Plasma C Peptide Level and Cognitive Function Among Older Women Without Diabetes Mellitus

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Background: Growing evidence suggests that type 2 diabetes mellitus and hyperinsulinemia may be related to diminished cognition. To help differentiate between the effects of diabetes and insulin, we examined the relation of insulin to cognitive function among nondiabetic participants of the Nurses’ Health Study.

Methods: We measured the C peptide level, representing insulin secretion, in blood samples provided by 718 women from June 14, 1989, to October 4, 1990, when they were aged 61 to 69 years. We administered telephone interviews an average of 10 years after blood collection, testing general cognition, verbal memory, category fluency, and attention; second cognitive assessments were conducted 2 years later. The primary outcomes were global cognitive function across all tests and verbal memory. We used regression models to estimate multivariable-adjusted mean differences in cognitive function and cognitive decline, and odds of cognitive impairment, across C peptide levels.

Results: Cognitive function was worse among women in the fourth C peptide quartile compared with those in the first quartile (eg, on the global score combining all cognitive tests, the multivariable-adjusted mean difference was −1.7 standard units [95% confidence interval, −2.9 to −0.6 standard units]; P = .002); the odds of cognitive impairment (defined as the worst 10% of the distribution) were 3-fold higher among women in the fourth quartile (95% confidence interval, 1.3-7.8). On verbal memory, women in the fourth quartile scored significantly worse than those in the first quartile; the odds of impairment were 2.8-fold higher (95% confidence interval, 1.1-7.0). Consistent findings were observed for cognitive decline.

Conclusion: Higher insulin secretion may be related to worse cognition, even among those without diabetes.

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DECREASING ALZHEIMER DISEASE is a public health priority. Identifying mutable factors in the early stages of preclinical dementia may be critical to effective prevention.1,2 A small impairment in cognition among healthy older individuals strongly predicts dementia development,3,5 and may be considered a marker of preclinical dementia.

Epidemiologic studies4 have identified type 2 diabetes mellitus as an important possible risk factor for diminished cognition. Growing evidence suggests the impact of diabetes on cognition may not be due entirely to cerebrovascular damage that often accompanies diabetes; for example, adjustment for vascular disease generally has little influence on results. Moreover, those with type 2 diabetes have increased hippocampal atrophy (the hippocampus is the key brain region for learning and memory), independent of vascular morbidity, on magnetic resonance imaging.5 Type 2 diabetes is initially characterized by elevated insulin levels, and insulin receptors are concentrated in the hippocampus.8 In animal and human models,5,11 an elevated insulin level increases the amyloid β (Aβ) level; Aβ accumulation is implicated in the pathogenesis of Alzheimer disease.12

To explore the effects of insulin independent of diabetes, we examined the relation of insulin in nondiabetic women in their early 60s to cognitive function in later life.

METHODS

NURSES’ HEALTH STUDY

The Nurses’ Health Study includes 121 700 US female nurses, aged 30 to 55 years at its inception in 1976. Participants complete biennial mailed questionnaires updating information on lifestyle and health. From June 1, 1988, to May 31, 1990, blood samples were requested from participants, and one third agreed to provide them.13 Health and lifestyle characteristics were similar between the whole cohort and those who returned blood samples (eg, 43% of the entire cohort vs 46.2% who provided blood never smoked and the mean alcohol intake was 5 g/d for both groups). Total follow-up for these women exceeds 98.0%.
COGNITIVE FUNCTION ASSESSMENT

From February 1995 to July 2001, Nurses' Health Study subjects 70 years and older, free of diagnosed stroke, participated in a telephone cognitive assessment. Of the 95.1% for whom we had telephone numbers, 93.3% completed the interview (n=19,395). A follow-up cognitive assessment began 2 years later and is virtually complete; so far, 92.9% completed follow-up and 7.1% refused or could not be located. Of these women, 6861 had provided a blood sample. Participation and follow-up in the cognitive study were similar in those who had and had not provided blood, suggesting little possibility for bias in examining associations within those providing blood samples.

In initial interviewing, we used only the Telephone Interview for Cognitive Status (TICS), a telephone version of the Mini-Mental State Examination. We gradually added 5 other cognitive tests; thus, the sample size differs somewhat for each test. We administered the following: immediate and delayed recalls of the East Boston Memory Test to assess verbal memory; a test of category fluency, in which women named animals during 1 minute; a delayed recall of the TICS 10-word list; and digit span backward, in which women repeated backward increasingly long series of digits, to evaluate attention and working memory.

The 2 primary outcomes were general cognition and verbal memory (studies have established that verbal memory is a strong predictor of Alzheimer disease development). For assessing general cognition, we considered the TICS, and calculated a global score combining our 6 cognitive tests. This global score was calculated by summing the z scores for each test. We calculated a verbal memory score by combining results of the immediate and delayed recalls of the East Boston Memory Test and the TICS 10-word list, using z scores. The global and verbal memory scores were only calculated for subjects who completed all component tests. Such composite scores are regularly used in cognitive research because they integrate information from various sources and, thus, provide a more stable representation of cognition than a single test.

VALIDITY AND RELIABILITY OF TELEPHONE ASSESSMENTS

We found a correlation of 0.81 comparing overall performance on our brief telephone interview with overall performance measured from an in-person interview in well-educated women, establishing high validity of our telephone method. We also found that the yearly rate of cognitive decline measured in our subjects was quite similar to that observed in a similar cohort using in-person interviews, indicating our measurement of decline is valid and accurate. In tests of instrument reliability, we administered the TICS twice to 61 Nurses' Health Study subjects at an interval of 1 month and found a correlation of 0.70 (P < .001).

ASCERTAINMENT OF INSULIN SECRETION

C peptide is cleaved in a 1:1 ratio in the conversion of proinsulin to insulin and provides an accurate representation of insulin secretion. Although insulin and C peptide are secreted in equimolar amounts, C peptide is not excreted by the liver and its half-life in the circulation is 2 to 5 times longer than insulin; therefore, C peptide is a more stable indicator of insulin secretion.

As part of nested case-control studies of type 2 diabetes, breast cancer, and hypertension, C peptide levels were measured in 791 women who also were subjects in the cognitive study. Blinded quality control specimens were included in each batch of samples to assess variation across batches. C peptide for all studies was assayed using reagents provided by Diagnostic Systems Laboratory, Webster, Tex. The C peptide level was measured using antiserum M1230 in an alcohol precipitation non-equilibrium assay. In blinded quality control tests, we found an intra-assay coefficient of variation of 2% and an interassay coefficient of variation of 7%.

POPULATION FOR ANALYSIS

Among women in the cognitive study who had C peptide measured in nested case-control studies of diabetes, breast cancer, or hypertension, we excluded 73 with diabetes: women who were cases in the diabetes case-control study and women in the case-control studies of hypertension and breast cancer who had been diagnosed as having diabetes as of the blood collection. Information on diabetes was provided via the biennial mailed questionnaires. Self-reported diabetes from these nurse participants was 98.2% accurate compared with medical records, and in 2 random samples of subjects without diagnosed diabetes, less than 2.0% had diagnostic evidence of diabetes in their blood sample. We did not exclude cases of breast cancer or hypertension, because neither was related to cognition in our data and because analyses excluding these cases yielded similar results to analyses including them. Thus, analyses presented herein are based on 718 subjects.

The characteristics of these 718 women were similar to those of the entire cognitive study. For example, mean age at cognitive assessment was 74 years in both groups and median body mass index (calculated as weight in kilograms divided by the square of height in meters) was 25 in both. For educational attainment, 71.9% had an associate’s degree and 7.1% an advanced graduate degree among those with C peptide measured, compared with 77.8% and 5.7%, respectively, in the overall cognitive cohort. Thus, despite using a convenience sample for these analyses, we did not find a likelihood of any meaningful bias.

STATISTICAL ANALYSIS

In primary analyses, we examined cognition across quartiles of C peptide. Because of batch-to-batch variation, we calcu-
Cognitive performance approximately 10 y after blood draw†

<table>
<thead>
<tr>
<th>Variable</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category fluency (points)</td>
<td>18.1 (5.0)</td>
<td>16.6 (4.5)</td>
<td>17.3 (5.2)</td>
<td>17.1 (4.5)</td>
</tr>
<tr>
<td>Digit span backward (points)</td>
<td>7.3 (2.5)</td>
<td>6.5 (2.2)</td>
<td>7.0 (2.4)</td>
<td>6.7 (2.5)</td>
</tr>
<tr>
<td>East Boston Memory Test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate recall (points)</td>
<td>9.6 (1.7)</td>
<td>9.4 (1.9)</td>
<td>9.6 (1.8)</td>
<td>9.3 (1.9)</td>
</tr>
<tr>
<td>Delayed recall (points)</td>
<td>9.2 (1.9)</td>
<td>8.9 (2.1)</td>
<td>9.2 (2.3)</td>
<td>8.8 (2.2)</td>
</tr>
<tr>
<td>TICS Overall (points)</td>
<td>34.3 (2.6)</td>
<td>33.8 (2.7)</td>
<td>33.9 (2.8)</td>
<td>33.6 (2.9)</td>
</tr>
<tr>
<td>10-Word list</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate recall (points)</td>
<td>4.9 (1.7)</td>
<td>4.6 (1.6)</td>
<td>4.7 (1.7)</td>
<td>4.5 (1.7)</td>
</tr>
<tr>
<td>Delayed recall (points)</td>
<td>2.9 (2.1)</td>
<td>2.3 (2.0)</td>
<td>2.1 (2.0)</td>
<td>2.2 (2.0)</td>
</tr>
</tbody>
</table>

Abbreviation: TICS, Telephone Interview for Cognitive Status.

†Data are given as mean (SD).

RESULTS

There was a broad distribution of C peptide levels among our subjects (Table 1); the median C peptide level in the fourth quartile was more than 4 times greater than that in the first quartile. Characteristics, including age and educational attainment, were generally similar across C peptide quartiles. However, the prevalence of hypertension increased with increasing C peptide level, and women in the third and fourth quartiles of C peptide used hormone therapy less often than those in the lower quartiles. Compared with women in the first quartile of C peptide, women in the fourth quartile had worse mean performance on all our cognitive tests.

After adjustment for age and educational attainment (Table 2), we found statistically significantly worse performance on the verbal memory and global scores in the second through fourth quartiles of C peptide, compared

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lateral separate quartile cut points for each assay batch. In analyses of cognitive function, we used linear regression to estimate multivariable-adjusted mean differences in performance across C peptide quartiles. We also calculated odds ratios of cognitive impairment using logistic regression; cognitive impairment was defined as a TICS score of less than 31 (based on a preestablished cut point) or the bottom 10% of the distribution for the global and verbal memory scores. Such a population-based 10% cut point is commonly used in cognitive research and has high sensitivity and specificity for cognitive impairment. We also examined C peptide as a continuous variable; the unit was a batch-specific 1-SD difference in C peptide level (average SD, 1.32 ng/mL [0.44 nmol/L]).

We also examined cognitive decline over 2 years. However, this short follow-up is in contrast to the average 10 years between blood collection and initial cognitive testing; thus, we likely underestimate relations between baseline C peptide level and cognitive decline. We used linear regression to calculate adjusted mean differences in cognitive decline across quartiles of C peptide.

In regression models, we included the following potential confounding variables: age, educational attainment, history of hypertension, postmenopausal hormone therapy, vitamin E supplementation, cigarette smoking, antidepressant use, and alcohol intake. In analyses of cognitive decline, we also adjusted for cognitive performance at the initial interview and for the interval between interviews. Information on potential confounding variables was determined as of the time of blood collection.

Several secondary models were constructed. In one, we adjusted for body mass index; this was not included in primary analyses because body mass index is among the strongest determinants of insulin levels and, thus, inclusion in models would amount to partial adjustment for our exposure. In other analyses, we adjusted for fasting status at blood draw (75.4% of samples were fasting) and excluded women diagnosed as having diabetes after the blood collection. We also constructed an alternative model, controlling for symptoms of depression using continuous scores from a validated mental health index.

Finally, to consider the influence of C peptide level on subsequent covariate status, we conducted an analysis updating information on potential confounding variables through the initial cognitive interview, rather than the time of blood draw.
Table 2. Mean Differences in Cognitive Function, According to Plasma C Peptide Quartile

<table>
<thead>
<tr>
<th>Cognitive Test†</th>
<th>Per SD Increase in C Peptide‡</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C Peptide Quartile</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.3 (−0.5 to −0.1)</td>
<td>0.0</td>
<td>−0.9 (−1.6 to −0.3)</td>
<td>−0.7 (−1.3 to 0.0)</td>
<td>−1.1 (−1.8 to −0.4)</td>
</tr>
<tr>
<td></td>
<td>Multivariable adjusted$§</td>
<td>0.0</td>
<td>−0.9 (−1.6 to −0.3)</td>
<td>−0.6 (−1.3 to 0.0)</td>
<td>−1.0 (−1.7 to −0.3)</td>
</tr>
<tr>
<td></td>
<td>$P$ value for trend</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>TICS (n = 718)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age and education adjusted</td>
<td>−0.2 (−0.4 to 0.0)</td>
<td>0.0</td>
<td>−0.5 (−1.0 to 0.1)</td>
<td>−0.4 (−0.9 to 0.2)</td>
</tr>
<tr>
<td></td>
<td>Multivariable adjusted$§</td>
<td>0.0</td>
<td>−0.5 (−1.1 to 0.1)</td>
<td>−0.3 (−0.9 to 0.3)</td>
<td>−0.6 (−1.2 to 0.0)</td>
</tr>
<tr>
<td></td>
<td>$P$ value for trend</td>
<td>0.05</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Global score (n = 574)</td>
<td>−0.5 (−0.9 to −0.1)</td>
<td>0.0</td>
<td>−1.9 (−2.9 to −0.8)</td>
<td>−1.3 (−2.3 to −0.2)</td>
</tr>
<tr>
<td></td>
<td>Multivariable adjusted$§</td>
<td>−0.4 (−0.8 to 0.0)</td>
<td>0.0</td>
<td>−1.9 (−3.0 to −0.9)</td>
<td>−1.2 (−2.2 to −0.1)</td>
</tr>
<tr>
<td></td>
<td>$P$ value for trend</td>
<td>0.03</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: NA, data not applicable; SD, standard deviation; TICS, Telephone Interview for Cognitive Status.
*Data are given as mean difference (95% confidence interval) unless otherwise indicated.
†The verbal memory score combines the results of immediate and delayed recalls of the East Boston Memory Test and the 10-word list; and the global score combines the results of the TICS, the category fluency test, digit span backward, and immediate and delayed recalls of the East Boston Memory Test and the 10-word list.
‡The average SD for C peptide is 1.32 ng/mL (0.44 nmol/L).
§Adjusted for age (in years), education (associate’s degree, bachelor’s degree, or master’s or doctoral degree), high blood pressure (yes or no), postmenopausal hormone therapy (current, past, or never), vitamin E use (yes or no), cigarette smoking (current, past, or never), use of antidepressants (yes or no), and alcohol intake (in tertiles).

with the first quartile. On the TICS, findings were slightly weaker, but mean scores among women in the fourth quartile of C peptide were significantly lower than among women in the first quartile. Further adjustment for various potential confounding factors had relatively little impact on results. For example, on the global score, after multivariable adjustment, women in the fourth C peptide quartile scored 1.7 standard units lower than those in the first quartile ($P = .002$). To help interpret these mean differences, we calculated the effect of age on cognitive performance. In our subjects, women 6 years apart in age had a mean difference of approximately 1.5 standard units on the global score; thus, being in the highest quartile of C peptide seemed cognitively equivalent to aging by 6 years. Furthermore, multivariable-adjusted analyses of C peptide as a continuous variable also indicated significant trends of worse cognition with increasing C peptide level. Each 1-SD increase in C peptide was associated with a mean difference of −0.4 standard units ($P = .03$ for trend) on the global score, or the approximate equivalent of aging by 2 years.

Analyses of the odds of cognitive impairment (Table 3) suggested the potential clinical importance of these differences in cognitive performance. Women in the fourth C peptide quartile had statistically significant, roughly 3-fold greater odds of impairment on verbal memory and the global score, compared with women in the first quartile. In analyses of C peptide as a continuous measure, we found significant trends of 30% to 50% increased odds of cognitive impairment with each 1-SD increase in C peptide level on the verbal memory and global scores.

In a secondary analysis, we evaluated whether eventual development of diabetes might explain our findings. We excluded all women who developed type 2 diabetes (an additional 35 women) between blood draw and the initial cognitive assessment, although in fact most such women were excluded in the primary analyses because they would have been cases in the nested case-control study of diabetes; results remained identical. In a separate analysis, we further adjusted for fasting status: C peptide measures are equally valid in fasting and nonfasting samples, but the absolute values of nonfasting samples may be slightly higher. However, these results were similar to primary findings, as were results excluding nonfasting samples. In analyses adjusted for body mass index, results were attenuated, as expected; nevertheless, there were significant differences in cognitive performance between those in the fourth vs first C peptide quartile (eg, on the global score, the multivariable-adjusted mean difference was −1.3 standard units [95% confidence interval, −2.4 to −0.1 standard units]). In addition, in a model further adjusting for depression using continuous scores from the mental health index, results were again identical. Finally, in models using covariates updated through the initial cognitive interview, findings were unchanged.

We had a short period for measuring cognitive decline (2 years), compared with an average 10 years between blood collection and initial cognitive testing. Thus, we likely underestimate the relation of C peptide level to cognitive decline. However, there was a suggestion of greater decline in performance with increasing C peptide level, generally supporting our findings from the initial cognitive testing. On the global score, after multivariable adjustment, there was a mean difference in decline of −0.5 standard units (95% confidence interval, −1.4 to 0.4 standard units) comparing the fourth with the first C peptide quartile, with a borderline significant trend of worse rates of decline with increasing C peptide quartile ($P = .1$) (data not shown).
COMMENT

Among women without diabetes, we found significantly worse cognitive function for those with higher compared with lower levels of C peptide. These findings persisted after adjustment for covariates, at blood draw and subsequent to blood draw, and persisted after exclusion of women who developed diabetes during the average 10 years between blood draw and cognitive testing. Specifically, being in the highest C peptide quartile seemed cognitively equivalent to aging by 6 years, and was associated with up to a 3-fold increased odds of cognitive impairment.

There are several possible explanations for these findings. An elevated insulin level and diminished cognition may share some common underlying cause, rather than having a direct relation to each other. Alternatively, higher insulin secretion, even before the development of diabetes, may lead to vascular damage and, thus, be an indirect source of cognitive impairment. However, accumulating evidence suggests a direct link between insulin level and cognition. A high insulin level may inhibit neuron firing and decrease the activity of choline acetyltransferase, an enzyme involved in forming neurotransmitters’ regulating memory and learning. In vitro studies indicate insulin causes a 3- to 4-fold increase in extracellular Aβ levels. The insulin-degrading enzyme provides a further possible link between insulin and Aβ levels. The insulin-degrading enzyme is primarily responsible for insulin degradation, but also degrades Aβ. In mice, insulin-degrading enzyme deficiency was associated with greater than 50% reduction in Aβ degradation, and cerebral Aβ accumulation was increased by up to 64% (the mice also developed a diabetic phenotype). Insulin binds more readily to insulin-degrading enzyme than other substrates, and is a competitive inhibitor of Aβ degradation; a recent investigation of 16 healthy older adults found that, on infusion of insulin, cerebrospinal fluid Aβ levels increased by 15% (P = .02).

Although relatively few large-scale epidemiologic studies have addressed this issue, existing data are consistent with our finding of a relation of elevated insulin level to cognition in nondiabetic persons. In a prospective cohort, 297 prediabetic women (fasting glucose level, >110 but <126 mg/dL [>6.1 but <7.0 mmol/L]) had a significantly elevated risk of developing cognitive impairment (odds ratio, 1.64; 95% confidence interval, 1.03-2.61) compared with women with a normal glucose level. Among 386 nondiabetic men, those in the highest quartile of fasting insulin level had 25% more errors on the Mini-Mental State Examination compared with those in the lowest quartile (95% confidence interval, 4%-50%; P = .02 for trend across quartiles). Stolk and colleagues observed a steady and significant decrease in mean Mini-Mental State Examination score with increasing post-load serum insulin level among 3278 women without dementia; results remained similar after excluding diabetic persons. Among 1897 older women without diabetes, there was a 30% increased risk of developing cognitive impairment with each 1% increase in glycosylated hemoglobin level, a measure of glucose control.

Several limitations of our study should be considered. We used self-reports to exclude those with diabetes and, consequently, may have included women with undiagnosed diabetes; thus, our findings could be driven in part by the association between diabetes and cognition. However, undiagnosed diabetes is likely rare in these health professionals; virtually all our participants have access to health care. In a random sample of 200 participants who never reported diabetes, we found only 1 had a plasma fasting glucose or fructosamine level in the diabetic range; in another such test, only 1.8% had elevated levels of glycosylated hemoglobin. In addition, we found strong rela-
tions of C peptide level to cognition in analyses excluding anyone reporting diabetes in the 10 years subsequent to blood draw, likely eliminating most women who may have had undiagnosed diabetes at blood draw.

An additional weakness was the brief period for assessing change in cognition. Nevertheless, our findings on cognitive decline, although nonsignificant, generally supported results from the initial cognitive testing, and suggested adverse cognitive effects of elevated C peptide level. Moreover, C peptide measures were taken among young-old women—an average 10 years before initial cognitive testing—and follow-up of the blood cohort was more than 98.0% complete; thus, analyses of the initial cognitive scores can be considered a reasonable representation of change in cognition since blood draw.

Finally, confounding is an important concern. However, we collected detailed data on potential confounding factors over many years, and adjusted for a wide array of covariates. Multivariable adjustment had relatively little impact on effect estimates, rendering it less likely that residual or uncontrolled confounding could completely explain the substantially worse cognitive performance we observed with higher C peptide levels. In addition, the relative homogeneity of the cohort reduces the potential influence of some unmeasured confounders (eg, access to health care or health knowledge). Finally, random misclassification of cognitive performance is possible. However, we have established high validity of our telephone instrument. Furthermore, composite scores (averaging several cognitive test results) help reduce random misclassification. Nevertheless, findings for the TICS, our only primary outcome that was not a composite, may underestimate effects of C peptide.

Overall, increasing evidence suggests that insulin level may have a direct effect on cognitive function. Further research is clearly necessary to confirm findings in our study and others, and to establish whether the apparent effects of insulin on cognition are indeed direct. Such research could have a large influence on public health, especially given the growing epidemic of obesity, which is often accompanied with a large influence on public health, especially given the growing epidemic of obesity, which is often accompanied by diabetes and increased insulin levels.

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