Salivary alpha-amylase activity and mild cognitive impairment among Japanese older adults: The Toon Health Study

Naho Yamane[1], Ai Ikeda PhD[1], Kiyohide Tomooka PhD[1], Isao Saito MD, PhD[2], Koutatsu Maruyama PhD[3], Eri Eguchi PhD[4], Keiko Suyama PhD[5], Akiko Fujii MNS[5], Tamami Shiba MNS[5], Kumiko Tanaka PhD[5], Akiko Kooka MNS[5], Satsuki Nakamura PhD[6], Masaru Kajita MNS[6], Ryoichi Kawamura MD, PhD[7], Yasunori Takata MD PhD[7], Haruhiko Osawa MD, PhD[7], Andrew Steptoe DPhil[8], Takeshi Tanigawa MD PhD[9]

1. Department of Public Health, Juntendo University Faculty of Medicine, Tokyo, Japan
2. Department of Public Health and Epidemiology, Faculty of Medicine, Oita University, Yufu, Japan
3. Laboratory of Community Health and Nutrition, Special Course of Food and Health Science, Department of Bioscience, Graduate School of Agriculture, Ehime University, Toon Japan
4. Department of Epidemiology, Fukushima Medical University School of Medicine, Fukushima City, Japan
5. Department of Community Health Systems Nursing, Graduate School of Medicine, Ehime University, Toon, Japan
6. Department of Nursing, Faculty of Human Health and Welfare, St. Catherine University, Matsuyama City, Japan
7. Department of Diabetes and Molecular Genetics, Graduate School of Medicine, Ehime University, Toon, Japan
8. Department of Behavioural Science and Health, University College London,

London, UK

9. Department of Public Health, Juntendo University Graduate School of Medicine, Tokyo, Japan

Address for correspondence:

Takeshi Tanigawa, MD, PhD

Department of Public Health, Juntendo University Graduate School of Medicine,

2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan.

Phone: +81 (3) 5802-1049 Fax: +81 (3) 3814-0305

Email: tataniga@juntendo.ac.jp

Abstract

Background: There is growing interest in examining objective markers for early identification and behavioral intervention to prevent dementia and mild cognitive impairment in clinical and community settings.

Objective: To investigate the association between salivary alpha-amylase as an objective measure of psychological stress response and mild cognitive impairment for the implication of psychological stress in the development of mild cognitive impairment.

Design, Setting, and Participants: This cross-sectional study involved 865 participants aged ≥ 65 years. A saliva sample was collected in the morning, and the levels of salivary alpha-amylase were assayed. Mild cognitive impairment was evaluated using the Japanese version of the Montreal Cognitive Assessment; a score < 26 was indicative of mild cognitive impairment. A multivariable logistic regression model was used to examine the association of salivary alpha-amylase and mild cognitive impairment after adjusting for age, sex, current drinking status, current smoking status, body mass index, hypertension, diabetes mellitus, physical activity, education, social support, social network, and heart rate variability.

Results: Salivary alpha-amylase was associated with mild cognitive impairment (the multivariable-adjusted odds ratio [95% confidence interval] for the 1-standard deviation increment of log-transformed salivary alpha-amylase was 1.24 [1.07–1.44]). This significant association persisted after adjusting for various confounding factors.

Conclusion: Elevation of salivary alpha-amylase was associated with mild cognitive impairment among Japanese community-dwelling older adults. This suggests that salivary alpha-amylase is a useful objective marker of psychological stress responses associated with mild cognitive impairment.

Keywords

Salivary alpha-amylase, mild cognitive impairment, psychological stress, cross-sectional study

Introduction

Approximately 50 million individuals have dementia worldwide, with nearly 10 million new cases diagnosed annually (1). Hence, the prevention of dementia is an important challenge. Mild cognitive impairment (MCI), a state in between normal cognition and dementia, often progresses toward dementia. This has generated a growing interest in identifying MCI and intervening lifestyle-related behavior of the patients in clinical and community settings against its further development (2-4).

Previous studies have shown associations between psychological stress and a deterioration in cognitive function (5), increased risk of developing MCI (6), and dementia (7). Two major systems are involved in the human stress response (8, 9). Upregulation of the hypothalamic-pituitary-adrenal (HPA) axis increases secretion of stress hormones such as cortisol (10). Corticosteroids can cause cognitive deficits by a mechanism that is independent of hippocampal neural loss (11). HPA axis disturbance has been found among patients with MCI (12), and a previous study has suggested that morning salivary cortisol levels could be a marker for MCI (13). The other system of the human stress response involves the activation of the sympathetic-adrenal-medullary (SAM) axis (8, 9). For instance, a previous cross-sectional study has found low heart rate variability (HRV) among MCI patients, which reflects dysfunction of the autonomic nervous system (14, 15). Moreover, salivary alpha-amylase (sAA) is a biomarker for psychological stress that indicates SAM axis activation (16). Psychological stress increases beta-adrenergic activity through the activation of the SAM axis, which may lead to elevated sAA (16). Previous studies also showed that increased beta-adrenergic activity leads to the production of amyloid beta (Aβ) peptide (17, 18). The production and deposition of Aβ peptide is important in the pathogenesis of Alzheimer's disease (AD) (19). Thus, sAA, an obfective marker

of the SAM axis, may be associated with MCI via beta-adrenergic activity, suggesting the contribution of psychological stress to cognitive decline. However, thus far, there have been no studies examining the association between sAA and MCI. Therefore, we conducted a large cross-sectional study to investigate the association between sAA and MCI in older adults.

Materials and Methods

Participants

This cross-sectional study was part of the Toon Health Study – a prospective cohort study initiated in Toon City, Ehime Prefecture, Japan in 2009. The Toon Health Study involved > 2,000 middle-aged and older adult community residents and aimed to determine risk factors for the development of cardiovascular diseases (20). For the present study, we recruited 1,778 participants between 2014 and 2017. We excluded participants aged < 65 years (n = 858). In addition, participants with missing data on sAA (n = 11) or the Japanese version of Montreal Cognitive Assessment (MoCA-J) (n = 3) or heart rate variability (HRV) (n=2), and those who had history of stroke (n = 19) or receiving medication for stroke (n = 20) were excluded. Finally, 337 men and 528 women were eligible for inclusion in the analysis. This study was approved by the Institutional Review Board of Ehime University Hospital, Toon, Japan and the Ethics Committee of Juntendo University, Tokyo, Japan. All participants provided informed consent.

Measurement of sAA

A saliva sample was collected from each participant in the morning with stimulation provided using a chewing gum. The participants chewed 1 g of bland and flavorless Salivar Gum (Tokyo Shizaisha, Tokyo, Japan) for 5 min. While chewing, their saliva was collected into plastic tubes.

Collected saliva was stored at −80 °C. An sAA kinetic enzyme assay kit (Salimetrics, LLC, State College, PA, USA) was used to measure the levels of sAA. A plate reader (Vmax PowerWave XS; BioTek Instruments, Tokyo, Japan) was used for salivary determination with 405-nm filters for sAA. An amylase intra-assay

coefficient of variation of 5.47 ± 1.49% and inter-assay reproducibility of 4.7 ± 0.15% were accepted.

Assessment of MCI

The MoCA-J was used to evaluate the cognitive function of participants. The MoCA-J includes measurements for attention, working memory, short-term memory, delayed recall, visuospatial abilities, executive functioning, language, and orientation to time and place (21, 22). The MoCA-J consists of the following 12 tasks: a five-item delayed recall task, a clock-drawing task, a cube-copying task, a trail-making task, a phonemic fluency task, a two-item verbal abstraction task, a target-tapping task, a serial subtraction task, a two-item digit-reading task, a three-item naming task, a two-item sentence-repeating task, and a six-item temporal and locational orientation task. Furthermore, for individuals with ≤ 12 years of formal education, 1 point was added to the total score of the cognitive tasks. The full score of the test was 30 points. A MoCA-J score < 26 points was indicative of MCI (21).

Assessment of confounding factors

Height and weight were measured in stocking feet and light clothing, and the body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). Blood pressure was measured twice in the sitting position after a rest of ≥ 5 min using an automatic sphygmomanometer (BP-103iII; OMRON Colin Co, Tokyo, Japan). The mean of the two measurements was used for analysis. The study physicians also questioned participants regarding their history and any current medications taken for hypertension, diabetes mellitus (DM), or stroke. Participants taking antihypertensive medication or having a systolic blood pressure ≥ 140 mmHg or a diastolic blood

pressure ≥ 90 mmHg were defined as hypertensive. We carried out an oral glucose tolerance test after at least a 10-h fast. Participants taking antidiabetic medication or having a fasting and 2-h-postload glucose ≥ 7.0 mmol/L and ≥ 11.1 mmol/L, respectively, at the oral glucose tolerance test were defined as diabetic. Investigators asked questions to determine the drinking status (current/non-drinker), smoking habits (current/non-smoker), educational level, physical activity, social support, and social networks of participants. Social support was assessed using the ENRICHD Social Support Instrument, which consists of seven items evaluating the four defining attributes of social support: emotional, instrumental, informational, and appraisal (23). Social networks were assessed using the Social Network Index, which includes four items related to the number of ties and their relative importance (24). The individual items of the ENRICHD Social Support Instrument and the Social Network Index were summed to obtain the total score. Scores in the lowest quartile denoted low social support and small social networks. To evaluate physical activity, the Japan Arteriosclerosis Longitudinal Study Physical Activity Questionnaire, which assesses the metabolic equivalents of different activities including occupational, household, and leisure-time was administered (25). Participants reported the frequency and duration of physical activity, and each physical activity was expressed as metabolic equivalents-hours per day. A noninvasive cardiac autonomic control assessment tool (TAS9; YKC Co. Ltd, Tokyo, Japan) was used to analyze HRV. Pulse rate was recorded with a fingertip pulse wave sensor for 5 minutes. The power spectrum was decomposed into frequency components and quantified by the relative intensity (power) of each component. The power spectrum was divided into high-frequency (HF; 0.15–0.40 Hz) and low-frequency (LF; 0.04–0.15 Hz) bands. The natural log-transformed LF/HF ratio was used for the analysis.

Statistical analysis

We computed the concentration of sAA divided by the collected volume of saliva (U/mL). In this study, women showed significantly higher sAA levels than men. Therefore, the participants were divided into sex-specific quartiles according to their sAA levels. The Kruskal–Wallis test and chi-squared test were used to compare the characteristics according to the sex-specific quartiles of sAA. The chi-squared test and *t*-test were used to compare the means or proportions of participants according to the diagnosis of MCI. The odds ratio (OR) and 95% confidence interval (CI) of MCI were calculated using multivariable logistic regression according to the sex-specific quartiles of sAA after adjusting for age (years), sex (model1), current drinking status (yes/no), current smoking status (yes/no), BMI (kg/m^2), hypertension, DM, physical activity (low/high), education (high school or lower/college or higher), social support (low/high), social network (small/large), and HRV (model2). Linear trends were tested using a 1-standard deviation increment of log-transformed sAA as an independent variable in the multivariable logistic regression model. P-values < 0.05 denoted statistically significant differences. The SAS statistical package version 9.4 (Statistical Analysis System, Cary, NC, USA) was used for these analyses.

Results

Participant's characterization

Table 1 shows the characteristics of participants according to the sex-specific quartiles of sAA. The mean age and proportion of those with MCI were higher in the highest sAA quartile compared to the lowest sAA quartile (72.4 ± 5.1 vs. 70.6 ± 4.3 years, and 52.3% vs. 38.4%, respectively). On the other hand, the proportion of smokers was lower in the highest sAA quartile compared to the lowest sAA quartile (4.6% vs. 7.9%). However, there were no differences in the other characteristics.

MCI and risk factors are associated

Table 2 shows the associations between MCI and possible risk factors. Risk factors that were higher in cases of MCI compared to controls included mean BMI (23.6 ± 3.2 vs. 23.0 ± 2.9), the proportion of males (48.4% vs. 32.1%), DM (23.9% vs. 17.2%), and those with low social support (30.2% vs. 20.0%). There were no differences between MCI cases and controls in the other risk factors.

Association between MCI and sAA

The multivariable-adjusted ORs and 95% CIs of MCI according to the sex-specific quartiles of sAA are presented in Table 3 and Figure1. The age- and sex-adjusted OR (95% CI) of MCI for the highest quartile group of sAA compared with the lowest quartile group was 1.56 (1.05–2.32) (Model 1). The age- and sex-adjusted OR (95% CI) for each 1-standard deviation increment of log-transformed sAA was 1.24 (1.07–1.43). Following further adjustments for current smoking status, BMI, hypertension, DM, physical activity, social support, social network, and HRV, the association between sAA and MCI remained statistically significant (Model 2).

Discussion

In the present study, significant associations between higher levels of sAA and MCI were found among Japanese community-dwelling older adults. These associations were independent of demographic, lifestyle, biological factors, and social relationships. This is a study to investigate the association between sAA and MCI and demonstrate the possible contribution of psychological distress in cognitive decline.

Our results are strongly congruent with those of previous studies that found associations of high levels of self-reported psychological distress with cognitive impairment (5) and dementia (7). The mechanisms underlying the association between the elevation of sAA as a consequence of high levels of psychological distress and MCI have not been completely elucidated. However, plausible explanations include engaging in unhealthy behaviors (i.e., smoking, heavy drinking, sedentary lifestyle, poor diet) (26), as well as through potentially toxic direct effects on the autonomic nervous system (17). High levels of psychological distress have been linked to activation of the sympathetic nervous system (8, 9). The activation of SAM axis elevates beta-adrenergic activity and increases the levels of sAA (16). The activated beta-adrenergic receptors increase the production of Aβ peptide (a major feature of AD pathogenesis) by enhancing gamma-secretase activity in the brain (17, 18). Moreover, a previous study revealed an increase in the activity of the glycogen-degrading enzyme alpha-amylase in hippocampal homogenates of patients with AD and the alpha-amylase immunoreactivity in astrocytes adjacent to $A\beta_{42}$ plaques (27). The increase in alpha-amylase activity occurs due to fibril $A\beta_{42}$ stimulation, which can be inhibited by treatment with a beta-adrenergic receptor antagonist (27). Luong et al. (2013) reported that long-term treatment of hypertensive patients with beta-adrenergic antagonists reduced the risk of AD (28). Taken together, the elevation of

sAA, which reflects the beta-adrenergic activity of the sympathetic nervous system, is associated with cognitive decline, probably due to the enhanced production of Aβ peptide.

Our study also suggests that psychological stress increases the risk of cardiovascular disease (CVD), which in turn causes vascular dementia (VaD). Psychological stress is an important risk factor for CVD (9). Previous studies and reviews have shown that vascular risk factors are associated with VaD, AD, mixed dementia, and amnestic MCI (29). Although this study excluded individuals with a history of stroke or taking stroke medication, and adjusted for major CVD risks, it has been hypothesized that subclinical cerebrovascular disease may lead to dysfunction of the neurovascular unit, which disrupts the ability of cerebrovascular microvessels to dilate in response to increased neuronal activity. This could lead to neuronal dysfunction and reduced neuronal activity, which causes cognitive dysfunction and Aβ accumulation (30).

sAA is a well-validated and reliable stress marker (31) compared to other stress markers in saliva, such as catecholamine and cortisol. This is because sAA can reflect exposure to not only acute but also chronic psychological stress, and is stable in collected saliva samples (32). Moreover, Nater et al (2006) found that stress elevated sAA levels; however, alpha-amylase responses were not closely related to catecholamine and cortisol responses under stressful condition (33). We also found that the association between sAA and MCI remained statistically significant after adjustment for HRV. Therefore, sAA can serve as a useful biomarker for stress in human studies, and has an independent association with MCI, as suggested in this study.

This study involved a large, well-characterized sample of men and women.

The collected data included amylase concentrations, MCI, social relationship measures, and numerous lifestyle variables. However, a major limitation of this study is its cross-sectional design, which limits our ability to draw causal inferences. Although we have interpreted the findings in relation to the possible role of psychological distress in the development of MCI, it is plausible that awareness of cognitive impairment elicits increased distress. Additionally, sAA was assessed on a single occasion; hence, we could not examine the dynamic relationship between the sympathetic nervous system and MCI in a longitudinal design. In addition to further longitudinal studies, behavioral interventions to reduce stress may also be necessary for patents with high sAA in clinical and community settings. In the analysis, we adjusted for various possible confounding factors. Nevertheless, there is a possibility of residual confounding by unmeasured variables, such as genetic factors (34), which may influence the MCI causality cascade. Finally, we used the MoCA-J rather than clinical diagnosis to measure MCI in the present study. According to Petersen's criteria, clinical diagnosis is established based on: 1) memory complaint, preferably corroborated by an informant; 2) impaired memory function for age and education; 3) preserved general cognitive function; 4) intact activities of daily living; and 5) absence of dementia.[3] The MoCA-J has been widely used in the epidemiological field, exhibiting good sensitivity (93.0%) and specificity (87.0%) (21).

In summary, we found that elevation in the levels of sAA was associated with MCI among Japanese community-dwelling older adults. This finding suggests that sAA is a useful measure of psychobiological stress responses related to MCI in population studies. The relationship between sAA and MCI was maintained after adjusting for a standard set of biological and behavioral factors, implying that other mechanisms should also be considered. Moreover, further studies are needed to

confirm the association between sAA and MCI in other populations (i.e., Alzheimer's patients) or in longitudinal settings.

Funding

This study was supported by JSPS KAKENHI Grant Numbers; JP16K09072, JP17KK0175, JP19K10670, 25293142, and 8020 Promotion Foundation, Japan in 2016, 2017. The sponsors had no role in the design and conduct of the study; in the collection, analysis, and interpretation of data; in the preparation of the manuscript; or in the review or approval of the manuscript.

Acknowledgments

The authors thank all the participants and staff members of the Toon Health Study for their substantial contribution.

Conflict of Interest

Dr. Ikeda reports grants from JSPS KAKENHI, during the conduct of the study. Dr. Saito reports grants from 8020 Promotion Foundation, during the conduct of the study. Dr. Tanigawa reports grants from JSPS KAKENHI, during the conduct of the study. The other authors have nothing to disclose.

References

1. World Health Organization (2021) Dementia. https://www.who.int/news-room/fact-sheets/detail/dementia. Last updated 2 September 2021, Accessed on 11 December 2021.
2. Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, Broich K, et al. International Psychogeriatric Association Expert Conference on mild cognitive impairment. *Lancet*. 2006;367(9518):1262-1270. doi:10.1016/S0140-6736(06)68542-5.
3. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol.* 1999;56(3):303–308. doi:10.1001/archneur.56.3.303
4. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *Lancet*. 2020;396(10248):413-446. doi:10.1016/S0140-6736(20)30367-6.
5. Freire ACC, Pondé MP, Liu A, Caron J. Anxiety and depression as longitudinal predictors of mild cognitive impairment in older adults. *Can J Psychiatry*. 2017;62(5):343-350. doi:10.1177/0706743717699175
6. Wilson RS, Schneider JA, Boyle PA, Arnold SE, Tang Y, Bennett DA. Chronic distress and incidence of mild cognitive impairment. *Neurology*. 2007;68(24):2085-2092. doi:10.1212/01.wnl.0000264930.97061.82
7. Johansson L, Guo X, Waern M, Ostling S, Gustafson D, Bengtsson C, et al. Midlife psychological stress and risk of dementia: a 35-year longitudinal population study. *Brain*. 2010;133(Pt 8):2217-2224. doi:10.1093/brain/awq116
8. Binder EB, Nemeroff CB. The CRF system, stress, depression and anxiety-

insights from human genetic studies. *Mol Psychiatry*. 2010;15(6):574-588. doi:10.1038/mp.2009.141

9. Cohen S, Janicki-Deverts D, Miller GE. Psychological stress and disease. *JAMA*. 2007;298(14):1685-1687. doi:10.1001/jama.298.14.1685
10. Hackett RA Steptoe A. Type 2 diabetes mellitus and psychological stress - a modifiable risk factor. *Nat Rev Endocrinol*. 2017;13(9):547-560. doi:10.1038/nrendo.2017.64
11. Lupien S, Lecours AR, Lussier I, Schwartz G, Nair NP, Meaney MJ. Basal cortisol levels and cognitive deficits in human aging. *J Neurosci*. 1994;14(5 Pt 1):2893-2903. doi:10.1523/JNEUROSCI.14-05-02893.1994
12. Lind K, Edman A, Nordlund A, Olsson T, Wallin A. Increased saliva cortisol awakening response in patients with mild cognitive impairment. *Dement Geriatr Cogn Disord*. 2007;24(5):389-395. doi:10.1159/000109938
13. Venero C, Díaz-Mardomingo C, Pereda-Pérez I, García-Herranz S, Utrera L, Valencia A, et al. Increased morning salivary cortisol levels in older adults with nonamnestic and multidomain mild cognitive impairment. *Psychoneuroendocrinology*. 2013;38(4):488-498. doi:10.1016/j.psyneuen.2012.07.007
14. Nicolini P, Ciulla MM, Malfatto G, Abbate C, Mari D, Rossi PD, et al. Autonomic dysfunction in mild cognitive impairment: evidence from power spectral analysis of heart rate variability in a cross-sectional case-control study. *PLoS One*. 2014;9(5):e96656. Published 2014 May 6. doi:10.1371/journal.pone.0096656
15. Collins O, Dillon S, Finucane C, Lawlor B, Kenny RA. Parasympathetic autonomic dysfunction is common in mild cognitive impairment. *Neurobiol*

Aging. 2012;33(10):2324-2333. doi:10.1016/j.neurobiolaging.2011.11.017

16. Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology*. 2009;34(4):486-496. doi:10.1016/j.psyneuen.2009.01.014
17. Yu NN, Wang XX, Yu JT, Wang ND, Lu RC, Miao D, et al. Blocking beta2-adrenergic receptor attenuates acute stress-induced amyloid beta peptides production. *Brain Res*. 2010;1317:305-310. doi:10.1016/j.brainres.2009.12.087
18. Ni Y, Zhao X, Bao G, Zou L, Teng L, Wang Z, et al. Activation of beta2-adrenergic receptor stimulates gamma-secretase activity and accelerates amyloid plaque formation. *Nat Med*. 2006;12(12):1390-1396. doi:10.1038/nm1485.
19. Tanzi RE. The synaptic Abeta hypothesis of Alzheimer disease. *Nat Neurosci*. 2005;8(8):977-979. doi:10.1038/nn0805-977
20. Saito I, Maruyama K, Eguchi E, Kato T, Kawamura R, Takata Y, et al. Low heart rate variability and sympathetic dominance modifies the association between insulin resistance and metabolic syndrome - the toon health study. *Circ J*. 2017;81(10):1447-1453. doi:10.1253/circj.CJ-17-0192
21. Fujiwara Y, Suzuki H, Yasunaga M, Sugiyama M, Ijuin M, Sakuma N, et al. Brief screening tool for mild cognitive impairment in older Japanese: validation of the Japanese version of the Montreal Cognitive Assessment. *Geriatr Gerontol Int*. 2010;10(3):225-232. doi:10.1111/j.1447-0594.2010.00585.x
22. Waldron-Perrine B, Gabel NM, Seagly K, Kraal AZ, Pangilinan P, Spencer RJ, et al. Montreal Cognitive Assessment as a screening tool: Influence of performance and symptom validity. *Neurol Clin Pract*. 2019;9(2):101-108. doi:10.1212/CPJ.0000000000000604

23. Vaglio J Jr, Conard M, Poston WS, O'Keefe J, Haddock CK, House J, et al. Testing the performance of the ENRICHD Social Support Instrument in cardiac patients. *Health Qual Life Outcomes*. 2004;2:24. Published 2004 May 13. doi:10.1186/1477-7525-2-24
24. Berkman LF, Syme SL. Social networks, host resistance, and mortality: a nine-year follow-up study of Alameda County residents. *Am J Epidemiol*. 1979;109(2):186-204. doi:10.1093/oxfordjournals.aje.a112674
25. Ishikawa-Takata K, Naito Y, Tanaka S, Ebine N, Tabata I. Use of doubly labeled water to validate a physical activity questionnaire developed for the Japanese population. *J Epidemiol*. 2011;21(2):114-121. doi:10.2188/jea.je20100079
26. McEwen BS. Protective and damaging effects of stress mediators: central role of the brain. *Dialogues Clin Neurosci*. 2006;8(4):367-381. doi:10.31887/DCNS.2006.8.4/bmcewen
27. Byman E, Schultz N, Netherlands Brain Bank, Blom AM, Wennström M. A Potential Role for α-Amylase in Amyloid-β-Induced Astrocytic Glycogenolysis and Activation. *J Alzheimers Dis*. 2019;68(1):205-217. doi:10.3233/JAD-180997
28. Lương Kv, Nguyen LT. The role of Beta-adrenergic receptor blockers in Alzheimer's disease: potential genetic and cellular signaling mechanisms. *Am J Alzheimers Dis Other Demen*. 2013;28(5):427-439. doi:10.1177/1533317513488924
29. Gorelick PB, Scuteri A, Black SE, Decarli C, Greenberg SM, Iadecola C, et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. *Stroke*. 2011;42(9):2672-2713. doi:10.1161/STR.0b013e3182299496
30. Nation DA, Hong S, Jak AJ, Delano-Wood L, Mills PJ, Bondi MW, et al. Stress,

exercise, and Alzheimer's disease: a neurovascular pathway. *Med Hypotheses*. 2011;76(6):847-854. doi:10.1016/j.mehy.2011.02.034

31. Ali N, Nater UM. Salivary Alpha-Amylase as a Biomarker of Stress in Behavioral Medicine. *International Journal of Behavioral Medicine*. 2020;*27*(3):337–342. doi: 10.1007/s12529-019-09843-x.
32. Booij SH, Bos EH, Bouwmans MEJ, et al. Cortisol and α-Amylase Secretion Patterns between and within Depressed and Non-Depressed Individuals. *PloS One*. 2015;*10*(7), e0131002. doi: 10.1371/journal.pone.0131002.
33. Nater UM, La Marca R, Florin L, et al. Stress-induced changes in human salivary alpha-amylase activity -- associations with adrenergic activity. *Psychoneuroendocrinology*. 2006;*31*(1), 49–58. doi: 10.1016/j.psyneuen.2005.05.010.
34. Mangialasche F, Kivipelto M, Solomon A, Fratiglioni L. Dementia prevention: current epidemiological evidence and future perspective. *Alzheimers Res Ther*. 2012;4(1):6. Published 2012 Feb 13. doi:10.1186/alzrt104

Table1. Participant's characterization.

	salivary alpha-amylase*/saliva volume (sex-specific)				
	Quartile†1	Quartile2	Quartile3	Quartile4	p value‡
N	216	216	217	216	
Median (IQR for men), U/mL	7.5 (1.6-10.8)	15.1 (10.9-20.0)	26.5 (20.0-34.0)	57.2 (34.5-464.8)	
Median (IQR for women), U/mL	13.9 (1.1-20.6)	27.1 (20.7-35.4)	42.5 (35.5-54.0)	90.8 (54.6-1437.8)	
Age§, years	70.6 ± 4.3	71.3 ± 4.8	71.9 ± 4.9	72.4 ± 5.1	0.001
Male‖	84 (38.9)	84 (38.9)	85 (39.2)	84 (38.9)	1.00
Current drinker	108 (50.0)	97 (44.9)	95 (43.8)	91 (42.1)	0.39
Current smoker	17 (7.9)	4 (1.9)	8 (3.7)	10 (4.6)	0.02
Physical activity, METs h/day	35.1 ± 5.5	35.0 ± 4.2	34.8 ± 4.1	34.9 ± 4.0	0.91
BMI	23.3 ± 3.1	22.9 ± 2.8	23.1 ± 3.1	23.7 ± 3.2	0.14

Hypertension	110 (50.9)	106 (49.1)	128 (59.0)	128 (59.3)	0.06
Diabetes mellitus	37 (17.1)	38 (17.6)	47 (21.7)	51 (23.6)	0.26
Low social support	54 (25.0)	58 (26.9)	46 (21.2)	52 (24.1)	0.58
Small social network	52 (24.1)	43 (19.9)	40 (18.4)	46 (21.3)	0.52
Heart rate variability{	1.08 ± 0.2	1.07 ± 0.2	1.10 ± 0.3	1.10 ± 0.3	0.65
≥College education	81 (37.5)	73 (33.8)	78 (35.9)	74 (34.3)	0.85
MoCA Score<26	83 (38.4)	76 (35.2)	92 (42.4)	113 (52.3)	0.002

*Salivary alpha-amylase was divided by total saliva volume; †Sex-specific quartile; ‡Statistical significance by Kruskal-Wallis and chi-square test; §mean ±SD for continuous variables; ‖N (%) for categorical variables; {natural log-transformed LF/HF ratio. Abbreviations: BMI, body mass index; IQR, inter quartile range; METs, metabolic equivalents; MoCA, Montreal Cognitive Assessment; SBP, systolic blood pressure.

Table 2. Association between MCI and its risk factors.

MCI	control	case	p value*
Male‡	161 (32.1)	176 (48.4)	<0.001
Current drinker	217 (43.3)	174 (47.8)	0.19
Current smoker	23 (4.6)	16 (4.4)	0.89
Physical activity†, METs h/day	35.2 ± 4.6	34.6 ± 4.3	0.05
BMI	23.0 ± 2.9	23.6 ± 3.2	0.01
Hypertension	268 (53.5)	204 (56.0)	0.46
Diabetes mellitus	86 (17.2)	87 (23.9)	0.01
Low social support	100 (20.0)	110 (30.2)	0.0005
Small social network	95 (19.0)	86 (23.6)	0.10
Heart rate variability	1.10 ± 0.3	1.07 ± 0.2	0.15
≥ College education	175 (34.9)	131 (36.0)	0.75

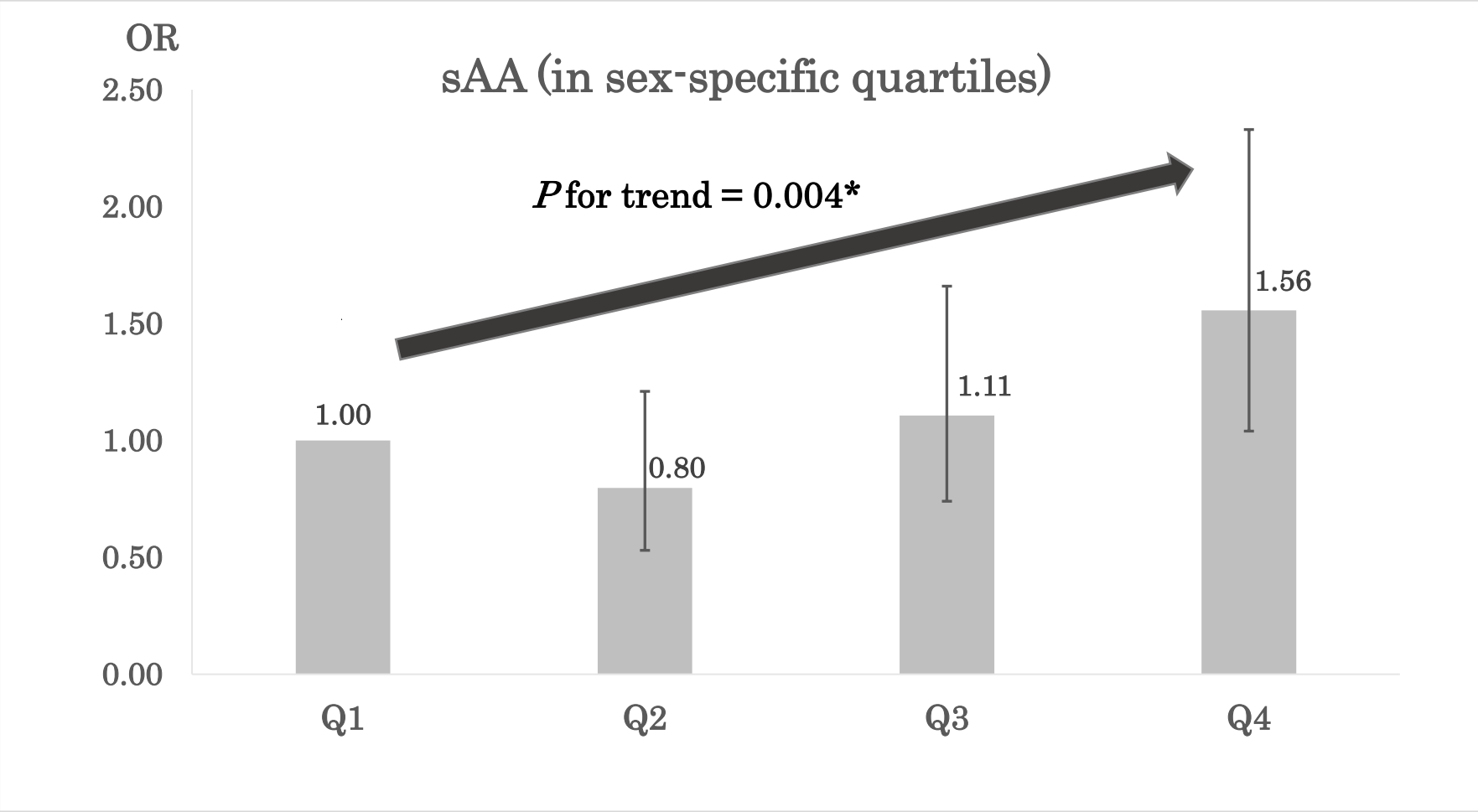
*Statistical significance by chi-square test or t-test; †mean ±SD for continuous variables; ‡N (%) for categorical variables. Abbreviation: BMI, body mass index; MCI, mild cognitive impairment; METs, metabolic equivalents; MoCA, Montreal Cognitive Assessment.

Table 3. Association between MCI and sAA.

	sAA*				
	Quartile†1	Quartile2	Quartile3	Quartile4	1SD increment for log-transformed sAA
N (case)	216 (83)	216 (76)	217 (92)	216 (113)	
Model1 OR‡ (95% CI)	1.00	0.81 (0.54,1.22)	1.07 (0.72,1.59)	1.56 (1.05,2.32)	1.24 (1.07,1.43)
Model2 OR (95% CI)	1.00	0.80 (0.53,1.21)	1.11 (0.74,1.66)	1.56 (1.04,2.33)	1.24 (1.07,1.44)

*Salivary alpha-amylase was divided by total saliva volume; †Sex-specific quartile; ‡Statistical significance by multivariable logistic regression; Adjustments for model1: age, sex; Adjustments for model2: age, sex, current drinker, current smoker, BMI, hypertension, diabetes mellitus, physical activity, education, social support, social network, heart rate variability. Abbreviation: BMI, body mass index; CI, confidence interval; OR, odds ratio; sAA, salivary alpha-amylase; SD, standard deviation.

Figure1. Association between MCI and sAA



*p for trend for 1SD increment for log-transformed sAA

Abbreviation: MCI, mild cognitive impairment; sAA, salivary alpha-amylase; OR, odds ratio.