UiT The Arctic University of Norway

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Risk factors associated with brain aging in the 7th Tromsø study

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Preface

This master thesis was written as a part of our fifth-year medical research paper.

The primary objective of this thesis was to investigate the relationship between lifestyle factors and brain aging in a population sample of people between the ages of 40 and 80 years, particularly, how the brain age gap (BAG = brain age -chronological age) could be associated with key variables such as age, sex, cardiovascular health, smoking, blood pressure, exercise and different lifestyle factors. Additionally, we investigated whether significant sex differences existed.

In preparing this master's thesis, it was deemed appropriate to provide some background information on the concept of brain age and the methodologies used to define predictors, thus resulting in a revision of the present understanding of brain age.

This master's thesis was supervised by associate professor Torgil Riise Vangberg. A second associate was Marte Christine Ørbo. Dr. Vangberg contributed to the research proposal, applications for obtaining data from the Tromsø study and in the advanced part of the statistical analysis. My contribution has been to review relevant literature, interpreting the data and writing this thesis.

Sincere gratitude to my supervisors Torgil Riise Vangberg and Marte Christine Ørbo for their time, effort, and supervision.

Nabila Ali 01.06.2022

Abbreviations

BAG: Brain age gap (chronological age – biological age estimated by neuroimaging techniques)

GVIF: Generalized Variance Inflation Factor

MRI: Magnetic resonance imaging

FLAIR: Fluid-attenuated inversion recovery

BMI: Body mass index

HDL- C: High-density lipoprotein cholesterol

LDL- C: Low-density lipoprotein cholesterol

SES: Socio-economical index

AD: Alzheimer's disease

NIAAA: National Institute on Alcohol Abuse and Alcoholism

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Abstract

Introduction: Aging is a natural biological process characterized by a deterioration of functions on multiple levels: molecular, cellular, organ-specific, and systemic. There is considerable variation between individuals in aging based on biological and functional markers. This variation forms the basis for the idea of "biological age," which measures a more representative age than chronological age. Biological age is clinically and scientifically interesting as it summarizes a complex aging process into a single number that can be a valuable instrument to assess an individual's health risks and conditions. A novel marker for biological age is "Brain age," which estimates the age of the brain based on MRI images. The difference between a person's brain age and chronological age is referred to as the "brain age gap" (BAG; brain age minus chronological age). A positive BAG indicates that a brain resembles an older average brain, while a negative BAG suggests that the brain resembles a younger average brain. BAG is what is scientifically relevant in this study, regardless of whether an individual's biological age is greater or less than their chronological age.

Methods: We analyzed data from the 7th Tromsø survey including 1.864 MRI scans. The data set consisted of people between 40 - 87 years, an average age of 65 years, and about the same number of participants from both sexes. Amongst exclusion criteria were people with infarction, tumors, and other major structural malformations in the brain that may affect the brain age estimate. We investigated how brain age could be associated with different variables. We corrected for education and socioeconomic status associated with brain health.

Results: Multiple regression model was applied to see what factors are associated with brain age. Resting heart rate, sex-differences, HDL cholesterol, diabetes, and smoking were significantly associated with BAG.

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Conclusion: Our findings suggest that males are likely to have higher brain age compared to females. In addition, resting heart rate, HDL-C, smoking and diabetes were significantly associated with BAG. Smoking, whether previous or current was found to accelerate brain age significantly. Also, having diabetes increased brain age by 2.4 years. Our study found none of the interaction terms for sex differences to be significant. A low heart rate may be advantageous for healthy brain aging. In addition, self-reported physical activity and self-reported sleep problems were not associated with BAG.

1 Introduction

1.1 On aging

Aging is a natural biological process that is characterized by the deterioration of function on multiple levels, including the molecular, cellular, organ-specific, and systemic (Kirkwood, 2011). Age is also a risk factor for the development of neurodegenerative disorders such as Alzheimer's disease or Parkinson's disease. However, age-related cognitive decline can also occur in the absence of obvious neurodegenerative abnormalities (Ridderinkhof & Krugers, 2022). From an evolutionary perspective, the disposable soma theory attempts to address the aging process as a gradual accumulation of faults within the organism despite cellular and molecular protection mechanisms (Kirkwood, 2005). Organisms that lack specific somatic cells, such as the hydra and various mollusks, do not age and only perish as a result of extreme environmental changes, such as predation, malnutrition, or temperature fluctuations (Ridderinkhof & Krugers, 2022). Organisms that have acquired specialized somatic cells nonetheless age and eventually perish. This specialization, therefore, comes at the price of aging and degeneration. Hence Kirkwood describes somatic cells as disposable.

Important findings reveal that DNA damage, dysregulated glucose metabolism, oxidative stress, metabolic alterations, and inflammatory processes may explain aging in general. Apart from this, there are age-related neural changes that do not act independently but are frequently interconnected and may explain age-related changes in brain cognitive function. Importantly, the age-related loss in cognitive function may not have a single neurobiological reason (Ridderinkhof & Krugers, 2022). It is also hypothesized that the genetic control of longevity is mediated by fine-tuning multiple mechanisms for the somatic maintenance and repair of the organism thereby suggesting a polygenic influence on longevity (Kirkwood, 2011).

The health status of the aging population is a major concern not only for elderly people but also for society as a whole. The percentage of adults aged 65 and older in Western nations is increasing to >25%, followed by a reduction in mental health and well-being (Ridderinkhof & Krugers, 2022).

In this study, we intend to examine the relationship between lifestyle factors and brain aging in a population sample of persons between the ages of 40 and 80, as well as whether

significant gender differences exist. In addition, identify lifestyle factors that are essential for healthy brain aging in both sexes.

1.2 Biological aging

There is considerable variation in aging based on biological and functional markers (Jylhava et al., 2017). This variation forms the basis for the concept of "biological age," which entails predicting a more accurate age based on biological indicators (Butler et al., 2004). These estimations must be minimally invasive and more accurate at predicting mortality than chronological age, according to the two primary criteria for their acceptance. (Butler et al., 2004; Johnson, 2006). Biological age can be calculated by telomere length (Lopez-Otin et al., 2013; Muezzinler et al., 2013). DNA methylation with a so-called "epigenetic clock" or a combination of several markers (Horvath, 2013; Le et al., 2018).

1.3 Brain Age

A novel marker of biological age is "brain age", which estimates the age of the brain based on MRI images (Cole & Franke, 2017; Franke & Gaser, 2019). Studies have found that this organ-specific marker is sensitive to brain health. However, it is important to emphasize that it is not estimated brain age per se that is of primary importance, but the difference between brain age and chronological age is called the "brain age gap" (BAG = brain age - chronological age). A positive BAG indicates that a brain resembles an older average brain, while a negative BAG indicates that the brain resembles a younger average brain.

It has been demonstrated that brain age correlates with cognitive aging and numerous elements of physiological aging, as well as predicting the risk of neurodegenerative disorders and mortality in the elderly population(Cole & Franke, 2017). Based on recent research, BAG is a more accurate predictor of mortality and dementia than chronological age (Franke & Gaser, 2019).

Population-based studies have also demonstrated that lifestyle factors like smoking (Cole, 2020), alcohol consumption (Pfefferbaum et al., 2001), obesity (Ronan et al., 2016), and diabetes (Franke et al., 2013) are correlated with higher BAG whereas exercise and education were associated with lower BAG (Steffener et al., 2016). These findings indicate that the estimations of brain age are sensitive to age-related changes in the brain and can be used to better understand how genes, the environment, and lifestyle influence brain aging processes

1.3.1 Predicting brain age using neuroimaging

Brain age can be predicted in individuals using neuroimaging data and machine learning techniques. Figure 1 illustrates roughly the pipeline process from neuroimaging studies to using 'supervised' machine learning algorithm to predict brain age. Next, multiple regression models are used to describe the association between BAG and selected variables.

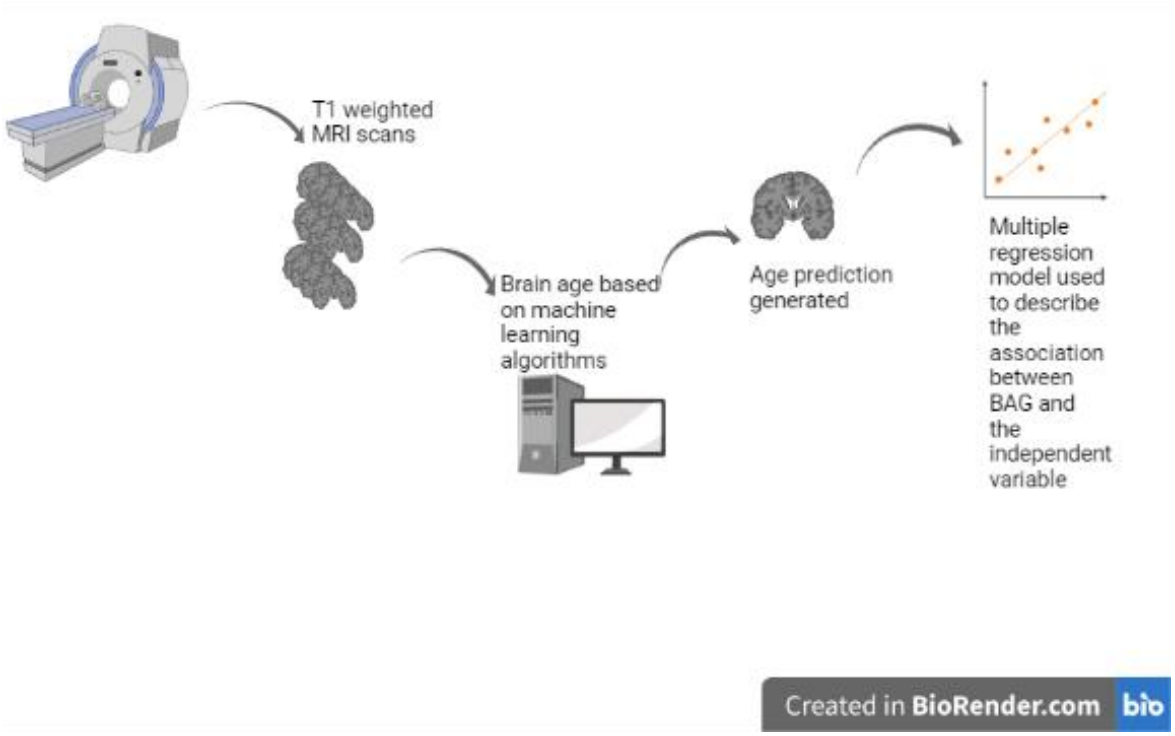

Figure 1. Schematic illustration of the brain age estimation process (Biorender). Neuroimaging data (T1-weighted structural MRI scans) from healthy individuals (training set) are placed into a machine learning algorithm to predict brain age. The projected brain age is than compared with the chronological age of test-set participants. The difference between brain age and chronological age (BAG) can then be utilized as a metric to statistically correlate with other participants variables

The vast majority of methods for determining brain age employ one or more types of brain MRI scans (Cole & Franke, 2017; Cole et al., 2018). T1-weighted scans, which have a high contrast between tissue types and hence accurately depict age-related morphological changes, are the most prevalent (Jonsson et al., 2019; Wang et al., 2019). Others utilize a variety of MRI contrasts, including FLAIR (a good marker for cerebrovascular pathology) and diffusion MRI (a marker for micro-structural changes) (Peng et al., 2021). Some studies employ so-called "features" generated from the MRI scans, such as gray matter volume or ventricular

volume (Wang et al., 2019), while others use the complete MRI image as a predictor (Bashyam et al., 2020).

There are advantages and disadvantages to both methods. Utilizing "features" (i.e., distinct quantitative measures such as gray matter volume, cortical thickness, etc.) to determine brain age requires extensive processing of MRI images but has the advantage of making it easier to identify individual factors that influence brain age (Smith et al., 2019).

Methods that utilize the complete MRI image have the benefit of requiring minimal preprocessing, but it is difficult to ascertain which features in the images influence the estimated brain age.

Most brain age estimation approaches are based on machine learning algorithms that involve training a model on a data set (essentially optimizing the model parameters in order to minimize the discrepancy between predicted and chronological age), validating the model, and then estimating brain age on independent data (Cole & Franke, 2017; Le et al., 2018). The best methods have an accuracy of ± 5 years (Le et al., 2018).

1.3.2 Effects of Gender-Specific health variables on Brain Aging

Exercise and education have been shown to have a protective effect on brain age, whereas unhealthy habits such as smoking, drinking, or obesity accelerate brain aging, as predicted by research on the effects of lifestyle and the environment on brain age. However, it is unclear whether environmental and lifestyle variables impact men's and women's brain ages differently, but some findings suggest this may be the case. Despite the fact that women have a longer life expectancy than males, smoking (Prescott et al., 1998) and drinking (Ceylan-Isik et al., 2010; Pfefferbaum et al., 2001) is more detrimental to women than men. Additionally, women have a higher number of age-related lesions in cerebral white matter (Vangberg et al., 2019) and a higher risk of Alzheimer's disease (Andersen et al., 1999).

In addition, it has been demonstrated that the rate of brain atrophy in women with dementia is up to 1.5% higher than in men (Ardekani et al., 2016).

Studies suggest that childbirth and estrogen levels in women are associated with brain age (Ryan et al., 2014), and some data indicate that estrogen may be responsible for the increased prevalence of Alzheimer's disease in women. (de Lange et al., 2020; Ryan et al., 2014).

Menopause is accompanied by endocrine changes that include a progressive, somewhat unpredictable, reduction in estrogen levels over several years (Dennerstein et al., 2007). It has been hypothesized that fluctuations in estrogen levels are responsible for the rise in memory problems during this time period. Experiment data suggest that estrogen has neuroprotective and neurotrophic effects (Li et al., 2014; Pike et al., 2009).

It has also been established that gender disparities exist in the degree to which physical activity protects against age-related alterations in the brain, with some data suggesting that women have more benefits than men do from engaging in physical activity (Barha et al., 2017; Barha & Liu-Ambrose, 2018)

2 Aims and objectives

The aim of this thesis is three-fold. First, we aim to examine the relationship between lifestyle factors and brain aging in a population sample of persons between the ages of 40 and 80. Second, we will assess whether significant sex differences exist. And lastly, identify lifestyle factors that are essential for healthy brain aging in both sexes.

This study will hopefully provide a deeper understanding of the relationship between lifestyle and brain health as we age.

3 Materials and methods

3.1 The Tromsø study

This study is based on data from the seventh survey of the Tromsø Study (Tromsø 7). It is a national project intended to collect data from inhabitants above the age of 40 in Tromsø municipality. Tromsø's study obtains a wide range of qualitative and quantitative survey questionnaires from participants over the course of one or more visits in addition to physical check-ups, blood chemistry, and imaging studies. The study was launched in 1974 in an effort to tackle the high mortality rate of cardiovascular diseases in Northern Norway, which was particularly prominent among middle-aged men. However, there has lately been a growing emphasis on additional chronic diseases and ailments. Inviting a wide variety of academic research groups to participate in research projects based on data from these past surveys (Njolstad et al., 2016).

3.2 Study participants

We analyzed data from participants in the seventh Troms study (N = 1876) who underwent brain MRI as part of the study (N = 1864). The data set consisted of people between 40 - 87 years, with an average age of 65, and about the same number of participants from both sexes (**Table 1**). Excluded from the study were those with brain disease (cysts, tumors, vascular malformations, and lesions caused by stroke or trauma), self-reported stroke, and abnormalities on the T1 imaging. There were n = 1618 participants in our final dataset (Figure 2).

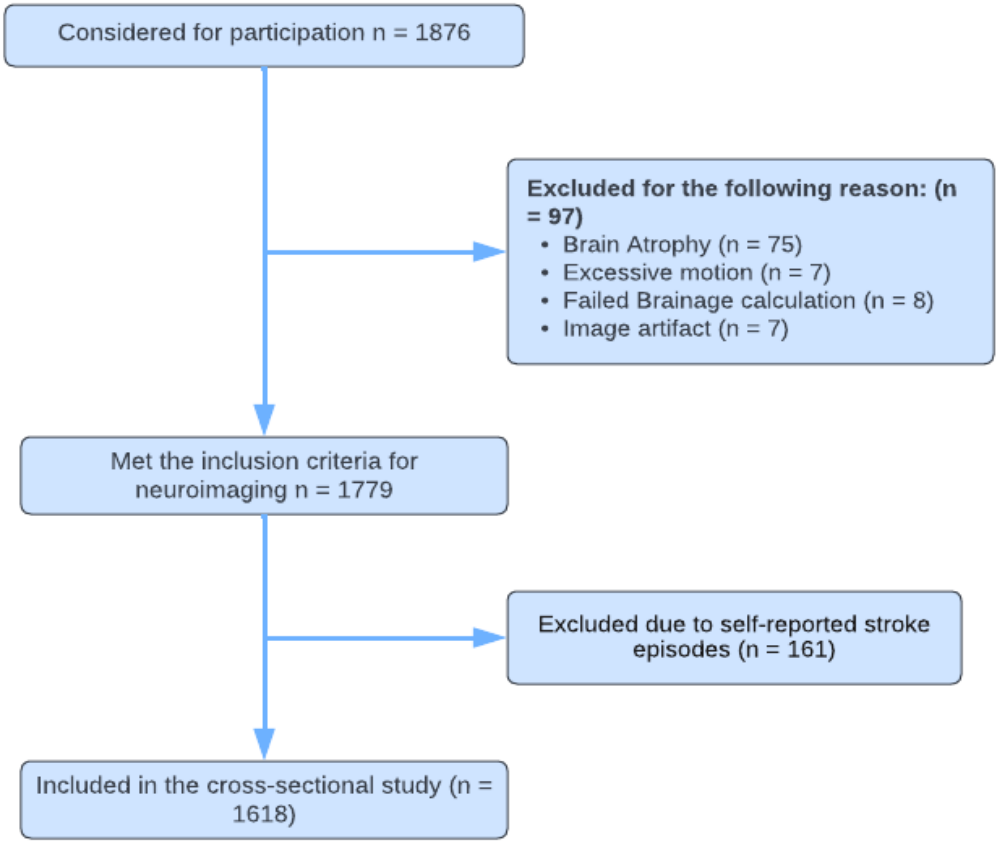

Figure 2. Flowchart of the selection of participants

3.3 MRI data

Participants were scanned at the University Hospital North Norway with the same 3T Siemens Skyra MR scanner (Siemens Healthcare, Erlangen, Germany). In the majority of examinations, a 64-channel head coil was used, but in 32 examinations, a slightly larger 20-channel head coil was required to suit the participants' heads. The MRI protocol consisted of T1-weighted, T2-weighted fluid-attenuated inversion recovery (FLAIR), TOF angiography, and susceptibility-weighted sequences. In the current project, only the T1-weighted images were used, which were acquired sagittally with 1 mm isotropic resolution using a 3D magnetization prepared rapid acquisition gradient-echo (MPRAGE) sequence (flip angle = 9°, TR/TE/ TI = 2300 /4.21 /996 ms) generalized auto-calibrating partially parallel acquisition (GRAPPA) acceleration factor 2, a field of view of 256 mm, 176 slices, 1 mm slice thickness, and 256 × 256 image matrix.

3.4 Brain age estimate

Brain age was estimated using a model developed by Johnson and colleagues (Jonsson et al., 2019). Briefly, the method used 3D convolutional neural networks trained on four features from T1-weighted images (T1 image, Jacobian map, gray and white matter densities, all in MNI space) from an Icelandic dataset of 1264 healthy subjects (18 - 75 years) to predict brain age. The model was further refined on 440 images from the IXI dataset (https://brain-development.org/ixi-dataset/) using transfer learning and tested on 12,395 images of British ancestry from the UK Biobank (Sudlow et al., 2015). The authors utilized the mean predicted age of the four features since it had similar accuracy as a linear regression fit and was easier to implement. The model yielded a mean absolute error (MAE) of 3.63 and $R^2 = 0.61$ on the UK biobank dataset (47 - 80 years) using the mean age prediction from the four features.

Image processing on the Tromsø 7 MRI data was done in the same manner as in the paper by Johnson and colleagues (Jonsson et al., 2019). The CAT12 toolbox v. 1092 (Gaser & Dahnke, 2022) was used with the default settings to compute the normalized T1 image, Jacobian map, gray matter, and white matter densities for each participant. Next, brain age was estimated from the four features using the publicly available code and model for the Johnson method (https://github.com/benniatli/BrainAgePredictionResNet), and the BAG was calculated as predicted age – the age at MRI. In our data, the brain age prediction had an MAE = 4.20 and $r^2 = 0.74$ relative to the age at the MRI examination. Finally, BAG was corrected for age to remove any residual correlation between BAG and age.

3.5 Variable selection

Variables were selected or derived from the raw data on blood chemistry, clinical measurements, and questionnaires to best reflect the primary factors of interest, i.e., basic health measurements, blood lipids, cardiovascular disease risk, lifestyle-related disease, physical activity, tobacco/alcohol use, and sleep quality. In addition, we intended to account for previously identified factors associated with BAG or brain health but not of primary interest; education and socioeconomic status (Hackman et al., 2010) and hypothyroidism (Begin et al., 2008).

Table 1. Summary of measurements available for variable selection.

Variable group*	Available measurements
Basic demographics and health	Age, sex, body mass index (BMI), resting pulse, systolic blood pressure (SBP), diastolic blood pressure (DBP), use of blood pressure medication
Cardiovascular disease	Age of heart first attack, age of first heart failure, arterial fibrillation, angina pectoris
Blood lipids	High-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), total cholesterol, triglycerides
Lifestyle-related disease	Diabetes, HBA1C, serum glucose.
Physical activity	Level of physical activity at work, level of physical activity outside work, hours sitting in weekdays, hours sitting in weekends, frequency of exercise, intensity of exercise and duration of exercise
Tobacco and alcohol	Frequency of drinking, number of alcohol units, smoking status and use of chewing tobacco
Sleep quality	Total score on Bergen insomnia scale, sleep duration on work days and on weekends, duration of insomnia, shift work
Confounders	Income, education hypothyroidism, age, loneliness

* Variable grouping reflects the current research questions, not potential casual associations, as for example smoking is often viewed as a risk factor for cardiovascular disease.

3.5.1 Basic demographics and clinical information

In this variable group, we included age, gender, BMI, and resting pulse rate (mean of reading two and three). Blood pressure is also important, but the use of blood pressure medication complicates the representation. We, therefore, computed a new dichotomous variable for hypertension, defined as true if at least one of the following conditions were true, SBP > 140, DBP > 90, or self-reported use of blood pressure lowering medication. Figure 3 illustrates the pair-wise associations between these variables (including SBP and DBP, which were not included in the statistical model).

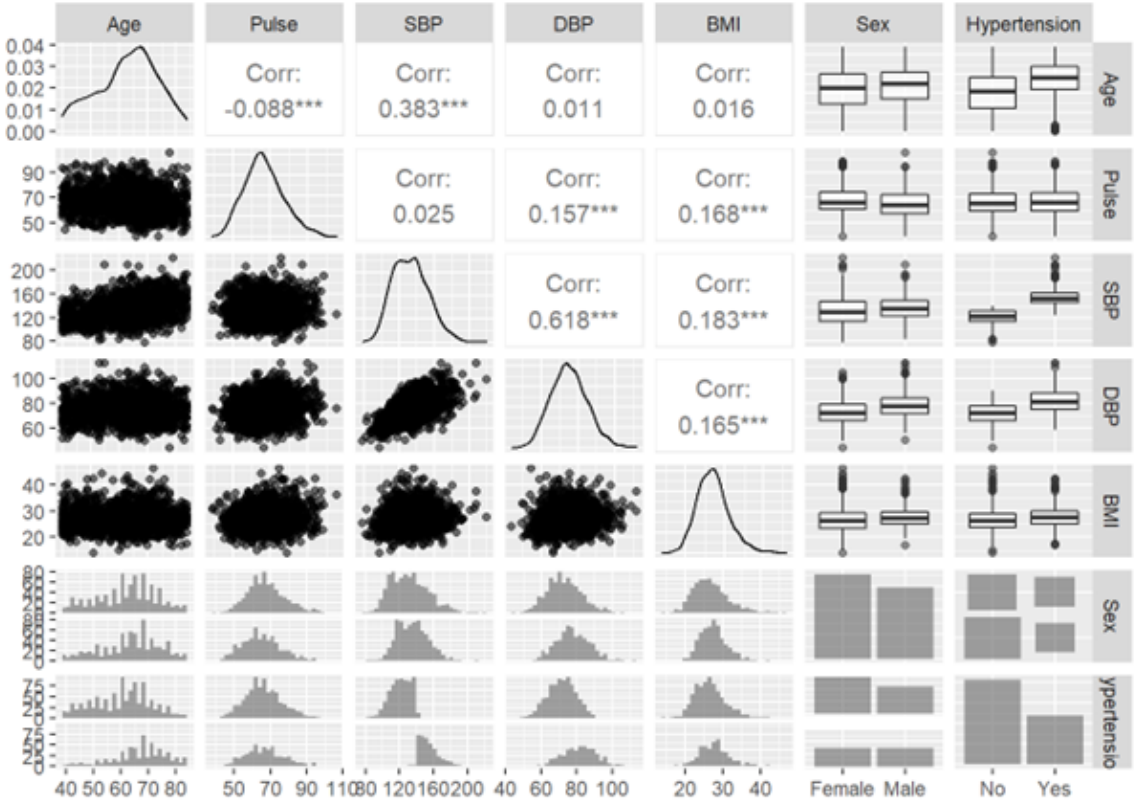

Figure 3. Overview of the basic demographic and clinical variables evaluated.

3.5.2 Cardiovascular disease

The dataset contained self-reported data on the age of the first heart attack, the age of the first heart failure, arterial fibrillation, and angina pectoris, with the latter two variables separated into "never," "previously," and “currently." We, therefore, computed a new "heart disease" variable that was true, if age was recorded for heart attack or heart failure, or whether arterial fibrillation or angina pectoris was "currently" or "previously."

3.5.3 Blood lipids

Blood lipids levels may partly reflect lifestyle choices such as one's diet. High blood cholesterol levels are commonly associated with an increased risk of cardiovascular disease. Blood lipids may be associated with brain health since HDL, LDL cholesterol and triglyceride levels in adults have been associated with gray matter volumes in adults (Moazzami et al., 2020; Ward et al., 2010). Nonetheless, a recent large population study failed to find an association between lipids and BAG (Beck et al., 2022). In our data, we had information on HDL, LDL, total cholesterol, and triglycerides. Figure 4 summarizes the association between these measures. It is apparent that total cholesterol is highly correlated with LDL and that there is a moderate correlation between HDL and triglycerides. We chose to include HDL, LDL, and triglycerides in the statistical model.

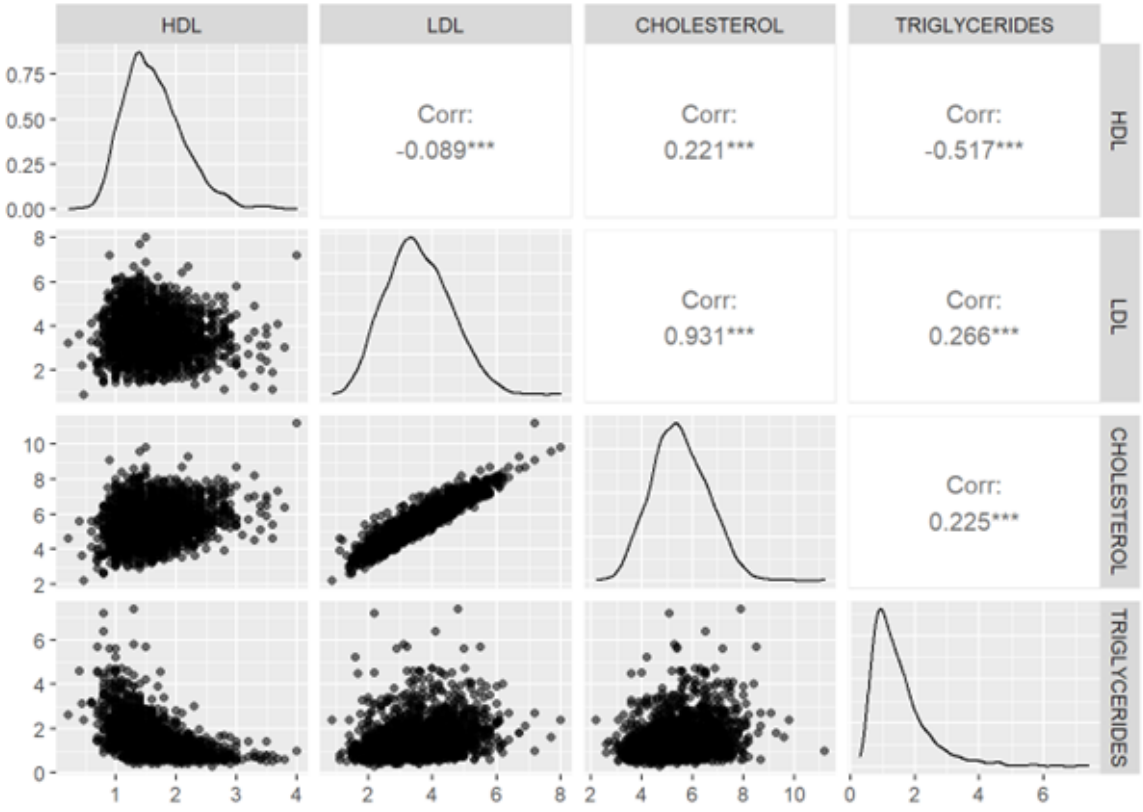

Figure 4. Overview of the blood lipid measurements that were available.

3.5.4 Lifestyle-related disease

We had data on two lifestyle-related diseases, obesity and diabetes type 2. Obesity is characterized by BMI (under general health), although this is not a very precise measure of obesity. Only 67 of the 1618 (4%) participants that were included in the statistical analysis were obese by standard definitions of obesity and BMI (BMI > 35). The other lifestyle-related disease we had information on was diabetes 2. Information on diabetes was self-reported, where participants could answer “no, “yes”, and “previously”. Unfortunately, the questionnaire did not differentiate between diabetes type 1 and diabetes type 2, but approximately 90% of all diabetes cases in Norway are due to diabetes type 2 (Stene et al., 2020), and therefore the diabetes variable will mostly reflect on diabetes 2. In addition, 6 of the 1,618 patients replied "previously" in regards to diabetes. To avoid statistical instability connected with factor levels with few observations, we recorded the “previously” responses to “yes”. One could also argue that previous diabetes could have an impact on brain age. Six participants (of 1618) omitted to answer the question about diabetes. Five of these had hemoglobin A1c levels (HBA1c) below 6.5%, and one had HBA1c = 6.5%. Since HBA1c ≥ 6.5% is indicative of diabetes (https://nhi.no/sykdommer/hormoner-og-naring/diabetes-generelt/hba1c/), we recorded these six cases according to this definition. Although HBA1c could have provided more fine-grained information on diabetes and pre-diabetes, these levels may be confounded by medication. Therefore, we chose to only include the “diabetes” variable in the statistical analysis.

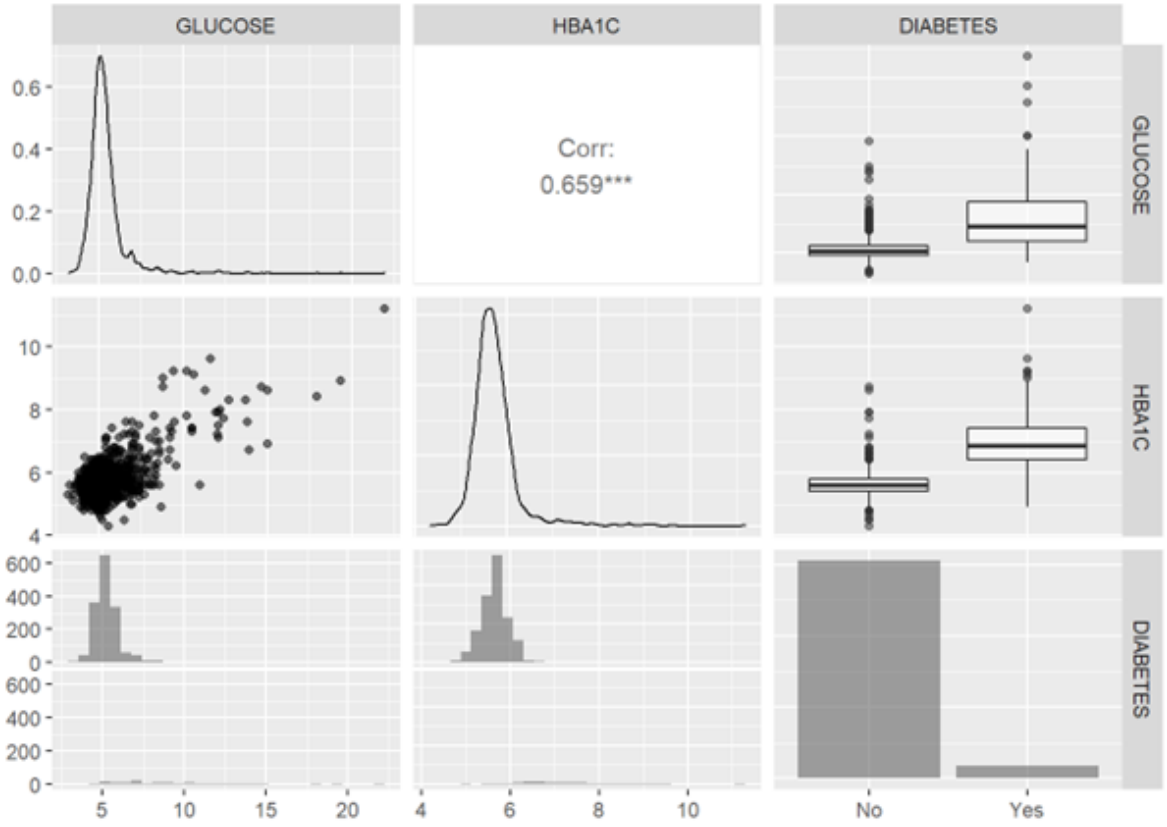

Figure 5. Overview of the available information related to diabetes.

3.5.5 Physical activity

There were seven variables related to physical activity:

1. Level of physical activity at work
2. Level of physical activity outside work
3. The number of hours spent during the weekdays
4. Hours sitting on weekends
5. Frequency of exercise
6. The intensity of exercise
7. Duration of exercise

However, the level of physical activity at work had 513 missing observations, perhaps due to the large number of participants that were retired. It has also been found that the level of physical activity outside work correlates well with two objective measures of physical fitness,

resting heart rate and maximal oxygen uptake (Emaus et al., 2010), suggesting that reported physical activity outside work could be a good indicator of physical activity. The questions on hours of sitting on weekdays and weekends have 14% and 15% missing data, respectively, which, even after imputation, may be problematic. Finally, there are three questions about exercise frequency, duration, and intensity, but 6 - 7% of the data on duration and intensity are missing, complicating the possibility of augmenting the exercise data. We, therefore, consider utilizing either the data on physical activity outside work or exercise duration (Table 2), but not both, since these were moderately correlated (Spearman's rho = 0.4). Since a large proportion of the participants were elderly, who do not necessarily exercise but may have varying amounts of physical activity by other means, we only used the variable physical activity outside work to quantify the physical activity in the statistical analyses.

Table 2. Responses on physical activity outside work and exercise frequency

Characteristic	N = 1,618*
Describe your exercise and physical exertion in leisure time over the last year. If your activity varies throughout the year, give an average.	
Reading, watching TV/screen or other sedentary activity	198 (13%)
Walking, cycling, or other forms of exercise at least 4 hours a week	940 (60%)
Participation in recreational sports, heavy gardening, snow shoveling etc at least 4 hours a week	387 (25%)
Participation in hard training or sports competitions, regularly several times a week	34 (2.2%)
Missing	59
How often do you exercise (i.e walking, skiing, swimming or training/sports)?	
Never	61 (3.8%)
Less than once a week	194 (12%)
Once a week	216 (13%)
2-3 times a week	673 (42%)
Approximately every day	457 (29%)
Missing	17

*n (%); Median (IQR)

3.5.6 Tobacco and alcohol

Excessive tobacco and alcohol use may affect brain health, directly or indirectly, e.g., through cardiovascular disease.

For alcohol, information was provided on drinking frequency (never, monthly or less, 2-4 times a month, 2-3 times a week, and 4 times a week), and the number of alcohol units generally consumed when drinking (1 - 2, 3 - 4, 5 - 6, 7 - 9, 10 or more). From this, we calculated the number of alcohol units pr. week using the averaged frequencies (e.g., 1.5 for range 1 - 2, 3.5 for range 3 - 4 etc.) and 4.35 for the average number of weeks in a month. We then classified the alcohol consumption into "none", "moderate" and "heavy", following the U.S. National Institute on Alcohol Abuse and Alcoholism's gender-adjusted norms (https://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/moderate-binge-drinking).

There were two sets of identical questions on smoking and the use of chewing tobacco (part 1 and 2 of the questionnaire), quantifying use into "currently", "previously" and "never". We combined the part 1 and 2 questions such that if there were missing data in part 1, answers in part 2 were used, giving complete data on smoking and only one missing observation for chewing tobacco.

The variables on smoking and tobacco use are summarized in Table 3, and the Spearman's rho correlation is plotted in Figure 6, showing a low correlation between the variables but, interestingly, a small age correlation.

Table 3. Summary of the variables for smoking, use of chewing tobacco and alcohol consumption used in the analyses

Characteristic	**N = 1,618**
Do you/did you smoke daily? (part 1 and 2 combined)	
Never	637 (39%)
Currently	208 (13%)
Previously	773 (48%)
Have you used or do you use snuff or chewing tobacco daily? (part 1 and 2 combined)	
Never	1,491 (92%)

Currently	68 (4.2%)
Previously	57 (3.5%)
Missing	2
Alcohol consumption according to NIAAA definitions	
Never	117 (7.3%)
Moderate	1,283 (80%)
Heavy	207 (13%)
Missing	11

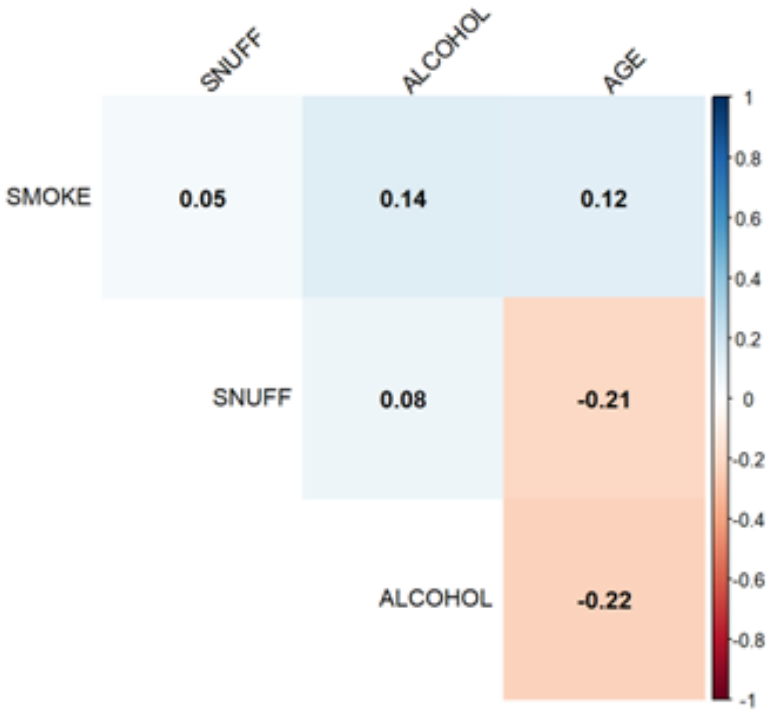

Figure 6. Spearman correlation between smoking, use of chewing tobacco ("snuff"), alcohol consumption, and age.

3.5.7 Sleep Quality

We evaluated 14 variables relevant to sleep quality. Six of these were part of the Bergen Insomnia Scale (Sivertsen et al., 2021), from which a compound score was calculated following the author's recommendations (https://helse-bergen.no/seksjon/sovno/Documents/BERGENINSOMNIASCALE

1.pdf). In addition, there was information on bedtime and awakening time on weekends and workdays, allowing us to compute sleep length on weekends and workdays. These values were moderately correlated (r = 0.6), thus we computed the average sleep length for the whole week (weighted average of the two sleep duration measures), which was used in the statistical analyses. The remaining sleep-related measures appeared to be uncorrelated to each other (Figure 7) and were therefore used in the statistical models (variables are summarized in Table 4). Note that insomnia duration was defined in the questionnaires as "none", "under a week", "1-3 weeks", "1 month", "2 months", "3 months" "4-6 months", "7 - 12 months", "1 - 5 years", "6 - 10 years", "more than 10 years", giving few observations in some of the categories, which may lead to inaccurate parameter estimates. Insomnia duration was therefore recorded into larger categories as shown in Table 4 .

Table 4. Available data on sleep characteristics. (Continuous variables reported as median (interquartile range))

Characteristic	**N = 1,618**
Average sleep duration (hours)	7.64 (7.00, 8.14)
Missing	72
Total score on the Bergen Insomnia Scale	5 (1, 14)
Missing	121
Number of years with sleep problems	
None	854 (56%)
Less than a year	220 (14%)
1- 5 years	199 (13%)
More than 5 years	264 (17%)
Missing	81
Do you usually work shifts or at night?	104 (6.8%)
Missing	80

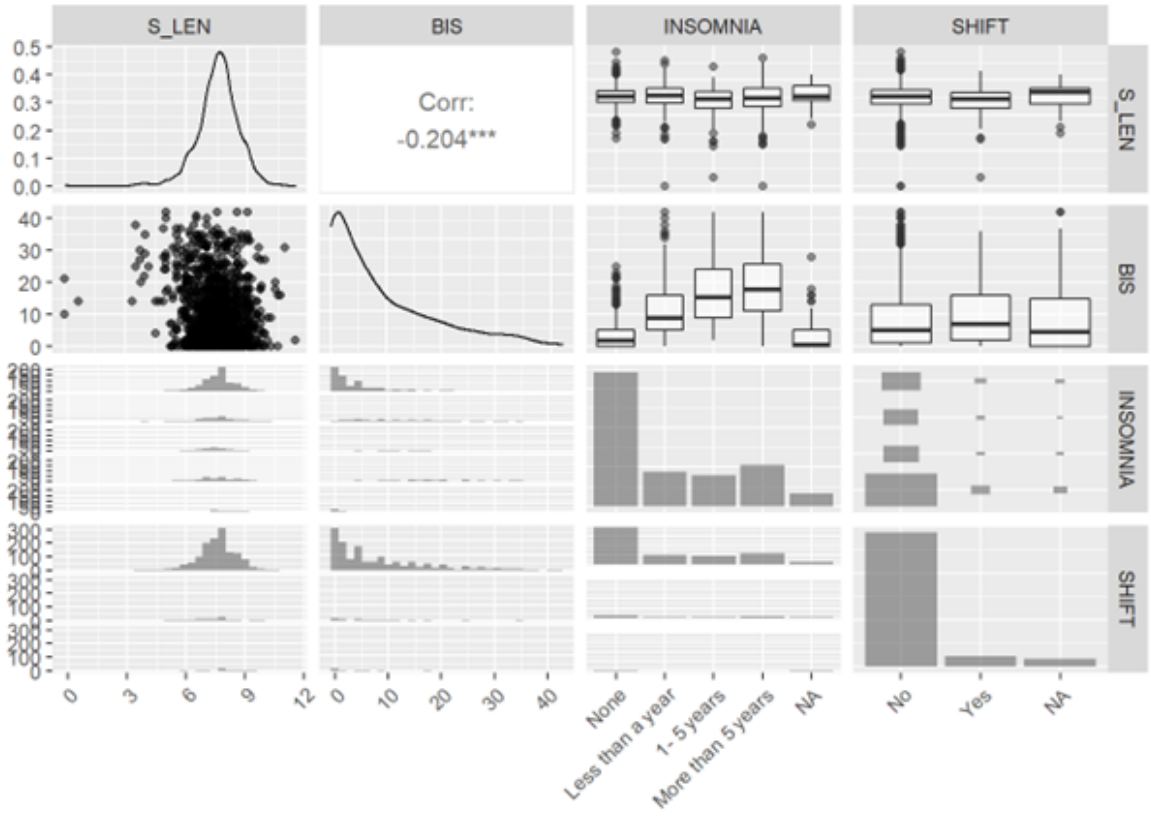

Figure 7. Associations between sleep-related parameters.

(S_LEN = average sleep length, BIS = total score on the Bergen Insomnia Scale, INSOMNIA = years of insomnia, SHIFT = work shift or nighttime).

3.5.8 Confounders

Several potential confounding parameters were identified, as discussed in the introduction. Age can also be regarded as a confounder and should be added to the model as it can adjust for subtle age effects in the variables of interest. Other confounders were socio-economical level (education and taxable household income), loneliness (whether one lives alone or together with others), number of childbirths, and hypothyroidism. For loneliness, we used two questions (1) whether one lives with a spouse (yes/no) and whether one lives with anybody else over the age of 18 (yes/no). Those answering “no” on both questions were classified as living alone. Information on hypothyroidism was based on a question on whether one used drugs for hypothyroidism (“currently”, “previously” and “never”). Since there were only 21 that answered “previously”, the variable was recoded to “currently” or “never”. Education and income were strongly correlated (Spearman's rho = 0.48), and also income and “live alone” (Spearman's rho = 0.48). Due to the association between education and income, a simple

"socio-economical index" (SES) was calculated as the numerical sum of education (4 levels) and income (income was here recoded from 8 levels to 4 levels by merging adjacent levels to give equal weight to each factor). There was still a high correlation between SES and "live alone" (Spearman's rho = 0.31). Due to potential collinearity issues, this may cause, and since "live alone" likely is an imperfect measure of loneliness, this parameter was dropped from the statistical analyses. Therefore, only age, SES and hypothyroidism were used as confounders in the regression models.

3.6 Statistical analysis

Statistical analysis was undertaken via a statistical software called "R" (version. 4.2.1). There were missing observations in the variables selected for statistical analysis (Table 5), and if complete observations should be used, there would only be 652 observations. Missing data were therefore imputed using the Multivariate Imputation by Chained Equations (MICE) method (van Buuren & Groothuis-Oudshoorn, 2011) using the default setting, including 5 imputed datasets and 5 interactions.

The associations between BAG and lifestyle factors were examined with multiple regression on the imputed data using pooled results across the five imputations. Multicollinearity was checked in the first permuted dataset using the generalized variance inflation factor (GVIF) (Fox & Monette, 1992), which also applies to categorical and nominal data. One can use the same rules of thumb as for the classical variance inflation factor with $(GVIF^{-2*DF})^2$ and values greater than 10 deemed problematic (DF = degrees of freedom).

We used two multiple regression models to address the research questions. A simple model examining the associations between lifestyle-related measures and BAG correcting for age, sex, and socio-economic status. The second model added interaction terms with sex to the lifestyle-related measures to examine whether associations between BAG and the lifestyle-related measures were different between females and males. Multiple comparisons were accounted for by Bonferroni correction dividing by the number of models, such that $p < 0.05/2 = 0.025$ was considered significant.

3.7 Ethical Approval

The approval was obtained by the regional committee for Medical and Health Research Ethics in Northern Norway (REK-Nord #241318) and conducted in compliance with the relevant policy and practices at UiT, the Arctic University of Norway. Before taking part in the study, all subjects submitted written informed consent.

4 Results

4.1 Subject characteristics

Table 5 outlines the characteristics and variables of the study population. Briefly, there were 743 males (45, 9%) and 875 females (54, 1%), the latter being slightly overrepresented in our final dataset. The overall sample had a mean age of 64. However, there was a substantial age gap between the sexes, as the average age of women was around 63 and the average age of men was 65 years.

The total mean LDL values were 3.50 (2.90 to 4.22). Women had somewhat higher LDL mean levels of 3.60 (2.90 to 4.30), whereas men had levels of 3.40. (2.80, 4.10). The mean HDL for women was 1.74 (1.40-2.10) and 1.40 (1.15-1.70) for men, also slightly higher for women in this case. The mean serum triglyceride level was 1.30 (0.90 to 1.80), slightly higher in males 1.40 (1.00, 2.00) compared to females 1.20 (0.90, 1.60). Nonetheless, figure 4 illustrates a slight negative correlation between HDL and triglycerides, which may suggest an inverse association.

13 % of the participants had reported having had or currently have heart disease of which 17 % were males and 9 % were females. Perhaps there is a higher usage of cholesterol medication among men in this study, given that a significant proportion of men reported heart disease, based on the data. Furthermore 41 % of men and 33 % of women from defined to be hypertensive or used antihypertension drugs.

We employed physical activity outside of work to quantify exercise. There was a total of 59 missing observations, majority being women around, 43. The questionnaire contained four classifications, ranging from minimum to maximal. The lowest category (reading includes watching TV/screen or other sedentary activity) comprised approximately 13% of the participants, with the sexes nearly evenly represented. The second category (Walking, cycling, or other kinds of exercise for at least 4 hours per week) was dominated by a majority of respondents (60 %), with 69% being females and 50% of males. Majority of men were in the third category (Participation in recreational sports, heavy gardening, snow shoveling etc. at least 4 hours a week), being 32% as can be seen in table 5. The fourth category had a total of x, where men dominated.

In both the previous and current groups of smokers, the distribution of male and female smokers was comparable. 13% were currently smoking while 48% had previously smoked.

The majority of the participants (80%) had a moderate consumption of alcohol according to NIAAA definition of whom 89% were men and 72 % were women. In addition, 13 % were categorized as heavy drinkers. The major portion of heavy drinkers were female (18%), meaning a 3:1 ratio compared to their male counterparts, who accounted for approximately 6.6 %. There were 11 missing data, eight of which represented females.

Most of the respondents (92%) had never used snuff or chewing snuff, with an overwhelming part being women (97%). Amongst men about 6,6% were currently using snuff.

In regard to "number of years with sleep problems", 56% of the participants reported no sleeping problems. This comprised 64% of males and 49 % of females, indicating that latter group reported more sleeping problems. The remaining 44% had sleeping problems of varying degrees equally distributed between males and females, with the exception of the last category where 22% of women reported having had sleeping problems for more than 5 years compared to men who reported 11%. There were a total of 80 missing data, 44 of which were females. In addition, the overall score on the Bergen Insomnia Scale was higher for females (7) than for males (4), with a mean of 5. Nevertheless, there were a total of 121 missing data, of whom females (86) contributed to the majority of missing data. Summarized females reported a higher level of sleeping problems compared to males but there were also more missing data on women compared to men.

Furthermore, data shows Shift work was equally distributed being 6, 9% for females and 6, 6 % males. A total of 80 missing observations, majority being females (44).

Socio-economical index, a measure for education and taxable income was categorized into seven groups, ranging from 1 to 7, with the lowest category representing low income/education and subsequent categories increasing in both respects. There were a greater number of females in first two groups (1, 2) 6, 6% and 14% compared to 4, 1% and 6, 2 % males, respectively. In contrast, the last category had a higher proportion of males (21%) than females (14%). The distribution of males and females in the remaining four groups (3-6) was roughly equivalent. There was total missing data of 96 whom females contributed to 61.

Finally, with reference to the drug usage question for hypothyroidism: 8, 7% of the study population were currently using medication, with a ratio of 3:1 for females (12%) to males (4, 2%). Women had a greater likelihood (ratio 3:1) of hypothyroidism medication usage irrespective of previous or current.

In general, it appears that females had more missing data for several variables such as physical activity, tobacco/alcohol use, and total score on the Bergen insomnia scale, number of years with sleep disorders, shiftwork, and socioeconomic status.

Table 5. Characteristics of participants

Characteristic	Overall, N = 1,618[1]	Female, N = 875[1]	Male, N = 743[1]
Age per 31.12.2015	64 (56, 71)	63 (54, 70)	65 (57, 71)
Body mass index (kg/m2)	26.8 (24.2, 29.4)	26.3 (23.5, 29.4)	27.3 (25.0, 29.5)
Missing	1	1	0
Pulse (mean of reading 2 and 3)	66 (60, 73)	66 (61, 74)	64 (58, 72)
Missing	5	3	2
Has, or have had, heart attack, heart failure, arterial fibrillation or angina	211 (13%)	83 (9.5%)	128 (17%)
Missing	1	1	0
Clinically defined hypertension, sbp > 140 and/or dbp > 90 or use of hypertension lowering medicine.	591 (37%)	290 (33%)	301 (41%)
Serum High density lipoprotein cholesterol (mmol/l)	1.60 (1.30, 1.90)	1.74 (1.40, 2.10)	1.40 (1.15, 1.70)
Missing	7	4	3
Serum low density lipoprotein cholesterol (mmol/l)	3.50 (2.90, 4.22)	3.60 (2.90, 4.30)	3.40 (2.80, 4.10)

Missing	7	4	3
Serum Triglycerides (mmol/l)	1.30 (0.90, 1.80)	1.20 (0.90, 1.60)	1.40 (1.00, 2.00)
Missing	7	4	3
Has, or have had diabetes.	91 (5.6%)	49 (5.6%)	42 (5.7%)
Describe your exercise and physical exertion in leisure time over the last year. If your activity varies throughout the year, give an average.			
Reading, watching TV/screen or other sedentary activity	198 (13%)	89 (11%)	109 (15%)
Walking, cycling, or other forms of exercise at least 4 hours a week	940 (60%)	575 (69%)	365 (50%)
Participation in recreational sports, heavy gardening, snow shoveling etc at least 4 hours a week	387 (25%)	155 (19%)	232 (32%)
Participation in hard training or sports competitions, regularly several times a week	34 (2.2%)	13 (1.6%)	21 (2.9%)
Missing	59	43	16
Do you/did you smoke daily? (part 1 and 2 combined)			
Never	637 (39%)	343 (39%)	294 (40%)
Currently	208 (13%)	119 (14%)	89 (12%)
Previously	773 (48%)	413 (47%)	360 (48%)
Have you used or do you use snuff or chewing tobacco daily? (part 1 and 2 combined)			
Never	1,491 (92%)	850 (97%)	641 (86%)
Currently	68 (4.2%)	15 (1.7%)	53 (7.1%)

Previously	57 (3.5%)	8 (0.9%)	49 (6.6%)
Missing	2	2	0
Alcohol consumption according to NIAAA definitions			
Never	117 (7.3%)	86 (9.9%)	31 (4.2%)
Moderate	1,283 (80%)	623 (72%)	660 (89%)
Heavy	207 (13%)	158 (18%)	49 (6.6%)
Missing	11	8	3
Total score on the Bergen Insomnia Scale	5 (1, 14)	7 (2, 16)	4 (0, 10)
Missing	121	86	35
Number of years with sleep problems			
None	854 (56%)	404 (49%)	450 (64%)
Less than a year	220 (14%)	121 (15%)	99 (14%)
1- 5 years	199 (13%)	121 (15%)	78 (11%)
More than 5 years	264 (17%)	184 (22%)	80 (11%)
Missing	81	45	36
Do you usually work shifts or at night?	104 (6.8%)	57 (6.9%)	47 (6.6%)
Missing	80	44	36
Socioeconomic index			
1	80 (5.3%)	51 (6.3%)	29 (4.1%)
2	156 (10%)	112 (14%)	44 (6.2%)
3	231 (15%)	128 (16%)	103 (15%)
4	271 (18%)	143 (18%)	128 (18%)
5	283 (19%)	137 (17%)	146 (21%)
6	246 (16%)	127 (16%)	119 (17%)
7	255 (17%)	116 (14%)	139 (20%)

Missing	96	61	35
Do you use, or have you used drugs for hypothyroidism (Levaxin or thyroxine)?			
Never	1,441 (90%)	739 (85%)	702 (95%)
Currently	139 (8.7%)	108 (12%)	31 (4.2%)
Previously	21 (1.3%)	18 (2.1%)	3 (0.4%)
Missing	17	10	7

[1] Median (IQR); n (%)

4.2 Association between BAG and risk factors independent of sex

As shown in Table 6, multiple linear regression models were used to investigate the association between BAG and risk factors, adjusting for baseline demographics, SES and hypothyroidism. Average GVIF for the independent variables were 1.3 (range 1.0 - 1.9), suggesting low multicollinearity in the model. We found that being male was significantly associated with higher BAG (β = 2.2; 95 % CI 1.7, 2.8; p <0.001). Furthermore, resting heart rate (pulse) was positively associated with BAG (β = 0.04; 95 % CI 0.01, 0.06; p <0.003). Put in context, an increase in resting heart rate of 10 beats would increase BAG by 0.4 years. Therefore, low resting heart rate would be considered beneficial. Serum HDL was significantly associated with higher BAG (β = 1.2; 95 % CI 0.60, 1.8; p <0.001), however no association was found between serum LDL and BAG. Diabetes was significantly associated with higher BAG (β = 2.2; 95 % CI1.1, 3.2; p <0.001)

Forty eight percent of the participants had previously smoked and 13% currently smoked. Current smokers (β = 1.4; 95 % CI 0.57, 2.2; p <0.001) exhibited a higher BAG than former smokers (β = 1.3; 95 % CI 0.76, 1.8; p <0.001), but both groups were statistically significant in terms of increased BAG. Usage of snuff/chewing tobacco was not significantly associated with lower BAG (β = -1.1; 95 % CI -2.3, 0.06; p <0.063)

Table 6. Results from regression of BAG for males and females. Results are reported as un-standardized beta, 95% confidence interval (CI) and p-value.

Characteristic	Beta	95% CI[1]	p-value
SEX			
Female	—	—	
Male	2.2	1.7, 2.8	**<0.001**
Age accurate	-0.02	-0.05, 0.01	0.2
BMI	-0.05	-0.11, 0.01	0.11
PULSE	0.04	0.01, 0.06	**0.003**
Has, or have had, heart attack, heart failure, arterial fibrillation or angina			
No	—	—	
Yes	-0.01	-0.74, 0.71	>0.9
Clinically defined hypertension, SBP > 140 and/or DBP> 90 or use of hypertension lowering medicine.			
No	—	—	
Yes	0.42	-0.08, 0.93	0.10
S_HDL	1.2	0.60, 1.8	**<0.001**
S_LDL	-0.17	-0.43, 0.08	0.2
S_TRIGLYCERIDES	0.31	-0.04, 0.66	0.084
Has, or have had diabetes.			
No	—	—	
Yes	2.4	1.1, 3.2	**<0.001**
Describe your exercise and physical exertion in leisure time over the last year. If your activity varies throughout the year, give an average.			
Reading, watching TV/screen or other sedentary activity	—	—	
Walking, cycling, or other forms of exercise at least 4 hours a week	0.25	-0.48, 1.0	0.5
Participation in recreational sports, heavy gardening, snow shoveling etc. at least 4 hours a week	-0.43	-1.3, 0.40	0.3

Participation in hard training or sports competitions, regularly several times a week	-0.55	-2.3, 1.2	0.5
Do you/did you smoke daily? (part 1 and 2 combined)			
Never	—	—	
Currently	1.4	0.57, 2.2	**<0.001**
Previously	1.3	0.76, 1.8	**<0.001**
Have you used or do you use snuff or chewing tobacco daily? (part 1 and 2 combined)			
Never	—	—	
Currently	-1.1	-2.3, 0.06	0.063
Previously	-0.61	-1.9, 0.71	0.4
Alcohol consumption according to NIAAA definitions			
Never	—	—	
Moderate	0.20	-0.73, 1.1	0.7
Heavy	0.88	-0.23, 2.0	0.12
Total score on the Bergen Insomnia Scale	0.02	-0.03, 0.06	0.4
Number of years with sleep problems			
None	—	—	
Less than a year	0.07	-0.66, 0.81	0.8
1- 5 years	-0.75	-1.6, 0.14	0.10
More than 5 years	0.01	-0.86, 0.88	>0.9
Do you usually work shifts or at night?			
No	—	—	
Yes	-0.18	-1.1, 0.74	0.7
Socioeconomic index	0.05	-0.15, 0.25	0.6
Do you use, or have you used drugs for hypothyroidism (Levaxin or thyroxine)?			
Never	—	—	

Currently	0.06	-0.77, 0.90	0.9
Previously	-0.63	-2.6, 1.4	0.5

[1] CI = Confidence Interval

Mean GVIF = 1.280807 (1.0420177 - 1.8812312)

4.3 Different associations between males and females

Table 7 outlines differences associated between males and females. In this model, the same variables were significant as in the previous model (Table 5), with the exception of PULSE, which was significant in the non-interaction model (p = 0.003) but not in this model (p = 0.045). In contrast to what we expected we found none of the interaction terms to be significant. Average GVIF for the independent variables were 1.3 (range 1.0 - 1.9), suggesting low multicollinearity in the model. Other factors were largely unaffected by the addition of interaction terms (i.e., beta estimates and p-values were similar), except for PULSE which was significant in the non-interaction model (b = 0.04, p = 0.003) but not in this model (b = 0.03, p = 0.045).

Table 7. Interaction terms between risk factors and sexes

Characteristic	Beta	95% CI[1]	p-value
SEX			
Female	—	—	
Male	1.5	-1.2, 4.1	0.3
AGE	-0.02	-0.05, 0.01	0.2
BMI	-0.05	-0.13, 0.02	0.15
PULSE	0.03	0.00, 0.06	0.045
Has, or have had, heart attack, heart failure, arterial fibrillation or agina			
No	—	—	
Yes	0.15	-0.89, 1.2	0.8
Clinically defined hypertension, sbp > 140 and/or dbp > 90 or use of hypertension lowering medicine.			
No	—	—	
Yes	0.23	-0.45, 0.91	0.5
S_HDL	1.5	0.72, 2.2	<0.001

S_LDL	-0.19	-0.52, 0.14	0.3
S_TRIGLYCERIDES	0.39	-0.15, 0.93	0.2
Has, or have had diabetes.			
No	—	—	
Yes	2.4	1.1, 3.8	<0.001
Describe your exercise and physical exertion in leisure time over the last year. If your activity varies throughout the year, give an average.			
Reading, watching TV/screen or other sedentary activity	—	—	
Walking, cycling, or other forms of exercise at least 4 hours a week	-0.02	-1.0, 1.0	>0.9
Participation in recreational sports, heavy gardening, snow shoveling etc at least 4 hours a week	-1.2	-2.4, 0.01	0.052
Participation in hard training or sports competitions, regularly several times a week	1.1	-1.5, 3.8	0.4
Do you/did you smoke daily? (part 1 and 2 combined)			
Never	—	—	
Currently	1.6	0.63, 2.6	0.001
Previously	1.4	0.72, 2.1	<0.001
Have you used or do you use snuff or chewing tobacco daily? (part 1 and 2 combined)			
Never	—	—	
Currently	-1.2	-3.5, 1.1	0.3
Previously	2.1	-1.1, 5.3	0.2
Alcohol consumption according to NIAAA definitions			
Never	—	—	
Moderate	0.04	-1.0, 1.1	>0.9
Heavy	0.55	-0.70, 1.8	0.4
Total score on the Bergen Insomnia Scale	0.03	-0.03, 0.09	0.3
Number of years with sleep problems			
None	—	—	
Less than a year	0.15	-0.83, 1.1	0.8
1- 5 years	-0.57	-1.7, 0.58	0.3
More than 5 years	-0.05	-1.2, 1.1	>0.9
Do you usually work shifts or at night?			
No	—	—	
Yes	-0.24	-1.2, 0.70	0.6

Socioeconomic index	0.05	-0.15, 0.25	0.6
Do you use, or have you used drugs for hypothyroidism (Levaxin or thyroxine)?			
Never	—	—	
Currently	0.10	-0.74, 0.94	0.8
Previously	-0.71	-2.7, 1.3	0.5
SEX * BMI			
Male * BMI	0.01	-0.12, 0.15	0.8
SEX * PULSE			
Male * PULSE	0.01	-0.03, 0.06	0.5
SEX * Has, or have had, heart attack, heart failure, arterial fibrillation or agina			
Male * Yes	-0.19	-1.7, 1.3	0.8
SEX * Clinically defined hypertension, sbp > 140 and/or dbp > 90 or use of hypertension lowering medicine.			
Male * Yes	0.47	-0.52, 1.5	0.4
SEX * S_HDL			
Male * S_HDL	-0.82	-2.1, 0.47	0.2
SEX * S_LDL			
Male * S_LDL	0.04	-0.48, 0.56	0.9
SEX * S_TRIGLYCERIDES			
Male * S_TRIGLYCERIDES	-0.23	-0.95, 0.50	0.5
SEX * Has, or have had diabetes.			
Male * Yes	-0.69	-2.8, 1.4	0.5
SEX * Describe your exercise and physical exertion in leisure time over the last year. If your activity varies throughout the year, give an average.			
Male * Walking, cycling, or other forms of exercise at least 4 hours a week	0.59	-0.87, 2.0	0.4
Male * Participation in recreational sports, heavy gardening, snow shoveling etc at least 4 hours a week	1.4	-0.32, 3.0	0.11
Male * Participation in hard training or sports competitions, regularly several times a week	-2.6	-6.1, 1.0	0.2
SEX * Do you/did you smoke daily? (part 1 and 2 combined)			
Male * Currently	-0.88	-2.5, 0.73	0.3
Male * Previously	-0.32	-1.4, 0.71	0.5

SEX * Have you used or do you use snuff or chewing tobacco daily? (part 1 and 2 combined)			
Male * Currently	-0.07	-2.8, 2.6	>0.9
Male * Previously	-3.2	-6.7, 0.34	0.077
SEX * Alcohol consumption according to NIAAA definitions			
Male * Moderate	0.63	-1.6, 2.9	0.6
Male * Heavy	1.0	-1.7, 3.7	0.5
SEX * Total score on the Bergen Insomnia Scale			
Male * Total score on the Bergen Insomnia Scale	-0.04	-0.12, 0.04	0.3
SEX * Number of years with sleep problems			
Male * Less than a year	-0.07	-1.6, 1.4	>0.9
Male * 1- 5 years	-0.29	-2.1, 1.5	0.7
Male * More than 5 years	0.41	-1.3, 2.2	0.6

[1] CI = Confidence Interval

Mean GVIF = 1.280807 (1.0420177 - 1.8812312)

5 Discussion

5.1 Associations between different variables and brain age

The purpose of the study was to determine the variables associated with brain aging. We anticipated that sex differences and other lifestyle factors previously associated with brain aging may be important. In this study the brain age was estimated using machine learning algorithm by Johnson based on four features (T1 image, Jacobian map, and the gray and white matter densities). BAG was calculated by subtracting the predicted age from the age at MRI. In addition, we examined certain variables individually and by combining different variables into one value. We investigated a total of 18 variables. From these we found five variables; sex, pulse, smoking, serum HDL levels and diabetes to be significantly associated with BAG. Smoking emerged as an important predictor of higher BAG in both men and women. Notably, current smoking was the most important contributor to higher brain age, while previous was somewhat less but still significant in terms of higher BAG. Furthermore, diabetes indicated a more "older" appearing brain pattern irrespective of sex-differences. There were no differences in the associations between males and females. However, being male was a significant contributor to higher brain age.

5.1.1 Association between sex and brain age

This study showed significant association between being male and higher brain age. These findings are to some extent aligned with prior studies demonstrating sex-differences in brain age (Bittner et al., 2021; Boyle et al., 2021; Goyal et al., 2019; Jahanshad & Thompson, 2017). Even though our results imply an association between being male and higher BAG, it is important to note that there was a substantial age gap between the sexes, as the average age of women was around 63 and the average age of men was 65 years. Females were also slightly overrepresented in the final dataset with about 10%.

Furthermore, males showed higher incidence of heart disease and hypertension in this sample population compared to females. Although cardiovascular disease and hypertension was not shown to be significantly associated with BAG in this study, it still is worth noting that males were somewhat overrepresented in terms of cardiovascular risk compared to females in this study. Previous studies have also demonstrated that cardiometabolic risk factors are linked to brain aging (Beck et al., 2022).

As stated in the introduction effects of childbirth and estrogen have been recognized in several studies to be beneficial for brain health. (Brann et al., 2007; Craig et al., 2008; Li et al., 2014; Ryan et al., 2014). Studies have also linked a prolonged reproductive period to improved cognition, especially verbal fluency in women (Ryan et al., 2009). It can be argued that females in this study show the protective effects of estrogen even long after menopause. While recent studies suggest that treatment with exogenous estrogen may help prevent age-related neurodegeneration and delay the onset of dementia, this has not been shown consistently. Moreover, there was no information on use exogenous estrogen in this study group.

Studies also suggest that estrogen can be synthesized locally in the brain, whereas circulating estrogen and its precursors (substrates for estrogen synthesis) can cross the blood–brain barrier and provide the required substrates for estrogen synthesis in the central nervous system (CNS)(Hojo et al., 2004; Kretz et al., 2004; von Schassen et al., 2006). The brain has also been shown to synthesize estrogen from cholesterol (Do Rego et al., 2009). In fact, it has been found that all enzymes necessary for the synthesis of estrogen, as well as essential intermediary metabolites, are present in different parts of the human brain. (Li et al., 2014; MacLusky et al., 1986). In particular, aromatase, a necessary enzyme for the final phase of synthesis, is extensively expressed in the brains of humans, rats, and primates in a region-specific manner. (Callard et al., 1978; Selmanoff et al., 1977). Age-matched males nevertheless undergo a rather slow drop in testosterone synthesis over the course of later life, and this testosterone can still be converted to estrogen to exert its neuroprotective effects (Li et al., 2014).

Sex-based disparities in brain age may exist. However, studies do not seem to show the same disparity in terms of neurodegenerative illnesses such as Alzheimer's disease (AD), on the contrary studies suggest women are more predisposed than men at certain age (Ullah et al., 2019) (Beinhoff et al., 2008). Compared to age-matched controls, female and male patients with AD had lower circulation levels of 17-estradiol and testosterone, respectively (Callahan et al., 2001; Barron and Pike, 2012), indicating a potential role of sex hormones in the epidemiology and pathophysiology of AD. A meta-analysis of population-based studies (beginning at age 65 and going onwards) found significant gender disparities in the incidence of AD after the age of 85 years, with women accounting for 81.7% and males accounting for

24%. (At 90 years). The cumulative risk of developing AD at the age of 95 for women who were 65 years old was 0.22, whereas the risk for men was 0.09 (Andersen et al., 1999).

Perhaps females have an advantage due to the surplus of circulating estrogen crossing blood-brain barrier? Thus upon cessation of circulating estrogen female brain might experience accelerated degeneration compared to age-matched males as several studies also suggest (Li et al., 2014).

These results partially supported our hypothesis, but it may possibly be explained by other factors than sex differences. More research is required in this area. Studies focusing solely on hormones or perhaps the brain vasculature in male versus female brains could provide valuable insight. Our study was multivariate, preventing us from establishing causality.

5.1.2 Associations between brain age and smoking, alcohol and snuff

We investigated the association of BAG with smoking, alcohol consumption and snuff/chewing tobacco. Our findings revealed that daily smoking was significantly associated with higher BAG. In addition, current smokers had a higher BAG than former smokers. Previous studies have also demonstrated a much higher rate of atrophy in some brain regions of smokers (Durazzo et al., 2012; Duriez et al., 2014).

In both the previous and current groups of smokers, the distribution of male and female smokers was comparable. There was also no significant difference in interaction terms between sexes. Current smokers (β = 1.4; 95 % CI 0.57, 2.2; p <0.001) exhibited an older brain of 1.4 years than non-smokers. Former smokers (β = 1.3; 95 % CI 0.76, 1.8; p <0.001) showed a brain that was 1.3 years older than non-smoker. Both groups were statistically significant in terms of aging brain. This shows that the negative effect of daily smoking on brain aging is lessened but not eliminated after cessation.

Usage of snuff/chewing tobacco appeared to be marginally associated with lower BAG (β = -1.1; 95 % CI -2.3, 0.06; p <0.063). Although not significant, his implies that the brain appeared 1.1 years younger than non-snuff users. However, it can be speculated whether snuff is predominantly used among those slightly younger subsets than the average age in this study population. Users were predominantly males. The negative BAG may be an indication that snuff use is primarily prevalent among newer generations. Some studies on animals have

suggested that nicotine (main component in smoking, snuff etc.) may have some anti-aging properties (Huang et al., 2015).

We did not find alcohol to be significantly associated with BAG. We employed gender-adjusted measures of alcohol consumption. Although findings were not significantly associated with BAG, beta estimate for heavy drinkers were four times higher compared to moderate drinkers. Previous findings have shown heavy alcohol consumption to be detrimental for brain health (Pfefferbaum et al., 2001; Shokri-Kojori et al., 2017). Majority of the subjects were moderate drinkers (80%), while 13 % were identified as heavy drinkers of whom females were majority, 18% compared to 6.6% males. This contradicts the findings of national surveys examining sex-differences in alcohol consumption although corrected. Men consumed substantially more alcohol than women throughout the entire period 2012–21. (Bye & Rossow, 2021; Folkehelseinstituttet, 2018) Calculations of alcohol consumption based on self-reporting in surveys typically yield significantly lower numbers than what appears from sales figures, typically between 40 to 50 % of sales figures (Knibbe & Bloomfield, 2001). It can be speculated whether there is some degree of underreporting amongst males which may affect our results. In sum, smoking appeared to age the brain significantly, while our study does not show an association with alcohol and snuff use. More research would be needed to examine the correlation more closely.

5.1.3 Association between BMI, cardiovascular disease, hypertension and brain age

We found no independent association between BAG and cardiovascular disease. Information on cardiovascular disease was based on self-reported data. Thirteen percent of the study subjects reported heart disease (17% males and 8.5 % females), males being slightly overrepresented. Previous studies have demonstrated an association between cardiovascular health and brain age (Beck et al., 2022; Sabia et al., 2019); however, we were unable to replicate this association. This may be due to our study design being overly specific, causing significant variables to be non-significant in and of themselves. Alternately, we could have combined multiple variables that have been found to raise cardiovascular risk into one variable and thus created a more comprehensive variable. Furthermore, self-reported cardiac disease is likely to be diagnosed and treated. The treatment would presumably minimize the disease's risk factor thus not appearing as harmful.

A substantial number of sample population (37%) reported hypertension which included those using antihypertensive. Previous studies have suggested a higher brain age among hypertensive subjects, especially males and showed that subjects with "*optimal BP defined as (MBP < 90, SBP < 115, DBP < 75) had a significantly lower BrainAGE than those who did not have an optimal BP".* However this study also found all BP measures were associated with higher brain age (Cherbuin et al., 2021) stating "*these effects were not uniquely driven by some extreme cases with poorly or un-controlled hypertension because sensitivity analyses showed similar associations between BP and BrainAGE in those who were normotensive, treated hypertensive, or untreated hypertensive indicating that a consistent effect was detected across the whole BP range*" We have speculated whether hypertension in our study could have been defined differently as to only include those manifesting high BP and not the one using antihypertensive; as usage would in theory protect from the harmful effects of hypertension.

It's perhaps safe to assume that if hypertension has been diagnosed than necessary measures would be in place to counteract the harmful effects. It may also be due to our study design not picking up the nuances of hypertension as a variable. This, also leaves open the possibility that, hypertension alone might not explain association. However, the harmful effects of hypertension on the brain have been well documented (Howard et al., 2019; Wajngarten & Silva, 2019). Although we did not find an association to brain age, it does not refute the harmful effects on brain health.

BMI was not found to be significantly associated with BAG. Average BMI for the sample population was about 26.8 kg/m^2. Females (26.3 kg/m^2) had on average slightly lower BMI than males (27.3 kg/m^2).

The BMI range for females (23.5 – 29.4) was higher than males (25.0 – 29.5). In addition, neither underweight nor obese participants were included. It is possible that a lack of range makes it difficult to observe the association between BMI and brain age. Low BMI amongst elderly women have been associated with mild cognitive impairment (Yuan et al., 2021) and obesity have shown to associated brain atrophy along with other risk factors (Ronan et al., 2016).

Furthermore, BMI does not differentiate between different mass compositions (muscle, fat, bone etc.). Generally, men are found to have higher BMI due to greater muscle mass. “Belly fat” is though found to be a risk factor in cardiovascular risk assessment; perhaps waist-hip ratio might have been a better measure since it better reflects on the “belly fat”(Hamer & Batty, 2019), and waist-hip ratio has previously also been shown to be associated with brain age (Beck et al., 2022).

5.1.4 Association between diabetes and brain age

Having diabetes was significantly associated with higher brain age. Having diabetes increased on average brain age by 2.4 years. This is consistent with previous studies where Type 2 diabetes have independently been shown to be associated with structural changes in the brain that reflect advanced aging(Cole, 2020; Franke et al., 2013). As previously noted, our data do not differentiate between Type 1 and 2 diabetes, but since the type 2 variant is most common, the findings largely reflect type 2 diabetes.

5.1.5 Association between lipid profile and brain age

We found a significant association between HDL-C and BAG, suggesting that high HDL-C levels increased the average age of the brain by 1.5 years. Females (1.74mmo/l) had on average higher mean HDL-C levels than males (1.40 mmol/l). Given its well-established significance in cardiovascular protection, plasma HDL-C has been the subject of substantial research (Rader & Hovingh, 2014), however the HDL-C theory has also been challenged by evidence gathered from human genetics research and randomized controlled trials. Hereditary disorders of low HDL-C have yielded significant insights. Mutations in apoA-I, ABCA1, and LCAT are known to be responsible for extremely low HDL-C. Even though HDL-C levels were below the 5th percentile, none of these mutations were clearly associated with coronary heart disease (Hovingh et al., 2005; Rader & deGoma, 2012). However, low HDL-C has been associated with lower gray matter (Ward et al., 2010). Additionally, research suggests that high levels of HDL-C were associated with decreased age-related cognitive deterioration and better memory (Barzilai et al., 2006; van Exel et al., 2002; Walter, 2009).

Furthermore, Moazzami found that higher LDL-C levels in late midlife were associated with larger brain volumes later in life, while higher triglyceride levels were associated with smaller brain volumes. Nonetheless, a recent large population study found no association between

lipids and BAG (Beck et al., 2022). Nor triglycerides or LDL-C were found to be significantly associated with BAG in our study.

It is unclear as to why HDL-C, which is normally associated with good cardiovascular health was correlated with increased BAG in this study. HDL-C and LDL-C levels were on average high in females, whereas they were on average low in males. Triglycerides were on average higher in males than females. It is well recognized that triglycerides are a risk factor for cardiovascular disease (Nordestgaard & Varbo, 2014) As mentioned earlier, a slight negative correlation between HDL-C and triglycerides was observed, which may suggest an inverse association.

An alternative approach could have been to examine if this inverse correlation was associated with BAG. HDL-C alone may not clarify the association between lipids and BAG; however, the association cannot be refuted either.

5.1.6 Association between physical activity, resting heart rate and brain age

We found no significant association between physical activity and BAG. Given past research, the absence of a significant association between physical activity and BAG was perhaps unexpected (Brown et al., 2013; Erickson et al., 2013; Erickson et al., 2010), as physical activity has been shown to improve cognition (Brown et al., 2013). Brown et al summarizes a large body of literature focusing on the effects of physical activity on the brain. It covers epidemiological studies, prospective cohorts, studies of animal models of AD, and interventional trials. They point unequivocally in the direction that increased physical activity is beneficial to cognition and slows the rate of cognitive decline. Angevaren et al. findings suggest that intensive physical activity, as opposed to total activity, was related to improved cognitive performance. Further suggesting there may be an intensity threshold over which cognitive improvements become more obvious (Angevaren et al., 2007). However, our study was unable to make a suitable intensity selection, and owing to missing data, we were compelled to leave out important information, which may have influenced the results.

Furthermore, the vast majority of studies evaluating the influence of physical activity on cognition often rely on subjective questionnaires or surveys (Middleton et al., 2008; Weuve et al., 2004) which are well-known for their poor validity and reliability (Shephard, 2003). People's recollections of physical exercise performed far back in time is unlikely to be

accurate which contributes to the poor accuracy of the data. Also, studies show that people have a tendency to inaccurately estimate their levels of physical activity (Aadahl et al., 2007). We have therefore speculated on whether the resting heart rate might be a better estimate for physical fitness.

To address this issue, we found the variable "physical activity outside work" correlates well with one objective measure of physical fitness (Emaus et al., 2010), namely resting heart rate, which we already possessed for our subjects. Resting heart rate was found to be significantly associated ($b = 0.04$, $p = 0.003$) with BAG suggesting that a 10-unit increase in resting heart rate would result in a 0.4-year rise in brain age. Moreover, in a recent study from the Karolinska Institute, a higher resting heart rate was found to be associated with an increased risk of dementia and an accelerated rate of cognitive decline independent of cardiovascular disease in a general population of elderly (Imahori et al., 2021). Those with a resting heart rate of 80 or above had a 55% increased chance of developing dementia than those with a resting heart rate between 60 and 69.

In addition, several studies show that sex hormones (testosterone and estrogen) may have neuroprotective qualities (Pike et al., 2009). At menopause, females undergo a significant decline in reproductive hormones, whereas men experience a more gradual decline in sex hormones (Li et al., 2014). It is theorized that physical activity raises the levels of such sex hormones, particularly testosterone, and that women benefit more from physical activity due to lower basal hormone levels (Li et al., 2014; Pike et al., 2009). For physical activity, we found no significant interaction terms. Even resting heart rate ($b = 0.03$, $p = 0.045$) became less significant when interaction terms were accounted for, which is difficult to explain.

As previously noted, we selected one of seven variables (level of physical activity outside work) related to physical activity. The large number of missing data for the other six variables compelled us to exclude them altogether. As previously speculated, the missing data may be related to a larger proportion of retirees. Also, majority of the missing data were amongst females compared to males. It also seemed higher percentage of males participated in high intensity compared to females.

Summarized, the absence of data may explain why physical activity was not significant, despite the fact that resting heart rate was significantly associated with BAG, albeit to a lesser

extent when interaction terms were taken into consideration. The dataset was large and accounted for numerous variables; not specifically designed to look for physical activity as the only variable. The questionnaire may not be the optimal method for assessing the effects of physical activity on BAG.

5.1.7 Association between SES and brain age

We found no association between the confounder SES and BAG, implying that education and taxable income were not significantly associated with brain age. Education have been shown to be associated with higher brain age (Arenaza-Urquijo et al., 2013), however the studies are inconsistent (Nyberg et al., 2021). Cross-sectional studies show only ambiguous support for an association between education and brain health (Walhovd et al., 2022) and a comprehensive review revealed that educational level did not reliably affect the progression of cognitive deterioration with aging (Lovden et al., 2020)

5.1.8 Association between sleep quality and brain age

Though prior research has indicated a link between sleep deprivation and brain age, particularly in relation to neurodegenerative illnesses (Jelicic et al., 2002; Sexton et al., 2017), we did not find a significant association between sleep quality and brain age. We examined three variables pertaining to potential sleep problems; a questionnaire for quantity ("number of years with sleeping issues"), a rating score for quality (Bergen insomnia scale score) and whether the subjects worked shift/night. Regarding the question "number of years with sleeping issues," the majority of respondents (56%) had no sleep problems, mostly males (64%) as opposed to 49% of females, indicating that more than half of the female respondents experienced sleep problems.

Seventeen percent of the sample population reported sleeping problems for more than 5 years, 22% being females and 11 % being males. The same pattern was seen among those with sleep problems for less than a year and between 1-5 years. Additionally, the variable “total score of Bergen insomnia scale” females had a higher score compared to males, 7 and 4 respectively. There were a total of 121 missing data, 86 of which were from females.

Studies suggest that lack of sleep leads to increased accumulation of beta amyloid plaques arguing that sleep may be a modifiable factor which can protect against cognitive decline (Yulug et al., 2017; Zhao et al., 2019). A small study using PET and 18F florbetaben,

measured beta- amyloid burden in 20 healthy controls after a night of restful sleep and then after a night of sleep deprivation. The study demonstrated that one night of sleep deprivation significantly increased the beta-Amyloid burden by 5% (Shokri-Kojori et al., 2018).

Ramduny and colleagues (2022) conducted a similar study to ours and found an association between sleep problems and brain age that we were unable to replicate. When we look closely at this other study (Ramduny et al., 2022), we can see that they used other objective (actigraphy-based assessment of sleep fragmentation) measures to quantify sleep problems. Furthermore, they used self-reported scale comprised of 19 questions designed to assess sleep quality and sleep problems over a one-month period. Compared to their self-reported scale our insomnia categories stretched from none to over five years. The span of time was quiet large which may make the questionnaire less specific

We derived brain age from four features: T1 -image Jacobian map, and the gray and white matter densities. While they derived brain age using other features such as whole brain morphometry and voxel-based morphometry - a neuroimaging technique used to study focal differences in brain anatomy. They also looked for microstructural changes in white matter (WM) as well as volumetric changes in grey matter. Although the same model was applied to calculate BAG, the features appear to have been slightly different. Therefore, our results regarding sleep quality were not significantly associated with BAG

5.2 Strengths and weaknesses

The study's strength was its relatively large sample size and well-characterized data set. However, some variables were not adequately defined (diabetes, hypertension i.e.), as discussed in previous sections. In addition, there were uncertainties over the applicability of the BrainAGE model to our dataset. Despite the fact that one of the criteria for a model is that it should be transferable to new datasets. Other studies utilizing diffusion tensor imaging or fluid attenuated inversion recovery (FLAIR) pictures may capture other features of brain aging which our model is insensitive to as T1-images were the sole method used to determine brain age in our study.

Furthermore, it is recognized that self-reported questionnaires may have poor validity and reliability, as people's recollection may be erroneous, which may contribute to poor accuracy. Missing data over 10% can skew results which compelled us to leave out certain variables. This may have influenced our findings.

6 Conclusions

Our findings suggest that males are likely to have higher brain age compared to females. In addition, resting heart rate, HDL-C, smoking and diabetes were significantly associated with BAG. Smoking, whether previous or current was found to accelerate brain age significantly. Also, having diabetes increased brain age by 2.4 years. Our study found none of the interaction terms for sex differences to be significant. Confounding variables such as education or economic status were not significantly associated with BAG. Despite previously contradictory findings, neither hypertension nor self-reported cardiovascular disease was significantly associated with BAG in this study. A low heart rate may be advantageous for healthy brain aging. In addition, self-reported physical activity and self-reported sleep problems were not associated with BAG; however, future studies should focus on these two areas and use objective measurements to assess their effects.

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