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The relation between insulin, insulin-related factors, and plasma amyloid beta peptide levels at mid-life in a population-based study

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Abstract

Little is known regarding factors associated with soluble amyloid beta peptide (Aβ) concentrations in humans at late midlife, when Aβ is likely most critical to Alzheimer disease pathogenesis. We examined the association between insulin, insulin-related factors, and plasma Aβ at late midlife. Plasma Aβ42, Aβ40, fasting insulin, and c-peptide were measured in 468 women without diabetes, aged 59–69 years (median 63 years). Prior to blood draw, participants reported body mass index, waist circumference, physical activity, alcohol intake, hypertension, and diabetes family history. Linear regression was used to calculate age-adjusted mean differences in Aβ42 to Aβ40 ratio, and Aβ42 levels, by insulin and insulin-related factors. The ratio of Aβ42 to Aβ40 was statistically significantly lower in women with diabetes family history, and Aβ42 was significantly lower with less physical activity, greater waist circumference, hypertension, and diabetes family history ($p<0.05$ for all). Aβ42 to Aβ40 ratio, and Aβ42 levels, appeared lower with higher c-peptide levels (p-trend=0.07 and 0.06, respectively), although these were not statistically significant. In summary, insulin-related factors appear associated with lower plasma Aβ42 to Aβ40 ratio, and Aβ42, at late mid-life, consistent with increased brain sequestration of Aβ42 (relative to Aβ40), suggesting insulin merits focus in strategies to prevent dementia.

Keywords

amyloid beta peptide; insulin; epidemiology

Introduction

Insoluble amyloid plaques in the brain regions important for memory and cognition are a pathologic hallmark of Alzheimer disease (AD). The plaques are predominantly composed of two forms of amyloid-beta peptide (Aβ), one with 42 amino acids (Aβ42) and the other with 40 amino acids (Aβ40); however, Aβ42 appears to be the key peptide involved in plaque initiation and growth.[1, 2] Cerebral accumulation of Aβ42 likely begins at least 10–15

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years before the onset of clinical symptoms.[3, 4] Therefore, identifying early determinants of Aβ42 accumulation may lead to ways to help stem the growing epidemic of AD.[2]

Surprisingly little is known regarding factors that may regulate Aβ levels in apparently healthy individuals. Several lines of evidence suggest that insulin and factors that affect insulin levels might mediate levels of circulating Aβ. For example, insulin degrading enzyme (IDE) preferentially binds to insulin over its other peptide substrates, including Aβ[5, 6]; thus, it is possible that high steady-state levels of insulin in the brain lead to greater Aβ42 accumulation and gradual deposition into insoluble plaques, and consequently lower levels of circulating Aβ42. In addition, several small clinical studies reported decreases in plasma Aβ42 levels, and in the ratio of plasma Aβ42 to Aβ40, in cognitively healthy adults who received either intravenous or intranasal doses of insulin, suggesting that high levels of insulin in the periphery and the brain might similarly impact Aβ homeostasis.[7, 8]

We investigated whether insulin and insulin-related factors were associated with Aβ42 levels and the ratio of Aβ42 to Aβ40 in plasma at late midlife in a cross-sectional study of 468 women without type 2 diabetes in the Nurses' Health Study (NHS). Although there are multiple sources contributing to Aβ levels in plasma, the brain is its principal source and evidence indicates that Aβ levels in plasma reflect those in the brain.[9–11] For example, studies administering anti-Aβ antibody to mice and humans both found reduced plaque deposition and increased plasma Aβ levels.[9, 11] Moreover, five of six large prospective studies of plasma Aβ[12–17] all found higher risks of cognitive decline and dementia among adults with lower plasma levels of Aβ42 relative to Aβ40, or a decrease in this ratio over time, likely reflecting a higher rate of sequestration of Aβ42 versus Aβ40 into insoluble deposits in the brain[18].

Methods

Nurses' Health Study

The NHS was initiated in 1976 when 121,700 female registered nurses ages 30–55 years returned a mailed questionnaire about their medical history and lifestyle. Participants have updated this information on biennial mailed questionnaires. This study was approved by the institutional review board of Brigham and Women's Hospital. Participants provided informed consent by returning their questionnaires.

From 1989 to 1990, 32,826 participants, aged 43–69 years, provided a blood sample. After arranging to have their blood drawn, women mailed the blood samples with an ice pack to our laboratory via overnight mail. Less than 5% of the samples were received more than 26 hours after being drawn. Whole blood samples were centrifuged and aliquotted into plasma, red blood cells, and white blood cells, and have been stored at −130° C in liquid nitrogen freezers. Women who provided blood samples were similar to the whole cohort in several key characteristics assessed at the time of blood draw, including mean age (56 vs. 55 years, respectively), mean body mass index (BMI; 25 kg/m^2 in both groups), mean alcohol intake (6 g/day in both groups), and family history of diabetes (30% vs. 26%, respectively).

Population for analysis

We conducted these analyses among women who were part of the NHS cognitive substudy. The cognitive substudy was initiated from 1995–2001, when 22,715 NHS participants aged 70 years and older and free of diagnosed stroke were invited to participate in a telephone cognitive assessment. We obtained the present study sample (n=500) by first oversampling participants from the top and bottom 20% of the distributions of cognitive decline in our population and then by selecting random participants from the remainder of the distribution. We also excluded women who reported type 2 diabetes for several reasons. Specifically,

insulin and insulin-related factors are complex in those with diabetes; many women make lifestyle changes in insulin-related factors (e.g., diet, weight, physical activity) in response to a diabetes diagnosis, which could lead to misclassification of long-term exposure status. In addition, insulin levels among adults with diabetes vary for multiple reasons, such as disease severity and duration, complications, and use of medications, and thus it is extremely difficult to disentangle insulin levels from disease state or medication use. Thus, overall, it would be difficult to interpret the direct, independent relation of insulin and insulin-related factors to Aβ levels in those with diabetes. . Finally, midlife levels of plasma Aβ40 or Aβ42 for 32 women were below the limit of detection; thus, the population for analysis included 468 women.

Detailed protocol for Aβ assays

We assayed plasma Aβ40 and Aβ42 by sandwich ELISA. Nunc MaxiSorp 384-well plates were coated with capture antibodies (2G3 for Aβ40 and 21F12 for Aβ42) in PBS and incubated for 4 hours at room temperature (RT), then blocked overnight at 4° C. Plates were washed 3 times with PBS-T, and samples were loaded into the wells and incubated for 4 hours at RT. Samples were then incubated with detector antibody (biotinylated 266 to the mid-region of Aβ) for 2 hours at RT. Finally, samples were incubated with streptavidin AP (Promega, Madison, WI, USA) in PBS for 1 hour at RT and washed 3 times with TBS. The signal was amplified with AttoPhos (Promega, Madison, WI, USA) and measured with a Victor2 fluorescent plate reader (PerkinElmer, Boston, MA, USA).

Reliability of plasma Aβ assays

Before beginning this study, we established that plasma Aβ measurements would yield reliable and valid results with the specific blood collection and storage procedures used in the NHS. For example, although only a small percentage of the NHS samples were processed more than 24 hours after blood draw, intraclass correlations were >0.95 for Aβ40 and Aβ42 after processing delays of up to 48 hours, indicating stability of Aβ levels with delayed processing.[19] During this study, a total of 7 plates were used for the ELISAs, and blinded duplicate QC pairs were included on each plate. High between-plate variability appeared to contribute to high overall CVs for the QC samples (45.0% for Aβ40 and 34.7% for Aβ42). However, after separating between- and within-plate variabilities, the average within-plate CV was only 10.2%. Thus, as described below, all analyses were conducted based on within-plate comparisons.

Measurement of plasma insulin and c-peptide and insulin-related variables

All of the women had provided fasting (≥ 8 hours) blood samples. Plasma insulin was measured using a radioimmunoassay (Linco, St Louis, MO). Plasma c-peptide, a marker of insulin production, was measured using antiserum M1230 in an alcohol precipitation non-equilibrium assay (Faber et al., 1978) with reagents provided by Diagnostic Systems Laboratory (Webster, TX). In blinded quality control tests that we conducted, mean intra-assay CVs were 5.1% for c-peptide and 5.9% for insulin.

Self-reported information on all insulin-related factors, except waist circumference, was current as of the questionnaire closest, but prior to, the date of blood draw (i.e., either the 1988 or 1990 questionnaire). For waist circumference, participants were given instructions in 1986 for taking this measurement; 375 women in this analysis provided this information. Physical activity, defined as metabolic equivalent (MET) hours per week, was derived from the average time per week spent participating in various recreational activities during the past year. Information on alcohol intake was collected using semi-quantitative food frequency questionnaires (FFQs). Items on typical beer, wine, and liquor consumption during the past year were used to calculate average grams of alcohol consumed per day.

Validation studies have established that these nurses' self-reports of various health indicators, including height, weight, waist circumference, physical activity, and alcohol consumption, are highly accurate.[20–23]

Statistical analysis

For these analyses, we examined plasma insulin, plasma c-peptide, waist circumference, and physical activity as categorical variables using quartile cut-points. We used standard categories for BMI (18.5–24.9, 25.0–29.9, ≥30 kg/m^2) and alcohol intake (0.0, 1.0–14.9, 15.0–30.0 g/day). History of high blood pressure and family history of diabetes were categorized as present versus absent.

We calculated age-adjusted mean differences (95% confidence intervals, CIs) in plasma Aβ42 levels and Aβ42 to Aβ40 ratio for insulin and each insulin-related variable in separate linear regression models (i.e., insulin and the insulin-related variables were not mutually adjusted for each other since this would have resulted in over-control for insulin). Although levels of Aβ42 and the ratio of Aβ42 to Aβ40 appear to be the most biologically relevant factors in AD, we also similarly considered plasma Aβ40 alone in a separate regression model. To improve the normality of the distributions, we calculated the natural logarithm of the plasma Aβ42 concentration, the ratio of the concentrations of Aβ42 to Aβ40, and Aβ40 concentration. Then, to correct for the high between-plate variability in amyloid beta concentrations (described above), we calculated plate-specific z-scores by dividing the difference between the participant's natural log Aβ42 (or Aβ42 to Aβ40 ratio, or Aβ40) value and the mean natural log Aβ42 (or ratio, or Aβ40) value among the samples on the same plate by the standard deviation.

Age was included as a continuous variable in all linear regression models. For all exposure variables except history of high blood pressure and family history of diabetes, we calculated p-values for linear trend by including the continuous variable in the model. We excluded the few women with a BMI less than 18.5 kg/m^2 (n=10) from the analyses of BMI and the few women who reported consuming more than 30 g of alcohol per day (n=19) from the analyses of alcohol consumption.

Results

Among the women in the study population, age at blood draw ranged from 59 to 69 years. Approximately 40% of the women had a BMI of 25 kg/m^2 or higher (Table 1). The mean waist circumference was 80 cm. Overall, 24% of women reported no alcohol intake. One third of the women reported a history of high blood pressure, and 30% had a family history of diabetes.

In a univariate model, older age was significantly associated with lower plasma Aβ42 to Aβ40 ratio (mean difference per 1-year change in age: −0.05, 95% CI −0.09, −0.02, p=0.001). The association between age and Aβ42 level was consistent with that for the ratio, but not statistically significant (mean difference: −0.02, 95% CI −0.06, 0.02, p=0.36). After adjusting for age, the average levels of plasma Aβ42 and Aβ42 to Aβ40 ratio were lower in women with higher levels of plasma insulin, but were not statistically significant (p for trend=0.50 and 0.14, respectively; Table 2). For plasma c-peptide, findings were somewhat stronger, indicating lower levels of Aβ42 and Aβ42 to Aβ40 ratio in those with higher c-peptide levels, although again the results did not reach statistical significance (p for trend=0.06 and 0.07, respectively).

Several insulin-related factors were significantly associated with lower levels of circulating Aβ42 and/or Aβ42 to Aβ40 ratio (Table 2). Specifically, women with a family history of

diabetes had significantly lower levels of both circulating Aβ42 (p=0.03) and the ratio of Aβ42 to Aβ40 (p=0.04) than women without a family history of diabetes. There was a significant trend of decreasing plasma Aβ42 with increasing waist circumference (p for trend=0.04). Women who were the least physically active had significantly lower plasma Aβ42 levels than the most physically active women (p=0.04 comparing the bottom to the top quartile of physical activity). Women with a history of high blood pressure had lower plasma levels of Aβ42 (p=0.02) than women without high blood pressure. Finally, plasma levels of Aβ42 were significantly lower in women consuming 1–14.9 g of alcohol per day versus abstainers. The magnitude of the association was similar among the small number of women who reported consuming 15–30 g of alcohol per day, although not significant.

There was a suggestion of lower plasma levels of Aβ42 in women with a BMI of 30 kg/m^2 or higher compared with lean women (BMI 18.5–24.9 kg/m^2, p=0.06) and a suggestion of decreasing circulating Aβ42 with increasing level of BMI (p for trend=0.11); however, these associations were not statistically significant.

In additional analyses, we examined the association between insulin, insulin-related factors, and plasma levels of Aβ40. Age was not associated with Aβ40 level in a univariate model (mean difference per 1-year change in age: 0.01, 95% CI −0.02, 0.05, p=0.46). There were no statistically significant associations between Aβ40 and insulin or any of the insulin-related factors (Table 2), although there was a suggestion of lower Aβ40 levels with increasingly higher physical activity (p for trend=0.07).

Discussion

Among women without diabetes at late midlife, lower circulating levels of Aβ42 or Aβ42 relative to Aβ40 were significantly associated with several factors related to insulin hypersecretion[24–27], including family history of diabetes, physical inactivity, high waist circumference, and high blood pressure, as well as moderate alcohol intake. Levels of Aβ42 or Aβ42 relative to Aβ40 tended to be lower with higher c-peptide levels and high BMI, but these associations did not reach statistical significance. Circulating levels of Aβ40 alone were not significantly associated with insulin or any of the insulin-related variables, although there was a suggestion of lower Aβ40 with higher physical activity.

These findings are consistent with research indicating a relationship between hyperinsulinemia and less degradation of Aβ in the brain as well as increased accumulation of Aβ onto insoluble plaques. First, IDE is known to degrade insulin and Aβ as well as several other small peptide hormones.[5] When insulin levels in the brain are high, typical of early stages of chronic peripheral hyperinsulinemia[28], degradation of Aβ is partially inhibited due to competition with insulin for IDE[5]. In addition, higher levels of advanced glycation end products are associated with insulin resistance and appear to enhance aggregation of Aβ onto plaques.[29] Both of these potential mechanisms could lead to accumulation of Aβ in the brain and gradual sequestration into insoluble deposits, which would be expected to lead to lower levels of Aβ42 and Aβ42 relative to Aβ40 in plasma.

Our data are consistent with small clinical research studies in which plasma Aβ42 levels were lower in healthy adults acutely receiving insulin versus saline, either intravenously or intranasally.[7, 8] For example, among 50 cognitively healthy older adults (mean age 70.5 years) who received an intravenous dose of insulin to produce a level typical of postprandial levels in insulin resistant adults, Kulstad et al. observed lower levels of plasma Aβ42 compared to levels after an infusion of saline (p=0.03).[8] In the same study, plasma Aβ40 appeared higher after insulin infusion (p=0.09), which is consistent with a lower ratio of plasma Aβ42 to Aβ40. These results suggest that elevated insulin levels in the periphery

(and also in the brain) may impact clearance of Aβ42 in the brain. Moreover, in a study of 530 adults aged 65 years and older, with and without AD, Mayeux et al. observed a modest inverse correlation between plasma Aβ42 and BMI (r=−0.1, p=0.05), which is consistent with our results.[30]

A unique strength of our study is its focus on factors related to Aβ in healthy women at midlife, when evidence suggests intervention to prevent Aβ accumulation in the brain will be most effective.[3, 31] However, several limitations of this study should also be considered. First, between-plate variability led to high overall CVs for the measurements of plasma Aβ42 and Aβ40, which could result in bias to the null when examining relations between plasma Aβ and insulin and insulin-related factors. In particular, since the ratio of Aβ42 to Aβ40 incorporates random error in two variables, this may explain the higher p-values generally observed for relations between insulin variables and the Aβ42 to Aβ40 ratio, as opposed to absolute Aβ42 levels. Second, because we excluded women with diabetes from these analyses, the range of insulin levels in our sample was narrower than in the general population, which could lead to underestimation of the influence of insulin variables on circulating Aβ levels. In addition, statistical power to detect associations was likely further limited because we conducted these analyses within a relatively small subset of women from the Nurses' Health Study cohort. Finally, since our study included primarily white women, it is possible that these findings are not generalizable to men or to minorities. In particular, further research to examine these associations in women and men of different racial and ethnic backgrounds is needed.

In conclusion, our findings suggest that peripheral insulin levels and/or factors associated with them may be important in regulating levels of soluble Aβ42 in healthy women at late midlife, providing evidence that early intervention on insulin-related factors could be valuable in preventing Aβ42 accumulation.

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Table 1

Characteristics of the study population (n=468)

Variable	Mean (SD) or No. (%)
Age, yrs	63.1 (2.4)
Insulin, μIU/mL	3.3 (2.6)
C-peptide, pmol/mL	0.66 (0.31)
Waist circumference, cm[a]	80.0 (9.9)
Physical activity, MET-hrs/wk	16.7 (17.9)
Family history of diabetes	142 (30.3)
Body mass index, kg/m^2 [b]	
18.5–24.9	261 (57.0)
25.0–29.9	150 (32.8)
≥ 30	47 (10.3)
High blood pressure	157 (33.6)
Alcohol intake, g/day[c]	
0	108 (24.1)
<15	303 (67.5)
15–30	38 (8.5)

MET, metabolic equivalent; SD, standard deviation.

[a] N=375.

[b] 10 women with body mass index <18.5 kg/m^2 were excluded from analyses of body mass index.

[c] 19 women who reported consuming >30 g of alcohol per day were excluded from analyses of alcohol consumption.

Table 2

Associations between insulin, insulin-related factors, and plasma Aβ peptide levels

	Age-adjusted mean difference (95% CI)		
Variable[a]	**Aβ42**[b]	**Aβ42:Aβ40**[b]	**Aβ40**[b]
Insulin, μIU/mL			
0.00–2.17 (0.00)	0.00 (reference)	0.00 (reference)	0.00 (reference)
2.18–3.05 (2.67)	−0.07 (−0.33, 0.19)	−0.12 (−0.38, 0.14)	0.04 (−0.21, 0.30)
3.06–4.42 (3.67)	−0.04 (−0.30, 0.22)	−0.15 (−0.40, 0.11)	0.08 (−0.18, 0.33)
4.43–16.56 (5.83)	−0.05 (−0.31, 0.21)	−0.15 (−0.41, 0.11)	0.07 (−0.19, 0.33)
Per 1 μIU/mL increment	−0.01 (−0.05, 0.02)	−0.03 (−0.06, 0.01)	0.00 (−0.03, 0.04)
P linear trend[c]	0.50	0.14	0.82
C-peptide, pmol/mL			
0.12–0.44 (0.36)	0.00 (reference)	0.00 (reference)	0.00 (reference)
0.45–0.57 (0.51)	−0.04 (−0.30, 0.22)	−0.07 (−0.32, 0.19)	0.01 (−0.25, 0.26)
0.58–0.80 (0.69)	−0.15 (−0.41, 0.10)	−0.03 (−0.29, 0.23)	−0.13 (−0.38, 0.13)
0.81–2.45 (0.97)	−0.17 (−0.43, 0.09)	−0.21 (−0.47, 0.05)	−0.04 (−0.30, 0.22)
Per 0.1 pmol/mL increment	−0.03 (−0.06, 0.00)	−0.03 (−0.06, 0.00)	−0.01 (−0.04, 0.02)
P linear trend[c]	0.06	0.07	0.47
Family history of diabetes	−0.22 (−0.42, −0.02)	−0.21 (−0.40, −0.01)	−0.11 (−0.30, 0.09)
Body mass index, kg/m^2			
18.5–24.9 (22.4)	0.00 (reference)	0.00 (reference)	0.00 (reference)
25.0–29.9 (26.6)	−0.05 (−0.25, 0.15)	−0.14 (−0.34, 0.06)	0.06 (−0.14, 0.26)
30.0–40.2 (32.1)	−0.30 (−0.61, 0.01)	−0.17 (−0.48, 0.14)	−0.18 (−0.49, 0.13)
Per 1 kg/m^2 increment	−0.02 (−0.04, 0.00)	−0.01 (−0.04, 0.01)	−0.01 (−0.04, 0.01)
P linear trend[c]	0.11	0.34	0.34
Waist circumference, cm			
55.3–72.3 (69.6)	0.00 (reference)	0.00 (reference)	0.00 (reference)
72.4–78.6 (75.6)	0.03 (−0.27, 0.32)	0.01 (−0.28, 0.30)	0.04 (−0.25, 0.33)
78.7–86.3 (81.3)	0.02 (−0.27, 0.31)	0.15 (−0.14, 0.43)	−0.07 (−0.35, 0.22)
86.4–121.9 (91.4)	−0.20 (−0.49, 0.09)	−0.12 (−0.41, 0.17)	−0.13 (−0.41, 0.16)
Per 1 cm increment	−0.03 (−0.05, −0.00)	−0.02 (−0.04, 0.01)	−0.02 (−0.04, 0.01)
P linear trend[c]	0.04	0.17	0.17
Physical activity, MET-hrs/wk			
21.2–145.0 (36.3)	0.00 (reference)	0.00 (reference)	0.00 (reference)
10.7–21.1 (15.2)	−0.01 (−0.26, 0.24)	−0.02 (−0.27, 0.23)	0.01 (−0.24, 0.26)
4.8–10.6 (7.6)	−0.19 (−0.45, 0.07)	−0.01 (−0.27, 0.25)	−0.20 (−0.46, 0.05)
0.2–4.7 (2.0)	−0.27 (−0.53, −0.01)	−0.10 (−0.36, 0.16)	−0.21 (−0.47, 0.04)
Per 5 MET-hr/wk increment	0.02 (−0.00, 0.05)	0.00 (−0.02, 0.03)	0.02 (−0.00, 0.05)
P linear trend[c]	0.06	0.84	0.07
High blood pressure	−0.24 (−0.43, −0.04)	−0.15 (−0.34, 0.04)	−0.17 (−0.36, 0.02)
Alcohol, g/day			

	Age-adjusted mean difference (95% CI)		
Variable[a]	**Aβ42**[b]	**Aβ42:Aβ40**[b]	**Aβ40**[b]
0.0	0.00 (reference)	0.00 (reference)	0.00 (reference)
1.0–14.9 (2.7)	−0.29 (−0.51, −0.07)	−0.18 (−0.40, 0.04)	−0.23 (−0.45, −0.01)
15.0–30.0 (19.7)	−0.28 (−0.65, 0.09)	−0.01 (−0.38, 0.36)	−0.37 (−0.73, 0.00)
Per 1 g/day increment	−0.01 (−0.02, 0.01)	0.00 (−0.01, 0.02)	−0.01 (−0.02, 0.01)
P linear trend[c]	0.42	0.93	0.22

MET, metabolic equivalent.

[a] For continuous variables that were categorized, we present the category range (median).

[b] Analyses used batch-specific z-scores of natural log Aβ values.

[c] P-values for linear trend were calculated by including the continuous variable in the linear regression model.