Cerebrovascular Function in Adults with Depression

by
Rauchelle E. Richey
B.Sc. San Diego State University, 2013

A Thesis Submitted in Partial Fulfillment
Of the Requirements for the Degree of

MASTERS OF SCIENCE
In the Department of Kinesiology
College of Nursing and Health Innovation

May 2020

Supervisory Committee
Cerebrovascular Function in Humans with Depression

by
Rauchelle E. Richey
B.Sc. San Diego State University, 2013

Supervisory Committee Members

Jody L. Greaney, PhD **Co-Supervisor**	**Department of Kinesiology** **College of Nursing and Health Innovation**
R. Matthew Brothers, PhD **Co-Supervisor**	**Department of Kinesiology** **College of Nursing and Health Innovation**
Christopher R. Martens, PhD **External Committee Member**	**Kinesiology and Applied Physiology** **University of Delaware**

ABSTRACT

Cerebral vasodilatory responsiveness is blunted in older adults (~45-70 yrs) with depressive disorders and thought to contribute to the link between depressive symptomology and increased risk for neurocognitive (e.g., dementia) and cerebral vascular (e.g., stroke) diseases. In young adults with major depressive disorder (MDD), peripheral vascular endothelial dysfunction is evident; however, to date, limited investigations have examined cerebral vasodilatory function in young healthy adults with MDD. We tested the hypothesis that cerebral vasodilatory responsiveness to a hypercapnic stimulus would be blunted in healthy young adults with MDD compared to healthy non-depressed adults (HA). Fourteen HA (22 ± 3yrs) and 14 adults with MDD (22 ± 3yrs; n=9 tested with moderate to severe depressive symptoms) participated. Beat-to-beat mean arterial pressure (MAP; finger photoplethysmography), middle cerebral artery blood velocity (MCAv; transcranial Doppler ultrasound), internal carotid artery (ICA) diameter and blood flow (Doppler ultrasound), and end-tidal carbon dioxide concentration (P_{ETCO_2}; capnograph) were continuously measured during baseline (i.e., normocapnia) and rebreathing-induced hypercapnia. Cerebral vascular conductance index (CVCi=MCAv•MAP $^{-1}$) and ICA blood flow (V_{mean}• $\pi(d$•$20^{-1})^2$•60) and conductance (CVC=ICA blood flow•MAP $^{-1}$) were calculated at baseline and at the highest common magnitude of hypercapnia achieved by all subjects during rebreathing (Δ9 mmHg P_{ETCO2}). At baseline and hypercapnia, there were no differences between groups ($p>0.05$). MAP (HA: 91 ± 9mmHg; MDD: 90 ± 7mmHg), MCAv (HA: 101 ± 17 cm·s^{-1} ; MDD: 103 ± 18 cm·s^{-1}), absolute CVCi (HA: 1.11 ± 0.21 cm·s^{-1}·mmHg^{-1}; MDD: 1.15 ± 0.21 cm·s^{-1}·mmHg^{-1}) relative CVCi $\%_{baseline}$ (HA: 36.5 ± 12.5%; MDD: 31.0 ± 13.9%). There were also no group differences in ICA diameter (HA: 4.8 ± 0.8mm; MDD: 5.0 ± 0.7mm) or blood flow responses (HA: 550.0 ± 144.1 mL·min^{-1}; MDD: 563.8 ± 168.61 mL·min-

$_{1}$), absolute CVC (HA: 6.13 ± 1.72 mL·min^{-1}·mmHg^{-1}; MDD: 6.17 ± 1.55 mL·min^{-1}·mmHg^{-1}) or relative CVC %$_{baseline}$ (HA: 33.9 ± 23.3%; MDD: 33.0 ± 19.0%). These data suggest that cerebrovascular function in young adults with MDD is preserved, intimating a protective mechanism that may not be present in older adults with MDD.

DEDICATION

There is no growth in the comfort zone and no comfort in the growth zone ~ Tony Brigmon

Troy, this could not have happened without you. Thank you for leading my support crew- from the loudest cheers to wiping my tears to shoving me back in the race- this happened because of you. Thank you for your understanding and forgiving heart.
Best Friend, I love you.

Harvey and Frost, I started this endeavor thinking about how much I could teach you about hard work and never giving up- but you both taught me so much more. You tried to understand the burdens I felt, you wiped my tears, you reassured me that you believed in me and that I could do this really hard thing. You were team Mama all the way. Thank you boys! You will change the world with your love and kindness. I love you with all my heart, a million cagillion, forever and always, no matter what!

TABLE OF CONTENTS

LIST OF FIGURES

Chapter 1

Chapter 2

Relationship between PHQ-9 Scores and Conductance in the MCA and ICA.
At a Δ9mmHg P_{ETCO2} magnitude of hypercapnia cerebral vascular conductance index % from baseline ($CVCi\%_{baseline}$; Panel A) in the middle cerebral artery (MCA) and cerebral vascular conductance % from baseline ($CVC\%_{baseline}$; Panel B) in the internal carotid artery (ICA) were correlated with patient health questionnaire-9 (PHQ-9) in all young adults. No correlation was observed in either the MCA or ICA conductance value and PHQ-9 scores.

LIST OF TABLES

Chapter 2

Chapter 1: Introduction to Cerebrovascular Function in Adults with Major Depressive Disorder

1:1 Definition and Prevalence of Major Depressive Disorder

Major depressive disorder (MDD) is defined as a distinct change in mood, motivation, pleasure and interest. A diagnosis of MDD is based on five or more symptoms (e.g., depressed mood, changes in weight or appetite, changes in sleep patterns, fatigue, anhedonia, suicidal ideation, psychomotor retardation, agitation, indecisiveness, feelings of worthlessness, feelings of guilt, decreased ability to concentrate) that must be present for at least a two-week period and represent a change in functioning (2, 31, 37). This clinical diagnosis must include both symptoms of anhedonia and depressed mood as to be classified as MDD. (31, 52, 56).

The prevalence of MDD has steadily increased in the last 10-15 years and affects ~6% of adults worldwide (37). Specifically, adults aged 18-25 years have shown an increase of ~73% in serious psychological distress (e.g., depression, anxiety) while older adults aged 65+ have shown ~30% increase from the years 2005-2017 (54). Indeed, Hasin and colleagues demonstrated that the lifetime occurrence of MDD for adults aged 18-29 years was ~20%, while a MDD occurrence in the last 12 months adults was ~10% (17). Meaning that although recent occurrences of MDD seem low, there is a greater prevalence of lifetime MDD in adults and these numbers are continuously increasing. These observations are supported by others who also show an increased incidence of MDD in young adults (13).

Furthermore, while MDD and other psychiatric disorders are rapidly increasing among the general population, young adults are showing the highest increases with upwards of 70% greater incidence of these diseases from 2005-2017 (54). Moreover, a World Health Organization study in college students from eight different countries revealed that one-third of the participants (13,984 participants total) reported a history of one or more mental disorders (e.g., depression, anxiety, PTSD) (3).

The upward trend of MDD and mental disorders makes it a leading contributor to disease burden worldwide (36, 37). These data demonstrate that not only are there deleterious effects of MDD due to symptomology (i.e., suicidality, substance abuse, etc.,) but, as will be discussed later in this chapter, MDD is also a non-traditional risk factor for the development of cardiovascular and cerebrovascular disease. Indeed, MDD is associated with an increased risk of morbidity and mortality, and its contribution to all-cause mortality is believed to be ~10% (10, 13, 59). Importantly, these observations are not exclusive to one population, as both clinical and non-clinical populations have a similar risk for mortality due to MDD (10).

1:2 Evaluation and Assessment of Major Depressive Disorder

Given the increasing disease burden and incidence, it is important to first understand how MDD is assessed and evaluated. There are several different assessments tools used clinically to evaluate MDD, some are used for diagnosis and others are used for symptom evaluation. Diagnosis allows primarily clinicians to determine whether psychiatric illness is present or not, while symptom evaluation is based on a continuum that can indicate presence of symptoms but not necessarily diagnosis. Symptom evaluation has also been used to determine remission of MDD as well.

The most widely accepted diagnostic tool is the Mini International Neuropsychiatric Interview (MINI). The MINI is a structured diagnostic interview used by clinicians to diagnose psychiatric illness and is compatible with the Diagnostic and Statistical Manual of Mental Disorders (DSM). The purpose of this interview is to allow clinicians an immediate and concise method of patient evaluation thereby leaving more time for treatment (46). Another means of clinician assessment is the Hamilton Depression Scale (HAM-D). There are different variations

of this assessment that measure both depression and symptomology, such as depressed mood, suicide, work and loss of interest, retardation, agitation, gastro-intestinal symptoms, general somatic symptoms, hypercondriasis, insight and loss of weight (16).

More commonly used in research are self-report depressive symptom severity questionnaires. One such questionnaire is the Patient-Reported Outcomes Measurement Information System (PROMIS). This 8-item questionnaire evaluates eight of the nine cardinal symptoms of major depressive disorder the patient felt in the last 7-days. Thus, providing a current perspective on a patient's emotional health. PROMIS scores have been recommended as a general review in accompaniment of the DSM-fifth edition (DSM-V) criteria of depressive symptomology (9, 43). The National Institute for Health (NIH) also, developed an emotion assessment as part of the NIH Toolbox for the Assessment of Neurological and Behavioral Function. The goal of this self-report assessment is to comprehensively measure a breadth of emotional health indices (42). The purpose of all the NIH Toolbox components are to assess comprehensive mental and emotional health instead of primarily symptomology. Emotional health indices that are measured by the NIH Toolbox emotion assessment have also been shown to be highly related to the PROMIS allowing both researchers and clinicians the ability to amalgamate multiple assessment methods of MDD evaluation (42). The Patient Health Questionnaire-9 (PHQ-9), similar to the PROMIS, allows a thorough assessment of the severity of depression symptoms in the last 7-days, as well as a continuum of severity evaluations allowing for sensitive evaluation of depression symptomology (9, 27).

Two other common self-report assessments, considered "legacy measures", Center for Epidemiologic Studies Depression Scale (CES-D) and the Beck Depression Inventory-II (BDI-II) (9). The CES-D was designed to be used for the general population but has also been useful in

evaluating clinical populations as well (9, 39). Similarly, BDI-II is used for both clinical and non-clinical populations (5, 25, 48). This 21-item assessment measures participant's emotional feelings within the last week, and thereby categorizing these scores on a continuum of symptomology (9). Beneficial work linking assessment scores from the PROMIS to the PHQ-9, CES-D and the BDI-II has allowed for misinterpretation reduction in varying methods of symptomology assessment (9).

Understanding these assessment methods is essential as classification of participants into depressed and non-depressed groups will be discussed. While methods of evaluations and symptomology sensitivity vary, these common evaluation methods have produced similar diagnosis characterizations between participants (9). It is important to note that interpretation of research using these assessments should delineate between whether symptom assessments or diagnostic assessments were used. This allows the reader to understand whether individuals were clinically diagnosed or whether symptomology classification alone occurred.

1:3 The Vascular Depression Hypothesis

Initially, the link between cerebrovascular disease and depression was thought to be related to chronic ischemic changes in the brain (24). *Starkstein and colleagues* discussed the relationship between affective disorders and cerebrovascular disease citing that those who suffer from a stroke are more likely to develop depression. They characterized this depression as "post-stroke depression (PSD)" and cited it as the most common emotional disorder to follow a stroke (47). While the exact mechanisms of PSD were not conclusive, it was postulated that due to the infarct and subsequent lesions produced, that these disruptions (i.e., lesions and infarct) within the brain may be the cause of depression development (24, 47).

Subsequently, the "vascular depression hypothesis" presented by Alexopoulos et. al indicated that depression development was highly prevalent among individuals who already had vascular disease (1, 22, 24, 47, 49). Additionally, individuals who had hypertension and coronary artery disease were also highly likely to develop depression (1, 49). A variety of mechanisms establishing the link between vascular risk factors and depression (Figure 1) reaffirms the assertions of *Alexopoulos and colleagues*, that vascular risk factors are linked with the development of depression (49).

Indeed, it's not surprising that when the brain is examined using MRI, structural markers of vascular disease are linked with a high prevalence of depression in older adults further substantiating these claims (22). Data from the *Rotterdam Study* (prospective population based study examining both chronic and disabling disease in the elderly) showed that reduced cerebral blood velocity and cerebral reactivity to a physiological stimulus was predictive of depression in elderly individuals (12). These data further supporting, the vascular depression hypothesis in older adults (12). Therefore, it would be safe to assume a link between vascular risk factors, cerebrovascular disease and depression development, exists in older adults (1).

However, it is unlikely that there is only one cause of elevated cerebrovascular disease risk, therefore, it has been suggested that this relationship is bi-directional, as suggested by Thomas et. al (50). They show that there are environmental factors and lifestyle choices that affect the development of various vascular diseases (6, 50). Likewise, individuals with mental illness (i.e., MDD) show that these illnesses are an independent risk factor for the development of cardiovascular and cerebrovascular disease (33, 50). Furthermore, depression severity may be an important modulator of this relationship (4, 14). Taken together, it is apparent that depression is a non-traditional risk factor for the development of cerebrovascular disease.

1:4 Major Depressive Disorder is a Risk Factor for Disease

While it is recognized that traditional risk factors such as, environmental, psychosocial and genetic factors play a role in disease development, it is important to appreciate that disease risk may also be attributed to non-traditional independent disease risk factors such as psychiatric illness. Since, MDD is a known independent non-traditional risk factor for disease it is critical to understand the link between MDD and disease development. As illustrated in Figure 2, MDD has been linked to a variety of cardiovascular, cerebrovascular and neurodegenerative diseases. Indeed, longitudinal study designs have demonstrated the increased risk that MDD poses on the development of diabetes mellitus, heart disease, stroke, hypertension, obesity, cognitive impairment, cancer and Alzheimer's disease (37). Meta-analysis and reviews on mortality have shown a significantly increased risk of 60-80% mortality due to disease in individuals who have MDD compared to non-depressed individuals (10, 37, 59).

Likewise, data from the *Netherlands Study of Depression and Anxiety (NESDA)* found that participants who were categorized as depressed were at an increased likelihood to have subclinical atherosclerosis (45). Specifically, these participants were 3-times more likely to have a low ankle brachial index (ABI) score, a low-score being an indicator of atherosclerosis and PAD (45). Similarly, associations of ABI score and depression have further exhibited that depression precedes the development of cardiovascular disease (60). Moreover, other investigations have shown that clinically diagnosed depression and smoking independently have the same elevated risk of cardiovascular disease development (58). Further, severity of depression and development of cardiovascular disease is a dose response relationship, although the extent of the relationship is still relatively unknown (58).

1:4:1 MDD is an Independent Risk Factor for Cerebrovascular Disease

MDD has been identified as an independent risk factor for various cardiovascular diseases and co-morbidities. In addition, there is also a significant aggregate of research further establishing MDD as an independent risk factor for cerebrovascular disease. Indeed, data from the *Alameda County Study* demonstrate in a group of middle-aged adults (aged 44 ± 17 years) who were all initially stroke free, those with depression were at an 8% increased risk for stroke mortality (14). Those with severe depression symptoms exhibit an even higher risk for mortality, even after accounting for co-morbidities (14).These data indicate that symptom severity serves as a predictor for cerebrovascular mortality, rather than that of a binary diagnosis of depression.

Data from the *NHANES-I Epidemiologic Follow-up Study,* presented similar data indicating that with depressive symptom evaluation and the exclusion of cardiovascular co-morbidities, depression correspondingly confirmed an increased risk for stroke (23). Participant age (mean age: 50 years) and other demographic factors were evaluated as to whether they played a role in increased disease risk. Results indicated that after controlling for potential confounding factors there was no change in stroke risk for individuals who had depression, thus, the conclusion that depression has a significant association with stroke incidence and mortality (23).

Investigations have continued to prove that depression is an independent risk factor for disease development, with those who have a lifetime history of depressive disorder being at an elevated risk for disease development (28). Additionally, risk of stroke is significantly related to depressive symptoms, the more severe the symptomology, the greater the risk for stroke (35). As these data have shown, individuals who have depression are at an increased risk for stroke incidence with risk being highly correlated with depressive symptomology. While longitudinal

studies provide valuable information, their main limitation lies within the follow-ups that take years to elucidate associations between MDD and stroke mortality. Within this time, these diseases may have already taken their toll, thus negating treatment and prevention strategies. Therefore, being able to delineate potential risk before mortality, is critical to the well-being of these patients.

1:5 Cerebrovascular Function in Humans with Depression

Despite the crucial information provided by the aforementioned longitudinal observation research, the mechanisms of the heightened disease risk are not elucidated. Researchers have sought to address this limitation and provide crucial disease risk information through testing cerebrovascular function. Due to the highly regulated nature of the cerebral circulation any changes in the arterial carbon dioxide concentration (P_{aCO2}) initiate concomitant changes in the cerebral blood flow (CBF) in response (18, 21). Cerebrovascular reactivity (CVR) illustrates how changes in $P_{aCO2,}$ are reflected in changes in CBF (8, 20, 40, 41). Changes in CBF are typically quantified using the middle cerebral artery blood velocity (MCAv), as measurement from the transcranial Doppler ultrasound (TCD) does not allow for diameter measurements (7, 19, 20, 26, 34, 38, 40, 41). Changes in CBF can be manipulated in the laboratory setting by having participants breathe known quantities of vasoactive gases (i.e., CO_2, N_2, O_2) (7, 15, 19, 20, 38, 55) or by infusion of pharmacological substances such as acetazolamide (ACZ) (29, 34, 41, 44). Impaired CVR and CBF has been shown to be indicative of cerebrovascular disease, namely stroke (8, 32, 61).

Using these methods of assessing CVR, research has shown how individuals with depression differ in their reactivity to a hypercapnic stimulus. Specifically participants with MDD were shown to have a lower MCAv, CBF, and CVR compared to their non-depressed

counterparts (11, 29, 30, 34, 51, 57). Interpretation of these data, however, is challenged by the presence of a number of confounding variables (e.g., PAD, high systolic blood pressure and antihypertensive medications) among the study population of older adults (51).

Other research using ACZ intravenous administration to induce dilation in the cerebral circulation found that in middle aged adults (~ 42-years) when measuring MCAv response that the depressed participants had a significantly reduced CVR when compared to healthy non-depressed participants (11, 29, 34). In contrast to other research showing the same result, the subjects in these studies presented with no other vascular disease co-morbidities (with the exception of smoking). These results indicate that with no other apparent vascular disease co-morbidities, individuals with depression are at an increased risk of developing cerebrovascular disease.

In addition to these data regarding a reduced CVR among depressed participants *Gómez-Carrillo de Castro and colleagues* also demonstrated a relationship between attenuated CVR, blood velocity response, and current depressive state. Their results indicate that participants who currently have acute depression, meaning they are currently in a depressive state or episode, demonstrated a decrement in CVR (11). These data were further corroborated by Vakilian et. al, who confirmed that participants currently suffering from a depressive episode have a reduced CVR (57). Thus, these results indicate that current depressive episode and symptomology may play a crucial role in cerebrovascular functioning.

1:6 Future Directions

These studies present sound evidence for a strong relationship between depression and the associated decrements in cerebrovascular function. One of the limitations of the

abovementioned data is the lack of a broad age-range of participants. Many participants who were tested were midlife and older adults (~42-72 years) which presents numerous potential confounding factors. Not only were some of these studies conducted on adults with other cardiovascular co-morbidities but, given that age, beyond 40 years, plays an important role in the reduction of cerebral blood velocity, flow and reactivity (53), it is important to consider that some of these cerebral responses in participants with depression, may have been impacted by age.

Further research into the mechanisms behind the reduced cerebrovascular response in depression are required to better understand disease progression and to identify novel therapeutic targets. Therefore, it is important to establish whether these same decrements that are present in middle aged and older adults are also present in healthy young adults with MDD. Elucidating whether cerebrovascular dysfunction exists in an otherwise healthy young population allows further understanding of disease risk in individuals with depression.

1:7 Figures

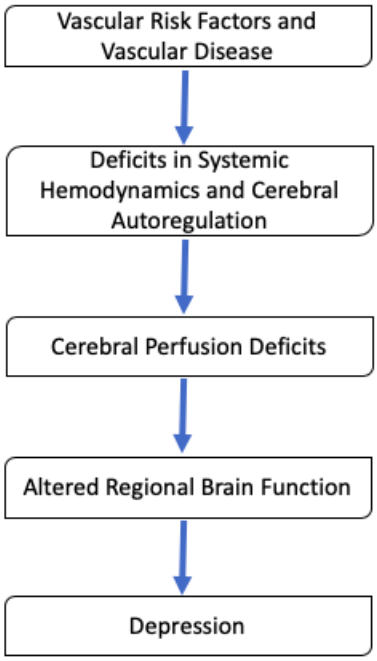

Figure 1.1: Vascular Depression Hypothesis Link. This model illustrates the hypothesized link from vascular risk factors and disease to the development of depression. It is important to note that alterations in brain matter (i.e., lesions, neural disruptions and altered connectivity) along with genetic and environmental factors influencing inflammation are also believed to play a role in the development of late life depression. *Adapted from Taylor et. al (2013) (49)*

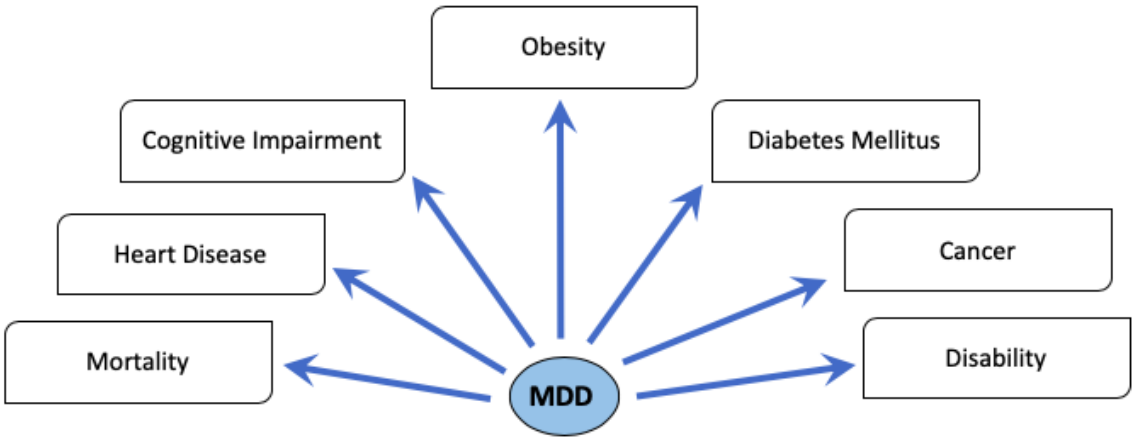

Figure 1.2: Relative Risk of the Development of Disease Due to MDD. Association of MDD with the development risk of various cardiovascular, cerebrovascular and neurodegenerative diseases. Illustrating that a patient who has MDD is at a higher likelihood for the development of these various diseases whether through the deleterious effects on physiology or through environmental factors and lifestyle choices. *Adapted from Otte et. al (2016) (37)*

CHAPTER 1: REFERENCES

1. **Alexopoulos GS, Meyers BS, Young RC, Campbell S, Silbersweig D, and Charlson M**. 'Vascular Depression' Hypothesis. *Archives of General Psychiatry* 54: 915-922, 1997.
2. **Association AP**. *Diagnostic and statistical manual of mental disorders (DSM-5®)*. American Psychiatric Pub, 2013.
3. **Auerbach RP, Mortier P, Bruffaerts R, Alonso J, Benjet C, Cuijpers P, Demyttenaere K, Ebert DD, Green JG, Hasking P, Murray E, Nock MK, Pinder-Amaker S, Sampson NA, Stein DJ, Vilagut G, Zaslavsky AM, and Kessler RC**. WHO World Mental Health Surveys International College Student Project: Prevalence and distribution of mental disorders. *J Abnorm Psychol* 127: 623-638, 2018.
4. **Baune BT, Stuart M, Gilmour A, Wersching H, Arolt V, and Berger K**. Moderators of the relationship between depression and cardiovascular disorders: a systematic review. *General Hospital Psychiatry* 34: 478-492, 2012.
5. **Beck AT, Ward CH, Mendelson M, Mock J, and Erbaugh J**. An inventory for measuring depression. *Arch Gen Psychiatry* 4: 561-571, 1961.
6. **Breslau N, Peterson EL, Schultz LR, Chilcoat HD, and Andreski P**. Major Depression and Stages of Smoking. *Archives of General Psychiatry* 55: 161, 1998.
7. **Brothers RM, Lucas RAI, Zhu Y-S, Crandall CG, and Zhang R**. Cerebral vasomotor reactivity: steady-stateversustransient changes in carbon dioxide tension. *Experimental Physiology* 99: 1499-1510, 2014.
8. **Burley CV, Lucas RAI, Whittaker AC, Mullinger K, and Lucas SJE**. The CO 2 - stimulus duration and steady-state time-point used for data extraction alters the cerebrovascular reactivity outcome measure. *Experimental Physiology* 2020.
9. **Choi SW, Schalet B, Cook KF, and Cella D**. Establishing a common metric for depressive symptoms: linking the BDI-II, CES-D, and PHQ-9 to PROMIS depression. *Psychol Assess* 26: 513-527, 2014.
10. **Cuijpers P, Vogelzangs N, Twisk J, Kleiboer A, Li J, and Penninx BW**. Comprehensive Meta-Analysis of Excess Mortality in Depression in the General Community Versus Patients With Specific Illnesses. *American Journal of Psychiatry* 171: 453-462, 2014.
11. **De Castro AG-C, Bajbouj M, Schlattmann P, Lemke H, Heuser I, and Neu P**. Cerebrovascular reactivity in depressed patients without vascular risk factors. *Journal of Psychiatric Research* 42: 78-82, 2008.
12. **Direk N, Koudstaal PJ, Hofman A, Ikram MA, Hoogendijk WJ, and Tiemeier H**. Cerebral hemodynamics and incident depression: the Rotterdam Study. *Biological psychiatry* 72: 318-323, 2012.
13. **Enewold L, Brinton LA, McGlynn KA, Zahm SH, Potter JF, and Zhu K**. Oral contraceptive use among women in the military and the general U.S. population. *J Womens Health* 19: 839-845, 2010.
14. **Everson SA, Roberts RE, Goldberg DE, and Kaplan GA**. Depressive Symptoms and Increased Risk of Stroke Mortality Over a 29-Year Period. *Archives of Internal Medicine* 158: 1133, 1998.
15. **Flück D, Beaudin AE, Steinback CD, Kumarpillai G, Shobha N, McCreary CR, Peca S, Smith EE, and Poulin MJ**. Effects of aging on the association between cerebrovascular responses to visual stimulation, hypercapnia and arterial stiffness. *Frontiers in Physiology* 5: 2014.

16. **Hamilton M**. A rating scale for depression. *Journal of neurology, neurosurgery, and psychiatry* 23: 56, 1960.
17. **Hasin DS, Sarvet AL, Meyers JL, Saha TD, Ruan WJ, Stohl M, and Grant BF**. Epidemiology of Adult DSM-5 Major Depressive Disorder and Its Specifiers in the United States. *JAMA Psychiatry* 75: 336, 2018.
18. **Hoiland RL, Fisher JA, and Ainslie PN**. Regulation of the Cerebral Circulation by Arterial Carbon Dioxide. *Compr Physiol* 9: 1101-1154, 2019.
19. **Hurr C, Kim K, Harrison ML, and Brothers RM**. Attenuated cerebral vasodilatory capacity in response to hypercapnia in college-aged African Americans. *Experimental Physiology* 100: 35-43, 2015.
20. **Hurr C, Patik JC, Kim K, and Brothers RM**. Blunted cerebral vascular responsiveness to hypercapnia in obese individuals. *Experimental Physiology* 102: 1300-1308, 2017.
21. **Ide K, Eliasziw M, and Poulin MJ**. Relationship between middle cerebral artery blood velocity and end-tidal PCO2 in the hypocapnic-hypercapnic range in humans. *Journal of applied physiology* 95: 129-137, 2003.
22. **Ikram MA, Luijendijk HJ, Vernooij MW, Hofman A, Niessen WJ, van der Lugt A, Tiemeier H, and Breteler MM**. Vascular brain disease and depression in the elderly. *Epidemiology* 21: 78-81, 2010.
23. **Jonas BS, and Mussolino ME**. Symptoms of Depression as a Prospective Risk Factor for Stroke. *Psychosomatic Medicine* 62: 463-471, 2000.
24. **Kales HC, Maixner DF, and Mellow AM**. Cerebrovascular Disease and Late-Life Depression. *The American Journal of Geriatric Psychiatry* 13: 88-98, 2005.
25. **Kendall PC, Hollon SD, Beck AT, Hammen CL, and Ingram RE**. Issues and recommendations regarding use of the Beck Depression Inventory. *Cognitive Therapy and Research* 11: 289-299, 1987.
26. **Kirkham Fe, Padayachee T, Parsons S, Seargeant L, House F, and Gosling R**. Transcranial measurement of blood velocities in the basal cerebral arteries using pulsed Doppler ultrasound: velocity as an index of flow. *Ultrasound in medicine & biology* 12: 15-21, 1986.
27. **Kroenke K, Spitzer RL, and Williams JBW**. The PHQ-9. *Journal of General Internal Medicine* 16: 606-613, 2001.
28. **Larson SL, Owens PL, Ford D, and Eaton W**. Depressive Disorder, Dysthymia, and Risk of Stroke: Thirteen-Year Follow-Up From the Baltimore Epidemiologic Catchment Area Study. 32: 1979-1983, 2001.
29. **Lemke H, Castro AG-CD, Schlattmann P, Heuser I, and Neu P**. Cerebrovascular reactivity over time-course – From major depressive episode to remission. *Journal of Psychiatric Research* 44: 132-136, 2010.
30. **Liotti M, Mayberg HS, McGinnis S, Brannan SL, and Jerabek P**. Unmasking Disease-Specific Cerebral Blood Flow Abnormalities: Mood Challenge in Patients With Remitted Unipolar Depression. *American Journal of Psychiatry* 159: 1830-1840, 2002.
31. **Malhi GS, and Mann JJ**. Depression. *Lancet* 392: 2299-2312, 2018.
32. **Markus H**. Severely impaired cerebrovascular reactivity predicts stroke and TIA risk in patients with carotid artery stenosis and occlusion. *Brain* 124: 457-467, 2001.
33. **Musselman DL, Evans DL, and Nemeroff CBJAogp**. The relationship of depression to cardiovascular disease: epidemiology, biology, and treatment. 55: 580-592, 1998.
34. **Neu P, Schlattmann P, Schilling A, and Hartmann A**. Cerebrovascular reactivity in major depression: a pilot study. *Psychosomatic medicine* 66: 6-8, 2004.

35. **Ohira T, Iso H, Satoh S, Sankai T, Tanigawa T, Ogawa Y, Imano H, Sato S, Kitamura A, and Shimamoto T**. Prospective study of depressive symptoms and risk of stroke among Japanese. *STROKE-DALLAS*- 32: 903-906, 2001.
36. **Organization WH**. *The global burden of disease: 2004 update*. World Health Organization, 2008.
37. **Otte C, Gold SM, Penninx BW, Pariante CM, Etkin A, Fava M, Mohr DC, and Schatzberg AF**. Major depressive disorder. *Nature Reviews Disease Primers* 2: 16065, 2016.
38. **Patik JC, Tucker WJ, Curtis BM, Nelson MD, Nasirian A, Park S, and Brothers RM**. Fast-food meal reduces peripheral artery endothelial function but not cerebral vascular hypercapnic reactivity in healthy young men. *Physiological Reports* 6: e13867, 2018.
39. **Radloff LS**. The CES-D Scale. *Applied Psychological Measurement* 1: 385-401, 1977.
40. **Ringelstein EB, Sievers C, Ecker S, Schneider PA, and Otis SM**. Noninvasive assessment of CO2-induced cerebral vasomotor response in normal individuals and patients with internal carotid artery occlusions. *Stroke* 19: 963-969, 1988.
41. **Ringelstein EB, Van Eyck S, and Mertens I**. Evaluation of Cerebral Vasomotor Reactivity by Various Vasodilating Stimuli: Comparison of CO2 to Acetazolamide. *Journal of Cerebral Blood Flow & Metabolism* 12: 162-168, 1992.
42. **Salsman JM, Butt Z, Pilkonis PA, Cyranowski JM, Zill N, Hendrie HC, Kupst MJ, Kelly MAR, Bode RK, Choi SW, Lai JS, Griffith JW, Stoney CM, Brouwers P, Knox SS, and Cella D**. Emotion assessment using the NIH Toolbox. *Neurology* 80: S76-S86, 2013.
43. **Schalet BD, Pilkonis PA, Yu L, Dodds N, Johnston KL, Yount S, Riley W, and Cella D**. Clinical validity of PROMIS Depression, Anxiety, and Anger across diverse clinical samples. *Journal of Clinical Epidemiology* 73: 119-127, 2016.
44. **Schwertfeger N, Neu P, Schlattmann P, Lemke H, Heuser I, and Bajbouj M**. Cerebrovascular reactivity over time course in healthy subjects. *Journal of the neurological sciences* 249: 135-139, 2006.
45. **Seldenrijk A, Vogelzangs N, Van Hout HPJ, Van Marwijk HWJ, Diamant M, and Penninx BWJH**. Depressive and anxiety disorders and risk of subclinical atherosclerosis. *Journal of Psychosomatic Research* 69: 203-210, 2010.
46. **Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, and Dunbar GC**. The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *The Journal of clinical psychiatry* 1998.
47. **Starkstein SE, and Robinson RG**. Affective disorders and cerebral vascular disease. 154: 170-182, 1989.
48. **Storch EA, Roberti JW, and Roth DA**. Factor structure, concurrent validity, and internal consistency of the beck depression inventory?second edition in a sample of college students. *Depression and Anxiety* 19: 187-189, 2004.
49. **Taylor WD, Aizenstein HJ, and Alexopoulos GS**. The vascular depression hypothesis: mechanisms linking vascular disease with depression. *Molecular Psychiatry* 18: 963-974, 2013.
50. **Thomas A**. Depression and vascular disease: what is the relationship? *Journal of Affective Disorders* 79: 81-95, 2004.
51. **Tiemeier H**. Cerebral haemodynamics and depression in the elderly. *Journal of Neurology, Neurosurgery & Psychiatry* 73: 34-39, 2002.
52. **Tolentino JC, and Schmidt SL**. DSM-5 Criteria and Depression Severity: Implications for Clinical Practice. *Frontiers in Psychiatry* 9: 2018.

53. **Tomoto T, Riley J, Turner M, Zhang R, and Tarumi T**. Cerebral vasomotor reactivity during hypo- and hypercapnia across the adult lifespan. *Journal of Cerebral Blood Flow & Metabolism* 40: 600-610, 2020.
54. **Twenge JM, Cooper, A. B., Joiner, T. E., Duffy, M. E., & Binau, S. G.** Age, period, and cohort trends in mood disorder indicators and suicide-related outcomes in a nationally representative dataset, 2005–2017. *Journal of Abnormal Psychology* Advance online publication: 2019.
55. **Tymko MM, Ainslie PN, MacLeod DB, Willie CK, and Foster GE**. End tidal-to-arterial CO2 and O2 gas gradients at low- and high-altitude during dynamic end-tidal forcing. *Am J Physiol Regul Integr Comp Physiol* 308: R895-906, 2015.
56. **Uher R, Payne JL, Pavlova B, and Perlis RH**. MAJOR DEPRESSIVE DISORDER IN DSM-5: IMPLICATIONS FOR CLINICAL PRACTICE AND RESEARCH OF CHANGES FROM DSM-IV. *Depression and Anxiety* 31: 459-471, 2014.
57. **Vakilian A, and Iranmanesh F**. Assessment of cerebrovascular reactivity during major depression and after remission of disease. *Annals of Indian Academy of Neurology* 13: 52, 2010.
58. **Van Der Kooy K, Van Hout H, Marwijk H, Marten H, Stehouwer C, and Beekman A**. Depression and the risk for cardiovascular diseases: systematic review and meta analysis. *International Journal of Geriatric Psychiatry* 22: 613-626, 2007.
59. **Walker ER, McGee RE, and Druss BG**. Mortality in mental disorders and global disease burden implications: a systematic review and meta-analysis. *JAMA Psychiatry* 72: 334-341, 2015.
60. **Wattanakit K, Williams JE, Schreiner PJ, Hirsch AT, and Folsom AR**. Association of anger proneness, depression and low social support with peripheral arterial disease: the Atherosclerosis Risk in Communities Study. *Vascular Medicine* 10: 199-206, 2005.
61. **Yonas H, Smith HA, Durham SR, Pentheny SL, and Johnson DW**. Increased stroke risk predicted by compromised cerebral blood flow reactivity. *Journal of Neurosurgery* 79: 483-489, 1993.

Chapter 2: Cerebrovascular Function is preserved in Young Adults with Major Depressive Disorder

Major depressive disorder (MDD) is characterized by persistently depressed mood and/or anhedonia causing clinically significant functional impairment in daily life (1). MDD is a leading cause of worldwide disability and overall global disease burden (3, 28, 32). The World Health Organization has quantified disease burdens based on the difference between potential years lived in healthy good quality life and years of life that are lost based on disease instigated poor quality of life. It is based on the difference between these years that the total global burden of disease is illustrated, and is therefore indicating that MDD is quickly becoming the leading cause of global burden and disease (32). The prevalence of MDD is also growing, with some studies reporting increases of ~73% in young adults aged 18-25 years within the United States from the years 2005-2017 (48). However, the biological and physiological mechanisms underlying MDD are largely unknown, making it difficult to predict the detrimental health effects caused by MDD.

Older adults with MDD exhibit an increased risk for cerebral vascular disease. Indeed, several studies have shown that older adults with depression exhibit decrements in cerebral blood flow and cerebral vascular reactivity (11, 14, 27, 30, 33). Although, it is unclear if these changes are attributable to conventional cardiovascular risk factors, such as, age-related declines in vascular function (47), other comorbidities such as atherosclerosis (40), or, are a direct result of MDD-induced pathophysiology. Alternatively, others have demonstrated that young adults with MDD, who are free of comorbidities, also exhibit significant decrements in vascular function, as defined by a blunted dilatory response to a hyperemic challenge compared to young adults without depression (9, 17, 36). Although these data suggest a relationship between vascular dysfunction and MDD in young adults, it is important to note that these findings are not universal, nor do they represent a lasting potential risk (16, 46). However, whether cerebrovascular decrements also exist in healthy young adults with MDD is presently unknown.

Cerebral blood flow is highly sensitive to changes in the partial pressure of arterial carbon dioxide (P_{aCO_2}). Given its potent vasodilatory effect on the cerebral circulation, carbon dioxide (CO_2) is frequently used to test the reactivity of the cerebral circulation, Ide et al. illustrated this concept by showing that inducing hypercapnia (increased P_{ET,CO_2}) and hypocapnia (decreased P_{ET,CO_2}) elicited increases and decreases in cerebral blood flow respectively (21). Indeed, cerebral reactivity to CO_2 is a well-established test to assess cerebral vascular and neurodegenerative disease risk (4, 11, 19, 20, 27, 30, 49). Multiple studies have demonstrated that large increases in cerebral blood flow and/or velocity in response to an increase in CO_2 (i.e., hypercapnic stimulus) are associated with cerebrovascular health. Whereas a blunted increase is an indicator of the inability of the vasculature to respond appropriately to changes in P_{aCO_2} thus indicating a potential increased risk for cerebrovascular and neurodegenerative disease (5, 19, 20, 30, 34, 37).

Given these considerations, the aim of the present study was to assess cerebrovascular function in otherwise healthy, young adults with MDD, in response to a hypercapnic stimulus. We hypothesized that cerebral vasodilatory responsiveness to hypercapnia would be blunted in young adults with MDD compared to healthy non-depressed young adults. We further hypothesized that the severity of depressive symptoms would be negatively related to the magnitude of cerebral vasodilation.

METHODS

The Institutional Review Board at The University of Texas at Arlington approved the experimental procedures. The study was conducted in accordance with the guidelines set forth by the Declaration of Helsinki. The study benefits, procedures and risks were explained to the

subjects, and verbal and written informed consent were obtained voluntarily from all participants prior to participation.

Screening Assessment

Participants were recruited from the University of Texas at Arlington and surrounding Dalla-Fort Worth area. Participants screened for eligibility prior to enrollment. All participants underwent the Mini-International Neuropsychiatric Interview (42), a structured clinical interview, to detect clinically significant depression. Fifty-five otherwise healthy non-medicated adults were screened for MDD. Fourteen individuals (10 women) met the strict criteria for inclusion and were enrolled in the MDD cohort. Fourteen healthy adults (HA) (9 women) without any history or evidence of major psychiatric illness served as the control group. Subject demographics and baseline characteristics are expressed in Table 1. Exclusion criteria included any of the following: co-morbid current psychiatric disorders (e.g. schizophrenia, bipolar disorder, psychosis, post-traumatic stress disorder, panic disorder, etc.), the recent or current use of psychoactive or psychopharmacological drugs, and active suicidal ideation.

Depressive symptom severity was evaluated by both the National Institutes of Health Patient-Reported Outcomes Measurement Information System (PROMIS; emotional distress – depression, short-form) (39) and the Patient Health Questionnaire-9 (PHQ-9) (43). The PROMIS survey consists of 8 items assessed on a 5-point scale (1= never to 5=always) and is limited to the past 7 days; item content focuses on emotional, cognitive, and behavioral manifestations of depression (7). Raw scores converted to T-scores allow for standardization of scores with respect to the U.S. general population mean and standard deviation (50 ± 10) (7). Thus, a PROMIS depression T-score of 60 indicates depressive symptoms one standard deviation higher than the national average. The PHQ-9 is a nine-item instrument based directly on the diagnostic criteria

for depressive disorders outlined in the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) (25). Participants rated depressive symptoms referencing the previous 2 weeks using a 4-point scale (0=not at all to 3=nearly every day). Scores of 5, 10, 15, and 20 represent cut points for mild, moderate, moderately severe, and severe depression, respectively. Both the PROMIS and PHQ-9 provide a valid and sensitive index of depressive symptomology (7, 25, 43)

All participants completed a medical health history questionnaire and assessment of anthropometric measurements, resting blood pressure, heart rate measurements (Connex Spot Monitor; Welch Allyn, Skaneateles Falls, NY, USA), blood chemistry and lipid profile (LabCorp; Arlington, TX). Participants were free of cardiovascular, metabolic, or renal disease, were recreationally active, non-obese (body mass index < 30 kg/m2), did not use tobacco products, and were not taking prescription medications, with the exception of hormonal contraception (n=1 HA). All MDD subjects were tested within ~ 1 week of enrollment in order to facilitate expedient follow-up with a mental healthcare provider. Consequently, it was not feasible, following these criteria, to control for phase of menstrual cycle. A urine pregnancy test confirmed the absence of pregnancy at the experimental visit. Fifteen women were tested in the follicular phase (days 1-13: n=8 MDD, n=7 HA) and four women were tested during the luteal phase (days 16-28: n=2 MDD, n=2 HA) of the menstrual cycle. All subjects underwent familiarization with the study procedures following enrollment at the screening visit.

Assessment of Emotional Health, Physical Activity and Cognitive Function

In addition to the aforementioned surveys, participants also completed the National Institute of Health Toolbox (NIH-TB) cognition battery. This thorough assessment evaluated multiple aspects of cognitive function such as, memory, reaction time, executive function, and attention. (50). Additionally, participants also completed the NIH -TB emotion battery. This

battery was used as a supplementary assessment of negative affect, psychological well-being, and stress, among other measures (38). Participants were also asked to complete the International Physical Activity Questionnaire (10) to estimate habitual physical activity. These measures allowed for a robust overview of each participants physical, cognitive, emotional and psychological well-being on the day of testing.

Experimental Visit – Assessment of Cerebrovascular Function

Participants were asked to fast for a minimum of 3 hours preceding their experimental visit and abstain from caffeine, alcohol, strenuous exercise, and over the counter medications for 24 hours prior to testing. Upon arrival to the laboratory, the participants were positioned supine on an examination bed and instrumented as follows: heart rate (HR) and cardiac rhythm were monitored via four-lead ECG (Cardio Card, Nasiff Associates; Central Square, NY), arterial blood pressure was continuously monitored via finger photoplethysmography (Finometer Pro, Finapres Medical Systems; Enschede, NL), and pulmonary respiration was measured via a strain gauge pneumograph fitted around the abdomen (Pneumotrace II, UFI; Morro Bay, CA).

Left middle cerebral artery velocity (MCAv) was measured using transcranial Doppler ultrasonography (TCD) with a 2MHz Doppler probe (Neurovision TOC, Multigon Industries Inc; Yonkers, NY) positioned over the temporal window of the left temple. The TCD probe was fixed in position using a headband preventing the probe from shifting throughout the experimental procedure. Duplex ultrasound (Logiq P5, GE; Milwaukee, WI) was used to measure the right internal carotid artery (ICA) diameter and velocity continuously during the protocol. P_{ETCO2} was measured continuously through a cannula connected to a capnograph (Capnocheck Plus, Smiths Medical; Dublin, OH).

In accordance with a well-validated modified rebreathing technique (4, 19, 34) participants were fitted with a nose clip and a mouthpiece attached to a Y-valve (Hans Rudolph; Shawnee, KS), with one port of the Y-valve open to ambient air and the other attached to a 5-Liter bag. Immediately prior to the baseline period, the 5-liter bag was filled with subjects expired air. To fill the bag, participants were asked to deeply inhale ambient air, then at end inspiration the investigators rotated the stopcock on the Y-valve opening the port to the 5-L bag. Subjects were then instructed to exhale into the bag filling it. This process was repeated until the 5-liter bag was full, after which the stopcock was returned back to the ambient air position. Following a 3-minute baseline period of quiet rest (i.e., normocapnia), the stopcock was rotated, and participants were asked to breathe their own expired gas mixture from the 5-liter bag for approximately 3-minutes.

During rebreathing oxygen saturation was maintained by bleeding a known concentration of oxygen into the bag according to the Harris-Benedict formula (4, 8, 19, 20, 34). Oxygen saturation levels were constantly monitored using pulse oximetry (Capnocheck Plus, Smiths Medical; Dublin, OH). Following rebreathing, participants had 3-min period of quiet, resting recovery. ICA, MCAv, P_{ETCO2}, arterial blood pressure, and HR were measured continuously throughout rest, rebreathing, and recovery.

Data and Statistical Analysis

During baseline, rebreathing, and recovery, hemodynamic data was sampled at 40-1,000Hz via a data acquisition system (Powerlab, ADInstruments; NZ) and stored for offline analysis (LabChart, ADInstruments; NZ). MCAv, ICA diameter, and P_{ETCO2} were measured and mean arterial pressure (MAP), ICA flow, cerebral vascular conductance index (CVCi; MCAv/MAP) and cerebral vascular conductance (CVC; ICA_{flow}/MAP) were calculated on a

breath by breath basis. Data were averaged during the first 90-seconds of baseline and the last 90-seconds of recovery. During rebreathing MCAv, ICA diameter, and $P_{ET,CO2}$ were measured and MAP, ICA flow, CVCi and CVC were calculated on a breath by breath basis over a 2.5-3-minute period (6). Percent change from baseline was calculated for CVCi and CVC, while the absolute change in MAP, ICA flow, ICA diameter, MCAv, HR and P_{ETCO2} were determined by calculating the 3-breath average at each previously determined magnitude of P_{ETCO2} (Δ3 mmHg, Δ6, Δ9, etc.,). All data are reported to the highest common magnitude that all participants reached (P_{ETCO2} Δ 9mmHg). Edge detection software (CardioSuite, Quipu; Pisa, IT) was used post-testing for analysis of ICA ultrasound derived images. Diameter and velocity measurements were taken across a region of interest where there were clearly defined walls of the ICA. Blood velocity measurements were used to calculate blood flow ($Flow = \pi r^2 \cdot Vmean \cdot 60$). ICA images were obtained in 24 participants (HA=12, MDD=12), 4 individuals (n=2 MDD, n=2 HA) were excluded from analysis due to inadequate image quality.

An *a priori* power analysis ($\alpha = 0.05$, $\beta = 0.80$) indicated a sample size of 10 participants per group would be required to detect a meaningful physiological difference based on an effect size of 1.51 (19). Student's unpaired t-tests were used to compare participant characteristics. Differences in cardiovascular and cerebrovascular outcome variables were analyzed using two-way (group x magnitude of hypercapnia) mixed-model ANOVA, with post hoc corrections (Tukey) applied for comparisons (SAS v9.4; Cary, NC). Data are presented as mean ± standard deviation and significance was set at $\alpha < 0.05$.

RESULTS

The groups were closely matched for age, anthropometrics, resting hemodynamics, blood biochemistry and cognitive functioning (Table 1). Depression and emotional assessment scores (PHQ-9, PROMIS, NIH emotion assessment) were significantly different between groups demonstrating that depression symptom severity (PHQ-9 and PROMIS) and emotional affect were significantly elevated in the MDD group compared to HA group ($p< 0.01$) indicating worse overall psychiatric health.

There were no differences ($p>0.05$, all) between groups at baseline (P_{ETCO2} Δ0 mmHg) in MAP (HA: 87 ± 7mmHg; MDD: 87 ± 4mmHg), MCAv (HA: 72 ± 16 cm·s^{-1}; MDD: 77 ± 15 cm·s^{-1}), CVCi (HA: 0.83 ± 0.21 cm·s^{-1}·mmHg^{-1}; MDD: 0.88 ± 0.19 cm·s^{-1}·mmHg^{-1}), Diameter (HA: 4.7 ± 0.7 mm; MDD: 5.0 ± 0.8 mm), Flow (HA: 401.8 ± 101.8 mL·min^{-1}; MDD: 414.7 ± 123.2 mL·min^{-1}), and CVC (HA: 4.6 ± 1.2 mL·min^{-1}·mmHg^{-1}; MDD: 4.7 ± 1.3 mL·min^{-1}·mmHg^{-1}).

Although there were significant hypercapnia dependent changes during rebreathe, there were no group differences during hypercapnia ($p>0.05$, all) between HA and MDD in MAP (HA: 91 ± 9mmHg; MDD: 90 ± 7mmHg), MCAv (HA: 101 ± 17 cm·s^{-1} ; MDD: 103 ± 18 cm·s^{-1}), CVCi (HA: 1.11 ± 0.21 cm·s^{-1}·mmHg^{-1}; MDD: 1.15 ± 0.21 cm·s^{-1}·mmHg^{-1}) and ΔCVCi% (HA: 36.5 ± 12.5%; MDD: 31.0 ± 13.9%) despite increasing ET_{CO2} (0-9mmHg CO_2) (Fig. 2.1).

There were also no significant group differences in ICA during hypercapnia (Fig. 2.2). ICA diameter (HA: 4.8 ± 0.8mm; MDD: 5.0 ± 0.7mm), flow (HA: 550.0 ± 144.1 mL·min^{-1}; MDD: 563.8 ± 168.61 mL·min^{-1}), and CVC (HA: 6.13 ± 1.72 mL·min^{-1}·mmHg^{-1}; MDD: 6.17 ± 1.55 mL·min^{-1}·mmHg^{-1}). Moreover, $\Delta CVCi\%_{baseline}$ (HA: 36.5 ± 12.5%; MDD: 31.0 ± 13.9%) and $\Delta CVC\%_{baseline}$ (HA: 33.9 ± 23.3%; MDD: 33.0 ± 19.0%) are also shown to have no

differences between groups (Fig. 2.3). Changes in blood pressure, respiratory rate, and heart rate are summarized in Table 2.

When we looked at depression symptom severity as illustrated by the PHQ-9, we found significant group differences ($p<0.01$) in PHQ-9 responses. This confirmed that there was an elevated depressive symptom severity in our MDD participants, and a lack of depressive state of HA participants on the day of testing. Further PHQ-9 responses in all subjects showed no correlation with either $\Delta CVCi\%_{baseline}$ (slope= -0.63 ± 0.39, R^2= 0.09, p= 0.12) or $\Delta CVC\%_{baseline}$ (slope= 0.17 ± 0.66, R^2= 0.003, p= 0.80) (Fig. 2.4).

DISSCUSSION

The major finding of the present study, contrary to our hypothesis, is that cerebrovascular function is similar in young adults with MDD compared to HA. These results conflict with previous research in older adults with MDD, suggesting that cerebrovascular decrements are not yet apparent in healthy young adults with MDD. Moreover, we found no correlation between depressive symptom severity and cerebrovascular function. Together, these data could indicate a protective mechanism in the cerebral circulation of young adults with MDD that may be attenuated with age.

Previous research hypothesized that the development of depression may be due to vascular risk and disease, emphasizing that, particularly in the brain, there were indices of vascular disease that predated the incidence of depression in these individuals (2). Indeed, individuals with markers of cerebrovascular disease were at a higher prevalence for the development of depression later on (13, 22). Contrary to these findings, our data suggests no cerebrovascular impairment in young adults with MDD indicating that our results do not follow this hypothesis. Given our findings, it raises the question of whether depression was previously

present but not tested for until cerebrovascular disease manifested in these participants (13, 22), making it necessary to consider the alternate hypothesis, that depression is a trigger for cerebrovascular disease.

Likewise, various prospective studies indicate that depression and depressive symptoms are indicative of stroke development later in life (15, 23, 26, 31). Jonas et. al demonstrated a significant relationship between depressive symptoms and stroke. They substantiated their claims by excluding early stroke cases and other cardiovascular diseases (in the last 10-years) in their cohort. Thus, through case exclusion they determined that vascular risk or injury did not lead to the development of depression (23). Similar results have provided further evidence that baseline depression evaluations confirming depression are an indicator for cerebrovascular disease development (26, 31). Given this prospective evidence, further investigations sought to establish whether there were indicative decrements in cerebrovascular function in adults with depression that could be an early vascular indicator of cerebrovascular disease. Cerebrovascular function was thereby tested in cohorts of midlife and older adults (~42-72 yrs) using both CO_2 induced hypercapnia and acetazolamide infusions (11, 27, 30, 45). These results demonstrated a blunted reactivity and blood velocity (as measured by the TCD) in their depressed participants compared to their non-depressed participants. (11, 27, 30, 45).

In light of this evidence, we hypothesized on the belief we would observe a similar blunted blood velocity and dilation in our participants during the modified rebreathing test. However, our results did not support this hypothesis, we speculate that this may be due to the age of our cohort and associated age-related protective mechanisms. Consistent with this notion, *Tomoto and colleagues* recently showed that aging is associated with decreases in cerebral blood

velocity during a modified rebreathing protocol, lending further evidence that age-dependent vascular adaptations played a role in prior findings surrounding cerebrovascular function (47).

Indeed, evidence has shown that aging has a profound impact on the brain overall, and that these changes are associated with depression. An investigation by Desmidt et. al revealed that cerebral blood velocity correlated with brain volumes in individuals with depression and remitted depression. Their results showed that those with depression had smaller brain volumes and thereby lower blood velocity than those with remitted depression. Moreover, they found that those with remitted depression indicated increased brain size and thereby increased blood velocity thus, indicating a difference in brain perfusion between individuals with depression and in remission (12). These results correspond with the aforementioned studies, when we examine the age of their cohort (42 ± 12 years) we again see that the participants are midlife and older adults. Furthermore, others have demonstrated that brain volumes begin to decline at a rate of ~5% around the age of 40-years (35). These findings support the general hypothesis that advancing age contributes to decreases in brain volume and thereby decreases in cerebral blood flow, velocity as well as altered reactivity due to changes in perfusion.

In addition to age, lifestyle factors have been shown to negatively affect brain volumes and blood flow, including alcoholism (35). However, none of our participants indicated alcoholism with most participants indicating that they drank sparingly or abstained completely from alcohol. With these data we would suggest that our cohort of participants were potentially both too young and healthy to exhibit any decrements in cerebrovascular function.

Limitations and Future Directions

Despite the best efforts to perform rigorous screening protocols and well-established validated methodologies, there were limitations to this study. While TCD is a well validated

measurement of the MCAv, it is important to note that this measurement is valid based on the prevailing assumption that the diameter of the MCA does not change (24, 41). Therefore, given that there is evidence to suggest the diameter of MCA does change marginally (18), future studies should determine the potential impact of MCA dilation on measurements of cerebrovascular function in young adults with MDD.

Future work is needed to replicate this study design in individuals a decade older than our cohort. Given that research has established this decrement in older adults it is important to help establish the age at which depression induces a blunted flow and dilatory effect in the cerebral circulation. Therefore, an additional cross-sectional study design composed of adults across the spectrum of youth and age would yield crucial evidence characterizing the point at which these blunted cerebral circulation responses begin to appear. Furthermore, longitudinal research testing would allow for a more definitive assessment of a more precise age when cerebrovascular decrements occur in individuals with MDD. This would provide both causal and mechanistic insight into the potential differences in cerebrovascular function in participants with MDD and non-depressed adults as well as the potential role of other lifestyle and environmental factors that may influence these cerebrovascular decrements.

Finally, it would also be beneficial to assess other indices of brain microvascular function (aside from the MCA) in this cohort of young adults, as prefrontal cortex perfusion has been shown to have diminished flow and velocity in depressed older adults (29). Future research should also examine sex differences and the effect of depression on the cerebral circulation. Recent research has shown differential responses in cerebral perfusion which necessitates the further investigation into the variance of effects depression may have on cerebral perfusion between sexes (44). As we lacked sufficient statistical power to analyze sex differences, we did

not assess sex as a variable within this study. Further investigation into potential differences in depression incidence and cerebrovascular function between sexes is warranted as not much is known regarding sex differences in depression and cerebrovascular function in young adults.

Conclusion

Although no differences in cerebral blood velocity in the MCA or flow in the ICA between our young adults with MDD and HA were observed, these data show important evidence regarding individuals with MDD. Indeed, these data may indicate there is a protective mechanism in the brain not present in older adults. These results are a positive indicator that young adults with MDD are currently protected from stroke development and cerebrovascular disease as indicated by their preserved cerebrovascular function. Further investigation is critical to elucidating both the mechanisms and age-related changes in cerebrovascular function in adults with MDD, especially to distinguish the time point for when these individuals begin to increase their risk for stroke and cerebrovascular disease. Addressing these gaps in the literature is critical to developing treatments for depression that can prevent the devasting effects that accompany cerebrovascular disease.

Chapter 2: Tables and Figures

Table 2.1. Subject Characteristics.

Characteristic	HA	MDD	p-value
N (M/F)	14 (5/9)	14 (4/10)	
Age (yr)	22 ± 3	22 ± 3	1.00
Height (cm)	164 ± 9	162 ± 6	0.57
Mass (kg)	67 ± 11	62 ± 11	0.20
BMI (kg/m2)	24.8 ± 2.5	23.4 ± 4.4	0.34
Heart Rate (bpm)	71 ± 8	74 ± 10	0.35
Systolic BP (mmHg)	117 ± 11	115 ± 14	0.56
Diastolic BP (mmHg)	73 ± 5	71 ± 8	0.52
Habitual Physical Activity (MET-mins/wk)	8228 ± 7202	5041 ± 5935	0.22
Blood Biochemistry			
HbA1c (%)	5.2 ± 0.2	5.2 ± 0.3	0.76
Total Cholesterol (mg/dl)	164 ± 30	161 ± 23	0.77
HDL (mg/dl)	57 ± 13	61 ± 12	0.40
LDL (mg/dl)	88 ± 27	84 ± 21	0.62
Triglycerides (mg/dl)	96 ± 48	86 ± 35	0.54
Depression Assessment			
PROMIS (raw score)	15 ± 5	25 ± 6 *	<0.01
PROMIS (T-score)	52 ± 7	63 ± 6 *	<0.01
PHQ-9 (au)	3 ± 3	11 ± 7 *	<0.001
Emotional Assessment			
Negative Affect (T-score)	48 ± 13	64 ± 9 *	<0.01
Social Satisfaction (T-score)	52 ± 10	39 ± 9 *	<0.01
Psychological Well-Being (T-score)	54 ± 9	39 ± 11 *	<0.01
Cognition Assessment			
Total Composite Score	111 ± 8	109 ± 6	0.36
Fluid Composite Score	116 ± 9	113 ± 9	0.35
Crystallized Composite Score	104 ± 7	103 ± 4	0.83

HA, healthy adults; MDD, Major Depressive Disorder; BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PROMIS, patient-reported outcome measurement information system; PHQ-9, Patient Health Questionnaire (symptom severity: 0-4, minimal; 5-9, mild; 10-14, moderate; 15-19, moderately severe; 20-27, severe) (7). Emotional and Cognition Assessments were derived from the NIH Toolbox measures (38, 50). Values are mean ± SD. *P<0.05 v. HA.

Table 2.2 Hemodynamic Responses to Hypercapnia.

Variable	HA	MDD	group	hypercapnia	interaction
SBP (mmHg)					
baseline	123 ± 12	121 ± 9			
$P_{ET}CO_2$ = Δ3 mmHg	128 ± 18	122 ± 11	p=0.64	p<0.01	p<0.01
$P_{ET}CO_2$ = Δ6 mmHg	132 ± 15	123 ±10			
$P_{ET}CO_2$ = Δ9 mmHg	133 ± 17 *	123 + 11			
DBP (mmHg)					
baseline	70 ± 6	71 ± 5			
$P_{ET}CO_2$ = Δ3 mmHg	67 ± 8 *	71 ± 5	p=0.45	p<0.01	p=0.05
$P_{ET}CO_2$ = Δ6 mmHg	72 ± 7 *	73 ± 5 *			
$P_{ET}CO_2$ = Δ9 mmHg	73 ± 7 *	74 ± 6 *			
RR (breaths·min^{-1})					
baseline	14 ± 4	12 ± 5			
$P_{ET}CO_2$ = Δ3 mmHg	12 ± 3	12 ± 5	p=0.58	p=0.42	p=0.44
$P_{ET}CO_2$ = Δ6 mmHg	12 ± 4	12 ± 5			
$P_{ET}CO_2$ = Δ9 mmHg	12 ± 3	12 ± 3			
Heart Rate (beats·min^{-1})					
baseline	69 + 6	67 ± 11			
$P_{ET}CO_2$ = Δ3 mmHg	73 ± 7 *	71 ± 13 *	p=0.56	p<0.01	p=0.11
$P_{ET}CO_2$ = Δ6 mmHg	72 ± 6	68 ± 12			
$P_{ET}CO_2$ = Δ9 mmHg	71 ± 7	71 ± 13 *			

HA, healthy adults; MDD, major depressive disorder; SBP, systolic blood pressure; DBP, diastolic blood pressure; RR, respiratory rate. Values are mean ± SD. *p<0.05 vs. baseline.

FIGURE 2.1

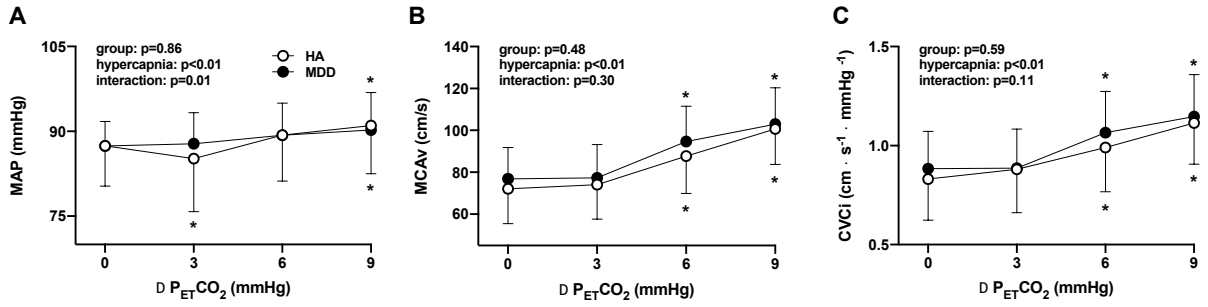

Figure 2.1: Blood Pressure and MCAv Response to Hypercapnia in Young Adults with MDD and HA. The increases in mean arterial pressure (MAP; Panel A) middle cerebral artery velocity (MCAV; Panel B) and absolute cerebral vascular conductance index (CVCi; Panel C) during hypercapnia in adults with major depressive disorder (MDD; closed circles) and healthy adults (HA; open circles). Hypercapnia triggered increases in both groups across all variables; however, there were no differences between MDD and HA in their response to hypercapnia. *p<0.05 v. baseline (Δ 0mmHg P_{ETCO2})

FIGURE 2.2

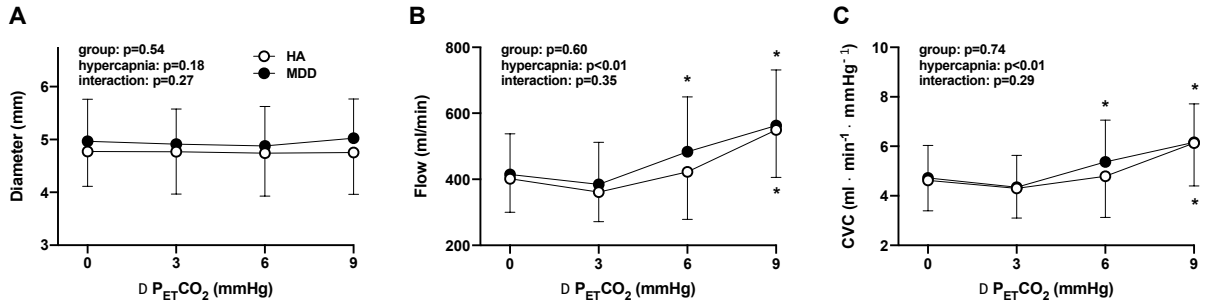

Figure 2.2: ICA Response to Hypercapnia in Young Adults with MDD and HA. Diameter remained the same during hypercapnia (Panel A) while flow (Panel B) and cerebral vascular conductance (CVC; Panel C) in the internal carotid artery (ICA) increased during hypercapnia in adults with major depressive disorder (MDD; closed circles) and healthy adults (HA; open circles). Hypercapnia triggered increases in both groups in their flow and CVC response; however, there were no differences between MDD and HA in their response to hypercapnia. *p<0.05 v. baseline (Δ 0mmHg P_{ETCO2})

FIGURE 2.3

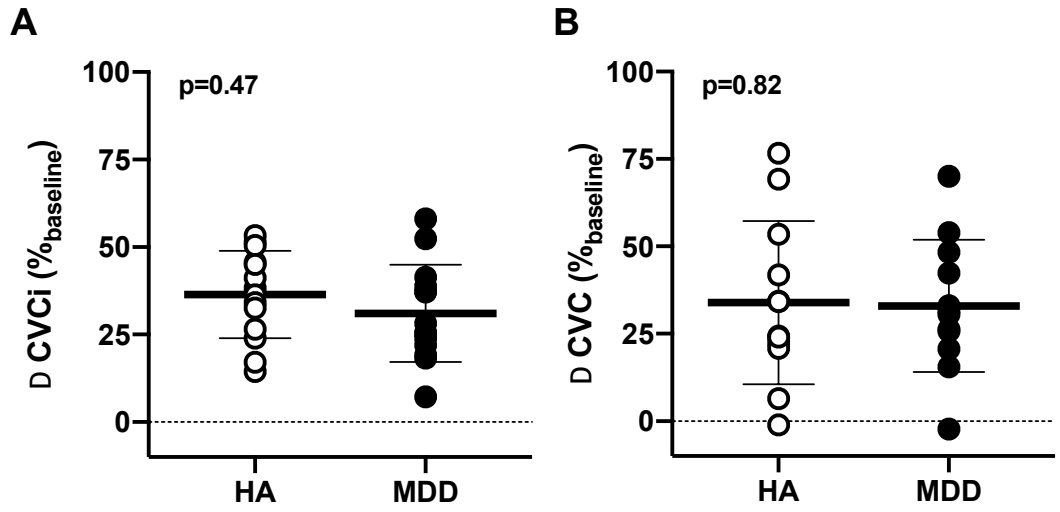

Figure 2.3: MCA and ICA Response to Hypercapnia in Young Adults with MDD and HA. At a Δ9mmHg P_{ETCO2} magnitude of hypercapnia cerebral vascular conductance index % from baseline ($CVCi\%_{baseline}$; Panel A) in the middle cerebral artery (MCA) and cerebral vascular conductance % from baseline ($CVC\%_{baseline}$; Panel B) in the internal carotid artery (ICA) exhibited no differences between adults with major depressive disorder (MDD; closed circles) and healthy adults (HA; open circles). P-values are shown above; $p>0.05$ between groups.

FIGURE 2.4

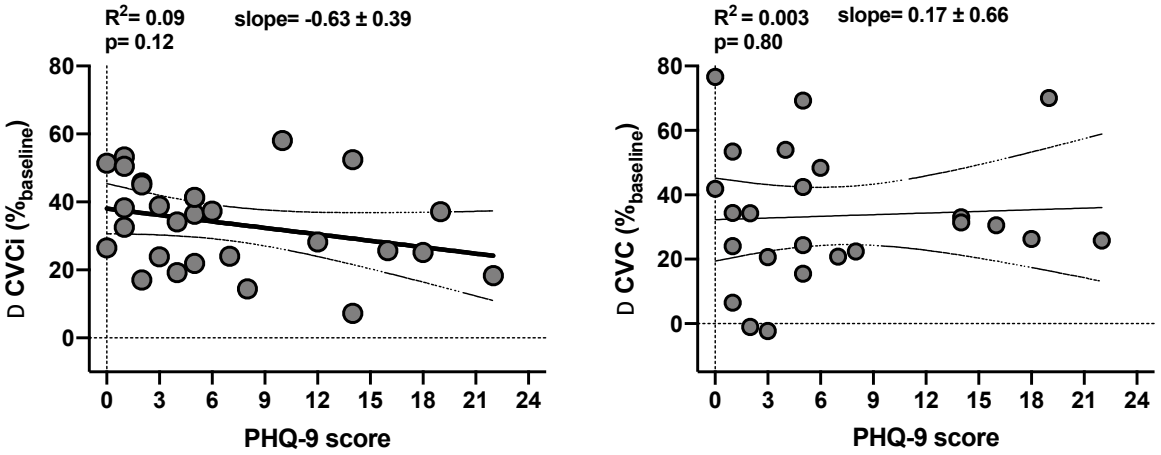

Figure 2.4: Correlation between PHQ-9 Scores and Conductance in the MCA and ICA. At a Δ9mmHg P_{ETCO2} magnitude of hypercapnia cerebral vascular conductance index % from baseline ($CVCi\%_{baseline}$; Panel A) in the middle cerebral artery (MCA) and cerebral vascular conductance % from baseline ($CVC\%_{baseline}$; Panel B) in the internal carotid artery (ICA) were correlated with patient health questionnaire-9 (PHQ-9) in all young adults. No correlation was observed in either the MCA or ICA conductance value and PHQ-9 scores.

Chapter 2: References

1. *American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders*. Arlington, VA: American Psychiatric Association, 2013.
2. **Alexopoulos GS, Meyers BS, Young RC, Campbell S, Silbersweig D, and Charlson M**. 'Vascular Depression' Hypothesis. *Archives of General Psychiatry* 54: 915-922, 1997.
3. **Brody DJ, Pratt LA, and Hughes JP**. Prevalence of Depression Among Adults Aged 20 and Over: United States, 2013-2016. *NCHS Data Brief* 1-8, 2018.
4. **Brothers RM, Lucas RAI, Zhu Y-S, Crandall CG, and Zhang R**. Cerebral vasomotor reactivity: steady-stateversustransient changes in carbon dioxide tension. *Experimental Physiology* 99: 1499-1510, 2014.
5. **Brothers RM, Wingo JE, Hubing KA, and Crandall CG**. The effects of reduced end-tidal carbon dioxide tension on cerebral blood flow during heat stress. *Journal of Physiology* 2009.
6. **Burley CV, Lucas RAI, Whittaker AC, Mullinger K, and Lucas SJE**. The CO 2 - stimulus duration and steady-state time-point used for data extraction alters the cerebrovascular reactivity outcome measure. *Experimental Physiology* 2020.
7. **Choi SW, Schalet B, Cook KF, and Cella D**. Establishing a common metric for depressive symptoms: linking the BDI-II, CES-D, and PHQ-9 to PROMIS depression. *Psychol Assess* 26: 513-527, 2014.
8. **Claassen JAHR, Zhang R, Fu Q, Witkowski S, and Levine BD**. Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing. *Journal of Applied Physiology* 102: 870-877, 2007.
9. **Cooper DC, Milic MS, Tafur JR, Mills PJ, Bardwell WA, Ziegler MG, and Dimsdale JE**. Adverse Impact of Mood on Flow-Mediated Dilation. *Psychosomatic Medicine* 72: 122-127, 2010.
10. **Craig CL, Marshall AL, Sj??Str??M M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, and Oja P**. International Physical Activity Questionnaire: 12-Country Reliability and Validity. *Medicine & Science in Sports & Exercise* 35: 1381-1395, 2003.
11. **De Castro AG-C, Bajbouj M, Schlattmann P, Lemke H, Heuser I, and Neu P**. Cerebrovascular reactivity in depressed patients without vascular risk factors. *Journal of Psychiatric Research* 42: 78-82, 2008.
12. **Desmidt T, Andersson F, Brizard B, Cottier J-P, Patat F, Gissot V, Belzung C, El-Hage W, and Camus V**. Cerebral blood flow velocity positively correlates with brain volumes in long-term remitted depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 81: 243-249, 2018.
13. **Direk N, Koudstaal PJ, Hofman A, Ikram MA, Hoogendijk WJ, and Tiemeier H**. Cerebral hemodynamics and incident depression: the Rotterdam Study. *Biological psychiatry* 72: 318-323, 2012.
14. **Dotson VM, Beason-Held L, Kraut MA, and Resnick SM**. Longitudinal study of chronic depressive symptoms and regional cerebral blood flow in older men and women. 24: 809-819, 2009.
15. **Everson SA, Roberts RE, Goldberg DE, and Kaplan GA**. Depressive Symptoms and Increased Risk of Stroke Mortality Over a 29-Year Period. *Archives of Internal Medicine* 158: 1133, 1998.

16. **García RG, Zarruk JG, Barrera C, Pinzón A, Trillos E, Arenas WD, Luengas C, Tomaz C, and López-Jaramillo P**. Plasma Nitrate Levels and Flow-Mediated Vasodilation in Untreated Major Depression. *Psychosomatic Medicine* 73: 344-349, 2011.
17. **Greaney JL, Saunders EFH, Santhanam L, and Alexander LM**. Oxidative Stress Contributes to Microvascular Endothelial Dysfunction in Men and Women With Major Depressive Disorder. *Circulation Research* 124: 564-574, 2019.
18. **Huber P, and Handa J**. Effect of Contrast Material, Hypercapnia, Hyperventilation, Hypertonic Glucose and Papaverine on the Diameter of the Cerebral Arteries. *Investigative Radiology* 2: 17-32, 1967.
19. **Hurr C, Kim K, Harrison ML, and Brothers RM**. Attenuated cerebral vasodilatory capacity in response to hypercapnia in college-aged African Americans. *Experimental Physiology* 100: 35-43, 2015.
20. **Hurr C, Patik JC, Kim K, and Brothers RM**. Blunted cerebral vascular responsiveness to hypercapnia in obese individuals. *Experimental Physiology* 102: 1300-1308, 2017.
21. **Ide K, Eliasziw M, and Poulin MJ**. Relationship between middle cerebral artery blood velocity and end-tidal PCO2 in the hypocapnic-hypercapnic range in humans. *Journal of applied physiology* 95: 129-137, 2003.
22. **Ikram MA, Luijendijk HJ, Vernooij MW, Hofman A, Niessen WJ, Van Der Lugt A, Tiemeier H, and Breteler MMB**. Vascular Brain Disease and Depression in the Elderly. *Epidemiology* 21: 78-81, 2010.
23. **Jonas BS, and Mussolino ME**. Symptoms of Depression as a Prospective Risk Factor for Stroke. *Psychosomatic Medicine* 62: 463-471, 2000.
24. **Junejo RT, May S, Alsalahi S, Alali M, Ogoh S, and Fisher JP**. Cerebrovascular carbon dioxide reactivity and flow-mediated dilation in young healthy South Asian and Caucasian European men. *American Journal of Physiology-Heart and Circulatory Physiology* 318: H756-H763, 2020.
25. **Kroenke K, Spitzer RL, and Williams JBW**. The PHQ-9. *Journal of General Internal Medicine* 16: 606-613, 2001.
26. **Larson SL, Owens PL, Ford D, and Eaton W**. Depressive Disorder, Dysthymia, and Risk of Stroke: Thirteen-Year Follow-Up From the Baltimore Epidemiologic Catchment Area Study. 32: 1979-1983, 2001.
27. **Lemke H, Castro AG-CD, Schlattmann P, Heuser I, and Neu P**. Cerebrovascular reactivity over time-course – From major depressive episode to remission. *Journal of Psychiatric Research* 44: 132-136, 2010.
28. **Malhi GS, and Mann JJ**. Depression. *Lancet* 392: 2299-2312, 2018.
29. **Matsuo K, Onodera Y, Hamamoto T, Muraki K, Kato N, and Kato T**. Hypofrontality and microvascular dysregulation in remitted late-onset depression assessed by functional near-infrared spectroscopy. *NeuroImage* 26: 234-242, 2005.
30. **Neu P, Schlattmann P, Schilling A, and Hartmann A**. Cerebrovascular reactivity in major depression: a pilot study. *Psychosomatic medicine* 66: 6-8, 2004.
31. **Ohira T, Iso H, Satoh S, Sankai T, Tanigawa T, Ogawa Y, Imano H, Sato S, Kitamura A, and Shimamoto T**. Prospective study of depressive symptoms and risk of stroke among Japanese. *STROKE-DALLAS*- 32: 903-906, 2001.
32. **Organization WH**. *The global burden of disease: 2004 update*. World Health Organization, 2008.

33. **Pan A, Sun Q, Okereke OI, Rexrode KM, and Hu FB**. Depression and Risk of Stroke Morbidity and Mortality. *JAMA* 306: 1241, 2011.
34. **Patik JC, Tucker WJ, Curtis BM, Nelson MD, Nasirian A, Park S, and Brothers RM**. Fast-food meal reduces peripheral artery endothelial function but not cerebral vascular hypercapnic reactivity in healthy young men. *Physiological Reports* 6: e13867, 2018.
35. **Peters R**. Ageing and the brain. *Postgrad Med J* 82: 84-88, 2006.
36. **Rajagopalan S, Brook R, Rubenfire M, Pitt E, Young E, and Pitt B**. Abnormal brachial artery flow-mediated vasodilation in young adults with major depression. *The American Journal of Cardiology* 88: 196-198, 2001.
37. **Ringelstein EB, Sievers C, Ecker S, Schneider PA, and Otis SM**. Noninvasive assessment of CO2-induced cerebral vasomotor response in normal individuals and patients with internal carotid artery occlusions. *Stroke* 19: 963-969, 1988.
38. **Salsman JM, Butt Z, Pilkonis PA, Cyranowski JM, Zill N, Hendrie HC, Kupst MJ, Kelly MAR, Bode RK, Choi SW, Lai JS, Griffith JW, Stoney CM, Brouwers P, Knox SS, and Cella D**. Emotion assessment using the NIH Toolbox. *Neurology* 80: S76-S86, 2013.
39. **Schalet BD, Pilkonis PA, Yu L, Dodds N, Johnston KL, Yount S, Riley W, and Cella D**. Clinical validity of PROMIS Depression, Anxiety, and Anger across diverse clinical samples. *Journal of Clinical Epidemiology* 73: 119-127, 2016.
40. **Seldenrijk A, Vogelzangs N, Van Hout HPJ, Van Marwijk HWJ, Diamant M, and Penninx BWJH**. Depressive and anxiety disorders and risk of subclinical atherosclerosis. *Journal of Psychosomatic Research* 69: 203-210, 2010.
41. **Serrador JM, Picot PA, Rutt BK, Shoemaker JK, and Bondar RL**. MRI Measures of Middle Cerebral Artery Diameter in Conscious Humans During Simulated Orthostasis. *Stroke* 31: 1672-1678, 2000.
42. **Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, and Dunbar GC**. The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *The Journal of clinical psychiatry* 1998.
43. **Spitzer RL**. Validation and Utility of a Self-report Version of PRIME-MD<SUBTITLE>The PHQ Primary Care Study</SUBTITLE>. *JAMA* 282: 1737, 1999.
44. **Tallon CM, Barker AR, Nowak-Flück D, Ainslie PN, and McManus AM**. The influence of age and sex on cerebrovascular reactivity and ventilatory response to hypercapnia in children and adults. *Experimental Physiology* 2020.
45. **Tiemeier H**. Cerebral haemodynamics and depression in the elderly. *Journal of Neurology, Neurosurgery & Psychiatry* 73: 34-39, 2002.
46. **Tomfohr LM, Murphy MLM, Miller GE, and Puterman E**. Multiwave Associations Between Depressive Symptoms and Endothelial Function in Adolescent and Young Adult Females. *Psychosomatic Medicine* 73: 456-461, 2011.
47. **Tomoto T, Riley J, Turner M, Zhang R, and Tarumi T**. Cerebral vasomotor reactivity during hypo- and hypercapnia across the adult lifespan. *Journal of Cerebral Blood Flow & Metabolism* 40: 600-610, 2020.
48. **Twenge JM, Cooper, A. B., Joiner, T. E., Duffy, M. E., & Binau, S. G.** Age, period, and cohort trends in mood disorder indicators and suicide-related outcomes in a nationally representative dataset, 2005–2017. *Journal of Abnormal Psychology* Advance online publication: 2019.

49. **Vakilian A, and Iranmanesh F**. Assessment of cerebrovascular reactivity during major depression and after remission of disease. *Annals of Indian Academy of Neurology* 13: 52, 2010.
50. **Weintraub S, Dikmen SS, Heaton RK, Tulsky DS, Zelazo PD, Bauer PJ, Carlozzi NE, Slotkin J, Blitz D, Wallner-Allen K, Fox NA, Beaumont JL, Mungas D, Nowinski CJ, Richler J, Deocampo JA, Anderson JE, Manly JJ, Borosh B, Havlik R, Conway K, Edwards E, Freund L, King JW, Moy C, Witt E, and Gershon RC**. Cognition assessment using the NIH Toolbox. *Neurology* 80: S54-S64, 2013.