**Supplementary Material**

**EXTENDED METHODS**

*Covariates*

Information on educational level, medical history, and lifestyle factors was obtained in an interview with the patient and his/her caregiver[1]. Eight degrees of educational level were distinguished and classified as low (i.e., no education, primary education, or lower vocational education), intermediate (i.e., intermediate general secondary education, intermediate vocational education, or higher general secondary education), or high (i.e., higher vocational education or university).

Cardiovascular disease was defined as (a history of) hypertension, hyperlipidemia, cardiac arrhythmias, angina pectoris, myocardial infarction, coronary artery disease, carotid artery stenosis, and/or peripheral artery disease.Cerebrovascular disease was defined as a history of transient ischemic attack, reversible ischemic neurologic deficit, and/or stroke. Patients were asked whether they had been diagnosed with diabetes of either type.

Current alcohol consumption was classified as none or any. Smoking behavior was categorized into current, former, or never smoking.

Clinicians conducted a standardized psychical examination, which included a single blood pressure measurement and recording of height and weight to determine the body mass index. Genomic DNA was extracted from blood samples with the polymerase chain reaction technique and used to determine the apolipoprotein E ε4 (APOEε4) genotype[1].

**REFERENCE**[1] Aalten P, Ramakers IH, Biessels GJ, de Deyn PP, Koek HL, OldeRikkert MG, Oleksik AM, Richard E, Smits LL, van Swieten JC, Teune LK, van der Lugt A, Barkhof F, Teunissen CE, Rozendaal N,Verhey FR, van der Flier WM(2014) The Dutch Parelsnoer Institute--Neurodegenerative diseases; methods, design and baseline results. *BMC Neurol***14**, 254.

**Supplementary Table 1**

Characteristics of individuals included and excluded from the present analyses

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Included  (n=138) | Excluded  (n=22) | | Missing\* | p-value# |
|  |  | |
| Age (y) | 66 ± 9 | 63 ± 11 | | 0 | 0.232 |
| Male | 90 (65.2%) | 17 (77.3%) | | 0 | 0.265 |
| Educational level  Low  Middle  High | 32 (23.2%)  52 (37.7%)  54 (39.1%) | 6 (27.3%)  7 (33.3%)  8 (38.1%) | | 1 | 0.853 |
| SBP (mmHg) | 145 ± 19 | 139 ± 17 | | 4 | 0.163 |
| DBP (mmHg) | 84 ± 10 | 79 ± 9 | | 4 | 0.065 |
| BMI (kg/m2) | 25.2 ± 3.2 | 25.7 ± 1.6 | | 13 | 0.684 |
| Diabetes | 16 (11.6%) | 1 (7.1%) | | 8 | 0.615 |
| Cardiovascular disease | 43 (31.2%) | 5 (27.8%) | | 4 | 0.770 |
| Cerebrovascular disease | 17 (12.3%) | 4 (22.2%) | | 4 | 0.247 |
| Smoking behavior  Never  Former  Current | 58 (43.0%)  56 (41.5%)  21 (15.6%) | 7 (35.0%)  8 (40.0%)  4 (25.0%) | | 2 | 0.549 |
| Alcohol consumption | 110 (80.3%) | 14 (70.0%) | | 2 | 0.291 |
| CSF insulin (pmol/L) | 3.79 [3.37-4.52] | 38 [3.4-4.8] | | 0 | 0.194 |
| CSF Aβ1-42(ng/L) | 678 ± 281 | 753 ± 313 | | 0 | 0.254 |
| CSF Tau (ng/L) | 421 [229-633] | 336 [187-492] | | 0 | 0.215 |
| CSF p-Tau (ng/L) | 55 [36-77] | 44 [28-73] | | 0 | 0.281 |
| MMSE (score) | 26 ± 3 | 27 ± 3 | | 0 | 0.164 |
| AVLT (words)  Total immediate recall  Delayed recall | 30 ± 11  4 ± 4 | 29 ± 11  4 ± 4 | | 0 | 0.575  0.884 |

Data are presented as n (%), mean± SD, or median [IQR]. \*Number of missings for a specific variable. #-value for difference between individuals being included and excluded from the present analyses as evaluated with use of independent samples t-tests for continuous variables and Chi-square tests for categorical variables.SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; CSF, cerebrospinal fluid; MMSE, Mini-Mental State Examination; AVLT, 15 Word-Auditory Verbal Learning Test.

**Supplementary Table 2**

Association of CSF insulin levels with cognitive performance, excluding individuals with diabetes

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Total  (n=118) | SCI  (n=41) | aMCI  (n=39) | AD  (n=38) | Male  (n=74) | Female  (n=44) |
|  |  | | | |  |  |
| **MMSE** | | | | |  |  |
| Model 1 | 0.013  (-0.172; 0.197) | 0.055  (-0.275; 0.385) | 0.197  (-0.126; 0.519) | 0.008  (-0.336; 0.351) | 0.172  (-0.062; 0.406) | -0.251  (-0.556; 0.054) |
| Model 2 | -0.038  (-0.214; 0.139) | 0.089  (-0.259; 0.436) | 0.201  (-0.123; 0.525) | -0.092  (-0.439; 0.255) | 0.154  (-0.074; 0.381) | -0.267  (-0.540; 0.006) |
| Model 3 | -0.054  (-0.239; 0.131) | 0.173  (-0.201; 0.547) | 0.210  (-0.160; 0.580) | -0.057  (-0.419; 0.305) | 0.164  (-0.074; 0.381) | -0.335\*  (-0.619; -0.052) |
|  |  |  |  |  |  |  |
| **Composite memory score** | | | | |  |  |
| Model 1 | -0.030  (-0.215; 0.155) | -0.336\*  (-0.628; -0.043) | 0.163  (-0.168; 0.494) | 0.216  (-0.119; 0.550) | 0.134  (-0.101; 0.370) | -0.187  (-0.496; 0.123) |
| Model 2 | -0.038  (-0.208; 0.132) | -0.272  (-0.565; 0.021) | 0.233  (-0.068; 0.534) | 0.144  (-0.214; 0.502) | 0.103  (-0.109; 0.314) | -0.202  (-0.482; 0.077) |
| Model 3 | -0.005  (-0.182; 0.172) | -0.113  (-0.402; 0.175) | 0.365\*  (0.038; 0.693) | 0.180  (-0.193; 0.554) | 0.163  (-0.053; 0.380) | -0.213  (-0.511; 0.086) |
|  |  |  |  |  |  |  |

Data are presented as standardized regression coefficients, which reflect the standard deviation change in cognitive performance per standard deviation increase in cerebrospinal fluid insulin levels. Insulin levels were transformed with the natural logarithm prior to analysis. Stratified analyses according to sex were performed as interaction analyses revealed statistically significant interaction of this factor on the association between CSF insulin and cognitive performance. \*p<0.05. Model 1: adjusted for UMC; Model 2: additionally adjusted for age and sex (the latter not in case of stratified analysis according to sex); Model 3: additionally adjusted for BMI. CSF, cerebrospinal fluid; SCI, subjective cognitive impairment; aMCI, amnestic mild cognitive impairment; AD, Alzheimer’s disease; UMC, University Medical Center; BMI, body mass index.

**Supplementary Table 3**

Associations of CSF insulin levels with levels of CSF Aβ1-42 and (p-)Tau, excluding individuals with diabetes

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Total  (n=122) | SCI  (n=43) | aMCI  (n=39) | AD  (n=40) | Male  (n=78) | | | Female  (n=44) | | | APOEε4 carrier  (n=73) | | | APOEε4  non-carrier  (n=49) | | |
|  |  | | | | | |  | | |  | | |  | | |  | | |
| **CSF Aβ1-42** | | | | | |  | | |  | | |  | | |  | | |
| Model 1 | 0.017  (-0.164; 0.198) | -0.008  (-0.327; 0.312) | 0.087  (-0.250; 0.425) | 0.187  (-0.116; 0.489) | … | | | … | | | … | | | … | | |
| Model 2 | -0.011  (-0.187; 0.165) | -0.045  (-0.369; 0.279) | 0.137  (-0.203; 0.476) | 0.166  (-0.164; 0.495) | … | | | … | | | … | | | … | | |
| Model 3 | -0.083  (-0.262; 0.097) | -0.026  (-0.381; 0.329) | -0.095  (-0.439; 0.250) | 0.124  (-0.217; 0.465) | … | | | … | | | … | | | … | | |
|  |  |  |  |  |  | | |  | | |  | | |  | | |
| **CSF Tau** | | | | | |  | | |  | | |  | | |  | | |
| Model 1 | -0.075  (-0.256; 0.106) | 0.145  (-0.172; 0.462) | -0.285  (-0.593; 0.022) | -0.273  (-0.593; 0.047) | -0.187  (-0.414; 0.040) | | | 0.097  (-0.217; 0.411) | | | -0.242\*  (-0.473; -0.010) | | | 0.199  (-0.094; 0.493) | | |
| Model 2 | -0.049  (-0.216; 0.117) | 0.097  (-0.176; 0.371) | -0.385\*  (-0.655; -0.114) | -0.265  (-0.600; 0.070) | -0.165  (-0.367; 0.036) | | | 0.113  (-0.171; 0.397) | | | -0.229\*  (-0.453; -0.005) | | | 0.207  (-0.044; 0.458) | | |
| Model 3 | 0.014  (-0.156; 0.184) | 0.208  (-0.076; 0.492) | -0.307\*  (-0.610; -0.004) | -0.250  (-0.601; 0.101) | -0.117  (-0.324; 0.091) | | | 0.203  (-0.087; 0.492) | | | -0.204  (-0.446; 0.038) | | | 0.276\*  (0.042; 0.511) | | |
|  |  |  |  |  |  | | |  | | |  | | |  | | |
| **CSF p-Tau** | | | | | |  | | |  | | |  | | |  | | |
| Model 1 | -0.050  (-0.229; 0.129) | 0.132  (-0.184; 0.449) | -0.292  (-0.616; 0.032) | -0.188  (-0.503; 0.128) | -0.184  (-0.408; 0.040) | | | 0.141  (-0.166; 0.448) | | | -0.185  (-0.415; 0.044) | | | 0.174  (-0.121; 0.469) | | |
| Model 2 | -0.033  (-0.203; 0.137) | 0.088  (-0.208; 0.385) | -0.401\*  (-0.700; -0.101) | -0.195  (-0.528; 0.139) | -0.168  (-0.378; 0.043) | | | 0.157  (-0.122; 0.435) | | | -0.182  (-0.413; 0.049) | | | 0.173  (-0.087; 0.433) | | |
| Model 3 | 0.040  (-0.132; 0.213) | 0.220  (-0.084; 0.524) | -0.325  (-0.662; 0.011) | -0.166  (-0.514; 0.181) | -0.097  (-0.310; 0.116) | | | 0.238  (-0.048; 0.524) | | | -0.127  (-0.374; 0.121) | | | 0.240  (-0.006; 0.487) | | |
|  |  |  |  |  |  | | |  | | |  | | |  | | |

Data are presented as standardized regression coefficients, which reflect the standard deviation change in cerebrospinal fluid levels of Aβ1-42 and (p-)Tau per standard deviation increase in cerebrospinal fluid insulin levels. Insulin and (p-)Tau levels were transformed with the natural logarithm prior to analysis. \*p<0.05. Stratified analyses according to sex and APOEε4 genotype were performed as interaction analyses revealed statistically significant interaction of these variables on the association between CSF insulin and CSF (p-)Tau.Model 1: adjusted for UMC; Model 2: additionally adjusted for age and sex (the latter not in case of stratified analysis according to sex); Model 3: additionally adjusted for BMI. CSF, cerebrospinal fluid; SCI, subjective cognitive impairment; aMCI, amnestic mild cognitive impairment; AD, Alzheimer’s disease; Aβ1-42, amyloid-β1-42; Tau, total Tau; p-Tau, phosphorylated Tau; UMC, University Medical Center; BMI, body mass index.