Healthy Lifestyle Index and Caloric Intake Associated with Brain Structure and Function in an Elderly Swedish Population

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ABBREVIATIONS

7MS – Seven-Minute Screening
AD – Alzheimer’s Disease
ANOVA/ANCOVA – Analysis of (Co)Variance
BA – Brodmann Area
BMI – Body Mass Index
CDR – Cognitive Drug Research
DBM – Deformation-Based Morphometry
DHA – Docosahexaenoic Acid
DM2 – Type 2 Diabetes Mellitus
EPA – Eicosapentaenoic Acid
FOV – Field of View
FWE – Family-Wise Error
FWHM – Full Width Half Maximum
HAP – Healthy Aging Phenotype
HDI – Healthy Diet Indicator
HLI – Healthy Lifestyle Index
MMSE – Mini-Mental State Examination
MNI – Montreal Neurological Institute
MRI – Magnetic Resonance Imaging
MS or SyndX – Metabolic Syndrome
PIVUS – Prospective Investigation of the Vasculature in Uppsala Seniors
SPM – Statistical Parametric Mapping
TBM – Tensor-Based Morphometry
TR/TE – Repetition Time/Echo Time
TFE – Turbo Field Echo
TMT – Trail Making Test
VBM – Voxel-Based Morphometry
WFU – Wake Forest University
Abstract

**Background:** Research into healthy aging and improved quality of later life continues to be on the rise as the world’s life expectancy is anticipated to continue to increase. However, the world’s population is growing both older and growing heavier. Obesity is an international concern and is associated with a lower quality of life and increased risk for a host of comorbidities. The classical cause of obesity is increased energy consumption coupled with decreased energy expenditure. Calorie-restricting elderly, such as the Okinawans of Japan, have been documented to have the longest lifespans. Studies of Swedish centenarians found that extreme lifespans were also a factor of physical and cognitive reserves. This thesis project investigates the lifestyle and dietary practices of a selected sample of cognitively healthy, non-diabetics from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) cohort. This project aims to associate differences in regional gray matter volume and cognitive performance to lifestyle and dietary habits within a selected sample of elderly Swedish subjects.

**Methods:** Selected subjects from the PIVUS cohort were assessed for a healthy lifestyle index (HLI) and for their approximated daily average caloric intake (n = 292 and n = 310, respectively). HLIs were assigned based on BMI, dietary habit, smoking status, alcohol consumption and physical activity. Caloric intake was approximated from seven-day food diaries which were confirmed to have accurate reporting. Unified segmentation voxel-based morphometry (VBM) was used in this project to locate regional volumes of gray matter which may be affected by overall lifestyle (sub-sample n = 147) or caloric intake (n = 156).

**Results:** VBM regression models showed no indication that a HLI could be significantly tied to regions of gray matter volume differences. Univariate ANOVA confirmed that cognitive performance on the verbal fluency task was only significantly greater in those who had the highest HLI. Caloric intake was negatively associated with regional gray matter volume of the left parahippocampus and had a positive trend with the right superior frontal gyrus volume after family-wise error correction. Gray matter volumes from these identified regions were extracted from the magnetic resonance images and correlated to various cognitive performance measures. The left parahippocampus was not significantly correlated to any cognitive task. However, an increase in the right superior frontal gyrus gray matter volume correlated to reduced completion times for trail making tasks.

**Conclusion:** These results suggested that a high HLI may be protective to cognitive health in an elderly Swedish population, specifically in verbal fluency. High caloric intake may attribute to decreased gray matter volumes in regions of the brain associated with memory encoding and recall, namely the parahippocampus. The decreased recall memory performance and simultaneous improved working memory correlated to increased caloric intake prompt the need for further investigation into the macro- and micronutrient components of these individuals’ diets to determine if the dietary energy sources may have helpful or hurtful effects on brain structure and cognition.
1. Introduction

The proportion of the elderly continues to increase as the average life expectancy grows longer in many industrialized countries. The annual report from the Swedish National Board of Health and Welfare (Socialstyrelsen, in Swedish) reported a one percent reduction in deaths among the 75+ age group between 2008 and 2009 in addition to a general decline in death rates since 1987 (2011). This increasingly prominent demographic of seniors is accompanied with a similar increase in age-related research. A concurrent theme in contemporary research is the investigation of the underlying mechanism of obesity as the affliction reaches epidemic proportions. Obesity has numerous comorbidities including Type II diabetes mellitus (DM2), hypertension and hyperlipidemia which together comprise the metabolic syndrome (MS or SyndX) (JAMA 2001). Obesity and its comorbidities affect vascular health and subsequently can affect behavior, mood and cognition (Elias et al. 2005, Scott et al. 2008, Maayan et al. 2011).

The Healthy Aging Phenotype (HAP) is described as one in which a living person may have highly preserved metabolic, hormonal and neuro-endocrine systems; cardiovascular, neuronal and skeletal systems similar to a younger body (Franco et al. 2009). The purpose of this project is to determine how the lifestyles of elderly Swedish adults affect cognitive function and brain gray matter volume. A healthy lifestyle index (HLI) will be defined based on data collected from an elderly Swedish population and a crude analysis of dietary habits will also be assessed.

1.1 The Aging Process

Aging can be characterized as a process of increasing mortality and decreasing physiological function (Kirkwood and Austad 2000). Kirkwood and Austad summarized that longevity is modulated by genes responsible for repair activities. Aging is an organism’s inability to maintain equity between daily cell damage and repair processes. Reduced enzymatic capacities are subsequently a likely secondary cause for age-related reduction of various tissues. Additionally, sarcopenia—wasting away of lean muscle—has been shown to be increasing with age and can be compounded with obesity to result in “sarcopenic obesity” (Baumgartner 2000).

Alzheimer’s disease (AD) and other dementias are responsible for a significant portion of disability in the global elderly population—especially in developing countries (Ferri et al. 2005). Alzheimer’s disease is characterized by the diminished cognitive capabilities resulting from plaque accumulation in the brain (PubMed Health 2010). As one’s age progresses, risk for cognitive impairment increases. Some lifestyle factors among the elderly have also been linked to cognitive decline. For example, limited “life-space”—the area in which day-to-day activities occur—has been shown to increase risk for cognitive decline over a four year period (Crowe et al. 2008).

In a population-based study of East Asians, Chee et al. reported cognitive processes declined with increasing age including speed of processing, executive function, visuospatial memory, language, attention and verbal memory (2009). This study of East Asian brains also resulted in a significant age-related decline in hippocampal and overall cerebral volumes. Another study of cognitively healthy elderly individuals found that automated temporoparietal brain volumes could be used to accurately predict those at risk for future memory decline (Chiang et al. 2011).

1.2 Dietary Intake

Dietary habits are most often assessed by use of self-reported values on questionnaires. Comparisons of diet self-reporting methods have shown that dietary journals and food recall interviews differ from one another in evaluating micronutrients (Ortiz-Andreuccetti et al. 2009) but agree on approximate caloric intake (Mahalko et al. 1985).
Caloric restriction and its association to healthy, long lives among Japanese populations have been documented (Willcox et al. 2007). Furthermore, caloric intake has been postulated to affect metabolic stability (Demetrius 2004). Increased caloric intake is often conducive to an increase in one's body mass index (BMI) barring compensatory physical activity or metabolic disorders, and BMI has been previously linked to a global decrease in brain volume (Ho et al. 2010). Cognitive dysfunction can be a secondary effect of dietary habits; DM2 can be induced by unhealthy eating practices, and Kerola et al. (2010) summarized the negative impact of such cardiovascular disorders on cognition.

Macronutrient and micro-nutrient intake have also been controversially linked to cognitive performance in the literature. Kaplan et al. (2001) showed that energy intake after a fasting period improved cognitive performance in elderly subjects independent of the macronutrient energy source. Other studies cite that diets high in omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) improve mood cognitive function in human (Freemantle et al. 2006, McNamara 2009) and rat models (Hashimoto et al. 2002, Hashimoto et al. 2009). A study in rats showed that a high-fat/high-stress diet impaired hippocampus-dependent memory (Alzoubi et al. 2009). One study even connected approximated long-chain omega-3 fatty acid intake with regional gray matter volume (Conklin et al. 2007). Given the range of beneficial and harmful effects dietary intake has been shown to have on cognitive function and brain structure, it is worth investigating how dietary patterns may influence physical and cognitive decline in a healthy elderly population.

This project will focus only on the absolute average daily caloric intake of an elderly Swedish sample populations; macronutrient energy sources and micronutrients will not be considered at this time.

1.3 Voxel-based Morphometry
Voxel-based morphometry (VBM) is an unbiased and mostly automated computational neuroimaging technique in which high-resolution magnetic resonance images (MRIs) are compared for localized gray matter concentration differences. Voxels are the volumetric base units of 3D images just as a pixel describes an elementary area of a two-dimensional image. This technique involves several steps including spatial normalization of the images, segmentation of the tissue types and smoothing of segmented tissues before performing statistical analysis (Ashburner and Friston 2000). VBM differs from other neuroanatomical imaging techniques, such as deformation- or tensor-based morphometry (DBM or TBM) in that it quantifies localized tissue type concentrations rather than macroscopic structural differences (Mechelli et al. 2005). The output from VBM is known as a statistical parametric map which illustrates local regions of gray or white matter differences based on a voxel-by-voxel comparison of brain images.

VBM analysis of 465 healthy aging brains showed a decline in global gray matter volume; this decline was further specified into local areas of gray matter loss (Good et al. 2001). Identified local regions of gray matter concentration decline were parietal lobes, pre- and post-central gyri, insula, anterior cingulate cortices and other temporal lobe regions. It is intuitive to believe that function follows structure; therefore, gray matter volume deficits should be linked to proportional cognitive function. Indeed, it has been shown that greater regional gray matter volumes were positively linked to positive performance (Taki et al. 2010).

1.4 Aims
The current project aims to determine whether a HLI determined for subjects at the age of 70 can significantly predict regional gray matter differences and cognitive performance assessed at the age of 75. This project, furthermore, looks into the dietary component of lifestyle to link caloric intake with differences in regional gray matter volume and cognitive performance. The hope is that this
investigation will provide insight into the importance of a healthy lifestyle and temperate energy intake in an elderly Swedish population.

2. Materials and Methods

2.1 Subjects
This project used data from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) is a longitudinal study conducted by Uppsala University consisting of approximately one thousand participants recruited at the age of 70 years (Lind 2011). Anthropometric, metabolic, cognitive and lifestyle data were collected and revisited when the subjects were 70 and 75 years of age.

2.1.1 Selection Criteria
The PIVUS cohort consists of 1016 participants; however, for the purpose of this study, further exclusion criteria were assessed. The Table 1 below details the exclusion steps carried out for this project.

Table 1. Exclusion criteria and participant count from the PIVUS cohort. The number of female subjects is listed in parentheses. Subjects who also participated in MRI scanning were analyzed for regional gray matter volume differences by VBM.

<table>
<thead>
<tr>
<th>Participant Count</th>
<th>Exclusion Criteria and Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIVUS study cohort, n = 1016 (509)</td>
<td>Unreliable or unreported dietary data, n = 377 (184)</td>
</tr>
<tr>
<td></td>
<td>Data not available from medical questionnaire, n = 128 (62)</td>
</tr>
<tr>
<td></td>
<td>Cognitive battery unavailable or not given, n = 10 (7)</td>
</tr>
<tr>
<td></td>
<td>MMSE score ≤21, n = 1 (0)</td>
</tr>
<tr>
<td></td>
<td>Clinically diagnosed dementia, n = 8 (3)</td>
</tr>
<tr>
<td></td>
<td>Stroke, n = 14 (6)</td>
</tr>
<tr>
<td></td>
<td>Diabetes, n = 46 (25)</td>
</tr>
<tr>
<td></td>
<td>Use of anti-hyperlipidemia medications, n = 98 (49)</td>
</tr>
<tr>
<td></td>
<td>Systolic blood pressure ≥180 mmHg, n = 24 (16)</td>
</tr>
<tr>
<td>Cognitive healthy with dietary data, n = 310 (157) Scanned, n = 156 (71)*</td>
<td>Data missing from lifestyle questionnaire, n = 18 (8)</td>
</tr>
<tr>
<td>Comprehensive lifestyle data, n = 292 (149) Scanned, n = 147 (68)*</td>
<td></td>
</tr>
</tbody>
</table>

*Extra exclusion criteria for scanned sub-sample
Corrupted scan, n = 1 (0)
White matter lesions, n = 2 (1)
Bilateral hygroma, n = 1 (0)

2.1.2 Cognitive Tests
The subjects were administered a battery of cognitive performance tests which included: the Mini-Mental State Examination (MMSE), the seven minute screen (7MS), and Trail Making Tests A and B (TMT-A and TMT-B). The MMSE is a widely used clinical cognitive health assessment test (Folstein et al. 1975). The 7MS is comprised of four sub-tests (1) the Benton temporal orientation task, (2) category fluency, (3) enhanced cued recall and (4) clock-drawing task (Solomon et al.1998). Both the MMSE and the 7MS were designed for clinical dementia and Alzheimer’s disease screening among elderly subjects. The TMTs are useful in assessing spatial and task-switching abilities and are not specific for age; although, TMT performance is known to be negatively affected by age. TMT-A requires the subject to connect sequential digits and TMT-B requires that alternating digits and letters be connected sequentially (Lezak 2004). The subjects were permitted to decline cognitive tests with
which they did not feel comfortable; for this reason, there were fewer participants reported for verbal fluency, trail making A or trail making B tasks.

2.2 Healthy Lifestyle Index
The healthy lifestyle index used in this project was modified from that which was presented in Flöel et al. (2008). The original lifestyle index used the same five elements detailed below, but the present project modified the component strata to better fit with available PIVUS data. Higher scores correspond to healthier practices for all HLI component categories. All data for the HLI scoring were derived from information collected from the subjects upon recruitment to the study at the age of 70 with the exception of smoking status. The HLI was derivative of the composite point total which had a range of 5 – 20 points. HLIs reported in this thesis were based on the following cut-off: composite score of 9 or 10, HLI 1; composite score of 11 or 12, HLI 2; composite score of 13 or 14, HLI 3; composite score of 15 or 16, HLI 4; and composite scores of 17 to 19 were assigned HLI 5.

2.2.1 Body Mass Index
The BMI component was scored as described in the Flöel et al. study with no modifications. The ascending points were assigned to the following BMI classes: ≥35.0; 30.0 – 34.9; 25.0 – 29.9; 22.0 – 24.9; and <22.0.

2.2.2 Physical Activity Level
The lifestyle questionnaire given to the PIVUS cohort included a self-reported physical activity score from one to four. These self-reported figures were used for this section. The levels of physical activity were described as little or no activity, mild physical activity, moderate physical activity, and high physical activity. The latter two activity levels further stipulate that the activity should be strenuous enough to have caused sweating.

2.2.3 Dietary Habits
Macro- and micronutrient intakes were approximated from self-reported seven-day food diaries maintained by the subjects as described in Sjögren et al. (2010). These approximations were subsequently corrected for unreliable reporting using the Goldberg equation cut-off described by Black (2000). Although comprehensive dietary information was available for the subjects, this exploratory project uses a dietary pattern to summarize dietary behavior.

The dietary component of this modified lifestyle index was scored out of three points. The points corresponded to the level of adherence the subjects had to dietary practices referred to as the Healthy Diet Indicator (HDI) defined by Sjögren et al. Dietary analyses and scoring were performed by Uppsala University’s Department of Public Health and Caring Sciences division of Clinical Nutrition and Metabolism.

2.2.4 Smoking Status
The smoking component for this project was scored out of three points, and these points were determined by smoking status rather than smoking frequency. Those who were never smokers were given one point; those who were previous smokers but no longer did at the time of the MRI were given two points; three points went to those who were currently smokers at the time of the MRI.

2.2.5 Alcohol Intake
Alcohol intake had modified cut-off values based on average grams of alcohol consumed per day. The healthiest amount of alcohol intake was described as the mean value for the population as per Flöel et al. The alcohol intake cut-offs were defined for this project as (in order of increasing point value): non-drinkers; 0 – 3 g/d; 3.0 – 9.9 g/d; more than 25 g/d; and 10.0 – 25 g/d.
2.3 MRI

2.3.1 Image Acquisition
High resolution 3D T1-weighted Turbo Field Echo (TFE) MRI images for the subjects were obtained using a Philips 1.5 Tesla scanner (Gyroscan NT, Philips Medical Systems, Best, The Netherlands). The scan parameters were TR/TE/Flip = 8.6 ms/4.0 ms/8°. Sagittal slice dimensions were dx = 0.94 mm, dy = 0.94 mm, and dz = 1.2 mm with a field of view (FOV) of 240 mm. The same scanner was used for all subjects in this study.

2.3.2 Unified Segmentation Voxel-based Morphometry
Voxel-based morphometry using the unified segmentation approach (Ashburner and Friston 2005) was performed during this study. All MR data were processed with Statistical Parametric Mapping software package (SPM8, Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk/spm) with MATLAB 7.12 R2011a (MathWorks, Natick, MA, USA; http://www.mathworks.com). Gray matter, white matter and cerebral spinal fluid were segmented in native space. The segmented gray matter was subsequently modulated to correct for changes made by spatial normalization. The spatially normalized gray matter probability maps were then normalized in a voxel-wise manner into Montreal Neurological Institute (MNI) standard space for later multiple regression analysis and then converted from MNI to Talairach space using the ‘mni2tal’ script (http://imaging.mrc.cbu.cam.ac.uk/imaging/MniTalairach). MR images were then smoothed using an 8 mm full width half maximum (FWHM) Gaussian kernel prior to SPM contrast or regression analyses.

Multiple regression general linear model batches were generated for HLI and caloric intake regressions (Friston 2005) covaried for gender and global gray matter volume. The caloric intake regression included BMI as an additional covariate. The peak threshold was set to P = 0.0005 and the voxel threshold was 100; clusters which had family-wise error corrected P values of less than 0.10 were considered regions of interest (ROI) in this project. Identified significant brain regions volumes were extracted using a combination of WFU PickAtlas 3.0.3 (Maldjian et al. 2003, Maldjian et al. 2004, and Tzourio-Mazoyer et al. 2002) and the SPM ROI extraction tool 'MarsBaR' (MARSeille Boite À Région d'Intérêt), version 0.42 (http://www.marsbar.sourceforge.net). Region of interest masks were defined using the automated anatomical labeling (aal) atlas within WFU PickAtlas tool and subsequent extraction from modulated, warped, corrected MR images was performed using the MarsBaR tool.

2.4 Statistics
Statistical analyses were performed with IBM SPSS Statistics Standard 19 software for Windows (SPSS Inc., Chicago, IL, USA; http://www.spss.com). Univariate analysis of variance (ANOVA) was used where warranted to compare the means of several groups. Partial correlations were performed correcting for confounding variables when associating cognitive performance to the HLI, dietary caloric intake or extracted gray matter volumes from regions of interest.

3. Results

3.1 Sample Population Description
Analyses were carried out on selected subjects from the PIVUS cohort on two levels: (1) the entire selected sample and (2) MRI-scanned sub-sample. Select attributes of these groups are described below in Tables 2 and 3.
Table 2. Selected demographic data for subjects assessed for healthy lifestyle indices from the PIVUS cohort. Mean values reported are given with standard deviations.

<table>
<thead>
<tr>
<th></th>
<th>HLI (n = 292)</th>
<th>MRI HLI (n = 147)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male / Female, n</td>
<td>143 / 149</td>
<td>79 / 68</td>
</tr>
<tr>
<td>University education, n (n%)</td>
<td>88 (30%)</td>
<td>41 (28%)</td>
</tr>
<tr>
<td>BMI age 70</td>
<td>25.7 ± 3.5</td>
<td>25.7 ± 3.3</td>
</tr>
<tr>
<td>BMI age 75</td>
<td>25.6 ± 3.5</td>
<td>25.5 ± 4.0</td>
</tr>
<tr>
<td>Calorie intake, kcal</td>
<td>2041 ± 432</td>
<td>2048 ± 428</td>
</tr>
<tr>
<td>Gray matter volume, ml</td>
<td>N/A</td>
<td>576 ± 61</td>
</tr>
</tbody>
</table>

Table 3. Selected demographic data for subjects assessed for caloric intake from the PIVUS cohort. Mean values are reported with standard deviations.

<table>
<thead>
<tr>
<th></th>
<th>Caloric Intake (n = 310)</th>
<th>MRI Caloric Intake (n = 156)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male / Female, n</td>
<td>153 / 157</td>
<td>85 / 71</td>
</tr>
<tr>
<td>University education, n (n%)</td>
<td>95 (31%)</td>
<td>43 (28%)</td>
</tr>
<tr>
<td>BMI age 70</td>
<td>25.7 ± 3.4</td>
<td>25.5 ± 3.3</td>
</tr>
<tr>
<td>BMI age 75</td>
<td>25.5 ± 3.8</td>
<td>25.5 ± 3.8</td>
</tr>
<tr>
<td>Calorie intake, kcal</td>
<td>2047 ± 444</td>
<td>2032 ± 389</td>
</tr>
<tr>
<td>Gray matter volume, ml</td>
<td>N/A</td>
<td>578 ± 60</td>
</tr>
</tbody>
</table>

3.2 Lifestyle Index

The total subject sample size available for HLI classification was 292 of which 149 were female. Figure 1 is an overview of the PIVUS subjects’ distribution across the HLI spectrum. The composite scores and the subsequent indices had normal Gaussian distributions. The HLI composite score range for this sample population was 9 – 19 points. Table 4 describes the HLI component demographics for the selected PIVUS subjects for both investigation levels. The scanned sub-sample is representative based on the component score frequencies.

Figure 1. Histogram of the PIVUS sample population’s healthy lifestyle index (HLI), n = 292. An index of five corresponds to individuals that carry out the healthiest lifestyles as assessed by their BMI, adherence to a healthy diet, alcohol intake, physical activity level and smoking status at the age of 70.
Table 4. Summarized HLI component score distribution for the selected sample (n = 292) and the MRI-scanned sub-sample (n = 147) of cognitively healthy elderly. Frequency percentages are listed in parentheses next to each value.

<table>
<thead>
<tr>
<th>HLI Component</th>
<th>Points</th>
<th>Description</th>
<th>HLI Sample (n = 292)</th>
<th>MRI-scanned (n = 147)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>≥35.0</td>
<td>4 (1%)</td>
<td>1 (&lt;1%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30.0 – 34.9</td>
<td>32 (11%)</td>
<td>16 (11%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>25.0 – 29.9</td>
<td>122 (42%)</td>
<td>65 (44%)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>22.0 – 24.9</td>
<td>97 (33%)</td>
<td>45 (31%)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>&lt;22.0</td>
<td>37 (13%)</td>
<td>20 (14%)</td>
</tr>
<tr>
<td><strong>Physical activity level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Low activity</td>
<td>24 (8%)</td>
<td>11 (7%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Mildly active</td>
<td>176 (60%)</td>
<td>91 (62%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Moderately active</td>
<td>75 (26%)</td>
<td>37 (25%)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Highly active</td>
<td>17 (6%)</td>
<td>8 (5%)</td>
</tr>
<tr>
<td><strong>HDI adherence</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Low adherence</td>
<td>31 (11%)</td>
<td>14 (9%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Moderate adherence</td>
<td>220 (75%)</td>
<td>116 (79%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>High adherence</td>
<td>41 (14%)</td>
<td>17 (12%)</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Current smoker</td>
<td>18 (6%)</td>
<td>9 (6%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Previous smoker</td>
<td>103 (35%)</td>
<td>47 (32%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Non-smoker</td>
<td>171 (59%)</td>
<td>91 (62%)</td>
</tr>
<tr>
<td><strong>Alcohol intake</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Non-drinker</td>
<td>50 (17%)</td>
<td>28 (19%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0 – 3 g/d</td>
<td>64 (22%)</td>
<td>31 (21%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.0 – 9.9 g/d</td>
<td>101 (35%)</td>
<td>49 (33%)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>&gt;25 g/d</td>
<td>11 (4%)</td>
<td>5 (3%)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10.0 – 25 g/d</td>
<td>66 (23%)</td>
<td>34 (23%)</td>
</tr>
</tbody>
</table>

3.2.1 Lifestyle Index and Regional Gray Matter Volume
The lifestyle indices assigned to the sample group were not significantly associated with any global or regional gray matter volumes when a multiple regression model was used. Subsequent VBM contrasts comparing the extreme quintiles of the HLI were also not significantly associated with regional gray matter volume differences. In an attempt to improve the sensitivity of the multiple regression model, the lifestyle component scores, ranging from 5–20 points, were used as predictors; however, this also yielded no significant regions of interest (data not shown).

3.2.2 Lifestyle Index and Cognition
In the sample population of n = 292, uncorrected Spearman correlations resulted in trends of improved performance on the clock drawing and trail making B tasks (data not shown). HLIs showed a positive trend with verbal fluency performance when correcting for gender (P = 0.054, one-tailed). The addition of the systolic blood pressure as a covariate did not strongly affect the P value (P = 0.056, one-tailed). The mean verbal fluency score for those with the healthiest lifestyle, as indicated by having a HLI of 5, was significantly higher than mean scores for lower lifestyle indices (Figure 2), controlling for gender and systolic blood pressure. HLI cognitive mean performance for each cognitive task assessed is listed in Table 5.
Table 5. Mean scores (± SD) for cognitive tasks in the HLI-selected PIVUS subjects. Fewer subjects than specified in the table heading were administered the verbal fluency and trail making tasks; for these tasks, n values are reported in parentheses beside the mean score.

<table>
<thead>
<tr>
<th>Cognitive Task</th>
<th>Ideal Score</th>
<th>HLI Sample (n = 292)</th>
<th>MRI Sub-sample (n = 147)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMSE</td>
<td>30</td>
<td>28.9 ± 1.2</td>
<td>28.7 ± 1.3</td>
</tr>
<tr>
<td>Benton temporal orientation</td>
<td>0</td>
<td>0.6 ± 3.6</td>
<td>0.7 ± 5.0</td>
</tr>
<tr>
<td>Free recall</td>
<td>16</td>
<td>9.9 ± 2.3</td>
<td>9.6 ± 2.3</td>
</tr>
<tr>
<td>Cued recall</td>
<td>0</td>
<td>5.9 ± 2.1</td>
<td>6.2 ± 2.2</td>
</tr>
<tr>
<td>Clock drawing</td>
<td>7</td>
<td>6.4 ± 0.9</td>
<td>6.4 ± 0.8</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>Higher is better</td>
<td>21.0 ± 5.5, (291)</td>
<td>21.1 ± 5.6</td>
</tr>
<tr>
<td>TMT-A (s)</td>
<td>Lower is better</td>
<td>55.5 ± 21.2, (288)</td>
<td>54.7 ± 19.1, (145)</td>
</tr>
<tr>
<td>TMT-B (s)</td>
<td>Lower is better</td>
<td>158 ± 110, (287)</td>
<td>164 ± 117, (144)</td>
</tr>
</tbody>
</table>

Figure 2. Mean verbal fluency scores grouped by HLI, covaried for gender and systolic blood pressure. Those with an HLI of 5 exhibited significantly greater performance on the verbal fluency task than all other indices, (* P ≤ 0.02) as confirmed by univariate analysis of variance with post-hoc Bonferroni correction.

3.3 Caloric Intake

3.3.1 Caloric Intake and Regional Gray Matter Volume

Caloric intake approximations were significantly negatively associated with left parahippocampal gray matter volume when covaried for gender, BMI and total gray matter volume (P_{FWE-corr} = 0.047; k_E = 301). A positive trend was determined between caloric intake and the right superior frontal gyrus, Brodmann area 6 (P_{FWE-corr} = 0.071; k_E = 268)—see Figure 3 below. Summarized peak voxel statistics are listed in Table 6.
Table 6. Peak voxel cluster statistics and average regional volume (ml) from SPM8 multiple regression analysis of caloric intake as a predictor of gray matter volume in an elderly Swedish sample population. The design matrix was covaried for gender, global gray matter volume and BMI.

<table>
<thead>
<tr>
<th>Approximate Brain Region</th>
<th>Talairach Coordinates</th>
<th>P_{uncorr}</th>
<th>P_{FWE-corr}</th>
<th>kE</th>
<th>Direction of Association</th>
<th>Average Volume (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left parahippocampus</td>
<td>-14 -12 -18</td>
<td>0.004</td>
<td>0.047</td>
<td>301</td>
<td>Negative</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>Right superior frontal gyrus (BA6)</td>
<td>20 14 46</td>
<td>0.006</td>
<td>0.071</td>
<td>268</td>
<td>Positive</td>
<td>8.6 ± 1.1</td>
</tr>
</tbody>
</table>

P_{uncorr} (Uncorrected P value); P_{FWE-corr} (Family-wise error corrected P value); kE (voxel cluster size)

Figure 3. “Glass brain” representations of regions of significant gray matter difference which were predicted by average daily caloric intake (n = 156). The top row of the panels are the sagittal and coronal views, respectively. The second row is the axial point of view. The peak voxel P value threshold was set to 0.0005 and the cluster extent was 100 contiguous voxels. The multiple regression model in SPM8 was covaried for gender, BMI and global gray matter volume. A.) Depiction of regional volumes where there is an inverse relationship with caloric intake. The region indicated by the circle is best approximated as the left parahippocampal gyrus (P_{FWE-corr} = 0.047; kE = 301). B.) Darker regions in this panel correspond to regional volumes where there is a positive trend with caloric intake. The selected region is best approximated as the right superior frontal gyrus, Brodmann area 6 (P_{FWE-corr} = 0.071; kE = 268).

3.3.2 Caloric Intake and Cognition

Correlations were performed on both the sample population as a whole and the scanned sub-sample. The cohort-wide correlation showed a relationship between caloric intake and recall memory detailed in Figures 4 and 5. The MRI-scanned sub-sample, less those with corrupted scan images, showed an inverse trend with free recall (r = -0.151; P = 0.063, two-tailed) and positive associations with the completion time for the TMT-A (r = 0.247; P = 0.002, two-tailed). The overall mean performance for each cognitive test assessed is detailed in Table 7.

Table 7. Mean scores (± SD) for cognitive tasks in the caloric intake-selected PIVUS subjects. Fewer subjects than specified in the table heading were administered the verbal fluency and trail making tasks; for these tasks, n values are reported in parentheses beside the mean score.

<table>
<thead>
<tr>
<th>Cognitive Task</th>
<th>Ideal Score</th>
<th>Sample (n = 310)</th>
<th>MRI Sub-sample (n = 156)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMSE</td>
<td>30</td>
<td>28.9 ± 1.2</td>
<td>28.7 ± 1.3</td>
</tr>
<tr>
<td>Benton temporal orientation</td>
<td>0</td>
<td>0.6 ± 3.5</td>
<td>0.8 ± 4.9</td>
</tr>
<tr>
<td>Free recall</td>
<td>16</td>
<td>9.83 ± 2.3</td>
<td>9.5 ± 2.3</td>
</tr>
<tr>
<td>Cued recall</td>
<td>0</td>
<td>5.9 ± 2.1</td>
<td>6.2 ± 2.2</td>
</tr>
<tr>
<td>Clock drawing</td>
<td>7</td>
<td>6.4 ± 0.9</td>
<td>6.4 ± 0.8</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>Higher is better</td>
<td>21.0 ± 5.5, (309)</td>
<td>21.0 ± 5.6, (155)</td>
</tr>
<tr>
<td>TMT-A (s)</td>
<td>Lower is better</td>
<td>55.4 ± 21.0, (306)</td>
<td>55.0 ± 19.1, (155)</td>
</tr>
<tr>
<td>TMT-B (s)</td>
<td>Lower is better</td>
<td>116 ± 108, (305)</td>
<td>163 ± 115, (154)</td>
</tr>
</tbody>
</table>
Figure 4. Scatter plot with regression line relating average dietary intake (kcal) with free recall score in an elderly Swedish sample population \((n = 292)\). The correlation coefficient of \(-0.184\) \((P = 0.001\), two-tailed\) has been corrected for gender and BMI.

Figure 5. Scatter plot with regression line relating average dietary intake (kcal) with cued recall score in an elderly Swedish sample population \((n = 292)\). The correlation coefficient of 0.138 \((P = 0.017\), two-tailed\) has been corrected for gender and BMI.
3.4 Isolated Brain Regions and Cognition
Two regions of gray matter volume variance were associated with daily caloric intake. The negative association between caloric intake and left parahippocampal volume was the only significant region after family-wise error correction ($P_{\text{FWE}} = 0.047$). The right superior frontal gyrus was not significant but did have a positive trend with caloric intake ($P_{\text{FWE}} = 0.071$). The extracted gray matter volume for the left parahippocampal region was not significantly correlated to any of the cognitive tasks when controlling for global gray matter volume, gender and body mass index at the time of MRI scan. The extracted right superior frontal gyrus gray matter volumes, however, show a significant correlation to the completion time for TMT-A. The right superior frontal gyrus had a weak trend with the trail making B completion time, but the correlation’s significance was lost after correcting for gender, global gray matter volume and BMI (data not shown).

![Figure 6. Correlation of extracted right superior frontal gyrus volume to the TMT-A completion time (s), corrected for gender, BMI and global gray matter volume. The corrected correlation coefficient is equal to -0.163 with $P = 0.046$ (two-tailed), n = 147.](image)

The modified HLI derived in this project was not capable of significantly predicting regional gray matter volume differences in a regression model and as such no correlations to cognitive performance were performed.

4. Discussion

4.1 Lifestyle Index and Regional Gray Matter Volume
The HLI was unable to significantly predict any regions of gray matter volume differences in the 147 subjects assessed. The secondary analysis using the HLI composite scores was performed in order to provide a more sensitive scale for predicting. Neither of these regression analyses nor a two-sample contrast yielded regional gray matter volume differences. The HLI used for this project is a simplified version of a lifestyle index used in a study of elderly Germans (Flöel et al. 2008). It was necessary to
simplify the scoring components in order to accommodate the data which was available from the PIVUS cohort. The cut-off values for the HLI component scores are almost completely arbitrary and unverified. In order to increase confidence in the predictive capability of this HLI for regional gray matter volumes, it would be necessary to define cut-off values which corresponded to actual observational differences in the subjects.

Data for the PIVUS study was collected with a particular scope of interest which did not include volumetric analyses of brain structures. Had the study’s initial scope considered VBM analysis, the questionnaires should include more detailed information such that a more sensitive and accurate HLI described above scoring system could be generated.

4.2 Lifestyle Index and Cognitive Performance

Compared to the Flöel et al. study on an elderly German cohort, this current study on an elderly Swedish cohort was larger (n = 292 compared to n = 198); yet maintained a similar distribution of the subjects into the respective HLIs with frequencies differing less than five percent. Unlike the reference study, the HLIs were correlated directly to cognitive performance scores and not the standardized residual values. Differing covariates were used between these studies because the PIVUS cohort was initially selected for age; therefore, it was not necessary to include this as a confounding term. The modified HLI devised for evaluation of selected PIVUS participants did not result in a near-linear relationship with verbal memory.

Subjects with a lifestyle index of five performed significantly higher on average on the verbal fluency task when controlling for gender and systolic blood pressure. This point suggests interplay among all five elements of the HLI such that scoring low in just one lifestyle area could be deleterious to cognitive performance in verbal fluency tasks. However, many of the HLI component elements have been shown to independently affect cognition. For example, gait speed—a physical ability measure—has been shown to have a direct relationship to verbal fluency (Soumaré et al. 2009). A review article from 2002 reported that the HDI score, not adherence as assessed in this project, and moderate alcohol consumption were associated with improved cognitive performance as evaluated by the MMSE (Jorissen and Riedel 2002). The HLI defined in this study needs to be evaluated and the influence of the component measures on cognitive function should be quantified. At the present time, the effect of the individual HLI components have not been assessed, and therefore it cannot be made certain if the result observed in this study is explained by a single factor or an interaction of multiple HLI factors.

4.3 Caloric Intake and Regional Gray Matter Volume

Caloric intake was associated with a significant reduction in gray matter volume in the left parahippocampal region. The parahippocampal region has been shown in fMRI studies to be associated with place, or scenic, learning but not recollection (Aguirre et al. 1996, Epstein et al. 1999). Subjects with reduced gray matter volume in their left parahippocampi would be expected to perform worse on tasks which require them to encode and recount spatial memory tasks. No cognitive tasks required the subjects to learn scenic or spatial details; as a result, no significant associations between extracted left hippocampal gray matter volume and any cognitive measures were expected.

Additionally, an increase in caloric intake produced a positive trend in the right superior frontal gyrus in the sixth Brodmann area. The superior frontal gyrus has been reported to affect memory performance but on the left cerebral hemisphere assessed by white matter lesions, contrary to the present finding (Boisgueheneuc et al. 2006); subjects who had white matter lesions in their left superior frontal gyri did not perform as well on working memory tasks compared to controls.
### 4.4 Caloric Intake and Cognitive Performance

Dietary energy intake was negatively correlated with free recall and positively correlated with cued recall performance controlling for gender and BMI. Declarative recall memory is associated with the medial temporal lobes (Squire 1992). In a recent study of controlled high-energy diets in rats, increased body weight over time and reduced performance on memory retention tasks were observed (Kanoski and Davidson 2010). Short-term high-fat diets in healthy humans showed decreased attention and speed of memory as assessed by Cognitive Drug Research (CDR) computerized assessment batteries (Holloway et al. 2011). Assuming that excess dietary energy is from fat, the present results agree with other human and animal model studies.

Absolute energy intake was not associated with any other cognitive task assessed in this study; however, due to the complexity and variability of dietary nutrients and their respective physiological impacts, significant associations with cognitive performance could be masked by competing effects. Morley and Banks summarized in a review that circulating cholesterol and triglycerides could negatively impact cognition; conversely, long-chain essential fatty acids, such as the omega-3 fatty acids, were referenced to have beneficial effects on cognitive performance (2010). Diets which had increased energy from protein resulted in improved reaction time in healthy young men (Jakobsen et al. 2011). The possibility of masking effects or macronutrient energy source interactions needs to be addressed in future studies by means of individual macronutrient intake analysis on cognition.

### 4.5 Isolated Brain Regions and Cognition

High calorie intake was associated with reduced performance on recall memory, and VBM regression analysis showed a significant decrease in gray matter volume in the left parahippocampal region in a MRI-scanned sub-sample. There was not a significant association between left parahippocampal gray matter volume and any cognitive task in the scanned sub-sample. There are two possible explanations for this disparity—power and plasticity. The scanned sub-sample was approximately half the size of the subjects considered for this project and may not have been a good representation of the larger sample. Additionally, it may be the case that function does not always follow structure. Neural plasticity could explain how individuals with smaller regional gray matter volumes could exhibit cognitive performance equal to that of others with greater regional gray matter volumes. It is also possible that some subjects could have developed compensatory pathways to account for gray matter deficits in regions normally associated with particular cognitive functions.

The increase in right superior frontal gyrus volume, which had a positive trend with caloric intake, was negatively correlated with the TMT-A completion time. This region of interest could be associated with complex motion planning as it is proximal to the premotor cortex and supplementary motor area. The observed correlation between the right superior frontal gyrus and improved efficiency in this connecting-the-dots task could be attributed to dexterity. The prefrontal cortex is ubiquitous for executive function and working memory (Baddeley and Della Salla 1998), but usually on the left hemisphere.

### 4.6 Limitations

The PIVUS cohort was devised initially to explore the progression of cardiovascular health in an aging Swedish population. Thus, the current project should only be considered as a speculative and exploratory cross-sectional study, and as such no results herein can be regarded as causal relationships. The present analysis was performed with the assumption that the dietary behavior and lifestyle did not significantly change in the five-year span between the start of the PIVUS study and the MRI-scanning session. Furthermore, since this study did not record all the same measures at both 70 and 75 years of
age, an observed rate of brain matter erosion or cognitive decline cannot be determined. The lack of repeated measures for many variables in the PIVUS study makes it difficult to add a longitudinal element connecting the two time points. This study would benefit most if future planned examinations of the PIVUS cohort included repeated measures for cognition and brain MRI. If such measures were to be recorded at the cohort’s re-evaluation at the age of 80, data from this present study could serve as a baseline from which an more reliable model of lifestyle and cognitive decline could be designed.

5. Conclusion
This project suggests that a healthy lifestyle for 70 year-old Swedish seniors may be associated with greater verbal fluency at the age of 75, but lifestyle indices may not be a sensitive enough measurement to be associated with regional brain matter volumes. HLI sensitivity and reliability would benefit from a less arbitrary scoring protocol. A future HLI based on relative rather than absolute measures may produce natural stratification of the component categories. Multivariate modeling techniques may also be employed to define a healthy lifestyle model from which a more accurate index system could be derived.

Self-reported dietary caloric intake could predict differences in regional gray matter volumes of the left parahippocampus (significantly) and the right superior frontal gyrus (trend). Consumption of excess dietary energy may lead to impaired recall memory; however, the improved working memory by way of increased superior frontal gyral volume confuses the story slightly. These conflicting data may indicate the importance of the dietary energy source. It warrants further investigation as to whether macro- or micronutrients of particular combinations can decelerate regional brain matter erosion and improve cognition in the elderly.

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I would like to take the opportunity to thank my project supervisors Christian Benedict, Samantha Brooks and Helgi Schiöth. I extend further gratitude to Lars Lind, Per Sjögren, Erika Ax and all others with whom I have worked on the PIVUS project. A final thank you is due to my colleagues at the Functional Pharmacology group for making me feel welcomed to a truly remarkable group of researchers.
References


