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# Higher glucose levels associated with lower memory and reduced hippocampal microstructure

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## ABSTRACT

**Objectives:** For this cross-sectional study, we aimed to elucidate whether higher glycosylated hemoglobin (HbA1c) and glucose levels exert a negative impact on memory performance and hippocampal volume and microstructure in a cohort of healthy, older, nondiabetic individuals without dementia.

**Methods:** In 141 individuals (72 women, mean age 63.1 years  $\pm$  6.9 SD), memory was tested using the Rey Auditory Verbal Learning Test. Peripheral levels of fasting HbA1c, glucose, and insulin and 3-tesla MRI scans were acquired to assess hippocampal volume and microstructure, as indicated by gray matter barrier density. Linear regression and simple mediation models were calculated to examine associations among memory, glucose metabolism, and hippocampal parameters.

**Results:** Lower HbA1c and glucose levels were significantly associated with better scores in delayed recall, learning ability, and memory consolidation. In multiple regression models, HbA1c remained strongly associated with memory performance. Moreover, mediation analyses indicated that beneficial effects of lower HbA1c on memory are in part mediated by hippocampal volume and microstructure.

**Conclusions:** Our results indicate that even in the absence of manifest type 2 diabetes mellitus or impaired glucose tolerance, chronically higher blood glucose levels exert a negative influence on cognition, possibly mediated by structural changes in learning-relevant brain areas. Therefore, strategies aimed at lowering glucose levels even in the normal range may beneficially influence cognition in the older population, a hypothesis to be examined in future interventional trials.

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## GLOSSARY

**AD** = Alzheimer disease; **BDI** = Beck Depression Inventory; **BMI** = body mass index; **DTI** = diffusion tensor imaging; **FIRST** = FMRIB's Integrated Registration and Segmentation Tool; **HbA1c** = glycosylated hemoglobin; **ICV** = intracranial volume; **IGT** = impaired glucose tolerance; **MD** = mean diffusivity; **T2DM** = type 2 diabetes mellitus.

Deleterious effects of diabetic glucose levels on brain structure, particularly the hippocampus, have been reported in both animal<sup>1</sup> and human<sup>2</sup> studies. Moreover, impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM) are associated with lower cognitive function and higher incidence of dementia, including Alzheimer disease (AD) and vascular dementia.<sup>3</sup> Even in individuals without IGT or T2DM, higher glucose levels may exert negative effects on memory performance and the volume of the hippocampus.<sup>4,5</sup> However, a more detailed delineation of hippocampal microstructure has not been conducted. Such an approach is feasible by multimodal acquisition of high-resolution T1-weighted MRIs and diffusion tensor images (DTIs) to estimate mean diffusivity (MD) within the hippocampus.<sup>6-8</sup> Analyses of hippocampal MD, as a measure of neuronal integrity,<sup>9,10</sup> may add substantial information about potential mechanisms underlying associations between glucose metabolism and hippocampal function. Also, a comprehensive model for the association of not only short-term, but also long-term

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markers of glucose regulation and memory performance or hippocampal structure in nondiabetic individuals has not been generated. Therefore, we assessed the association between peripheral short- and long-term glucose metabolism and memory performance in a well-characterized cohort of nondiabetic older adults without IGT, using comprehensive multiple regression models. Second, we conducted detailed analyses of both hippocampal volume and microstructure including MRI-based volume and MD. Third, we used simple mediation models to evaluate whether decline in hippocampal volume or microstructure mediated the effect of glucose levels on memory performance.

**METHODS** **Participants.** Participants aged 50 to 80 years were recruited via advertisements in Berlin, Germany. Individuals with a history of T2DM, severe untreated medical disease, major neurologic or severe or untreated psychiatric diseases were excluded. Also, a body mass index (BMI)  $<25 \text{ kg/m}^2$  or  $>30 \text{ kg/m}^2$ , daily consumption of  $>50 \text{ g}$  alcohol,  $>10$  cigarettes, or  $>6$  cups of coffee, and not native German speaking led to exclusion. The Mini-Mental State Examination<sup>11</sup> was used to rule out preexisting cognitive impairment (a score  $<26$  points led to exclusion).

**Standard protocol approvals, registrations, and patient consents.** The study was approved by the Ethics Committee of the Charité University Medicine Berlin, Germany, and was in accordance with the declaration of Helsinki. All subjects provided informed written consent before participation in the study, and received a small reimbursement at the end.

**Fasting blood parameters and APOE genotyping.** Venous blood was collected after an overnight fast of at least 10 hours. Blood measurements included glycosylated hemoglobin (HbA1c) (in mmol/mol), fasting glucose (in mmol/L), and insulin (in  $\mu\text{U/mL}$ ). HbA1c and glucose were considered as the primary parameters of glucose metabolism. To assess individual APOE status, DNA was extracted from whole blood using a Blood Mini Kit (Qiagen, Hilden, Germany) and stored at  $-80^\circ\text{C}$  until analysis. Genotyping was performed at the laboratory of Prof. Dr. Dan Rujescu (University of Halle, Germany) following procedures as previously described.<sup>12</sup> For 3 samples, genotyping was missing because of failed DNA extraction. See appendix e-1 on the *Neurology*<sup>®</sup> Web site at [www.neurology.org](http://www.neurology.org) for detailed information on blood parameters, anthropometric assessments, and questionnaires.

**Memory performance.** To evaluate individual learning and memory performance, subjects were tested using the German version of the Rey Auditory Verbal Learning Test.<sup>13</sup> We evaluated the following 3 subtasks: delayed recall (primary outcome parameter), learning ability, and memory consolidation (for details see appendix e-1).

**MRI.** MRI was performed on a 3T Magnetom Trio system, using a 12-channel head coil (see appendix e-1). Analyses were done using the software packages FSL ([www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl)) and FreeSurfer (<http://surfer.nmr.mgh.harvard.edu/>). To estimate hippocampal volume, FMRIB's Integrated Registration and Segmentation Tool (FIRST) was applied to high-resolution

T1-weighted images to perform a model-based segmentation of the left and right hippocampus.<sup>14</sup> Nonbrain tissue was deleted using brain extraction tools and the brains were segmented using FMRIB's Automated Segmentation Tool into gray matter, white matter, and CSF, which added up to intracranial volume (ICV). Individual hippocampal volumes were adjusted for head size according to previous studies<sup>7,15</sup> using the following formula: adjusted volume = raw volume  $- b \times (\text{ICV} - \text{mean ICV})$ . The coefficient  $b$  represents the slope of regression of a region-of-interest volume on ICV.

Microstructure of the hippocampus was assessed by MD estimated using diffusion tensor imaging (DTI), in line with previous studies.<sup>7,8</sup> Therefore, a tensor model was fitted to the motion-corrected DTI data to create individual 3-dimensional images of MD. Then, individual T1-weighted images were coregistered to the b0 images using rigid-body transformations. These registrations were used to transform masks of the left and right hippocampus (derived by FIRST on the T1 images) to the MD maps, for extraction of the mean individual hippocampal MD values. Ten subjects had to be excluded from MD analysis because of image artifacts.

**Statistical analysis.** To detect associations among glucose metabolism, memory performance, and hippocampal parameters, we ran bivariate correlations (univariate analyses). To clarify whether associations with hippocampal parameters were region-specific, we additionally assessed total gray matter volume and MD within the thalamus. Further analyses included correlations between memory performance and demographic parameters and independent  $t$  tests for sex and APOE genotype groups ( $\epsilon 4$  carrier vs noncarrier). To test whether glucose metabolism correlated with memory performance after controlling for other known factors related to cognition, we performed multiple regression analyses with dependent variable delayed recall, learning ability, or consolidation, and independent variables HbA1c, glucose, insulin, hippocampal volume and MD, age, sex, BMI, Beck Depression Inventory (BDI), APOE status, physical activity, systolic blood pressure, and smoking, using a stepwise selection procedure. A 2-sided significance level of  $\alpha = 0.05$  was considered.

All variables were sufficiently normally distributed (unimodal,  $|\text{skewness}| < 1$ ) and are reported using means and SDs, except insulin, BDI, physical activity, and smoking. For these, we report medians and interquartile ranges and used Spearman rank coefficients instead of Pearson coefficients for univariate analyses. Before entering those variables in our regression models, we performed a log transformation or a square-root calculation to overcome the skewed distribution.

We next sought to determine whether HbA1c and glucose effects on memory performance were mediated by changes in hippocampal volume or microstructure using mediation analyses. To test this hypothesis, we estimated indirect effect sizes of simple mediation models using a bootstrapping method within the SOBEL toolbox,<sup>16</sup> which were not adjusted for age or sex, in line with previous studies using this analysis tool with similar sample sizes.<sup>17,18</sup> Here, we determined whether the influence of an independent variable  $x$  (HbA1c/glucose) on a dependent variable  $y$  (delayed recall/learning ability/consolidation) is accounted for by a mediator  $M$  (hippocampal volume/MD). This is the fact if the value of the direct path coefficient between  $x$  and  $y$  is reduced by the inclusion of  $M$ . Perfect mediation occurs when reduction decreases the value of the direct path to zero, partial mediation occurs if the effect decreases the value to a considerable amount, and no evidence for mediation occurs when the reduction is not significant. The term  $R^2_{4,5}$  is interpreted as the overlap of the variances of the independent variable (glucose markers) and the

Table 1 Participants' characteristics (n = 72 women)	
Age, y	63.10 (6.87)
Education, y	16.15 (3.06)
MMSE score	29.04 (1.10)
BMI, kg/m <sup>2</sup>	27.60 (1.77)
Systolic blood pressure, mm Hg	138.10 (16.10)
HbA1c, mmol/mol Hb	39.81 (2.93)
Glucose, mmol/L	5.03 (0.44) <sup>a</sup>
Insulin, $\mu$ U/mL	7.80 (6.10–9.70)
HC volume, mm <sup>3</sup>	3,834.34 (349.25)
MD, 10 <sup>-3</sup> mm <sup>2</sup> /s	1.06 (0.06)
BDI score	4.00 (3.00–8.75)
Physical activity, kcal/wk	4,678.39 (2,799.33–7,130.72)
Smoking, pack-years	1.00 (0.00–10.21)
Handedness, %	79.53 (22.60)
APOE $\epsilon$ 4 carrier, %	19.6

Abbreviations: BDI = Beck Depression Inventory; BMI = body mass index; HbA1c = glycosylated hemoglobin; HC = hippocampus; MD = mean diffusivity; MMSE = Mini-Mental State Examination.<sup>11</sup>

Data are mean (SD) or median (interquartile range).

<sup>a</sup>In 2 subjects with glucose levels >6.1 mmol/L, impaired glucose tolerance was not present according to a 2-hour oral glucose tolerance test.

dependent variable (memory performance) with the variance of the mediator (hippocampal parameters).<sup>19</sup> Statistical analyses were calculated using SPSS 20 (PASW, SPSS; IBM, Armonk, NY).

**RESULTS** In total, 141 healthy older subjects (72 women) were included in the study. For participants' characteristics, see table 1 and appendix e-1.

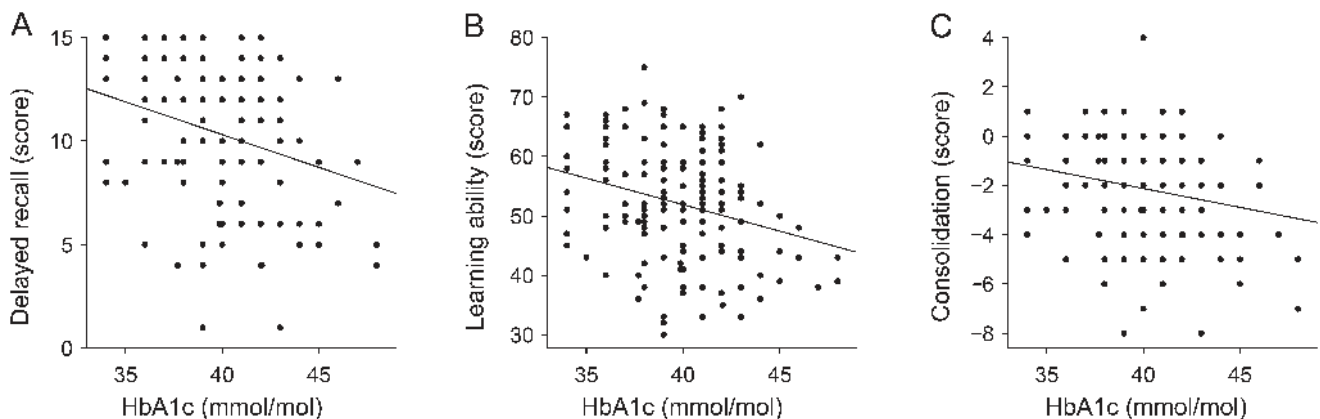
**Peripheral markers of glucose metabolism and memory performance (univariate analyses).** Lower performances in all 3 memory tasks (delayed recall, learning ability, consolidation) were associated with higher levels of both the long-term marker of glucose control, HbA1c (figure), and the short-term marker glucose (all  $r \leq -0.22$ , all  $p \leq 0.01$ ; for details see table 2). Correlations were less clear for insulin (all  $r < -0.15$ , all  $p < 0.07$ ).

Memory performance was also correlated with hippocampal volume (learning ability) and lower MD, lower age, and in part with lower blood pressure and female sex (for  $r$  and  $p$  values, see table 2). No significant associations were found between memory performance and *APOE* genotype, BMI, BDI, physical activity, and smoking (table 2).

**Peripheral markers of glucose metabolism and hippocampal parameters (univariate analyses).** Lower levels of HbA1c were also associated with larger hippocampal volume (nonsignificant trend,  $r = -0.16$ ,  $p = 0.06$ ). The association between lower fasting glucose levels and higher hippocampal volume reached significance ( $r = -0.22$ ,  $p = 0.01$ ). No significant relationship between hippocampal volume and insulin was found ( $r = -0.06$ ,  $p = 0.45$ ). We did not observe correlations between total gray matter volume and HbA1c or glucose (all  $|r| < 0.12$ , all  $p > 0.05$ ).

Turning to hippocampal microstructure, we found that lower levels of all markers of glucose metabolism significantly correlated with lower MD within the hippocampus (HbA1c:  $r = 0.22$ ,  $p = 0.01$ ; glucose:  $r = 0.37$ ,  $p < 0.01$ ; insulin:  $r = 0.26$ ,  $p < 0.01$ ). Note that lower MD indicates improved hippocampal barrier density.<sup>8</sup> We did not observe statistically significant correlations between average MD within the thalamus and HbA1c ( $r = 0.09$ ,  $p = 0.29$ ). For

**Figure** Scatterplots showing the correlations between HbA1c and memory performance



(A) Delayed recall score represents the number of words recalled after 30 minutes (best score: 15;  $r = -0.28$ ,  $p = 0.001$ ). (B) Learning ability score represents the total sum of correctly recalled words of 5 trials (best score: 75;  $r = -0.26$ ,  $p = 0.002$ ). (C) Consolidation score represents the number of recalled words after the fifth trial minus the number of recalled words after 30 minutes (best score: 0; results multiplied by factor  $-1$ ;  $r = -0.21$ ,  $p = 0.01$ ). HbA1c = glycosylated hemoglobin.

glucose, we observed a statistical trend ( $r = 0.15$ ,  $p = 0.08$ ).

**Multiple regression analyses.** Using stepwise regression, HbA1c and sex remained in model A (outcome: delayed recall,  $R^2 = 0.10$ , all  $|\beta| > 0.18$ , all  $p < 0.026$ ). HbA1c, sex, and hippocampal volume remained in model B (outcome: learning ability,  $R^2 = 0.16$ , all  $|\beta| > 0.22$ , all  $p < 0.005$ ). In model C (outcome: consolidation), HbA1c and age remained in the model ( $R^2 = 0.06$ , all  $|\beta| < 0.17$ , all  $p < 0.042$ ) (table 3).

We additionally conducted regression models using the enter procedure with memory performance as dependent variable, and glucose markers plus/minus hippocampal parameters as independent variables (for results see table e-1).

**Simple mediation model analyses.** Simple mediation models revealed that the association of glucose markers (HbA1c, glucose) with memory performance was in part mediated by hippocampal volume (learning ability, all  $R^2_{4,5} > 0.02$ ) and MD (delayed recall, learning ability, all  $R^2_{4,5} > 0.02$ ) explaining between 2% and 3% of the overlap in variances in the respective models (see table 4 for details).

**DISCUSSION** We found that higher levels of both long-term (HbA1c) and short-term (fasting glucose) blood glucose markers were significantly associated with poorer memory performance in a cohort of healthy older individuals. Moreover, higher levels of HbA1c and glucose correlated with decreased volume and microstructure of the hippocampus. Simple mediation models suggested that volume and microstructure of the hippocampus might have a role in mediating the effects of blood glucose on memory performance.

Our findings of detrimental effects of higher glucose levels on memory and hippocampal structure are in line with previous studies involving patients with T2DM or IGT. Patients with T2DM have a higher risk of developing dementia of both AD and vascular type, and in parallel, demonstrate structural brain abnormalities.<sup>20</sup> For example, brain imaging studies in patients with T2DM consistently reported a modest decrease of global brain volume and more pronounced atrophies in the hippocampus and amygdala compared with healthy subjects.<sup>3</sup> In line with our results, a relationship between higher levels of HbA1c and lower hippocampal volume has been demonstrated in middle-aged diabetic subjects.<sup>21</sup> For individuals with IGT, poorer glucose control was likewise associated with cognitive impairments.<sup>22,23</sup> However, other IGT studies could not confirm deleterious effects of nondiabetic glucose levels on cognition.<sup>24,25</sup> This may be attributable to divergent methods of classifying glucose levels and different types of cognitive tests, because, for example, domains such as verbal memory seem to be most sensitive to the effects of increased glucose levels (for discussion, see reference 22).

Even in individuals with blood glucose levels in the normal range, an association between higher short-term peripheral glucose markers and poorer memory performance as well as lower hippocampal volume has been observed.<sup>4</sup> Regarding brain structure, these findings were recently supported by a longitudinal study. Here, changes in plasma glucose levels correlated with volumetric changes in the hippocampus and amygdala at 4-year follow-up.<sup>5</sup> However, in contrast to our study, associations between higher glucose levels and lower hippocampal volume were not observed for the cross-sectional data in that study. This may be attributable to subtle variations in respective study cohorts, e.g., differences in age range and sex ratio and also methodologic differences in MRI acquisition. We used higher magnetic field strength that offers a higher sensitivity of hippocampal volumetry and thus greater statistical power to observe significant associations.

In the present study, we were not only able to confirm previous associations between nondiabetic glucose levels and memory performance and hippocampal volume put forward on the basis of cross-sectional<sup>4</sup> and

**Table 2** Univariate analyses and independent t tests for the associations of glucose metabolism, hippocampal parameters, demographics, and APOE genotype with memory performance

	Delayed recall		Learning ability		Consolidation	
	r	p	r	p	r	p
HbA1c	-0.28	0.001 <sup>a</sup>	-0.26	0.002 <sup>a</sup>	-0.21	0.011 <sup>a</sup>
Glucose	-0.25	0.003 <sup>a</sup>	-0.23	0.006 <sup>a</sup>	-0.22	0.008 <sup>a</sup>
Insulin <sup>b</sup>	-0.17	0.04 <sup>a</sup>	-0.20	0.02 <sup>a</sup>	-0.15	0.07
HC volume	0.09	0.28	0.27	0.001 <sup>a</sup>	-0.03	0.73
HC MD	-0.19	0.03 <sup>a</sup>	-0.22	0.01 <sup>a</sup>	-0.17	0.06
Age	-0.22	0.01 <sup>a</sup>	-0.26	0.002 <sup>a</sup>	-0.21	0.01 <sup>a</sup>
BMI	0.01	0.90	-0.06	0.50	0.04	0.61
BDI	-0.02	0.80	0.03	0.73	-0.07	0.42
Physical activity <sup>b</sup>	-0.05	0.53	-0.06	0.45	-0.06	0.45
Smoking <sup>b</sup>	-0.01	0.96	-0.03	0.75	-0.02	0.79
Systolic blood pressure	-0.21	0.01 <sup>a</sup>	-0.19	0.03 <sup>a</sup>	-0.12	0.14
	Mean (SD)	p	Mean (SD)	p	Mean (SD)	p
Sex						
M	9.7 (3.6)	0.03 <sup>a</sup>	49.5 (10.2)	0.002 <sup>a</sup>	-2.2 (2.2)	0.49
F	11.0 (3.0)		54.5 (9.0)		-2.0 (2.0)	
APOE						
ε4 carrier	10.3 (2.6)	0.50	51.0 (9.8)	0.87	1.7 (2.0)	0.23
Non-ε4 carrier	10.4 (3.5)		52.4 (9.9)		2.2 (2.1)	

Abbreviations: BDI = Beck Depression Inventory; BMI = body mass index; HbA1c = glycosylated hemoglobin; HC = hippocampus; MD = mean diffusivity.

<sup>a</sup> Significant according to  $p < 0.05$ .

<sup>b</sup> Spearman rank correlation coefficient.



**Table 3** Multiple regression analyses, separate models for delayed recall, learning ability, and consolidation

	$\beta^a$	$p^b$
<b>Model A: delayed recall (dependent variable), <math>R^2 = 0.10</math></b>		
<b>Independent variable</b>		
HbA1c	-0.27	0.001
Sex	0.18	0.026
<b>Model B: learning ability (dependent variable), <math>R^2 = 0.16</math></b>		
<b>Independent variable</b>		
HbA1c	-0.22	0.005
Sex	0.24	0.003
HC volume	0.22	0.005
<b>Model C: consolidation (dependent variable), <math>R^2 = 0.06</math></b>		
<b>Independent variable</b>		
HbA1c	-0.18	0.037
Age	-0.17	0.042

Abbreviations: HbA1c = glycosylated hemoglobin; HC = hippocampus.

All regression models were tested for HbA1c, glucose, insulin, hippocampal volume, mean diffusivity, age, body mass index, Beck Depression Inventory, physical activity, systolic blood pressure, smoking, sex, and APOE status using a step-wise selection procedure. A 2-sided significance level of  $\alpha = 0.05$  was considered.

<sup>a</sup>The  $\beta$  refers to the standardized  $\beta$  value (regression coefficient) of the predictor. It gives the number of SDs that the outcome will change as a result of 1 SD change in the predictor.<sup>40</sup>

<sup>b</sup>The  $p$  value refers to whether the regression coefficient is statistically significantly different from zero. It serves as a measure of whether the predictor is making a significant contribution to the model.

longitudinal<sup>5</sup> data, but also to substantially extend these findings by conducting more detailed neuroimaging protocols at 3-tesla MRI, yielding complementary data of both hippocampal volume and microstructure using MD estimates of the hippocampus. DTI-based assessment of MD reflects the diffusion of water molecules in tissue and is thus considered to be an inverse measure of the integrity of neuronal cell bodies, axons, and dendrites in cerebral gray matter. Loss of membrane integrity or synaptic contacts, for example, would lead to an expansion of the extracellular space where water diffusivity is faster and subsequently MD is higher (for further discussion, see references 9, 10, and 26).

A close association between microstructure of this brain area, measured using DTI-based MD, and memory performance was recently found in individuals without dementia.<sup>7</sup> Moreover, it was demonstrated that spatial navigation training in healthy adults led to significant decreases in hippocampal MD, associated with better functional scores.<sup>8</sup> Using MD of the hippocampus, we found a significant relationship with all 3 markers of glucose metabolism and memory performance,

indicating for the first time that higher long- and short-term glucose levels negatively affect hippocampal microstructure.

Mechanisms underlying this proposed neuropathologic pathway may include glucose-related increases in inflammatory responses and blood coagulation activation, leading to clinical and subclinical strokes and subsequent volume loss.<sup>5,27,28</sup> Moreover, animal studies suggested that impaired brain glucose transport may have a crucial role regarding deficits in hippocampal-dependent cognitive functioning<sup>29</sup> since the hippocampus emerged as being specifically vulnerable to disturbances in glucose supply and metabolism<sup>30,31</sup> (for review, see reference 32). Direct “toxic” effects of glucose on neuronal structures include disturbances of intracellular second messenger pathways, imbalance in the generation and scavenging of reactive oxygen species, or advanced glycation of important functional and structural proteins in the brain.<sup>33</sup> This in turn might negatively affect neuronal membrane integrity and lead to increases in extracellular water content,<sup>34</sup> a process indicated by higher hippocampal MD as observed in the present study. Because of its high vulnerability, the hippocampus may be one of the first targets of the deleterious processes set in motion by chronically elevated glucose levels. This interpretation is further supported by the lack of significant associations in our cohort of healthy older adults between elevated HbA1c/glucose and volume/MD of other brain areas, i.e., total gray matter volume and MD within the thalamus. Ultimately, these structural changes may lead to decreased neurotransmitter signaling and loss of synaptic contacts with subsequent memory impairment.<sup>22</sup> This hypothesis is supported by our results showing not only an association between higher glucose levels and poorer memory performance, but also a partial mediation of these effects by hippocampal volume and microstructure.

Some limitations should be considered when interpreting our findings. First, our study is of cross-sectional design, therefore it is difficult to draw definite conclusions about causalities. Also, our study sample was relatively small. In addition, it may not be completely representative of the population at large, because it was not drawn randomly from the population but rather recruited by advertisements. Although we thoroughly collected a large number of parameters known to be associated with cognition and added these to our statistical regression models, we cannot exclude that other parameters may also contribute to the observed effects. In addition, mediation effects failed to reach significance after adjusting for age and sex. The study contains several important strengths. First, we assessed both long- and short-term markers of peripheral glucose metabolism. In contrast to most of the nondiabetic studies discussed earlier,

**Table 4** Simple mediation models for the mediation of hippocampal parameters on the association between glucose markers and memory performance

Variables in simple mediation model				
Dependent variable y	Independent variable x	Mediator M	Mean indirect effect (95% CI)	Mean effect size, $R^2_{4,5}$ (95% CI)
Delayed recall	HbA1c	HC volume	-0.007 (-0.044, 0.026)	0.006 (-0.009, 0.024)
	Glucose		-0.053 (-0.362, 0.266)	0.007 (-0.01, 0.029)
	HbA1c	HC MD	-0.031 (-0.078, 0.006)	0.021 (0.0006, 0.058) <sup>a</sup>
	Glucose		-0.312 (-0.825, 0.135)	0.025 (0.0004, 0.063) <sup>a</sup>
Learning ability	HbA1c	HC volume	-0.126 (-0.288, 0.0007)	0.02 (0.0000, 0.05) <sup>a</sup>
	Glucose		-1.12 (-2.44, -0.144) <sup>a</sup>	0.023 (0.002, 0.055) <sup>a</sup>
	HbA1c	HC MD	-0.116 (-0.277, -0.002) <sup>a</sup>	0.025 (0.003, 0.061) <sup>a</sup>
	Glucose		-1.224 (-2.931, 0.162)	0.03 (0.003, 0.06) <sup>a</sup>
Consolidation	HbA1c	HC volume	0.008 (-0.01, 0.035)	-0.003 (-0.02, 0.009)
	Glucose		0.08 (-0.062, 0.365)	-0.005 (-0.026, 0.011)
	HbA1c	HC MD	-0.019 (-0.051, 0.007)	-0.014 (-0.001, 0.044)
	Glucose		-0.196 (-0.513, 0.101)	0.02 (-0.003, 0.06)

Abbreviations: CI = confidence interval; HbA1c = glycosylated hemoglobin; HC = hippocampus; MD = mean diffusivity. <sup>a</sup>Significant according to bootstrapping method within the SOBEL toolbox.

we additionally measured HbA1c levels, which reflect peripheral glucose levels of the preceding 8 to 12 weeks. Second, analyses of hippocampal MD provided first-time data of the association between hippocampal microstructure and glucose metabolism. Third, our mediation models allowed us to address the question of whether markers of glucose metabolism may or may not be independently associated with the observed improvements in memory performance. For this, we used a bootstrapping method, which produces statistical inference testing that is not based on large-sample theory, thus it can be applied even to relatively small sample sizes.<sup>16–18</sup> Finally, we conducted our cross-sectional study in a well-characterized cohort to curb the possibility that other than our observed factors might have led to the detected effects on memory performance. Therefore, individuals with diabetes, IGT, or neurologic disorders, and those taking antidepressive medication were excluded. Moreover, we carefully controlled our regression models for important other risk factors such as *APOE* genotype, BMI, depression, physical activity, and smoking.

The present findings might lead to a better understanding of the mechanisms underlying the effect of chronically elevated glucose brain function and structure, and the interaction between these factors. Moreover, our results indicate that lifestyle strategies aimed at long-term improvement of glucose control may be a promising strategy to prevent cognitive decline in aging. One example may be lower dietary caloric intake, which reduced peripheral glucose not only in diabetic individuals<sup>35</sup> but also in individuals with

glucose levels in the normal or high-normal range.<sup>36</sup> Importantly, diet-induced changes in markers of glucose metabolism directly correlated with increases in memory functions.<sup>37</sup> Also, physical activity could lower glucose levels in T2DM (see meta-analysis, reference 38) and in the nondiabetic range.<sup>39</sup> In summary, our study may provide the rationale for targeting glucose control in individuals who do not have T2DM and IGT with lifestyle interventions, a hypothesis to be evaluated in future trials.

#### AUTHOR CONTRIBUTIONS

L.K. coordinated the study, was responsible for data collection and management, worked out the statistical analyses, interpretation of the results, manuscript preparation, and submission. A.V.W. contributed to the design of the study, provided methodologic input and theoretical expertise, and contributed to data analysis and writing of the manuscript. A.W. contributed to data collection and management and revision of the manuscript. U.G. advised on statistical analyses and revision of the manuscript. D.R. contributed to study measurements and interpretation of results. A.F. contributed to the design of the study, provided methodologic input and theoretical expertise, advised on statistical analyses, and contributed to writing and editing of the manuscript.

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#### DISCLOSURE

The authors report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

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