, there is no longer a dose-response relationship, with relative risks for dementia for moderate or frequent plaques of 2.60, 2.45, and 2.53 across the three glucose exposure groups (p=0.99); however, we do lose almost 10% of our sample when requiring *APOE* genotype. The only apparently statistically significant relationships were for microinfarcts. Due to cells with sparse data, bootstrapping failed for these models. Microinfarcts were associated with a 1.76-fold higher risk for those with glucose levels <100, with a 4.95-fold increased risk among those with glucose levels 100–110, and, confusingly, with a risk reduction for those with glucose levels >110 (RR=0.68, 95% CI 0.23, 1.98). We are not aware of a reason that microinfarcts would be protective for people with the highest glucose levels, and thus likely attribute this finding to the small sample size and the corresponding high level of uncertainty in estimation.

4. Discussion

Here we report neuropathologic associations with glucose levels from a well-characterized community-based cohort. Based on findings in cohort studies, our *a priori* hypothesis was that glucose levels would be associated with the neuropathologic features characteristic of Alzheimer's disease, which are recorded as the Braak stage for neurofibrillary tangles and CERAD score for neuritic plaques. While higher levels of Braak stage and CERAD score were commonly seen in our autopsy cohort, we did not find any evidence of association between higher glucose levels and these findings, either in our primary analyses in which time prior to death was the time axis or for our secondary analyses which considered age bands as the time axis. We saw a J-shaped relationship between glucose exposures and risk of hippocampal sclerosis. These associations were statistically significant but caution is warranted because of sparse models due to low numbers of individuals with that neuropathologic outcome. Furthermore, in our secondary analyses, we did not appreciate consistent associations across multiple age groups between glucose levels and risk of hippocampal sclerosis, tempering our enthusiasm about whether those findings would replicate in another setting. We did not observe associations with other common neuropathologic findings, including microinfarcts or macroscopic infarcts including lacunar infarcts, in our primary analyses. In our secondary analyses, we did see the suggestion of an association between higher glucose exposure levels and Lewy bodies in the substantia nigra or locus ceruleus, a rarer neuropathologic finding, when we used five-year age bands, but did not find evidence of an association in our primary analyses that considered exposures in the

five years prior to death. We did not find convincing evidence of interactions between glucose levels in the five years prior to death, neuropathologic findings at autopsy, and dementia risk. Overall, we did not find evidence of an association between glucose levels and neuropathology that would explain the association we previously found between glucose levels and dementia risk among people who were not treated for diabetes.

There is increasing evidence that prolonged elevated glucose is associated with neuronal dysfunction (Mooradian, 1997). The mechanism by which this dysfunction may cause cognitive impairment or dementia is uncertain. The mechanism may occur through amyloid β or tau dependent or independent mechanisms (Sato and Morishita, 2014). The amyloid cascade hypothesis of Alzheimer's disease suggests that either increased production and/or reduced clearance of amyloid β species is a key mechanistic event in the development of Alzheimer's disease pathology. Elevated plasma glucose levels have been associated with changes in blood brain barrier transport function including through alterations in the receptor for advanced glycosylation end products (RAGE) enzyme (Prasad, et al., 2014). These investigations have primarily focused on much higher glucose loads than those observed in our study participants. Neurofibrillary tangles are a pathologic hallmark of Alzheimer disease and are highly correlated with dementia status. Animal models of diabetes have shown increased phosphorylation of tau, the primary protein component of neurofibrillary tangles (reviewed in (Sato and Morishita, 2014)). A study from Hisayama in Japan administered an oral glucose tolerance test to participants, and found associations between neuritic plaques and post-load plasma glucose, fasting insulin, and insulin resistance as assessed by the homeostasis model assessment of insulin resistance (HOMA-IR). No report was made of relationships with glucose levels over time (Matsuzaki, et al., 2010). A study in a highly selected memory clinic population found no relationship between HbA1c or fasting glucose and cerebrospinal fluid levels of tau, phosphor-tau^{181}, or A$\beta_{1–42}$ (Exalto, et al., 2010) . A pooled evaluation of several autopsy studies found that diabetes was associated with increased risk of cerebrovascular disease, but not plaques or tangles. That study did not analyze heterogeneity in glucose levels among people who were never treated for diabetes (Abner, et al., 2016). An interesting imaging study from the Mayo Clinic Study of Aging found that diabetes was associated with higher risk of AD-type patterns on FDG-PET scan, but no increased risk of amyloid deposition on PiB scanning. That group also evaluated associations between HbA1c levels among people without diabetes, and found in those individuals that higher levels of HbA1c were associated with increased risk of hypometabolism in patterns consistent with Alzheimer's disease, though this relationship only was statistically significant among people without dementia (Roberts, et al., 2014). Taken as a whole, these studies do not support a relationship between glucose levels among people without diabetes and the neuritic plaques and neurofibrillary tangles that are characteristic of Alzheimer's disease. Our findings similarly do not support associations between variability in glucose levels within the normal range and either neuritic plaques or neurofibrillary tangles.

The epidemiologic link between elevated glucose and vascular brain injury is well established (Abner, et al., 2016,Kissela, et al., 2005,Liu, et al., 2011,Saczynski, et al., 2009). Microinfarcts as a correlate of dementia are less well studied. We previously described an association between microinfarcts and diabetes (Sonnen, et al., 2009). There are many

mechanisms by which prolonged hyperglycemia alters brain microvasculature (Prasad, et al., 2014). Nevertheless, as for neuritic plaques and neurofibrillary tangles, we found no evidence of associations between variability in glucose levels within the non-diabetic range and any vascular outcome, including atherosclerosis, macroscopic infarcts including lacunar infarcts, or microinfarcts. These results suggest that this may not be the pathway through which higher glucose levels lead to higher dementia risk in the general population.

Although we did find some evidence of a relationship between glucose exposures and risk of hippocampal sclerosis in the five years preceding death, we found no associations across age bands. Very little is known about the pathogenesis of hippocampal sclerosis in the elderly (Zarow, et al., 2008); to our knowledge associations with glucose have not been previously reported. One paper evaluated relationships between diabetes and hippocampal sclerosis in three different autopsy series and did not find a relationship (Neltner, et al., 2014). Hippocampal sclerosis was associated with both low and elevated glucose levels. Clinically hippocampal sclerosis has been associated with hypoglycemia, although at levels that are well below those reported in this analysis. One could conjecture that the high and low glucose levels measured in this analysis could represent labile serum glucose that might be mechanistically associated. As more elderly study participants come to autopsy, we will likely have much greater statistical power to investigate the relationship between glucose exposures and hippocampal sclerosis.

Results from our secondary analyses using age bands suggested a possible relationship between glucose levels and Lewy body neuropathology, but these results were not statistically significant due primarily to the low frequency of Lewy body neuropathology in our sample. There have been studies evaluating associations between diabetes and clinical diagnoses of dementia with Lewy bodies (DLB) such as (Dugger, et al., 2016); the present study evaluated Lewy bodies at autopsy as opposed to clinical diagnoses made without knowledge of brain findings at autopsy. We are unaware of any study that has considered the variability of glucose levels within the non-diabetic range and risk for Lewy body neuropathology. However, there is some evidence that Lewy body disease and diabetes may share common pathophysiological mechanisms. Chronic systemic inflammation and mitochondrial dysfunction have been mechanistically implicated in both Lewy body disease and type 2 diabetes (Lima, et al., 2014). Intriguingly, however, a meta-analysis of case-control studies (Lu, et al., 2014) and a recent epidemiological study (Saaksjarvi, et al., 2015) suggest that diabetes may reduce risk for Parkinson’s disease, and a recent review does not mention diabetes as a risk factor for Parkinson’s disease (Kalia and Lang, 2015). Very little is known about variability of glucose levels within the non-diabetic range and risk for Lewy bodies.

It is important to consider limitations related to our findings. While this is the most extensive study of its kind, the sample size for some of the neuropathologic findings was somewhat limited, resulting in high levels of uncertainty around many of our relative risk estimates. This was particularly the case for rarer neuropathologic findings; however, neurofibrillary tangles and especially neuritic plaques as assessed by Braak stage and CERAD grade were common in our sample. Nevertheless, the association we previously found with dementia status was relatively modest, and even though plaques and tangles were common in our

sample, we could have missed small associations between glucose variation and risk for these neuropathologic outcomes. We had thousands of clinical glucose values to populate our exposure models, but these were clinical samples, and in many cases prandial status was not known. In our previous study we were able to show an association despite this source of variability, which tempered some concerns about potential bias towards the null. In this instance, however, we cannot rule out the possibility that unknown prandial status may influence our findings. We are not aware of large autopsy samples that have better characterization of chronic glucose levels; nevertheless, we are limited to the data we have available to us. One possibility to explain a lack of findings is insufficient power. Our previous analyses of dementia risk included the entire ACT cohort at risk for dementia outcomes, and we stratified analyses for people with treated diabetes vs. people without treated diabetes. Here we were limited to the subset of people who consented to autopsy and, of those, people who had actually died and come to autopsy and, of those, people who never developed diabetes requiring medications during their lives. These considerations led to analyses in a much smaller dataset. Nevertheless, we carried out our analyses with the thought that neuropathologic findings are mechanistically more proximate to the glucose levels than dementia status, which should increase power. Similar lines of argumentation support the use of neuropathology-derived endophenotypes for genetics research (Bennett, et al., 2009). In previous analyses in our cohort, we found associations between non-steroidal anti-inflammatory drugs (NSAIDs) and dementia risk (Breitner, et al., 2009) and, when considering neuropathology data, also found associations between NSAIDs and neuritic plaques (Sonnen, et al., 2010). If there is a relatioinship between glucose exposure and neuritic plaques, it is weaker than the relationship between NSAID exposure and neuritic plaques. Ultimately we cannot say whether we have adequate power, only that we were unable to detect a clear explanation the relationship between glucose levels and dementia with the data we had available to us. The racial and ethnic heterogeneity that characterize our sample is somewhat limited, and whether our findings can be extrapolated to other groups is uncertain. Additionally, as with any observational study, we must allow for the potential for confounding due to unmeasured or imperfectly ascertained covariates, or inadequate model adjustment due to limited sample size. Because we focused on people who never received treatments for diabetes during life, hypothesized impacts of antidiabetic medication on the brain should not influence our findings. Our results should not be extrapolated to people with diabetes, as we did not evaluate those individuals in our analyses. Furthermore, we used diabetes treatment to determine eligibility for our analytic sample. As such, some people who may have had a diagnosis of diabetes but not been treated with medications (e.g., diet-treated) could have been included in our analyses. We suspect that study results would have been similar had we been able to clearly identify and remove data from any such individuals. It is difficult to predict the effect of competing risks for death or differential mortality rates on our findings; our modeling made no attempt to account for any such effects. Alzheimer's disease neuropathology is imperfectly assessed by CERAD grade and Braak stage. We will re-evaluate Thal phasing when those data become available (Hyman, et al., 2012,Montine, et al., 2012). Furthermore, molecular quantification of Aβ and tau load may provide additional information on risk of dementia (Postupna, et al., 2015). We will evaluate glucose levels and Histelide values of these antibodies in the future.

In summary, we did not find clear and compelling associations between variation in glucose levels among people with no history of diabetes treatment and neuropathologic findings associated with dementia. Further studies will be needed to more firmly discern the mechanism or mechanisms underlying the previously observed association between higher glucose levels and greater dementia risk in such individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Mechanisms of associations between glucose levels and dementia risk are not known.
- Glucose levels in 5 years prior to death (primary) and 5-year bands (secondary).
- No associations between glucose and Braak or CERAD grade.
- Inconsistent suggestions of associations with hippocampal sclerosis and Lewy bodies.
- Further work needed to elucidate mechanisms of glucose's risk on dementia.

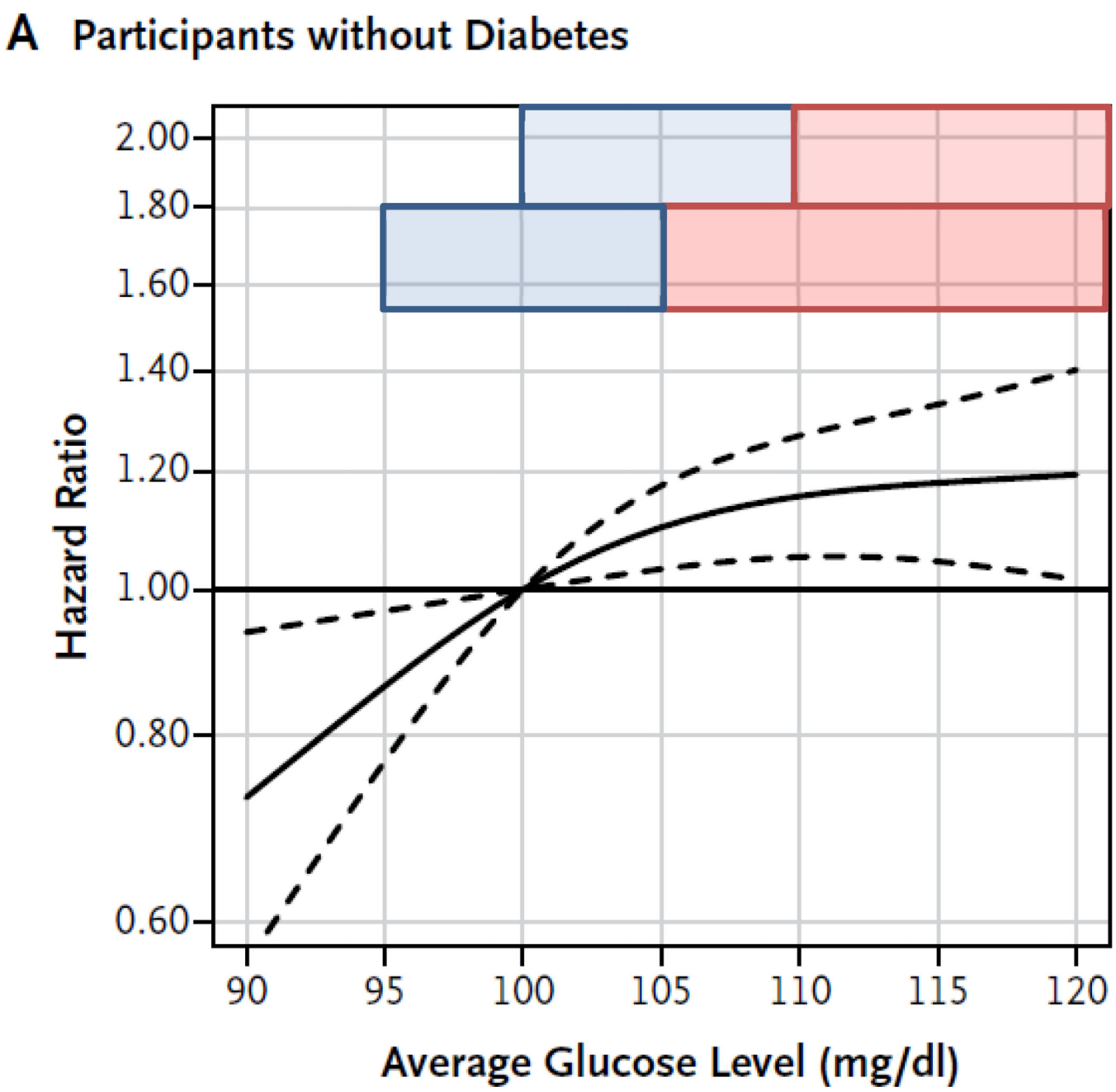

Figure 1. Summary of glucose exposure data superimposed on spline curve and confidence intervals summarizing association between glucose exposure and dementia risk as previously published

This graph shows the primary result from our earlier publication of the association between glucose levels and dementia risk (labeled “A”). We found a monotonically increasing relationship between glucose levels and dementia risk. The top blue and red figures show the glucose levels we used for our primary analyses, with the intermediate level of glucose exposure from 100–110 mg/dL depicted in blue, and the higher level of glucose exposures >110 mg/dL. The bottom blue and red figures show the glucose levels we used for our secondary analyses, with the intermediate level of exposures from 95–105 mg/dL, and the

higher level of exposures >105 mg/dL. The distribution of glucose levels in our autopsy cohort is shown in Supplemental Figure 1. The black and white figure labeled as “A” is from *New England Journal of Medicine*, Crane PK, Walker R, Hubbard RA, Li G, Nathan DM, Zheng H, Haneuse S, Craft S, Montine TJ, Kahn SE, McCormick W, McCurry SM, Bowen JD, Larson EB, Glucose Levels and Risk of Dementia, 369:6, p. 540–8. Copyright © 2013 Massachusetts Medical Society. Reprinted with permission.

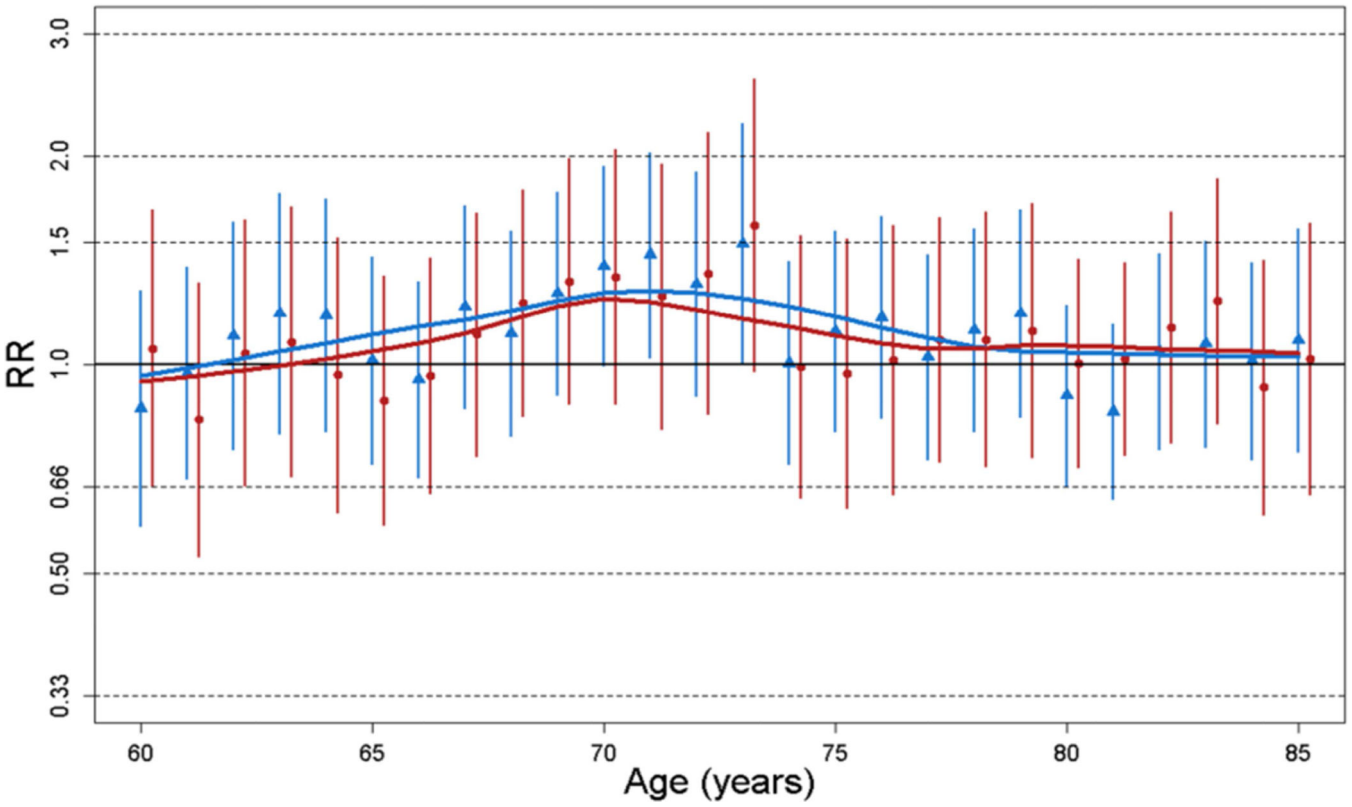

Figure 2. Association between glucose levels in five-year age bands and higher CERAD grades at autopsy

This graph summarizes results from 26 regression models. Each model considered glucose levels for five year age bands. For example, the red and blue bars above the number 60 on the x-axis show the relative risk (RR) point estimate and 95% confidence interval comparing risk of the higher CERAD grades in the 95–105 mg/dL exposure group (blue triangle and blue bar) and the >105 mg/dL exposure group (red circle and red bar) relative to a reference exposure group of <95 mg/dL. We superimposed lowess smoothed curves of the point estimates with the red and blue curves. Evidence of a dose-response relationship in this exploratory analysis would have been given support had the red curve (the highest exposure group) been consistently above the blue curve (the intermediate exposure group) and the blue curve consistently above RR=1 across many five year age bands. This graph does not lend support to such a relationship.

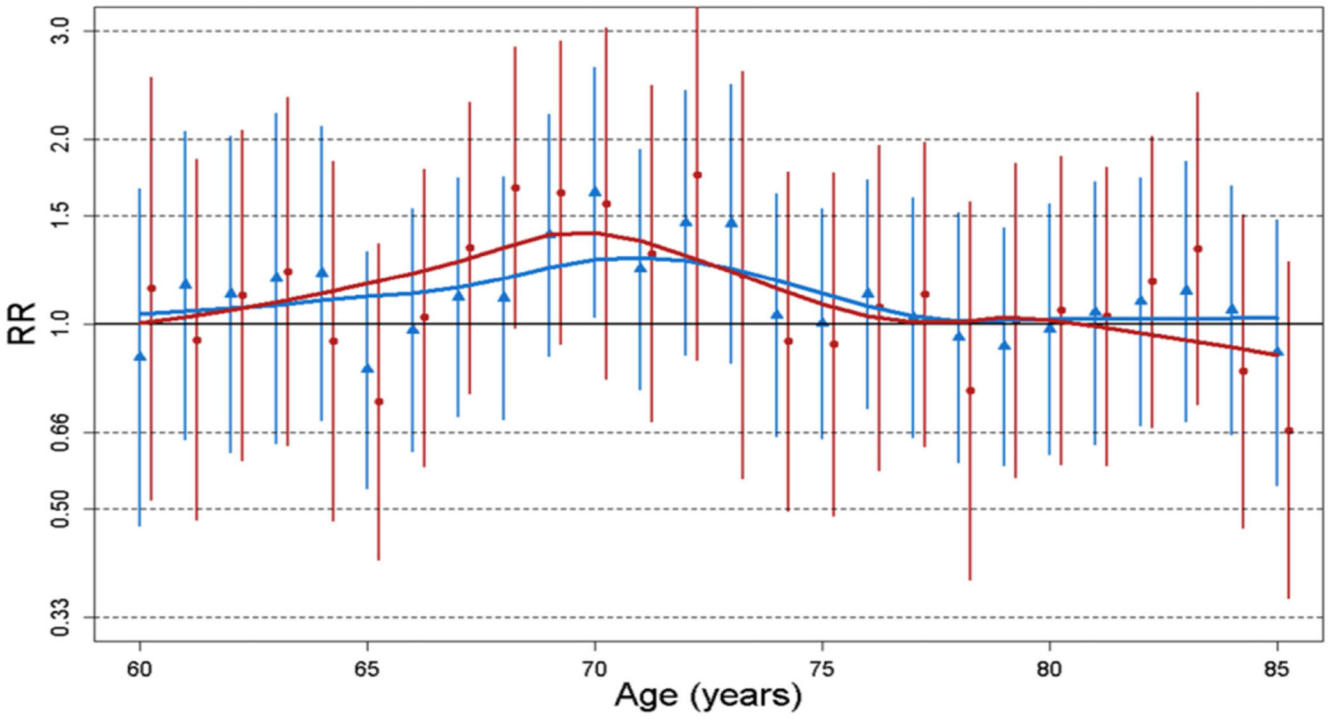

Figure 3.

Association between glucose levels in five-year age bands and Braak stage at autopsy. See note to Figure 2. This graph also does not lend support to a dose-response relationship between glucose levels and Braak stage.

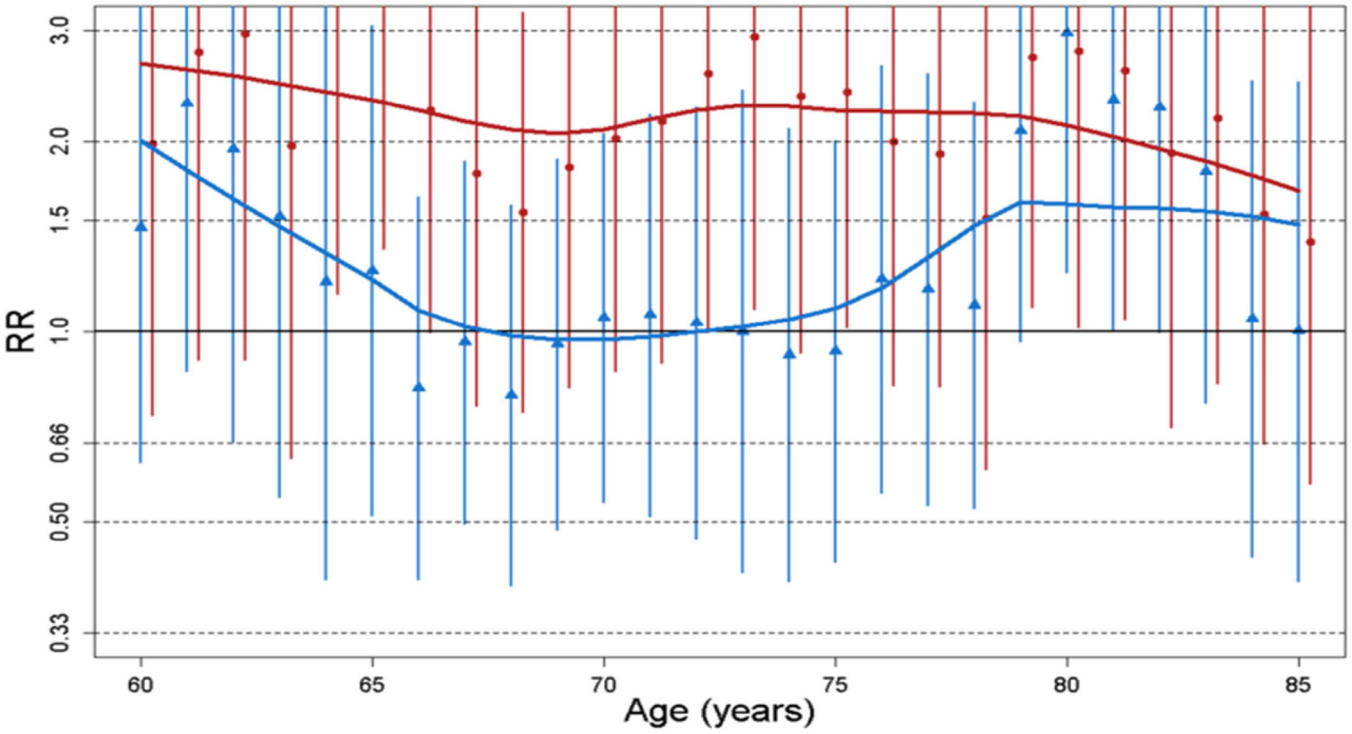

Figure 4.

Association between glucose levels in five-year age bands and presence of Lewy bodies in the substantia nigra or locus ceruleus at autopsy. See note to Figure 2. This graph may suggest some relationship between higher glucose levels and presence of Lewy bodies in the locus ceruleus or substantia nigra, as the red curves appear for most five year age bands to exceed the null. The small number of individuals with these Lewy bodies leads to the large confidence intervals, however, suggesting high uncertainty in these estimates and limited ability to draw strong conclusions regarding this relationship.

Table 1

Summary of two strategies for modeling glucose exposure

Characteristic	Biological Model (Primary)	Clinical Model (Secondary)
Time period	5 years prior to death	5 year age bands
Inclusion criteria	Autopsied ACT participants without a history of diabetes treatment	Autopsied ACT participants without a history of diabetes treatment
GH enrollment	Required continuous GH enrollment in the five years prior to death	At least some GH enrollment but no minimum
Glucose exposure measures	≥1 HbA1c or ≥3 glucose measures spanning ≥90 days within 5 years of death	≥1 HbA1c or glucose measure within the age band being analyzed
Glucose categories	<100, 100–110, >110	<95, 95–105, >105 (see Fig. 1)
Number of analyses	1 per neuropathologic outcome: 5 years prior to death	26 per neurological outcome: age 55–59, age 56–60, …, age 79–83, age 80–84
Sample size(s)	318	Low of 216 (age 55–59) to high of 388 (age 70–74) (see Supplemental Fig. 1)
Core adjustment covariates	ACT study cohort, age at death, sex, education	ACT study cohort, age at death, sex, education
Additional adjustment covariates	Average blood pressure levels, history of smoking, coronary artery disease, cerebrovascular disease, and atrial fibrillation	None
Accounting for selection	Inverse probability models, with all above covariates as well as dementia status (at last ACT visit)	Inverse probability models, with all above covariates as well as dementia status (at last ACT visit)
Bootstrapping	Bootstrapping performed	No bootstrapping
Analytic strategy	Modified Poisson regression models estimated via generalized estimating equations	Modified Poisson regression models estimated via generalized estimating equations
Outcomes	Neuritic plaques (CERAD), neurofibrillary tangles (Braak), amyloid angiopathy, Lewy bodies, microinfarcts, macroscopic infarcts including lacunar infarcts, atherosclerosis of the circle of Willis	Neuritic plaques (CERAD), neurofibrillary tangles (Braak), amyloid angiopathy, Lewy bodies, microinfarcts, macroscopic infarcts including lacunar infarcts, atherosclerosis of the circle of Willis

GH= Group Health.

Table 2

Demographic and clinical characteristics for the primary analyses (n=318)

	Average glucose <100 mg/dL in 5 years prior to death (n=102)	Average glucose 100–110 mg/dL in 5 years prior to death (n=110)	Average glucose >110 mg/dL in 5 years prior to death (n=106)	Total (n=318)
Demographic characteristics				
Age at death, median (IQR)	88.5 (84.2, 92.2)	88.0 (83.3, 92.1)	88.0 (82.5, 92.6)	88.1 (83.2, 92.3)
Female sex, n (%)	55 (54%)	62 (56%)	55 (52%)	172 (54%)
At least some college, n (%)	70 (69%)	74 (67%)	69 (65%)	213 (67%)
Vascular risk factors				
Current or former smoker, n (%)	53 (52%)	64 (58%)	58 (55%)	175 (55%)
Average SBP, median (IQR)[a]	135 (128, 144)	134 (124, 142)	132 (125, 143)	134 (125, 142)
Average DBP, median (IQR)[a]	71 (67, 76)	72 (68, 76)	71 (67, 76)	71 (67, 75)
Body mass index, median (IQR)[b]	24.2 (22.1, 26.5)	24.1 (22.2, 27.5)	25.6 (22.6, 28.0)	24.4 (22.3, 27.5)
Any regular exercise, n (%)[c]	34 (37%)	39 (40%)	34 (36%)	107 (38%)
***APOE* genotype**				
≥1 *APOE* ε4 allele, n (%)[d]	30 (32%)	27 (27%)	21 (22%)	78 (27%)
Clinical conditions				
Cerebrovascular disease, n (%)	60 (59%)	52 (47%)	50 (47%)	162 (51%)
Coronary artery disease n (%)	61 (60%)	65 (59%)	62 (58%)	188 (59%)
Atrial fibrillation	46 (45%)	47 (43%)	45 (42%)	138 (43%)
Glucose exposure				
Average glucose in 5 years prior to death, median (IQR)	96 (93, 98)	105 (103, 107)	117 (112, 123)	105 (99, 112

[a] Systolic and diastolic blood pressure data summarized in the table were ascertained based on data from medical record reviews, and represent the average values from the 5 years prior to death.

[b] Body mass index presented in the table was the value closest to age 65 for each participant.

[c] Exercise status was based on ACT study visit data during the 5 years prior to death and was missing for 35 participants overall, including 11 (11%) in the <100 mg/dL glucose exposure group, 12 (11%) in the 100–110 mg/dL glucose exposure group, and 12 (11%) of the >110 mg/dL glucose exposure group. Proportions shown in the body of the table reflect a denominator of the participants who had non-missing exercise data.

[d] APOE genotype was missing for 31 participants overall, including 8 (8%) of those in the <100 mg/dL glucose exposure group, 11 (10%) of those in the 100–110 mg/dL glucose exposure group, and 12 (11%) of those in the >110 mg/dL glucose exposure group. Proportions shown in the body of the table reflect a denominator of those who had non-missing APOE genotype data.

Abbreviations: IQR = inter-quartile range

Table 3

Neuropathology findings stratified by glucose level in the 5 years prior to death for the 318 people included in the primary analysis (left hand columns) and for the entire sample of 430 participants who came to autopsy and contributed data to at least one secondary analysis model (far right column)

Neuropathologic finding	Average glucose <100 mg/dL in 5 years prior to death (n=102)	Average glucose 100–110 mg/dL in 5 years prior to death (n=110)	Average glucose >110 mg/dL in 5 years prior to death (n=106)	Total sample for primary analyses (n=318)	Available sample for secondary analyses (n=430)
CERAD moderate or frequent	43 (42%)	54 (49%)	50 (47%)	147 (46%)	216 (50%)
Braak Stage V-VI	24 (24%)	32 (29%)	28 (26%)	84 (26%)	132 (31%)
Amyloid angiopathy[a]	28 (28%)	31 (28%)	27 (26%)	86 (27%)	127 (30%)
Cerebral microinfarcts[b]	12 (12%)	12 (11%)	14 (13%)	38 (12%)	60 (14%)
Macroscopic infarcts[c]	30 (30%)	29 (27%)	27 (26%)	86 (28%)	127 (30%)
Atherosclerotic disease[d]	59 (60%)	62 (58%)	62 (61%)	183 (60%)	256 (61%)
Lewy bodies					
Frontal or temporal cortex[e]	5 (5%)	6 (6%)	5 (5%)	16 (5%)	25 (6%)
Substantia nigra or locus ceruleus[f]	13 (13%)	14 (13%)	13 (12%)	40 (13%)	58 (14%)
Amygdala[g]	14 (15%)	17 (17%)	12 (12%)	43 (14%)	60 (15%)
Hippocampal sclerosis[h]	7 (7%)	3 (3%)	15 (15%)	25 (8%)	36 (9%)

CERAD = Consortium to Establish a Registry for Alzheimer's Disease

[a] Data missing for 3 of 430, all were among the 318 in the primary analyses, 1 in each glucose exposure category

[b] Data missing for 4 of 430, all were among the 318, 2 in <100 mg/dL, and 1 in each of the other categories

[c] Includes lacunar infarcts. Data missing for 9 of 430, 8 were among the 318, 3 in <100 mg/dL, 1 in 100–110 mg/dL, and 4 in >110 mg/dL category

[d] Data missing for 13 of 430, 12 were among the 318, 3 in <100 mg/dL, 4 in 100–110 mg/dL, and 5 in >110 mg/dL category

[e] Data missing for 2 of 430, both among the 318, 1 in 100–110 mg/dL and 1 in >110 mg/dL category

[f] Data missing for 1 of 430, who was among the 318 and was in the >110 mg/dL category

[g] Data missing for 25 of 430, 22 were among the 318, 9 in <100 mg/dL, 8 in 100–110 mg/dL, and 5 in >110 mg/dL category

[h] Data missing for 14 of 430, 13 were among the 318, 3 in <100 mg/dL, 5 in 110–110 mg/dL, and 5 in >110 mg/dL category

Table 4

Primary analyses: Associations between glucose exposures in the five years preceding death and neuropathology findings; average glucose levels <100 mg/dL serve as the reference category for all models

	Core adjustment[a]			Additional adjustment[b]		
	100–110 mg/dL	>110 mg/dL		100–110 mg/dL	>110 mg/dL	
Neuropathologic finding	**RR, 95% CI**	**RR, 95% CI**	**p value**	**RR, 95% CI**	**RR, 95% CI**	**p value**
CERAD moderate or frequent	1.23 (0.83,1.88)	1.01 (0.67, 1.51)	0.53	1.28 (0.87, 1.96)	1.07 (0.71, 1.59)	0.46
Braak Stage V-VI	1.22 (0.61, 2.46)	1.06 (0.53, 2.04)	0.85	1.32 (0.69, 2.80)	1.12 (0.53, 2.16)	0.75
Amyloid angiopathy	0.80 (0.42, 1.37)	0.87 (0.48, 1.53)	0.77	0.83 (0.43, 1.44)	0.97 (0.53, 1.72)	0.80
Cerebral microinfarcts	1.08 (0.41, 2.81)	1.59 (0.65, 4.63)	0.61	1.12 (0.36, 2.98)	1.52 (0.53, 4.44)	0.70
Macroscopic infarcts[c]	0.96 (0.54, 1.65)	0.92 (0.52, 1.67)	0.97	1.03 (0.59, 1.85)	0.95 (0.54, 1.70)	0.97
Atherosclerotic disease	1.18 (0.87, 1.64)	1.03 (0.75, 1.42)	0.55	1.26 (0.95, 1.73)	1.12 (0.82, 1.53)	0.30
Lewy bodies						
Frontal or temporal cortex	1.09 (0.25, 4.30)	1.03 (0.22, 4.94)	0.99	0.67 (0.09, 3.59)	0.95 (0.16, 5.65)	0.85
Substantia nigra or locus ceruleus	1.67 (0.68, 4.35)	1.20 (0.49, 3.11)	0.52	1.52 (0.65, 4.38)	1.06 (0.37, 2.80)	0.58
Amygdala	1.36 (0.58, 3.37)	1.17 (0.50, 3.27)	0.78	1.45 (0.62, 4.17)	1.05 (0.39, 3.02)	0.66
Hippocampal sclerosis	0.26 (0.05, 1.60)	1.89 (0.58, 5.36)	0.01	0.23 (0.05, 1.22)	2.07 (0.61, 5.94)	0.01

CERAD = Consortium to Establish a Registry for Alzheimer's Disease. CI = confidence interval. RR = relative risk.

[a]These models included terms for ACT study cohort (Original Cohort enrolled 1994–1996; Expansion Cohort enrolled 2000–2003, or Continuous Enrollment enrolled 2005-onwards), age at death, sex, and level of formal schooling. These models used inverse probability weighting to account for selection bias. The selection model included the following covariates as of the last ACT study follow-up visit: ACT study cohort age, sex, level of formal schooling, and dementia status. P values and confidence intervals were obtained using bootstrapping. The p values are for an omnibus test of whether the RR for the 100–110 mg/dL group and/or the RR for the >110 mg/dL group is statistically different from the null.

[b]These models were additionally adjusted for average systolic and diastolic blood pressure, history of coronary artery disease, cerebrovascular disease, smoking, and atrial fibrillation. Note that history of cerebrovascular disease was not included in models of macroscopic infarcts. Further, these models used inverse probability weighting to account for selection bias. The selection model included the following covariates as of the last ACT study follow-up visit: ACT study cohort, age, sex, level of formal schooling, dementia status, coronary artery disease, cerebrovascular disease, smoking, and atrial

fibrillation. There were five individuals missing one or more covariates from the expanded selection data; they were excluded from these analyses but not for the primary selection models from above. P values and confidence intervals were obtained using bootstrapping.

[c] Includes lacunar infarcts