Plasma C-peptide levels and rates of cognitive decline in older, community-dwelling women without diabetes

Olivia I. Okereke[a,b,*], Michael N. Pollak[c], Frank B. Hu[b,d],
Susan E. Hankinson[b,e], Dennis J. Selkoe[f], Francine Grodstein[a,b,e]

[a]Division of Aging, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA, USA
[b]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA 02115, USA
[c]Departments of Medicine and Oncology, Lady Davis Research Institute of the Jewish General Hospital and McGill University, Montreal, Canada
[d]Department of Nutrition, Harvard School of Public Health, Boston, MA, USA
[e]Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA
[f]Center for Neurologic Diseases, Department of Neurology, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA, USA

Summary

Background: Both type 2 diabetes and hyperinsulinemia have been related to diminished cognition. To address independent effects of increasing mid-life insulin secretion on late-life cognition, we prospectively examined the relation of plasma C-peptide levels to cognitive decline in a large sample of older women without diabetes or stroke.

Methods: Plasma C-peptide levels were measured in 1187 "young-old" women (mean age = 64 years) without diabetes in the Nurses' Health Study. Cognitive decline was assessed approximately 10 years later. Three repeated cognitive batteries were administered over an average of 4.4 years using telephone-based tests of general cognition, verbal memory, category fluency, and attention. Primary outcomes were general cognition (measured by the Telephone interview for Cognitive Status [TICS], as well as a global score averaging all tests) and a verbal memory score averaging four tests of word-list and paragraph recall. Linear mixed effects models were used to compute associations between C-peptide levels and rates of cognitive decline.

*Corresponding author at: Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA 02115, USA. Tel.: +1 617 525 2027; fax: +1 617 525 2008.

E-mail address: ookereke@partners.org (O.I. Okereke).

Results: Higher C-peptide levels were associated with faster decline in global cognition and verbal memory. Compared to those in the lowest C-peptide quartile, multivariable-adjusted mean differences (95% CI) in rates of decline for women in the highest quartile were -0.03 ($-0.06, -0.00$) units/year for the global score, and -0.05 ($-0.09, -0.02$) units/year for verbal memory. Each one standard-deviation increase in C-peptide was associated with significantly faster decline on the TICS (p-trend $= 0.05$), global score (p-trend $= 0.04$) and verbal memory (p-trend $= 0.006$).

Conclusions: Higher levels of insulin secretion in those without diabetes may be related to decline in general cognition and verbal memory.

1. Introduction

Large-scale epidemiologic studies (Ott et al., 1999; Peila et al., 2002) have identified strong associations between type 2 diabetes and increased risk of dementia. The early stages of type 2 diabetes are represented by insulin resistance and compensatory increases in insulin production and secretion. Recent attention has turned to the question of whether higher levels of insulin may directly increase the risk of cognitive decline, possibly in addition to vascular damage often associated with insulin resistance—even in the absence of clinical diabetes. Indeed, growing biologic and epidemiologic evidence suggests a more direct contribution of high insulin levels to the development of Alzheimer disease (AD) brain pathology (Watson et al., 2003; Farris et al., 2003) and risk of dementia (Peila et al., 2004; Luchsinger et al., 2004).

However, few studies to date have examined the effect of elevated mid-life insulin secretion on cognitive decline—independent of the presence of diabetes mellitus. Because cognitive impairment typically develops gradually (Linn et al., 1995), mid-life risk factors may be most relevant to identifying targets for prevention of late-life cognitive decline. In an earlier publication, we presented preliminary data indicating an association between higher mid-life levels of C-peptide, a measure of insulin secretion, and lower late-life cognitive performance in a sub-sample of 718 Nurses' Health Study (NHS) participants without diabetes (Okereke et al., 2005). However, in the previous publication, participants had only one to two assessments of cognition, with <2 years of follow-up; this was a very short period for detecting meaningful cognitive decline, and the trajectory of decline is not well-measured with only two datapoints (Morris et al., 1999). Thus, in the present study, we substantially extend our previous work by increasing the follow-up to 4.4 years, including three repeated measures of cognition, and substantially increasing the sample size to approximately 1200 women without diabetes.

2. Methods

2.1. Study population

The NHS included 121,700 US female registered nurses, aged 30–55 years at the study's inception in 1976. Since then, participants have completed biennial mailed questionnaires updating information on lifestyle factors and health outcomes. Between 1989 and 1990, 32,826 NHS participants provided blood samples; health and lifestyle characteristics were very similar between the whole cohort and those who returned blood samples (Okereke et al., 2005). Total follow-up for women who provided blood samples exceeds 98%.

From 1995 to 2001, participants aged 70 years and over, and free of diagnosed stroke, were invited to participate in a telephone-based study of cognitive function, and 19,395 (93.3% of those eligible) completed an initial cognitive assessment. Since then, two follow-up cognitive assessments have been performed an average of 2 years apart. Follow-up remains over 90% for cognitive study participants.

Using stored blood samples from 1989 to 1990, we performed C-peptide measurements in a random sample of 1195 cognitive study participants who had provided a fasting blood sample, did not have diagnosed diabetes at blood draw, and had completed at least one follow-up cognitive assessment. Participation rates in the cognitive study were similar in those who had and had not provided blood, suggesting little possibility for bias in examining associations in those providing blood samples (Okereke et al., 2005). We excluded eight women who reported high levels of alcohol intake ($\geqslant 60$ g daily), as this potentially has a strong influence on both C-peptide levels as well as cognitive performance. Thus, the population for analysis was 1187 women.

The study was approved by the Institutional Review Board of Brigham and Women's Hospital, Boston, MA.

2.2. Measurement of fasting plasma C-peptide

C-peptide is cleaved in a 1:1 ratio in the conversion of proinsulin to insulin and provides an accurate representation of insulin secretion (Polonsky and Rubenstein, 1984; Wahren et al., 2000). Blood samples from the 1989 to 1990 blood collection had been stored at $-130\,°C$ or colder; plasma C-peptide was measured using antiserum M1230 in an alcohol precipitation non-equilibrium assay (Faber et al., 1978) with reagents provided by Diagnostic Systems Laboratory (Webster, Texas). Assays were run in a single batch, and in blinded quality control tests, the mean intra-assay coefficient of variation was 5.1%. In addition, a previous study demonstrated a within-person correlation coefficient of 0.57 between C-peptide measurements taken 4 years apart from a sample of health professionals (Ma et al., 2004), suggesting that one-time measures of C-peptide

provide a reasonably stable representation of participants' mid-life C-peptide levels.

2.3. Assessment of cognitive function

Participants were administered: the Telephone Interview for Cognitive Status (TICS) (Brandt et al., 1988), a test of general cognition similar to the Mini-Mental State Examination (MMSE) (Folstein et al., 1975); immediate and delayed paragraph recalls of the East Boston Memory Test (EBMT) (Albert et al., 1991); a test of category fluency, in which women named as many different animals as possible during 1 min; a delayed recall of the TICS 10-word list; and a digit span task, in which women repeated backward increasingly long series of digits, to evaluate attention and working memory.

Primary outcomes were general cognition and verbal memory—verbal memory, in particular, is a strong predictor of early AD (Small et al., 2000; Chen et al., 2001). To assess general cognition, we considered the TICS, as well as a global cognitive score, calculated by averaging the z scores of all six cognitive tests. For the verbal memory score, we combined the results of the immediate and delayed recalls of both the EBMT and the TICS 10-word list, by averaging z scores of those four tests (Okereke et al., 2005). Global and verbal memory scores were only calculated for participants who had completed all component tests.

2.4. Reliability and validity of telephone-based cognitive assessments

In a test of instrument reliability, we administered the TICS twice to a sample of women at an interval of 1 month and found a Pearson correlation of 0.70 ($p < 0.001$). Examining inter-rater reliability (interviewers all scored the same cognitive assessment), we found intraclass correlations > 0.95 on each test.

In a validity study, 61 women who had completed an extensive in-person interview were administered our brief telephone-administered battery; we found a correlation of 0.81 comparing overall performance on our telephone-based tests to overall performance measured from the in-person interview. Furthermore, among 88 older health professionals, cognitive impairment as determined by our telephone method was strongly associated with clinically diagnosed dementia 3 years later: poor performance on the TICS and in verbal memory was associated with significant 8- and 12-fold increased risks, respectively, of dementia—demonstrating strong clinical validity of our telephone assessment (Kang et al., 2006).

2.5. Data analysis

To examine the association of C-peptide levels with cognitive decline, quartiles of C-peptide were constructed, and the lowest quartile was used as the reference category. We also examined C-peptide as a continuous variable with the unit of analysis as a one standard deviation increment in C-peptide level (standard deviation = 0.32 nmol/L).

We used linear mixed-effects models (Laird and Ware, 1982) to examine the relation of C-peptide levels to change in cognitive test scores across three assessments. In the basic mixed models, time since baseline interview (in years), age, highest attained education, C-peptide quartile, as well as interaction terms for time by age, and time by C-peptide quartile were included as fixed effects. Multi-variable-adjusted mixed models also included the following potential confounders as fixed effects: body mass index (BMI, in kg/m^2) categories (normal/overweight/obese), current smoking (yes/no), history of hypertension (yes/no), history of elevated cholesterol (yes/no), alcohol use (grams/day), physical activity (metabolic equivalents/week), current postmenopausal hormone use (yes/no) and history of antidepressant use (yes/no); all covariates were determined as of the time of blood draw, except age and antidepressant use, which were determined as of the baseline cognitive interview. Because depression is an important potential confounder of cognition, we evaluated multivariable models that further adjusted for depression using Medical Outcomes Short Form-36 (Stewart et al., 1988) mental health index scores, which were available in most participants; results were identical, and thus we did not include these scores in the final models. In addition to the fixed effects, we included two person-specific random effects in all models: baseline cognitive level (random intercept) and rate of change (random slope). In models using C-peptide as a categorical variable, the interaction terms for time and C-peptide quartile represent the annual rate of cognitive decline associated with that quartile, compared to the first quartile, across the three assessments (mean interval from first to third assessment = 4.4 years, range = 2.9–7.7 years). Similarly, in models treating C-peptide as a continuous variable, the interaction terms for time and C-peptide represent annual rates of cognitive decline associated with each one-SD increment in plasma C-peptide.

Three a priori, secondary analyses were conducted. First, due to the strong correlation between C-peptide level and BMI (Harris et al., 2002), we created multivariable models including and excluding BMI, to assess the impact of this variable on results. Second, to evaluate whether incipient diabetes, or "pre-diabetes," at the time of blood draw might explain any observed association between C-peptide and cognitive decline approximately 10 years later, we excluded all women who developed type 2 diabetes between blood draw and the initial cognitive assessment ($n = 41$). Finally, given the strong association between insulin resistance and cardiovascular risk (Hsueh and Quinones, 2003; Kernan and Inzucchi, 2004) and its potential influence on any observed relation between C-peptide and cognitive decline, we performed a secondary analysis excluding women who had any history of heart disease before or stroke as of the final cognitive assessment ($n = 62$).

3. Results

Table 1 shows the characteristics of the study population at blood draw, across quartiles of C-peptide. Women in the highest C-peptide quartile were generally less healthy than those in the lowest quartile: higher C-peptide was associated with higher BMI, prevalence of hypertension and

Table 1 Characteristics of the study population at blood draw, by quartiles of fasting C-peptide*

Characteristic	1st quartile	2nd quartile	3rd quartile	4th quartile
Mean C-peptide (nmol/L)	0.36	0.52	0.69	1.11
Mean age at interview (years)	73.7	73.7	73.7	74.0
Mean age at blood draw (years)	64.1	64.0	64.2	64.4
Mean body mass index (kg/m^2)	22.6	24.2	25.4	27.7
Master's degree or higher education	8.4	7.8	7.1	5.7
History of hypertension	22.2	25.0	33.3	49.2
History of elevated cholesterol	26.9	34.8	34.3	40.4
Current smoking	6.4	9.1	7.1	10.4
Past smoking	36.4	39.9	41.6	46.1
Current hormone use	43.0	37.4	31.4	26.2
Past hormone use	22.4	26.3	28.6	33.3
History of antidepressant use[†]	6.7	3.7	7.4	7.7
Mean exercise level (METS/week)	18.2	17.4	17.4	14.2
Mean alcohol intake (g/day)	4.9	4.8	5.4	4.1

*Figures are expressed as percentages, unless otherwise indicated.
[†]Antidepressant use is as of baseline cognitive testing.

elevated cholesterol, and with lower physical activity. Also, women in the fourth C-peptide quartile were less likely to be using postmenopausal hormones than those in the first quartile.

Results from the linear mixed-effects models (Table 2) demonstrated that higher C-peptide levels were associated with faster cognitive decline across three assessments (over an average of 4.4 years). Compared to the first quartile, the multivariable-adjusted annual rates of decline (95% CI) for women in the fourth quartile were lower by -0.03 $(-0.06, -0.00; p = 0.03)$ standard units/year on the global score and -0.05 $(-0.09, -0.02; p = 0.004)$ standard units/year on verbal memory; in addition, there were significant trends of increasing levels of C-peptide and greater rates of cognitive decline for TICS (p-trend = 0.05), global score (p-trend = 0.04), and verbal memory (p-trend = 0.006). To help interpret these effect estimates, we compared the relation of C-peptide with cognitive decline to the relation of age with rates of cognitive decline in our cohort. We found that each year of age was associated with a greater annual rate of decline by -0.01 units on the global score; thus, our finding of greater annual decline of -0.03 units comparing the highest to lowest C-peptide quartile would indicate that higher levels of C-peptide are cognitively equivalent to 3 years of aging.

In separate analyses, we constructed multivariable models that removed BMI as a covariate; results were unchanged compared to those presented above, where BMI was included in the model: e.g., the mean differences in annual rates of decline associated with being in the fourth C-peptide quartile, compared to the first quartile, were -0.03 $(-0.06, -0.00; p = 0.03)$ units/year on the global score and -0.05 $(-0.09, -0.02; p = 0.004)$ units/year on verbal memory. Findings were nearly identical in secondary analyses that excluded women who developed type 2 diabetes after blood draw ($n = 41$): for example, the mean differences in the multivariable-adjusted annual rates of decline for each one-SD increase in C-peptide were -0.04

points $(-0.09, -0.00; p$-trend $= 0.03)$ on the TICS, -0.01 units $(-0.02, -0.00; p$-trend $= 0.03)$ on global score, and -0.02 units $(-0.03, -0.01; p$-trend $= 0.005)$ on verbal memory. Finally, results were also similar after excluding the 62 women with any history of coronary disease or who were diagnosed with coronary disease or stroke as of the final cognitive assessment (data for secondary analyses not shown in tables).

4. Discussion

In this study of community-dwelling women without type 2 diabetes mellitus, we found that higher mid-life levels of plasma C-peptide—a marker of insulin secretion—were associated with significantly greater late-life decline in general cognition and verbal memory over an average of 4 years. Specifically, being in the highest C-peptide quartile appeared to be cognitively equivalent to aging by 3 years. Associations remained strong after adjustment for numerous potential confounding factors—including vascular factors, such as hypertension and dyslipidemia—and after exclusion of women who were diagnosed either with diabetes at any time after C-peptide was measured, with any history of coronary disease, or with coronary disease or stroke as of the final cognitive assessment.

Our findings are consistent with previous epidemiologic studies linking higher insulin levels to cognitive impairment (Kalmijn et al., 1995; Okereke et al., 2005, 2006) and cognitive decline (Luchsinger et al., 2004) in older adults. We previously reported significant cross-sectional relations between higher C-peptide levels and greater general cognitive and verbal memory impairment among 718 NHS participants (Okereke et al., 2005); similarly, in a cross-sectional study involving 386 elderly men without diabetes, Kalmijn et al. (1995) reported that those in the highest quartile of fasting insulin had 25% more errors on the MMSE compared with those in the lowest quartile, with a trend of

Cognitive test	1st quartile	2nd quartile	3rd quartile	4th quartile	Per SD* increase in C-peptide
Age and education-adjusted†					
TICS	0.00	0.03 (−0.08, 0.14) $p = 0.58$	0.03 (−0.08, 0.14) $p = 0.54$	−0.03 (−0.14, 0.07) $p = 0.53$	−0.03 (−0.07, 0.01) p-trend = 0.11
Global score	0.00	−0.02 (−0.05, 0.01) $p = 0.17$	−0.03 (−0.06, 0.00) $p = 0.05$	−0.03 (−0.06, 0.00) $p = 0.05$	−0.01 (−0.02, 0.00) p-trend = 0.07
Verbal score	0.00	−0.04 (−0.07, −0.01) $p = 0.01$	−0.05 (−0.08, −0.01) $p = 0.01$	−0.05 (−0.08, −0.01) $p = 0.01$	−0.02 (−0.03, −0.00) p-trend = 0.02
Multivariable-adjusted‡					
TICS	0.00	0.02 (−0.09, 0.13) $p = 0.70$	−0.04 (−0.15, 0.08) $p = 0.53$	−0.06 (−0.17, 0.05) $p = 0.31$	−0.04 (−0.08, −0.00) p-trend = 0.05
Global score	0.00	−0.02 (−0.05, 0.01) $p = 0.13$	−0.03 (−0.06, −0.00) $p = 0.03$	−0.03 (−0.06, −0.00) $p = 0.03$	−0.01 (−0.02, −0.00) p-trend = 0.04
Verbal score	0.00	−0.05 (−0.08, −0.01) $p = 0.007$	−0.05 (−0.08, −0.01) $p = 0.008$	−0.05 (−0.09, −0.02) $p = 0.004$	−0.02 (−0.03, −0.00) p-trend = 0.006

Table 2 Mean differences in annual rates of decline over 4 years, by fasting C-peptide levels ($n = 1187$).

*SD = standard deviation (0.32 nmol/L).

†Adjusted for age at baseline interview and education.

‡Adjusted for age at baseline interview and education; cigarette smoking, postmenopausal hormone use, hypertension, elevated cholesterol, body mass index, alcohol intake and physical activity level as of blood draw; and antidepressant use as of cognitive testing.

increasing errors with increasing fasting insulin (p-trend = 0.02). Other studies (Vanhanen et al., 1998; Yaffe et al., 2004) have suggested associations between indicators of abnormal insulin (e.g., impaired glucose tolerance) and cognitive impairment: for example, Yaffe et al. (2004) reported significantly higher risk of developing cognitive impairment among older women with impaired fasting glucose but without diabetes, compared to those with normal glucose. Finally, Luchsinger et al. (2004) reported an association between hyperinsulinemia and significantly greater decline in memory scores, but not in other cognitive domains, among 683 older, community-dwelling adults; however, this sample did include people with diabetes, and participants were older when insulin levels were obtained (mean age = 76.2 years). While consistent with these previous reports, the current study represents an important advance in the literature, as it is a large-scale prospective study, involves only participants without diabetes, and relates mid-life insulin secretion levels to later-life cognitive decline over repeated assessments.

Higher insulin levels may impact cognitive function indirectly through many mechanisms, including vascular damage associated with insulin resistance even in the absence of diabetes (Hsueh and Quinones, 2003; Geroldi et al., 2005); thus, despite our ability to account for major vascular factors such as coronary disease, stroke and hypertension in our analyses, the impact on cognition of the micro-vascular changes associated with high insulin levels cannot be excluded in the current study, or in the others mentioned above. Nevertheless, there is growing biological evidence that more direct effects of elevated insulin may exist. For example, a leading hypothesis suggests that high levels of insulin in the brain could interfere with metabolism of amyloid beta (Aβ, the primary component of neuritic plaques which are central to AD pathology) (Hardy and Selkoe, 2002) through their impact on the insulin degrading enzyme (IDE, the major enzyme responsible for insulin degradation and also an Aβ-degrading protease) (Farris et al., 2003). Because IDE binds more readily to insulin relative to other substrates (Duckworth et al., 1998), insulin acts as a competitive inhibitor of Aβ degradation; thus, higher insulin levels may interfere with Aβ clearance, resulting in higher Aβ concentrations in the brain (Watson et al., 2003).

Strengths of the current study include the large sample size, high rate of follow-up, and abundant information on potential confounding factors. In addition, participants had three repeated assessments of cognitive function over an average of > 4 years, allowing us to examine the relation of insulin secretion levels to paths of cognitive change over time. Finally, C-peptide was measured from the blood collected in mid-life—an average of 10 years prior to the start of cognitive testing; since cognitive impairment appears to take many years to develop (Linn et al., 1995), the level of exposure at these younger ages may be most relevant to dementia risk and ultimately to targeting dementia prevention.

Potential limitations of our study should also be considered. First, we had a single measurement of C-peptide, which may have increased measurement error; however, such random error would lead, if anything, to

underestimation of the association between the C-peptide and cognitive decline. Second, we utilized a telephone-based instrument rather than in-person testing; however, this cognitive battery has been demonstrated to have excellent reliability and validity. Third, we relied on self-report to exclude diabetic women; however, high validity of these participants' reports of diabetes have been established (Manson et al., 1991). Nevertheless, our sample may have included women with undiagnosed diabetes; thus, a strong association between diabetes and cognitive decline may partly explain our findings. This is unlikely, however, as our results remained similar when we excluded all women who were diagnosed with diabetes during approximately 10 years of follow-up after blood draw, likely excluding any women with undiagnosed diabetes at time of blood draw. Furthermore, in a validation sample of 200 randomly selected NHS participants who never reported diabetes, only 1 woman had a plasma fasting glucose or fructosamine level in the diabetic range. Fourth, the generalizability of our findings is a potential concern: although basic biological relations observed in this largely Caucasian sample of well-educated women are likely comparable to those seen among women in the general population, further research involving ethnic minorities would be necessary to address potential differences in other populations. Finally, although we were able to adjust for numerous potential confounders, residual confounding is still possible, as may occur in any observational study. However, the relative homogeneity of our cohort reduces potential influences of many unmeasured confounders, such as health knowledge and access to care.

In conclusion, our study provides evidence for an association between elevated mid-life insulin secretion—in the absence of diabetes mellitus or clinical stroke—and late-life cognitive decline measured over several years. This association clearly requires further investigation, as addressing insulin regulation and secretion levels could lead to exciting preventive tools to combat cognitive decline and potentially AD.

Role of the funding sources

Acknowledgments

References

Albert, M., Smith, L.A., Scherr, P.A., Taylor, J.O., Evans, D.A., Funkenstein, H.H., 1991. Use of brief cognitive tests to identify individuals in the community with clinically diagnosed Alzheimer's disease. Int. J. Neurosci. 57, 167–178.

Brandt, J., Spencer, M., Folstein, M.F., 1988. The telephone interview for cognitive status. Neuropsychiatry, Neuropsychol. Behav. Neurol. 1, 111–117.

Chen, P., Ratcliff, G., Belle, S.H., Cauley, J.A., DeKosky, S.T., Ganguli, M., 2001. Patterns of cognitive decline in presymptomatic Alzheimer disease: a prospective community study. Arch. Gen. Psychiatry 58, 853–858.

Duckworth, W.C., Bennett, R.G., Hamel, F.G., 1998. Insulin acts intracellularly on proteasomes through insulin-degrading enzyme. Biochem. Biophys. Res. Commun. 244, 390–394.

Faber, O.K., Binder, C., Markussen, J., Heding, L.G., Naithani, V.K., Kuzuya, H., Blix, P., Horwitz, D.L., Rubenstein, A.H., 1978. Characterization of seven C-peptide antisera. Diabetes 27 (Suppl 1), 170–177.

Farris, W., Mansourian, S., Chang, Y., Lindsley, L., Eckman, E.A., Frosch, M.P., Eckman, C.B., Tanzi, R.E., Selkoe, D.J., Guenette, S., 2003. Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. Proc. Natl. Acad. Sci. USA 100, 4162–4167.

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. Mini-mental state. A practical method for grading the cognitive state of patients for the clinician. J. Psychiatry Res. 12, 189–198.

Geroldi, C., Frisoni, G.B., Paolisso, G., Bandinelli, S., Lamponi, M., Abbatecola, A.M., Zanetti, O., Guralnik, J.M., Ferrucci, L., 2005. Insulin resistance in cognitive impairment: the InCHIANTI study. Arch. Neurol. 62, 1067–1072.

Hardy, J., Selkoe, D.J., 2002. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297, 353–356.

Harris, M.I., Cowie, C.C., Gu, K., Francis, M.E., Flegal, K., Eberhardt, M.S., 2002. Higher fasting insulin but lower fasting C-peptide levels in African Americans in the US population. Diabetes Metab. Res. Rev. 18, 149–155.

Hsueh, W.A., Quinones, M.J., 2003. Role of endothelial dysfunction in insulin resistance. Am. J. Cardiol. 92 (Suppl), 10J–17J.

Kalmijn, S., Feskens, E.J., Launer, L.J., Stijnen, T., Kromhout, D., 1995. Glucose intolerance, hyperinsulinaemia and cognitive function in a general population of elderly men. Diabetologia 38, 1096–1102.

Kang, J.H., Cook, N., Manson, J., Buring, J.E., Grodstein, F., 2006. A randomized trial of vitamin E supplementation and cognitive function in women. Arch. Intern. Med. 166, 2462–2468.

Kernan, W.N., Inzucchi, S.E., 2004. Type 2 diabetes mellitus and insulin resistance: stroke prevention and management. Curr. Treat. Options Neurol. 6, 443–450.

Laird, N.M., Ware, J.H., 1982. Random-effects models for longitudinal data. Biometrics 38, 963–974.

Linn, R.T., Wolf, P.A., Bachman, D.L., Knoefel, J.E., Cobb, J.L., Belanger, A.J., Kaplan, E.F., D'Agostino, R.B., 1995. The "preclinical phase" of probable Alzheimer's disease. Arch. Neurol. 52, 485–490.

Luchsinger, J.A., Tang, M.X., Shea, S., Mayeux, R., 2004. Hyperinsulinemia and risk of Alzheimer disease. Neurology 63, 1187–1192.

Ma, J., Giovannucci, E., Pollak, M., Leavitt, A., Tao, Y., Gaziano, J.M., Stampfer, M.J., 2004. A prospective study of plasma C-peptide and colorectal cancer risk in men. J. Natl. Cancer Inst. 96, 546–553.

Manson, J.E., Stampfer, M.J., Colditz, G.A., Willett, W.C., Rosner, B., Hennekens, C.H., Speizer, F.E., Rimm, E.B., Krolewski, A.S., 1991. Physical activity and incidence of non-insulin-dependent diabetes mellitus in women. Lancet 338, 774–778.

Morris, M.C., Evans, D.A., Hebert, L.E., Bienias, J.L., 1999. Methodological issues in the study of cognitive decline. Am. J. Epidemiol. 149, 789–793.

Okereke, O., Hankinson, S.E., Hu, F.B., Grodstein, F., 2005. Plasma C-peptide level and cognitive function among older women without diabetes mellitus. Arch. Intern. Med. 165, 1651–1656.

Okereke, O., Kang, J.H., Gaziano, J.M., Ma, J., Stampfer, M.J., Grodstein, F., 2006. Plasma C-peptide and cognitive performance in older men without diabetes. Am. J. Geriatr. Psychiatry 14, 1041–1050.

Ott, A., Stolk, R.P., van Harskamp, F., Pols, H.A., Hofman, A., Breteler, M.M., 1999. Diabetes mellitus and the risk of dementia: the rotterdam study. Neurology 53, 1937–1942.

Peila, R., Rodriguez, B.L., Launer, L.J., 2002. Honolulu-Asia Aging Study. Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: the Honolulu-Asia aging study. Diabetes 51, 1256–1262.

Peila, R., Rodriguez, B.L., White, L.R., Launer, L.J., 2004. Fasting insulin and incident dementia in an elderly population of Japanese-American men. Neurology 63, 228–233.

Polonsky, K.S., Rubenstein, A.H., 1984. C-peptide as a measure of the secretion and hepatic extraction of insulin: pitfalls and limitations. Diabetes 33, 486–494.

Small, B.J., Fratiglioni, L., Viitanen, M., Winblad, B., Backman, L., 2000. The course of cognitive impairment in preclinical Alzheimer disease: three- and 6-year follow-up of a population-based sample. Arch. Neurol. 57, 839–844.

Stewart, A.L., Hays, R.D., Ware Jr., J.E., 1988. The MOS short-form general health survey: reliability and validity in a patient population. Med. Care 26, 724–735.

Vanhanen, M., Koivisto, K., Kuusisto, J., Mykkanen, L., Helkala, E.L., Hanninen, T., Riekkinen Sr., P., Soininen, H., Laakso, M., 1998. Cognitive function in an elderly population with persistent impaired glucose tolerance. Diabetes Care 21, 398–402.

Wahren, J., Ekberg, K., Johansson, J., Henriksson, M., Pramanik, A., Johansson, B.L., Rigler, R., Jornvall, H., 2000. Role of C-peptide in human physiology. Am. J. Physiol. Endocrinol. Metab. 278, E759–E768.

Watson, G.S., Peskind, E.R., Asthana, S., Purganan, K., Wait, C., Chapman, D., Schwartz, M.W., Plymate, S., Craft, S., 2003. Insulin increases CSF A beta 42 levels in normal older adults. Neurology 60, 1899–1903.

Yaffe, K., Blackwell, T., Kanaya, A.M., Davidowitz, N., Barrett-Connor, E., Krueger, K., 2004. Diabetes, impaired fasting glucose, and development of cognitive impairment in older women. Neurology 63, 658–663.