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RESEARCH ARTICLE

The role of cerebrovascular reactivity on brain activation during a working memory task in type 2 diabetes

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Abstract

INTRODUCTION: Impaired cerebrovascular reactivity (CVR) is common in type 2 diabetes (T2D) patients and is a risk factor for dementia. However, most prior functional magnetic resonance imaging (fMRI) studies in T2D disregarded the impact of impaired CVR on brain activation patterns. This study investigated the relationship between CVR and brain activation during an fMRI task in T2D patients.

METHODS: Seventy-four T2D patients underwent a working-memory (WM) fMRI task. CVR was measured by the breath-holding index test using transcranial Doppler (TCD). Regression analyses examined associations between CVR and brain activation and between glycated hemoglobin (HbA1c) and activation with/without adjusting for CVR.

RESULTS: An association between CVR and brain activation was found in the left middle and inferior frontal gyri. Adjusting for CVR led to a different pattern of HbA1c-related activation.

DISCUSSION: The findings highlight methodological implications, emphasizing the importance of accounting for impaired CVR when analyzing and interpreting fMRI data in T2D patients.

KEYWORDS
Alzheimer's disease, brain, cerebrovascular reactivity, fMRI, type-2 diabetes, working memory

Highlights

- The study found that cerebrovascular reactivity impacts brain activation patterns during a working memory task in type 2 diabetes patients.
- Accounting for cerebrovascular reactivity altered the brain regions showing activation related to working memory and glycemic control.
- The findings highlight the importance of considering vascular factors when interpreting fMRI data in populations with vascular dysfunction.

1 | INTRODUCTION

Blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) is a method widely used to investigate brain activity, providing an indirect assessment of neural activation Click or tap here to enter text.[1] The measurement of the BOLD signal is enabled by the principle of neurovascular coupling, in which neural activation induces a local increase in cerebral blood flow (CBF), leading to an oversupply of oxygenated blood to the active brain region.[2] Although alterations in BOLD signal are commonly interpreted in terms of changes in neuronal activity, it is also influenced by underlying physiological factors such as resting perfusion, blood volume, and venous oxygenation.[3,4] Across populations with cerebrovascular impairments, alterations in BOLD signal may also reflect the impaired cerebral hemodynamics.[2,5] This phenomenon may potentially cause misleading interpretations of fMRI data, rendering fMRI findings less reliable, as demonstrated in recent fMRI literature both in healthy participants[6] and in clinical populations.[2,7–10]

Cerebrovascular impairments are associated with hyperglycemia,[8,11] the core clinical feature of type 2 diabetes (T2D),[9x] which may lead to decreased CBF and impaired cerebrovascular reactivity (CVR).[11,12] CVR, a reliable and established measure of brain vascular health,[13] reflects the ability of cerebral blood vessels to dilate or contract their caliber in response to vasoactive stimuli, such as neural activity.[8] Impaired CVR in T2D patients can potentially disrupt blood delivery to active brain regions due to the presence of pre-existing vasodilatation.[14] Therefore, impaired CVR has been advocated to explain impaired neurovascular coupling,[15] which has also been reported in T2D patients.[16] Accordingly, the link between the measured BOLD response and neural activity may be altered in T2D patients. More specifically, these BOLD signal alterations may reflect impaired CVR rather than impaired neuronal activity alone.

To accurately assess CVR various methods have been developed, including noninvasive neuroimaging techniques without exogenous contrast agents such as arterial spin labeling (ASL), and transcranial Doppler (TCD) ultrasound; as well as other methods which induce hypercapnic stress during fMRI such as CO_2 inhalation,[17] breath holding[18] and acetazolamide injection.[19] In addition, it has been suggested that since hypercapnia and neuronal activation cause similar changes in blood flow, then normalization of dividing activation-related changes by hypercapnia-related changes will remove vessel architecture effects,[20] revealing that CVR correction increased BOLD sensitivity.[4] This hypercapnia normalization has been examined in age-related cognitive tasks,[3] Alzheimer's disease,[21] and small vessel diseases.[22,23] Nonetheless, this has not become common practice in fMRI studies on T2D patients, despite the well-established link between T2D and cerebrovascular complications. Task fMRI literature on T2D patients has increased rapidly in recent years,[24–27] yet the potential cerebrovascular impairment confound has not been investigated thoroughly, with most studies overlooking this factor in their analyses and discussion. Previous task-based fMRI studies in T2D have yielded inconsistent results regarding increased,[26,27] decreased[28,29] or both increased and decreased[21,23] brain activation in task-relevant regions, compared to controls. In addition, fMRI resting-state functional connectivity (rsFC) studies in T2D patients, have also yielded contradicting results regarding hyper-[30,31] or hypo-connectivity patterns.[10,24,32–35] T2D patients have different physiological response curves compared to non-T2D controls when tested for fMRI signal changes in response to a visual speed discrimination task suggesting that fMRI studies with T2D patients should use a long trial event-related design, to allow the extraction of the real hemodynamic response function.[36] Moreover, less severe disease in T2D patients (measured by fasting blood glucose, HbA1c and disease duration) was correlated with higher coupling of CBF and rsFC measures.[37] Although direct assessment of neurovascular coupling was not performed in this study, its results implied the importance of considering the disruption of neurovascular coupling when the effects of T2D on brain function are investigated. The goal of the current study was to disentangle the effect of neurovascular coupling on BOLD signal in an fMRI study of older adults with T2D. We investigated the effect of CVR on brain activation during the performance of a block design n-back fMRI working-memory (WM) task. We hypothesized that higher CVR would be associated with increased BOLD signal while performing the WM task. In addition, to investigate the potential confounding effect of cerebrovascular impairment on brain activation, we examined whether the association of HbA1c, the primary measure of glycemic control in T2D, with brain activation, is altered when adjusting for CVR is applied.

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the relevant literature on cerebrovascular reactivity (CVR) impairments in type 2 diabetes, the link between CVR and cognitive dysfunction/dementia risk, and the implications of vascular dysfunction for functional neuroimaging studies using sources like PubMed. Several recent publications have highlighted the importance of CVR in functional neuroimaging and are appropriately cited.
2. **Interpretation**: The findings suggest an important interaction between CVR and brain activation patterns during an functional magnetic resonance imaging (fMRI) cognitive task in type 2 diabetes.
3. **Future directions**: The study proposes that CVR measurements should be incorporated into fMRI research in type 2 diabetes and other populations with vascular dysregulation. Specific areas for future research should include: (a) exploring how impaired CVR relates to neurovascular uncoupling and reduced brain activation, and (b) longitudinal studies examining how changes in CVR impact cognitive trajectories and the development of dementia.

2 | METHODS

2.1 | Participants

The current study is based on the Israel Diabetes and Cognitive Decline (IDCD) study, a collaboration of the Icahn School of Medicine, NY, the Sheba Medical Center, Israel, and the Maccabi Health Services, Israel, which aims to investigate the effects of long-term T2D-related characteristics on cognitive decline, as previously described in detail.[38] The study population consists of older adults (≥ 65 years old) T2D subjects, randomly recruited from the Maccabi Diabetes Registry. IDCD eligibility criteria include having T2D; normal cognition at entry; being free of severe neurological (e.g., Parkinson's disease, stroke), psychiatric (e.g., schizophrenia), or other diseases (e.g., substance abuse) that might affect cognitive status, fluent in Hebrew, living in central Israel, and having an informant. Participants were assessed by a physician experienced in dementia, and by a neuropsychologist, who administered a broad neuropsychological battery.

Entry criteria to the Diabetes Registry are any of the following: (1) glycated hemoglobin (HbA1c) > 7.25% (55.7 mmol/mol); (2) glucose > 11.10 mmol/L on two exams more than 3 months apart; (3) purchase of diabetic medication twice within 3 months supported by an HbA1c > 6.5% (47.5 mmol/mol) or glucose > 6.94 mmol/L within half a year; (4) diagnosis of T2D (ICD-9 code [www.icd9data.com/2007/Volume1]) by a general practitioner, internist, endocrinologist, ophthalmologist, or T2D advisor, supported by an HbA1c > 6.5% (47.5 mmol/mol) or glucose > 6.94 mmol/L within half a year.

2.2 | CVR assessment

All the examinations were performed in the Stroke Clinic at Sheba, by one of two qualified and experienced ultrasound technicians. CVR was assessed using a TCD examination, performed with Digi-Lite TCD device (Rimed, Raanana, Israel) with a 2 MHz probe. Patients were placed in a supine position on a hospital bed and rested for 5 min prior to vital signs examination. The ultrasound probe was positioned at the temporal ultrasonic window located above the zygomatic arch, from 1 to 5 cm in front of the ear. Flow velocities of the middle cerebral artery (MCA;depth range 40–65 mm) were recorded. Following, ultrasound probe was positioned at the transformational ultrasonic window, which is located at midline an inch below the edge of the skull.

2.2.1 | Breath-holding index method

The breath-holding index (BHI) method is a reproducible, non-invasive screening method to study cerebral hemodynamics by means of TCD.[39] It is considered an accurate, specific, and sensitive method for CVR evaluation in comparison with other methods[40]; higher BHI values are related to less CVR impairment. A decline in BHI has been reported among T2D patients.[40]

To assess BHI, patients were asked to hold their breath for as long as possible up to 30 s to create the dilatory effect of CO_2 which is mainly restricted to the peripheral arterial bed, particularly the small cortical vessels. With changing CO_2 concentrations, the relationship between flow velocity and volume flow within a large cerebral artery is linear, provided that the CO_2 level does not directly affect the diameter of the large proximal arterial segment. The length time of apnea was measured in seconds using a timer. Mean flow velocity at rest was obtained by continuous recording of a 1-min period of normal breathing. The resting period always preceded the breath-holding period, with a minimum interval of 10 min between measurements on different sides. BHI in the MCA was further calculated in a standardized manner, as percent increase in MCA mean blood velocity recorded by breath-holding divided by seconds of breath-holding. Right and left BHI values were averaged to a mean BHI.

$$\text{BHI} = \frac{100 \times (\text{MCA mean blood velocity after} - \text{MCA mean blood velocity before})}{\text{MCA mean blood velocity before} \times \text{time of breath holding}}$$

2.2.2 | MRI data acquisition

A random subsample of participants from the IDCD cohort underwent an fMRI scan. MRI scans were performed at the diagnostic imaging department at Sheba with a 3 Tesla scanner (GE Signa HDxt, version 16 VO2) equipped with an eight-channel head coil.

Structural imaging acquisition. High-resolution structural images (1 mm^3, matrix size 256 × 256, field of view [FOV] 25.6 cm, number of slices 156) were acquired with a standard 3D inversion recovery prepared fast gradient echo pulse (FSPGR) T1-weighted sequence with the following parameters: TR = 7.3 s; TE = 3.49 ms; flip angle = 20°; TI = 450 ms.

Functional MRI n-back task imaging acquisition. T2* weighted gradient-echo echo-planar images (GE-EPI) were acquired: TR = 3 s; TE = 35 ms; flip angle = 90; matrix size 64 × 64, FOV 22 cm, 40 contiguous oblique axial slices covering the whole brain (voxel size = 3 mm^3, 0.4 mm gap). Five dummy volumes were scanned and excluded from analysis to enable signal stabilization resulting in a total of 192 volumes collected. The n-back fMRI paradigm was presented using E-prime 2.0 software (Psychology Software Tools, Inc). Visual stimuli were back-projected by a radio-frequency shielded projector system and viewed through a mirror device. Responses were recorded using a Lumina response box (Lumina, Cedrus Corporation, CA, USA).

2.2.3 | Functional MRI n-back WM task description

The n-back is a well-established task, widely used in fMRI studies investigating WM. During the n-back procedure, for each stimulus in a continuous series, participants indicate whether the item matches a stimulus presented "n" (1, 2, or 3) stimuli previously. Similar to the

paradigm used in a previous study by our group,[41] our n-back task uses letter stimuli presented at two different memory load conditions (0-, 1-, 2-back). The experiment alternated between 1-back and 2-back tasks, each contrasted with a 0-back task that served as a control for basic attention and sensory input. During the 0-back task, participants were required to press a button whenever a predetermined target letter appeared. Responses were recorded using a Lumina response box (Lumina, Cedrus Corporation, CA, USA). For the 1-back task (low memory load), participants pressed the button when the current stimulus matched the immediately preceding one. In the 2-back task (high memory load), they responded when the current stimulus matched the one shown two steps earlier. The experiment design consisted of 12 condition repetitions (six per 0-back, and three per the 1-back and per the 2-back), with a 7-s slide presented between conditions. Every condition began with a 3-s instruction slide, followed by a 3-s fixation slide, before the stimuli were presented. In each condition 10 letters were presented for a total of 20 s at a rate of 0.5 s per stimulus separated by 1.5 s interval in which the screen was blank. Target stimuli were equally distributed across all conditions, appearing in 30% of the trials for each task. Prior to scanning, all participants were trained outside of the scanner on a desktop computer using a similar task training version. The fMRI n-back task recorded accuracy data, and participants were considered to have reached criterion if their task performance was at least 75% correct. This accuracy cutoff was implemented to ensure participant engagement and minimize performance based on chance, as previous research showed adults above the age of 60 achieve an average of above 85% accuracy on the 2-back condition of the n-back task[42].

2.2.4 | Functional MRI analysis

Functional MRI data was processed using Statistical Parametric Mapping software (SPM12, Wellcome Trust Centre for Neuroimaging, London, UK). The pre-processing of functional images included reorienting to the anterior commissure, realignment to the first image using affine transformation (with an exclusion criterion based on motion that exceeds maximum displacement of 2 mm movement), co-registration to the individual's 3D T1 images, normalization to the stereotactic space of the Montreal Neurological Institute (MNI) using a 12-parameter linear affine transformation and smoothing with a Gaussian kernel of 8 mm in order to minimize anatomical differences and increase signal-to-noise ratio. Following pre-processing, the data were analyzed individually for each subject using the general linear model convolved with the hemodynamic response function. Six motion regressors were included in the model to reduce the probability of obtaining false positives that could be attributed to residual movement-related artifacts. We did not include CSF and white matter signals as regressors due to the use of spatial smoothing which may introduce partial volume effects, thus regressing them out post-smoothing might inadvertently remove signals of interest. In addition, in the general linear model, we utilized the onset of stimuli as the timing variable and included incorrect button responses.

Since our research question does not seek to examine brain activation patterns under different memory loads, but rather brain activation in response to the WM task, we focused our analyses on the combined WM contrast of both low and high WM load conditions in comparison to baseline (1 and 2-back > 0-back conditions).

To examine the primary goal of this study, that is, associations between CVR and BOLD signal, a second-level whole brain analysis of regression between the combined WM load contrast maps and BHI scores was conducted. To further establish our finding regarding the effect of CVR on brain activation we conducted three secondary analyses: (1) one sample *t*-test of brain activation in the combined WM load with and without controlling for BHI; (2) linear regression to examine associations between the main clinical feature of T2D, that is, glycemic control (using the mean of all available HbA1c exams for each participant, provided by the Maccabi Diabetes Registry) and the combined WM load contrast, with and without adjustment for BHI scores; (3) partial Pearson correlation between BHI scores and mean HbA1c, adjusting for all the covariates detailed above using SPM.

All neuroimaging second-level analyses were conducted using the SnPM toolbox (https://warwick.ac.uk/fac/sci/statistics/staff/academic-research/nichols/software/snpm) for statistical non-parametric mapping of the data, with 10,000 permutations,[43] 8 mm Gaussian kernel smoothing and a significance level of $p < 0.05$ family-wise error (FWE) corrected, cluster defining threshold was set as 0.001. Results are displayed on a high-resolution template anatomical MRI (i.e. MNI152T1) using the xjview toolbox (http://www.alivelearn.net/xjview).

2.3 | Statistical analyses

Descriptive statistics, clinical characteristics, and behavioral performance variables were calculated using SPSS 23.0 for Windows (SPSS Inc., IBM, USA). All analyses were adjusted for the following covariates: (1) age; (2) sex; (3) duration of T2D; (4) time difference between MRI scan and TCD examination; (5) cardiovascular risk component derived from the first principal component of a factor analysis, comprising of systolic, diastolic, high-density lipoprotein (HDL), low-density lipoprotein (LDL), cholesterol and creatinine (for further details see Livny et al.)[44]; and (6) behavioral performance calculated as the average of the accuracy score of the 1- and 2-back conditions.

3 | RESULTS

One-hundred and eighty-three T2D participants underwent an fMRI scan, of whom 139 had also undergone the TCD examination. Sixty-five participants were excluded for the following reasons: 6 with missing BHI data, 18 due to missing data of one or more of the cardiovascular components, 34 participants did not reach the performance accuracy threshold of 75% in the 1- and 2-back conditions (1-back: M = 93.46%, SD = 8.47%; 2-back: M = 85.69%, SD = 7.65%), and 7 were excluded due to excessive movement. A total of 74 participants were included in

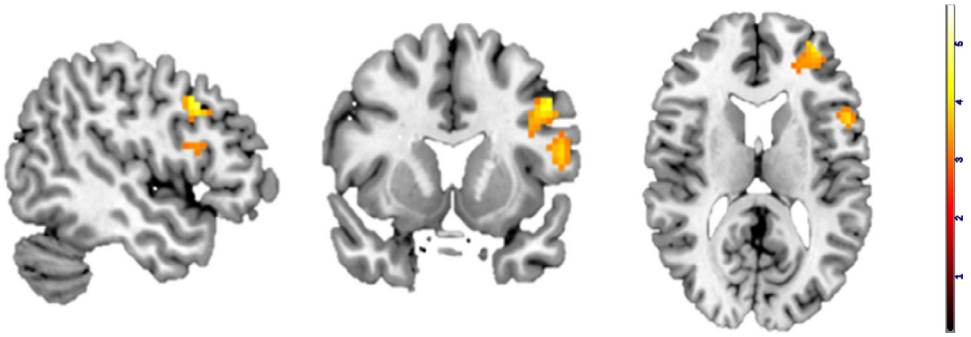

FIGURE 1 Significant clusters of combined WM load contrast in the n-back task which were associated with CVR. These maps of activation represent a significant positive correlation between BHI scores and brain activation in the middle and inferior frontal gyri at a threshold of FWE, $p < 0.05$, cluster-inference, using the SnPM Toolbox. The color scale shows *t*-values. BHI, breath-holding index; CVR, cerebrovascular reactivity; FWE, family-wise error; WM, working memory.

TABLE 1 Socio-demographic and clinical characteristics.

Parameter	Mean	SD	Range
Age (years)	71.39	4.45	64–84
Education (years)	14.38	3.48	8–24
BHI (% change in velocity)	0.86	0.43	0.194–1.9
Cardiovascular risk[a]	−0.03	1.02	−1.8–2.6
Duration of T2D (years)	8.04	2.7	2.13–8.93
Mean HbA1c (%)	6.55	0.87	4.00–9.28
n-Back accuracy score (%)	89.7	5.5	75–100

Abbreviations: BHI, breath-holding index; HbA1c, glycated hemoglobin; T2D, type 2 diabetes.
[a]Arbitrary units from the first component derived from a factor analysis with a Promax rotation.

this study (63.5% male). Socio-demographic and clinical characteristics are presented in Table 1.

The average time participants held their breath in the BHI task was 25.97 (SD = 21.54) s on the right side and 26.19 (SD = 22.93) s on the left side. The regression between the combined WM load contrast maps and BHI scores revealed that higher BHI values (i.e., less CVR impairment) were associated with increased BOLD signal in left frontal areas including the middle frontal and inferior frontal gyri (Table 2, Figure 1).

Our first secondary analysis focusing on the performance of the n-back task, in the combined WM load contrast, revealed that T2D patients activated four major clusters including parietal, temporal, frontal, and cerebellar regions. Although analyses of brain activation during the n-back task with and without BHI scores yielded a similar pattern, intensity values and cluster size were slightly different between the two analyses. In addition, when adjusting for BHI scores, an additional cluster, centered in the right putamen, became prominent (Table 3 and Figure S1).

Our second secondary analysis focusing on the associations between the main clinical feature of T2D and the combined WM load contrast demonstrated that higher levels of HbA1c were associated with higher WM load BOLD signal in the left and right caudate. After adjusting for the BHI scores, the pattern of BOLD signal changed so that the right caudate was no longer significant and an additional activation was found in the left putamen (Table 4 and Figure S2). Finally, our third secondary analysis of the partial Pearson correlation between BHI scores and mean HbA1c was not significant ($r = 0.129$, $p = 0.296$).

4 | DISCUSSION

Our study provides new evidence for the role of altered cerebral hemodynamics on task-related brain activation in older adults with T2D, a population known to have high prevalence of cerebrovascular disease.[2,15,39,45] We found that among T2D patients, CVR—an established measure of vascular health—was associated with frontal brain activation measured during a cognitive task. Our initial analysis of task-related activation in T2D participants revealed brain regions similar to those reported in other populations.[46] However, after adjusting for CVR, both the intensity and spatial pattern of these activations were significantly altered. Finally, HbA1c, the primary measure of glycemic control, was associated with higher activation of the left and right caudate nuclei; however, adjustment for CVR altered the results so that activation remained significant only in the left caudate nucleus with an additional peak found in the left putamen.

To date, prior research examining the effect of CVR on brain activation focused on various measures to assess CVR, such as hypercapnic normalization, breath-holding tasks, ASL, positron emission tomography and TCD[47–49] examined on simulated data,[2] resting-state fMRI in healthy participants,[6,50] and in other clinical populations with cerebrovascular diseases such as Moyamoya disease,[45] Alzheimer's disease,[7] and stroke.[51] Task induced BOLD signal also benefits from using CVR assessment in both healthy and clinical populations.[20,50,52–54] A previous study that examined T2D participants highlighted that using a standard canonical hemodynamic response function to model the BOLD response may result in misestimating the task induced BOLD response parameters.[36] Nonetheless,

TABLE 2 Association between CVR and brain activation during n-back task.

Cluster size	Peak intensity	Peak MNI coordinates			Region
No. of voxels	Pseudo *t*	x, y, z (mm)			Label (AAL)
90	**4.63**	**−48**	**14**	**35**	**L Inferior frontal gyrus**
		−54	11	14	L Frontal inferior operculum
83	**4.10**	**−33**	**50**	**14**	**L Middle frontal gyrus**
		−30	44	8	L Middle frontal gyrus

Abbreviation: AAL, automated anatomical labeling; CVR, cerebrovascular reactivity; L, left; MNI, Montreal Neurological Institute.

TABLE 3 Brain regions activated in the combined WM load contrast in T2D patients.

Condition	Cluster size	Peak intensity	Peak MNI coordinates			Region
	No. of voxels	Pseudo-t	x, y, z (mm)			Label (AAL)
One sample without adjusting for BHI scores	**3766**	**15.27**	**39**	**−61**	**47**	**R Angular gyrus**
			15	−67	53	R Superior parietal gyrus
			−30	−67	47	L Inferior parietal gyrus
	4855	**11.65**	**−42**	**29**	**32**	**L Middle frontal gyrus**
			−42	8	35	L Precentral gyrus
			33	14	53	R Middle frontal gyrus
	528	**11.11**	**−36**	**−73**	**−25**	**L Cerebellum Crus1**
			−54	−61	−10	L Inferior temporal gyrus
			−63	−34	−7	L Middle temporal gyrus
	415	**9.88**	**33**	**−70**	**−28**	**R Cerebellum Crus1**
			60	−55	−7	R Inferior temporal gyrus
			12	−70	−19	R Cerebellum 6
One sample adjusting for BHI scores	**3804**	**15.45**	**39**	**−61**	**47**	**R Angular gyrus**
			15	−67	53	R Superior parietal gyrus
			−30	−67	47	L Inferior parietal gyrus
	4897	**12.06**	**−42**	**29**	**32**	**L Middle frontal gyrus**
			−42	8	35	L Precentral gyrus
			33	14	53	R Middle frontal gyrus
	526	**11.05**	**−36**	**−73**	**−25**	**L Cerebellum Crus1**
			−54	−61	−10	L Inferior temporal gyrus
			−63	−34	−7	L Middle temporal gyrus
	411	**10.09**	**33**	**−70**	**−28**	**R Cerebellum Crus1**
			60	−55	−7	R Inferior temporal gyrus
			12	−70	−19	R Cerebellum 6
	92	**3.80**	**18**	**−4**	**14**	**R Putamen**

Note: This table presents regions of activation resulting from one sample *t*-test permutation analysis with and without adjusting for BHI scores at a threshold of FWE, $p < 0.05$, cluster-inference, using the SnPM Toolbox.
Abbreviations: AAL, automated anatomical labeling; BHI, breath-holding index; FWE, family-wise error; MNI, Montreal Neurological Institute; T2D, type 2 diabetes; WM, working memory.

the authors suggested that fMRI studies in T2D patients are only achievable with deconvolution in event-related experiments. This solution is somewhat problematic when the assessed cognitive function can only be measured using a block-design task, such as WM via n-back tasks. By incorporating TCD information, which is commonly available in clinical settings for various patient populations, our study was able to account for CVR in the interpretation of BOLD differences in brain responses of T2D patients.

TABLE 4 Association between mean HbA1c level and the combined WM load contrast brain activation.

	Cluster size	Peak intensity	Peak MNI coordinates			Region
Condition	No. of voxels	Pseudo t	x, y, z (mm)			Label (AAL)
Without adjusting for BHI scores	**181**	**3.81**	**−9**	**17**	**8**	**L Caudate nucleus**
			9	11	11	R Caudate nucleus
Adjusting for BHI scores	**115**	**3.61**	**−9**	**17**	**8**	**L Caudate nucleus**
			−21	14	8	L Putamen

Note: This table presents regions of activation resulting from regression permutation analysis with and without controlling for BHI at a threshold of FWE, $p < 0.05$, cluster-inference, SnPM.
Abbreviations: AAL, automated anatomical labeling; BHI, breath-holding index; FWE, family-wise error; HbA1c, glycated hemoglobin; MNI, Montreal Neurological Institute; WM, working memory.

Although cerebrovascular impairments are commonly reported among T2D patients,[11,13,16,37] a systematic study investigating the impact of CVR on brain activation while accounting for these cerebrovascular impairments has not been conducted in T2D patients, despite their known risk for cerebrovascular injury. Accordingly, the conflicting findings[25,27,28,55] in T2D task-based fMRI literature might be partly attributed to the effect of impaired CVR on BOLD signal. To the best of our knowledge, we are the first to report a possible confounding effect of cerebrovascular impairments on brain activation, both in task-based fMRI and in T2D patients. Our findings raise valuable methodological implications for BOLD imaging in T2D and other conditions affecting cerebrovascular health, and as well suggest caution in interpreting fMRI data in T2D patients where impaired CVR has not been considered.

Our main finding indicated a positive association between CVR and brain activation in the left middle and inferior frontal gyri, during the n-back WM task. The activation of these frontal regions is consistent with previous literature of regions activated during the performance of the n-back task.[28,29,55,56] The middle frontal gyrus plays a key role in maintaining information and supporting executive function during WM task,[28] and the inferior frontal gyrus is known to play a critical role in verbal WM.[56] Moreover, recent findings demonstrated lower activation in high WM load specifically in older adults with T2D compared to controls, specifically the left middle and inferior frontal gyri[28,29] consistent with our results. The observed regional specificity in frontal areas might be explained by their heightened vulnerability to age-related changes and neurodegenerative processes. Research has shown that these frontal regions have been shown to be particularly susceptible to cerebrovascular dysfunction,[57,58] and exhibit significant reductions in CBF with advancing age.[59] This vulnerability could potentially account for the more pronounced relationship between these regions and BHI, as compared to other task-associated brain areas. Moreover, the strong association between BOLD activation in the left middle and inferior frontal gyri and CVR may be attributed to their reliance on the MCA. The MCA is the primary blood supply to these lateral frontal regions, and variations in its reactivity could directly influence the observed BOLD signal changes.

Studies reporting decreased brain activation in T2D patients mostly linked this decrease to impaired neuronal activation without accounting for the potential confounding of cerebrovascular impairments. Impaired CVR has been implicated in impaired neurovascular coupling,[15] which in turn has been found in the left middle[16,37] and inferior frontal gyri[56] in T2D patients. Given that we found associations between CVR and brain activation, we propose that the attenuated BOLD response in frontal regions during the WM task exhibited by T2D patients with lower CVR may reflect not only decreased neuronal activity but also impairment of brain blood vessels to respond to neural activity as a result of disturbances in neurovascular coupling, rather than typically assumed abnormalities in neuronal activity. Further, the fact that CVR was associated with regional BOLD signal strongly suggests that the BOLD signal embodies pathological cerebral hemodynamics in addition to neural activity.

When examining BOLD response during the cognitive task, we found activations in brain regions commonly reported in the n-back working memory literature, including activation observed in right angular gyrus, superior parietal gyrus, middle frontal gyrus, inferior frontal gyrus, left middle temporal gyrus and the cerebellum.[28,29,55,56] When adjusting for CVR, minor differences in activation intensity and cluster size were observed in the same aforementioned regions. Moreover, an additional cluster of activation was found in the putamen, which has been shown to be activated among non-diabetic participants.[56] A possible explanation for the putamen activation after accounting for CVR is that most of the blood supply to the putamen region stems from the MCA, which is the main blood vessel supply assessed by our CVR measurement. MCA blood supply impairments have been shown to lead to abnormal neuronal activity in the putamen[60] suggesting that the additional activation in the putamen found after adjusting for CVR likely reflects impaired CVR rather than impaired neuronal activity in T2D. In addition, we have found an association between CVR and middle and inferior frontal gyri which also receive their blood supply from the MCA, further supporting the importance of accounting for CVR in fMRI studies of T2D patients. This finding suggests that CVR adjustments may be crucial for accurately interpreting fMRI activation patterns in regions supplied by the MCA, as failing to account for CVR could lead to misattribution of vascular effects to neuronal activity differences in T2D.

We found greater associations between CVR and middle and inferior frontal gyri in the left hemisphere. This left lateralization may reflect underlying vascular asymmetries or differences in cerebrovascular health between hemispheres in our T2D cohort. For example,

lateralization of CVR in the MCA during cognitive tasks[61,62] and during rest,[63–65] specifically left CVR predominance, has been suggested as a possible marker to identify patients at higher risk of hemodynamical stroke events. In addition, our n-back task used letter stimuli, which likely contributed to the observed lateralization in the BHI association findings. This is consistent with a previous meta-analysis, such as Owen et al.[46] comparing verbal and non-verbal n-back tasks which found greater left frontal activations in verbal tasks.

In order to further clarify the effect of CVR on brain activation, we also examined whether adjusting for CVR modifies the pattern of activation when investigating the association between a clinical characteristic of T2D, specifically mean HbA1c level, and brain activation in T2D patients. We found that accounting for CVR in this analysis modifies the positive association between mean HbA1c and brain activation such that activations in the left caudate nucleus remained significant, whereas the association with the right caudate nucleus was no longer significant. Also, an additional peak activation was found in the left putamen after adjusting for CVR. Abnormalities of subcortical nuclei, including the caudate nucleus and putamen, have been reported among T2D patients with brain pathology.[66] A significant association between HbA1c and gray matter atrophy in the caudate of T2D patients has also been reported.[66] Finally, the literature suggests contradicting findings regarding the association of HbA1c with activation and connectivity patterns measured with BOLD, with some studies reporting negative correlation[10,34,35,37] and others hyperactivation and greater connectivity.[24,30,55] According to these reports, a positive association between HbA1c and greater brain activation has been proposed to reflect a neuronal compensatory mechanism.[30,55] Given that higher HbA1c levels in the current study were associated with increased activation in the caudate nucleus, a possible interpretation within the scope of the existing literature is that this reflects a recruitment of compensatory neural resources to cope with the demand of a WM task due to the neurovascular inefficiency related to T2D. However, when we accounted for CVR, the apparent compensatory activation in the right caudate was no longer associated with HbA1c. These results suggest that accounting for cerebrovascular function is necessary when considering the impact of core clinical features of T2D on BOLD fMRI parameters.

A few limitations should be noted. First, the inclusion criteria for the IDCD includes no dementia combined with the criteria of the current study of people who performed the n-back above chance level. Thus, our results may not be generalizable for older T2D patients with cognitive impairment. Second, CVR was assessed using a BHI test which measures mean flow velocities in the MCA specifically. The use of BHI instead of end-tidal CO_2 measurements may have introduced some variability in our CVR assessments. While BHI is a validated method, especially suitable for older populations, future studies could benefit from incorporating end-tidal CO_2 measurements to provide more precise quantification of the CO_2 stimulus. To further explore the functional and regional impact of CVR, and to validate our findings, we encourage studies to assess whole-brain CVR dynamics using other modalities such as the ASL imaging method and BOLD based methods.[4,48] We used BHI rather than MRI based methods, which are more sensitive to age related differences.[67] Third, while we examined the impact of controlling for CVR in a working memory task, other paradigms could be used to examine activation during other tasks, or during resting-state BOLD acquisition to examine the impact of controlling for CVR. In addition, while we have thoroughly reported our preprocessing pipeline, we acknowledge that alternative preprocessing approaches such as controlling for tissue regressors, gray-matter volume, between condition motion regressors, and physiological signals, might yield different results, which should be considered when interpreting our findings. Finally, the emergence of putamen activation following CVR adjustment presents an intriguing finding, potentially indicative of impaired CVR. However, the absence of a direct association between BHI and putamen activity suggests that additional factors may be affecting this result. This complexity underscores the challenges inherent in interpreting fMRI data in populations with potential CVR impairments, such as those with T2D. This finding highlights the need for a cautious approach when interpreting fMRI results in the context of T2D and cerebrovascular health.

In the current study, we provided novel and unique evidence for the effect of CVR on brain activation in an fMRI task study in T2D patients. Similar to studies in other conditions impacting cerebrovascular health, the results imply that alterations in BOLD signal in T2D patients do not only reflect aberrant neural activity but also embody pathological cerebral hemodynamics, and thus accounting for impaired CVR is essential. To conclude, we believe that our findings have implications for the interpretability and reliability of findings from fMRI studies in T2D patients and are robustly applicable to other clinical and non-clinical populations such as patients with cerebrovascular diseases, stroke, dementia, and Alzheimer's disease. Finally, future studies are needed to clarify the relationship between CVR and neurovascular coupling and the potential mechanisms underlying it.

AUTHOR CONTRIBUTIONS

Yarden Oliel researched data and wrote the manuscript; Ramit Ravona-Springer researched data, reviewed the manuscript and contributed to the discussion; Maayan Harel researched data and reviewed the manuscript and contributed to the discussion; researched data and reviewed the manuscript; Chen Botvin Moshe researched data, and reviewed the manuscript; David Tanne reviewed the manuscript and contributed to the discussion; Salo Haratz reviewed the manuscript; Barbara B Bendlin reviewed the manuscript and contributed to the discussion; Michal Schnaider Beeri researched data, reviewed the manuscript and contributed to the discussion. Abigail Livny researched data, reviewed the manuscript and contributed to the discussion.

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CONFLICT OF INTEREST STATEMENT

The authors have no relevant conflict of interest to disclose. Author disclosures are available in the Supporting Information.

CONSENT STATEMENT

Participants provided informed consent prior to enrollment in the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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