A profile of impaired insulin degradation in relation to late-life cognitive decline: A preliminary investigation

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SUMMARY

Objective   Insulin degradation pathways may be related to Alzheimer's disease pathology. In preliminary analyses, we considered the relation of combined lower insulin secretion (c-peptide) and higher insulin—possibly a phenotype for impaired insulin degradation—to cognitive decline.

Method   Fasting plasma c-peptide and insulin were measured in 1,187 non-diabetic Nurses' Health Study participants (mean age = 64 years). Cognitive testing began 10 years later. Participants completed three repeated assessments (over an average span of 4.4 years) of verbal memory, a strong predictor of Alzheimer disease development. C-peptide and insulin distributions were dichotomized at their medians to create four cross-tabulated categories. Multivariable linear mixed effects models were used to relate c-peptide/insulin categories to cognitive decline.

Results   Compared to the lower c-peptide/lower insulin group, women with lower c-peptide/higher insulin had a significantly faster rate of verbal memory decline: the mean difference was -0.05 units/year (95% CI -0.09,-0.01). This mean difference was similar to that which we found for women 5 years apart in age, indicating that having a profile of lower c-peptide/higher insulin appeared cognitively equivalent to aging by five years on tests of verbal memory. For women with higher c-peptide/higher insulin, the estimated mean difference in decline compared to those in the lower c-peptide/lower insulin group was statistically significant, but slightly lower, at −0.04 units/year (95% CI: -0.07,-0.02).

Conclusion   These preliminary analyses of a possible phenotype of impaired insulin degradation provide supportive evidence that deficits in insulin degradation may be related to late-life verbal memory decline. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS — c-peptide; insulin-degrading enzyme; cognition; aging; Alzheimer disease

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INTRODUCTION

Basic science research has linked deficiencies in insulin breakdown to Alzheimer's disease (AD) pathology. Insulin-degrading enzyme (IDE), the major

protease responsible for insulin degradation, degrades amyloid-beta (Aβ) peptide, a central component in AD pathology (Hardy and Selkoe, 2002); furthermore, insulin acts as a competitive inhibitor for this enzyme (Farris et al., 2003). Thus, defects in IDE may result in higher Aβ concentrations in the brain. However, there are extremely limited data regarding the association of insulin clearance defects with late-life cognitive decline among healthy, community-dwelling adults. Thus, as a first attempt at exploring insulin degradation and cognition in humans, we conducted preliminary analyses, considering a possible phenotype of IDE dysfunction: the combination of lower plasma c-peptide [a marker of insulin secretion (Wahren et al., 2000)] and higher plasma insulin levels.

OBJECTIVES

Since there are no practical means of directly measuring impaired insulin degradation in large-scale epidemiologic studies, we conducted a preliminary investigation of a possible profile of impaired insulin degradation in relation to cognitive decline among approximately 1,200 older participants of the Nurses' Health Study. The profile was represented by subjects with lower levels of insulin secretion but higher levels of plasma insulin. Implications of study findings and suggestions of future epidemiologic research related to this topic are discussed.

METHODS

Participants

The Nurses' Health Study (NHS) included 121,700 U.S., female registered nurses, aged 30 to 55 years when the study began in 1976. Since then, participants have completed biennial mailed questionnaires updating health and lifestyle information. Between 1989–1990, one-third of participants provided blood samples. Beginning in 1995, women aged 70 + years and free of diagnosed stroke were invited to participate in a telephone-based study of cognitive function, and 19,395 women (93.3% of those eligible) completed an initial assessment. Two additional assessments have been performed approximately 2 years apart. Follow-up exceeds 90% for cognitive study participants.

Of the 19,395 women in the cognitive study, there were 4,399 participants who had provided fasting blood samples during the 1989–1990 collection and had also completed at least one follow-up cognitive assessment. Of note, 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 among those providing blood samples (Okereke et al., 2005). From the 4,399 participants, we generated a random sample of 1,195 women for measurement of plasma c-peptide and insulin levels, after excluding any women with diagnosed diabetes at blood draw. Finally, we excluded another eight women who were heavy alcohol users ($\geq$60 g/day), as this could potentially have a strong influence on both insulin clearance (Nygren et al., 1985) and cognitive performance. Thus, the sample for analysis included 1,187 women.

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

Measurement of c-peptide and insulin levels

C-peptide is cleaved in a 1:1 ratio in the conversion of pro-insulin to insulin and thus provides an representation of insulin secretion (Polonsky and Rubenstein, 1984). Although insulin and c-peptide are secreted in equimolar amounts, c-peptide is not rapidly extracted by the liver and its half-life in the circulation is between two and five times longer than that of insulin (Horwitz et al., 1975); therefore, c-peptide level is considered a reliable indicator of insulin production.

Blood samples had been obtained after $\geq$8-h fast, processed within 24 h of collection, and stored at $-30°$C. Using stored blood samples, we measured both plasma c-peptide, using antiserum M1230 in an alcohol precipitation non-equilibrium assay (Faber et al., 1978) with reagents provided by Diagnostic Systems Laboratory (Webster, TX), and plasma insulin, using a radioimmunoassay (Linco, St Louis, MO). In blinded quality control tests that we conducted, mean intra-assay coefficients of variation were 5.1% and 5.9%, respectively, for c-peptide and insulin.

Assessment of cognitive function

Our cognitive battery included the Telephone Interview for Cognitive Status (TICS) (Brandt et al., 1988), a telephone-based test of general cognition similar to the Mini-Mental State Examination (Folstein et al., 1975); immediate and delayed recall trials of the East Boston Memory Test (EBMT) (Albert et al., 1991); category fluency (naming as many different animals as possible during one minute); 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. High reliability and validity of this telephone method has been established (Kang *et al.*, 2006).

The primary outcome was verbal memory score. We emphasized verbal memory tasks because these are strong measures of episodic memory, and cognitive decline in episodic memory is one of the best-known neuropsychological predictors of Alzheimer disease risk (Chen *et al.*, 2000, 2001). Furthermore, because of potential biologic relations of insulin degradation pathways to risk of AD in particular, we felt it was important to focus on verbal memory. The verbal memory score was computed by averaging the *z*-scores of the immediate and delayed recall trials of both the EBMT and the TICS 10-word list. As a secondary outcome, we considered general cognition, using a global score averaging the *z*-scores of all six cognitive tests.

Statistical analyses

These are preliminary analyses of a possible phenotype of impaired insulin degradation, and there is no consensus method for defining deficits in insulin degradation in this setting. Thus, we chose to create four cross-tabulated categories of combined c-peptide/insulin status, after dichotomizing the c-peptide and insulin distributions by their median values (the median c-peptide level for the entire sample was 0.59 nmol/L, and the median insulin level was 3.0 uIU/mL). Thus, the low c-peptide/low insulin group consisted of women with below-median levels of both c-peptide and insulin; the low c-peptide/high insulin group consisted of women with below-median levels of c-peptide but above-median levels of insulin; and so on. First, we considered those with below-median c-peptide and above-median insulin to represent a possible phenotype of IDE dysfunction, since the contrast between c-peptide (i.e. insulin secretion) and insulin levels in this group was most likely to reflect a mismatch in peripheral clearance of insulin relative to production; more extreme groupings were also considered (e.g. below-median c-peptide/highest quartile insulin), but numbers of participants in such categories were too low. Second, the lower c-peptide/lower insulin category was chosen as the referent group, as this was most representative of normal secretion and adequate peripheral clearance of insulin. Finally, we also considered the two other categories in analyses: higher c-peptide/lower insulin and higher c-peptide/higher insulin; the latter category was of particular interest, as this group with both

higher insulin production and circulating insulin levels would be most consistent with the earliest stages of insulin resistance, which may also be associated with cognitive decline.

We used linear mixed effects models (Laird and Ware, 1982) to examine the relation of c-peptide/insulin levels to cognitive decline across three repeated assessments. The model included the following fixed effects: time since baseline assessment (in years), age, highest attained education, c-peptide/insulin category, interaction terms for time and age and time and c-peptide/insulin category, as well as the following potential confounders: body mass index (BMI) (kg/m^2), current smoking (yes/no), history of hypertension (yes/no), history of dyslipidemia (yes/no), alcohol use (grams/day), physical activity (metabolic equivalents/week), and current postmenopausal hormone use (yes/no), all of which were determined as of blood draw, and history of antidepressant use, which was determined as of baseline cognitive testing (yes/no). In addition to the fixed effects, we included two person-specific random effects: baseline cognitive level (random intercept) and rate of change (random slope). All statistical analyses were conducted using SAS$^®$ version 9.1 (SAS Institute, Cary, NC).

RESULTS

As expected, participants with higher c-peptide/higher insulin had generally worse health than the other groups; these women had the highest BMI and prevalence of hypertension at blood draw (Table 1). In contrast, although those in the lower c-peptide/higher insulin category also had higher levels of insulin, this group appeared healthier than those in the higher c-peptide/higher insulin group, with lower BMI, less hypertension, and lower prevalence of smoking. Table 2 presents the crude means and standard deviations on initial cognitive scores by c-peptide/insulin category; although baseline scores are generally similar, women in the lower c-peptide/higher insulin category appear to have slightly lower scores on most tests, compared to those in the lower c-peptide/lower insulin group.

After adjusting for potential confounders, we observed a significantly greater mean difference in decline in verbal memory of −0.05 standard units per year for women with lower c-peptide/higher insulin, compared to the referent group (lower c-peptide/lower insulin) (Table 3). To help interpret this estimate, we compared it to the effects of age on verbal memory decline: each year of age was associated with a mean

Table 1. Characteristics of the study population at blood draw, by categories of fasting c-peptide and insulin*

Characteristic	Lower c-peptide/ lower insulin ($n = 447$)	Higher c-peptide/ lower insulin ($n = 146$)	Lower c-peptide/ higher insulin ($n = 146$)	Higher c-peptide/ higher insulin ($n = 448$)
Median (range) c-peptide (nmol/L)	0.43 (0.1–0.6)	0.71 (0.6–1.9)	0.50 (0.3–0.6)	0.85 (0.6–2.5)
Median (range) insulin (uIU/mL)	2.1 (<2.0–3.0)**	2.4 (<2.0–3.0)**	3.7 (3.0–19.6)	4.9 (3.0–25.3)
Mean age (SD) (years)	64.0 (2.5)	63.9 (2.4)	64.3 (2.4)	64.4 (2.6)
Mean (SD) body mass index (kg/m^2)	23.1 (3.0)	25.7 (4.0)	24.4 (3.9)	26.8 (4.4)
Master's degree or higher education	7.4	8.9	10.3	5.6
History of hypertension	21.5	28.1	30.1	45.5
History of dyslipidemia	28.9	37.7	37.0	37.3
Smoking: Current	8.7	8.9	4.8	8.7
Past	39.8	44.5	32.9	43.6
Hormones: Current use	40.7	28.9	39.0	28.8
Past use	22.7	25.9	29.1	32.6
History of antidepressant use	5.4	6.9	4.8	7.8
Mean (SD) exercise level (METS/wk)	19.0 (20.3)	17.1 (21.4)	14.1 (16.2)	15.4 (17.8)
Mean (SD) alcohol intake (grams/day)	5.2 (8.3)	7.1 (10.6)	3.9 (6.8)	4.0 (8.1)

*Figures are expressed as percentages, unless otherwise stated. History of antidepressant use is as of cognitive testing.
**Low value of range is at the lower limit for insulin detection (2.0 uIU/mL).

decline of -0.01 units per year ($p < 0.0001$) in verbal memory; thus, the mean difference in decline in the lower c-peptide/higher insulin group was cognitively equivalent to 5 years of aging. When we examined the group with higher c-peptide/higher insulin, we also found significantly greater decline in verbal memory than the referent group, although to a somewhat lesser extent than observed for the women with lower c-peptide/higher insulin.

In the analysis of our secondary cognitive outcome, we found significantly worse declines in general cognition for women with higher c-peptide/higher insulin, compared to the referent group (Table 3). While the multivariable-adjusted effect estimate for cognitive decline was similar in the lower c-peptide/ higher insulin group, results were not statistically significant ($p = 0.12$).

DISCUSSION

In this preliminary investigation of nearly 1,200 community-dwelling women without diabetes, a profile of lower insulin secretion and higher plasma insulin—possibly reflecting poor systemic insulin breakdown or IDE dysfunction—was associated with significantly greater decline in verbal memory over an average of 4 years. In addition, although higher levels of insulin were generally associated with worse cognitive decline, the point estimate for verbal memory decline appeared slightly larger in the lower

Table 2. Unadjusted mean scores (SD) at baseline cognitive assessment, by c-peptide/insulin category

Cognitive test	Lower c-peptide/ lower insulin ($n = 447$)	Higher c-peptide/ lower insulin ($n = 146$)	Lower c-peptide/ higher insulin ($n = 146$)	Higher c-peptide/ higher insulin ($n = 448$)
TICS	34.3 (2.4)	34.2 (2.3)	34.1 (2.4)	34.3 (2.4)
Category Fluency	18.0 (4.8)	17.7 (4.3)	16.9 (4.5)	17.8 (4.4)
Digit Span Backwards	7.0 (2.4)	6.9 (2.4)	6.8 (2.3)	6.7 (2.3)
EBMT–immediate recall	9.6 (1.7)	9.6 (1.4)	9.5 (1.9)	9.8 (1.6)
EBMT–delayed recall	9.2 (2.0)	9.4 (1.5)	9.2 (1.8)	9.4 (1.7)
TICS 10-word list–immediate recall	4.8 (1.6)	4.7 (1.6)	4.7 (1.5)	4.8 (1.5)
TICS 10-word list–delayed recall	2.4 (1.8)	2.5 (1.8)	2.4 (2.0)	2.5 (2.0)

EBMT = East Boston Memory Test; TICS = Telephone Interview for Cognitive Status.

Table 3. Multivariable-adjusted* mean differences (95% CI) in annual rate of cognitive decline**, by categories of fasting C-peptide and insulin ($n = 1,187$)

Cognitive test	Lower c-peptide/ lower insulin ($n = 447$)	Higher c-peptide/ lower insulin ($n = 146$)	Lower c-peptide/ higher insulin ($n = 146$)	Higher c-peptide/ higher insulin ($n = 448$)
Verbal memory	0.00	−0.02 (−0.06, 0.02); $p = 0.35$	−0.05 (−0.09, −0.01); $p = 0.01$	−0.04 (−0.07, −0.02); $p < 0.01$
Global score	0.00	−0.01 (−0.05, 0.02); $p = 0.39$	−0.03 (−0.06, 0.01); $p = 0.12$	−0.03 (−0.06, −0.01); $p < 0.01$

*Models adjusted for: age at baseline cognitive testing; highest attained education; current smoking, current hormone use, hypertension, dyslipidemia, body mass index, alcohol intake and physical activity level as of blood draw; and antidepressant use as of cognitive testing.
**Participants were administered three repeated assessments of cognitive function over an average of 4.4 years.
CI = Confidence Interval.

c-peptide/higher insulin group than in the higher c-peptide/higher insulin group—despite the apparent better general health of the women with lower c-peptide/higher insulin. These preliminary results should be interpreted with caution however, as there were relatively few women with lower c-peptide/ higher insulin, and we do not report a statistically significant difference in the estimates between the two groups with higher insulin.

Our findings of a potential association between an impaired insulin degradation phenotype and verbal memory decline contribute uniquely to the existing literature, as comparable epidemiologic data on insulin degradation pathways and cognitive decline are very limited. Results from prior studies of cross-sectional associations between IDE polymorphisms and general cognitive performance have been mixed (Blomqvist *et al.*, 2005; Mueller *et al.*, 2007). However, in a longitudinal study (McQueen *et al.*, 2007) involving 365 community-recruited, older subjects, McQueen and colleagues reported a significant association between IDE polymorphisms and cognitive decline on neuropsychological tests repeated three times over an average of 4.6 years; associations between IDE polymorphisms and verbal memory were particularly strong. However, these genetic association studies did not directly measure IDE function. In the first functional study (Kim *et al.*, 2007) of IDE in AD families, Kim and colleagues reported that systemic insulin-degrading activity itself—in the absence of any differences in IDE gene expression—was significantly lower in AD affected *vs* unaffected family members. Thus, although evidence linking insulin-degrading pathways to cognitive decline or other 'AD-related phenotypes' (McQueen *et al.*, 2007) in older adults remains highly preliminary, emerging evidence supports a role for systemic deficits IDE function in AD pathogenesis (Kim *et al.*, 2007).

Our initial attempt at defining an easily-measurable phenotype of IDE impairment has limitations. Clearly, we did not measure IDE function directly but sought to combine plasma c-peptide and insulin measures to characterize a possible phenotype of IDE dysfunction; thus, we may have misclassified poor IDE function for some participants. Second, systemic clearance of insulin is a complex issue: e.g. insulin clearance depends to an extent on adequate hepatic and renal function (Henriksen *et al.*, 1987; Duckworth *et al.*, 1998)—both of which can influence cognition—but formal functional measures (e.g. liver enzymes and creatinine clearance) were not available in our sample. However, liver dysfunction sufficient to compromise insulin clearance would be exceedingly rare in this cohort of generally healthy women, and the exclusion of the small number of women with reported heavy alcohol consumption also reduces the likelihood of including hepatically-compromised participants in our sample; thus, it is unlikely that impaired hepatic function could explain these results. Similarly, c-peptide is substantially more dependent on renal function for its clearance, compared to insulin (Henriksen *et al.*, 1987; Duckworth *et al.*, 1998); thus, if undetected impaired renal function were the primary cause of higher peripheral insulin levels, it seems very unlikely that this would explain worse cognitive performance in the low c-peptide/high insulin group. In addition, other limitations should be considered. Residual confounding is always possible in observational studies, despite our inclusion of numerous potential confounders. Finally, the generalizability of findings may be a concern in our population of predominantly white women; further research in ethnically diverse populations will be important, given reported differences in insulin secretion levels and insulin resistance among African-Americans and Hispanics compared with non-Hispanic whites (Haffner *et al.*, 1996).

KEY POINTS

- Deficits in insulin degradation may be related to late-life cognitive decline.
- A profile of impaired insulin degradation may be associated with faster rates of decline in verbal memory.

CONCLUSION

This report provides preliminary evidence regarding a possible phenotype of poor insulin degradation in relation to verbal memory decline in generally healthy older women without diabetes. Overall, although higher levels of insulin were associated with worse cognitive decline, there was a possible suggestion that the combination of lower c-peptide/higher insulin may be related to more rapid rates of decline in verbal memory than the combination of higher c-peptide/ higher insulin. These early findings clearly require substantial further study—both to continue investigation of an easily-measurable marker of impaired insulin degradation, and to explore modulation of insulin-degrading pathways as a promising target for preventing cognitive decline and, ultimately, Alzheimer's disease.

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