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The role of peripheral β -amyloid in insulin resistance, insulin secretion, and prediabetes: *in vitro* and population-based studies

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Background: Previous experimental studies have shown that mice overexpressing amyloid precursor protein, in which β -amyloid (A β) is overproduced, exhibit peripheral insulin resistance, pancreatic impairment, and hyperglycemia. We aimed to explore the effects of A β on insulin action and insulin secretion *in vitro* and the association of plasma A β with prediabetes in human.

Methods: We examined the effects of A β 40 and A β 42 on insulin-inhibited glucose production in HepG2 cells, insulin-promoted glucose uptake in C2C12 myotubes, and insulin secretion in INS-1 cells. Furthermore, we conducted a case-control study (N = 1142) and a nested case-control study (N = 300) within the prospective Tongji-Ezhou cohort. Odds ratios (ORs) and 95% confidence intervals (CIs) for prediabetes were estimated by using conditional logistic regression analyses.

Results: In the *in vitro* studies, A β 40 and A β 42 dose-dependently attenuated insulin-inhibited glucose production in HepG2 cells, insulin-promoted glucose uptake in C2C12 myotubes, and basal and glucose-stimulated insulin secretion in INS-1 cells. In the case-control study, plasma A β 40 (adjusted OR: 2.00; 95% CI: 1.34, 3.01) and A β 42 (adjusted OR: 1.94; 95% CI: 1.33, 2.83) were positively associated with prediabetes risk when comparing the extreme quartiles. In the nested case-control study, compared to the lowest quartile, the highest quartile

of plasma A β 40 and A β 42 were associated with 3.51-fold (95% CI: 1.61, 7.62) and 2.75-fold (95% CI: 1.21, 6.22) greater odds of prediabetes, respectively.

Conclusion: Elevated plasma A β 40 and A β 42 levels were associated with increased risk of prediabetes in human subjects, which may be through impairing insulin sensitivity in hepatocytes and myotubes and insulin secretion in pancreatic β -cells.

KEYWORDS

peripheral β -amyloid, *in vitro* study, insulin resistance, insulin secretion, prediabetes, population-based study

1 Introduction

β -amyloid (A β), mainly including A β 40 and A β 42, is a natural product from enzymatic proteolysis of amyloid precursor protein (APP) and has been found to be deposited in the brains of Alzheimer's disease patients (1). Notably, A β is widely generated in the brain as well as various peripheral tissues (2–5). Accumulating evidence indicates that peripheral A β involves in regulating metabolism, especially glucose and insulin metabolism. Previous animal studies have demonstrated that mice overexpressing APP, in which A β is overproduced, exhibit peripheral insulin resistance and pancreatic impairment (6, 7). In addition, A β deposition has been found in human peripheral tissues taking part in glucose regulation, including skeletal muscle, liver, and pancreas (8–11). However, the effects of A β 40 and A β 42 on these peripheral tissues remain unclear.

Prediabetes, defined as an intermediate condition between normoglycemia and diabetes, is mainly manifested as peripheral insulin resistance and β -cell dysfunction (12). There is increasing number of individuals with prediabetes worldwide, with approximated 470 million people suffering from prediabetes by 2030 (13). Prediabetes individuals have been reported to exert greater risk of type 2 diabetes (T2D), cardiovascular disease (CVD), and mortality later in life (14, 15). Previous animal studies found that APP transgenic mice with high plasma A β concentrations displayed a prediabetes phenotype (16, 17). Yet, few epidemiological studies have explored the relationship between plasma A β and prediabetes.

Therefore, we examined the effects of A β 40 and A β 42 on insulin sensitivity in hepatocytes and myotubes as well as insulin secretion in pancreatic β -cells via *in vitro* studies. Furthermore, we evaluated the associations between plasma A β 40 and A β 42 and prediabetes in two independent populations, including a case-control study and a nested case-control study within the prospective Tongji-Ezhou cohort.

2 Materials and methods

2.1 Cell culture and A β treatment

HepG2 (human hepatoma) cell line was purchased from ATCC (Rockville, MD, USA); C2C12 (murine myoblast) and INS-1 (rat insulinoma) cell lines were purchased from the Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). All reagents were purchased from Gibco (Grand Island, NY, USA) unless otherwise mentioned. We cultured HepG2 cells in minimal essential medium supplemented with 10% (vol./vol.) fetal bovine serum (FBS), 1% (vol./vol.) non-essential amino acids, 1 mmol/l sodium pyruvate, and 1% (vol./vol.) penicillin-streptomycin. We maintained C2C12 cells in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (vol./vol.) FBS and 1% (vol./vol.) penicillin-streptomycin, and differentiated the cells into myotubes in the medium supplemented with 2% (vol./vol.) horse serum for 4 days when they reached confluence. We cultured INS-1 cells in RPMI 1640 medium supplemented with 10% (vol./vol.) FBS, 10 mmol/l HEPES, 1 mmol/l sodium pyruvate, 50 μ mol/l 2-mercaptoethanol (Genom, Hangzhou, China), and 1% (vol./vol.) penicillin-streptomycin. All cells were cultivated in a humidified condition (37 °C, 5% CO₂).

Synthetic A β 40, A β 42, A β 40-1, and A β 42-1 (Chinapeptides, Shanghai, China) were dissolved in dimethyl sulfoxide at 5 mmol/l and then diluted with phosphate buffered saline at 400 μ mol/l. They were further incubated at 37 °C, 220 rev/min for 48 h before use. Considering the deposition of A β in human peripheral tissues (8–11), A β concentrations in peripheral tissues may greatly exceed that in plasma. We treated cells with different doses (0, 2, 10, and 20 μ mol/l) of A β 40 or A β 42 according to previous studies (17–20). In addition, A β 40-1 and A β 42-1 were used as control peptides with reverse sequence, and cells were treated with 20 μ mol/l A β 40-1 or A β 42-1.

2.2 Cell viability assay

Cell viability of various cell lines was detected by CCK-8 (Dojindo, Kyushu Island, Japan). HepG2 cells, C2C12 myotubes, and INS-1 cells in 96-well plates were treated with different doses (0, 2, 10, and 20 $\mu\text{mol/l}$) of A β 40 or A β 42 for different periods of time (12, 24, and 48 h). Next, we added 10 μl CCK-8 reagent per well and measured optical density values by a microplate reader (Tecan, Männedorf, Switzerland) at 450 nm.

2.3 Glucose production assay

We performed glucose production assay according to the method described previously (21). We seeded HepG2 cells in 12-well plate and treated the cells with different doses of A β for 48 h. After treatment, we further incubated the cells with the medium for glucose production (glucose-free DMEM containing 2 mmol/l sodium pyruvate and 20 mmol/l sodium lactate) without or with 100 nmol/l insulin. After 3 h, the glucose level of supernatant was measured by a fluorescent glucose assay kit (Invitrogen, Carlsbad, CA, USA) and normalized to total protein amount.

2.4 Glucose uptake assay

Glucose uptake in C2C12 myotubes was determined as previously described (22). C2C12 myotubes in 12-well plate were treated with different doses of A β for 48 h. Next, C2C12 myotubes were glucose starved in glucose-free DMEM for 1 h and further incubated in phenol red-free DMEM supplemented without or with 100 nmol/l insulin for 2 h. Subsequently, we detected the glucose level of supernatant with the use of glucose assay kit (Invitrogen). Glucose intake was calculated by subtracting the remaining medium glucose from the fresh medium glucose and further normalized to the total protein amount.

2.5 Insulin secretion assay

We conducted insulin secretion assay according to the method described previously (23). We seeded INS-1 cells in 12-well plate and treated the cells with different doses of A β for 24 h. After treatment, the cells were glucose starved in the glucose-free RPMI 1640 media for 2 h and then incubated in the glucose-free RPMI 1640 media supplemented with 3 mmol/l or 15 mmol/l glucose for 2 h. After 2 h, the insulin level of supernatant was determined with a rat insulin assay kit (Mercodia, Uppsala, Sweden), and total protein amount was quantified to normalize insulin values.

2.6 Study design and population

The initial case-control study included 1142 participants (571 newly diagnosed prediabetes cases and 571 healthy controls) in

Wuhan, China. We recruited cases from patients for the first time diagnosed as prediabetes in Tongji Hospital from 2012 to 2015. Concurrently, healthy controls were recruited from a general population receiving a regular medical examination in Tongji Hospital and matched 1:1 to cases on basis of age (± 3 years) and gender. In this study, we included participants who were aged ≥ 30 and ≤ 80 years, body mass index (BMI) < 40 kg/m², no previous diagnosis of prediabetes or diabetes, and no use of antihyperlipidemic medication. Additionally, patients diagnosed as any other clinically systemic illness, infectious disease, chronic inflammatory illness, or acute disease were excluded.

To clarify the prospective association of plasma A β concentration with risk of prediabetes, we further conducted a nested case-control study within the Tongji-Ezhou cohort, a prospective cohort of 5533 participants from Echeng Stell, including 3101 retired employees and 2432 in-service employees. The cohort enrollment period was 2013–2015, and the first follow-up survey was completed by mid-2020. We conducted this study within the sub cohort of retired employees because the incidence rate of prediabetes was low in young in-service employees. At the first follow-up, 119 retired employees were diagnosed as new-onset prediabetes cases by detecting fasting plasma glucose (FPG). According to the same inclusion criteria as the case-control study, we excluded 2 new-onset prediabetes cases with age > 80 years. We also excluded 17 new-onset prediabetes cases without adequate blood samples. Two control participants were matched to each case on basis of age (± 3 years) and gender from retired employees without prediabetes or diabetes. Eventually, 100 cases and 200 age- and gender-matched controls were included for analysis.

These two population-based studies were approved by the Ethics Committee of Tongji Medical College and conducted by complying with the Declaration of Helsinki. Written informed consent was provided by the participants included in the study.

2.7 Assessment of prediabetes

In the case-control study, the definition of prediabetes was in accordance with the 1999 WHO criteria (24): FPG ≥ 6.1 and < 7.0 mmol/l or 2-h oral glucose tolerance test (OGTT) ≥ 7.8 and < 11.1 mmol/l. In the Tongji cohort, new-onset prediabetes was diagnosed when FPG was ≥ 6.1 and < 7.0 mmol/l.

2.8 Assessment of covariates

Sociodemographic characteristics of all participants, including age, gender, family history of diabetes, lifestyle habits (alcohol drinking, smoking, and physical activity), and history of diseases (CVD and hypertension) were obtained from a standardized questionnaire. The definition of physical activity was regular exercise for more than 60min/week in the past 6 months. The trained staff measured weight (kg) and height (m) and calculated BMI by dividing weight by the square of height. We determined fasting plasma insulin (FPI), FPG, total cholesterol, triacylglycerols, HDL-cholesterol (HDL-C), and LDL-cholesterol (LDL-C) using

fasting plasma samples as previously described (25). 2-h OGTT plasma samples were collected from subjects who were enrolled from Tongji Hospital and used to determine 2-h post-glucose load values. We calculated the index of homeostasis model assessment of insulin resistance (HOMA-IR) and β -cell function (HOMA- β) according to the following equations: $\text{HOMA-IR} = \text{FPI (pmol/l)} \times \text{FPG (mmol/l)} \div 156.3$; $\text{HOMA-}\beta = 2.88 \times \text{FPI (pmol/l)} \div [\text{FPG (mmol/l)} - 3.5]$. The triglyceride-glucose (TyG) index was calculated as: $\ln [\text{FPG (mg/dl)} \times \text{fasting TG (mg/dl)} \div 2]$.

2.9 Measurement of plasma A β levels

We simultaneously determined A β 40 and A β 42 levels in fasting plasma with the use of validated assay kits (Meso Scale Discovery, Rockville, USA). Both the within- and between-assay coefficients of variation of plasma A β 40 and A β 42 were <10%. Plasma A β levels of the included participants were above the limit of detection (A β 40: 20.0 ng/l; A β 42: 2.5 ng/l). The assays were performed by investigators who were blind to prediabetes status.

2.10 Statistical analysis

For the *in vitro* studies, all data are expressed as mean \pm standard error of mean (SEM) of three independent experiments. We assessed the differences among groups using one-way analysis of variance and further conducted multiple comparisons by least significant difference test.

For the population-based studies, characteristics of prediabetes cases and control subjects were compared using *t*-test, Mann-Whitney *U* test, or χ^2 test. We further evaluated the correlation between plasma A β and the variables of interest with Spearman correlation test. ORs and 95% CIs for prediabetes were estimated by conditional logistic regression analyses, in which plasma A β 40 and A β 42 levels were classified into quartiles according the distribution in control group or as continuous variables presented as an increment by 30 ng/l (A β 40) or 5 ng/l (A β 42). Adjustments were made for several potential confounders, including age (years), gender (female or male), BMI (<18.5, 18.5–23.9, 24–27.9, or \geq 28 kg/m²), family history of diabetes (yes or no), alcohol drinking habit (current, former, or never), smoking habit (current, former, or never), physical activity (yes or no), CVD (yes or no), and hypertension (yes or no). We conducted the linear trend test by assigning each quartile with the median of plasma A β 40 or A β 42 and using it as a continuous variable. Receiver-operating characteristic (ROC) curves were plotted to estimate the prediction of plasma A β levels on prediabetes. We compared area under the curves (AUCs) of model 1 including traditional risk factors (age, gender, BMI, family history of diabetes, alcohol drinking habit, smoking habit, physical activity, CVD, hypertension, FPG, and FPI) and model 2 with plasma A β 40 and A β 42 levels further added in.

All data analyses were conducted with the use of SPSS 20.0 (SPSS Inc., Chicago, IL) and R 4.2.2 (The R Foundation, <http://www.r-project.org>).

All presented *p* values are 2-tailed and considered as significance at level of 0.05.

3 Results

3.1 *In vitro* studies

Cells were treated with different doses of A β 40 and A β 42, and viability was detected at 12, 24, and 48 h. Exposure to 20 $\mu\text{mol/l}$ A β 40 and A β 42 induced a significant decrease in cell viability for all cell lines (Supplemental Figures 1–3). The viability of HepG2 cells and C2C12 myotubes were still above 80% after exposure to 20 $\mu\text{mol/l}$ A β 40 and A β 42 for 48 h. In INS-1 cells treated with 20 $\mu\text{mol/l}$ A β 40 and A β 42 for 48 h, cell viability decreased to 67% and 77%, respectively. To maintain high cell viability (>80%), INS-1 cells were treated with various doses of A β 40 and A β 42 for 24 h in insulin secretion assay. To rule out the effects of cytotoxicity of A β on subsequent experiments, the amounts of glucose production, glucose uptake, and insulin secretion were normalized to total protein amount of the remaining cells in the culture plate.

As expected, insulin significantly inhibited the glucose production in HepG2 cells and promoted the glucose uptake in C2C12 myotubes ($p < 0.001$) (Figures 1A–D). A β 40 and A β 42 dose-dependently attenuated the inhibitory effect of insulin on the glucose production in HepG2 cells (Figures 1A, B). The promotional effect of insulin on the glucose uptake in C2C12 myotubes gradually decline with increasing dose of A β 40 and A β 42 (Figures 1C, D). In addition, A β 40 and A β 42 dose-dependently suppressed the basal and glucose-stimulated insulin secretion in INS-1 cells (Figures 1E, F). Conversely, neither A β 40-1 nor A β 42-1 affected the insulin sensitivity in HepG2 cells and C2C12 myotubes and insulin secretion in INS-1 cells (Figures 1A–F).

3.2 Case-control study

General characteristics of the 1142 participants (571 newly-diagnosed prediabetes cases and 571 matched controls) are described in Table 1. Prediabetes cases showed higher BMI, higher levels of FPI, FPG, triglyceride, LDL-C, HOMA-IR and TyG index as well as lower levels of HDL-C and HOMA- β compared with control subjects. A higher proportion of prediabetes cases had a family history of diabetes, CVD, and hypertension. Additionally, prediabetes cases exhibited higher plasma A β 40 and A β 42 levels compared to control subjects.

Plasma A β 40 and A β 42 were significantly relevant to several parameters of glucose metabolism among healthy participants (Supplemental Table 1). After adjustment for age, gender, BMI, family history of diabetes, alcohol drinking habit, smoking habit, physical activity, CVD, and hypertension, plasma A β 40 was significantly related to FPG ($r = 0.220$), FPI ($r = 0.128$), HOMA-IR ($r = 0.175$), and TyG index ($r = 0.204$). Plasma A β 42 was also significantly correlated with FPG ($r = 0.145$), HOMA-IR ($r = 0.114$), and TyG index ($r = 0.106$) after these adjustments.

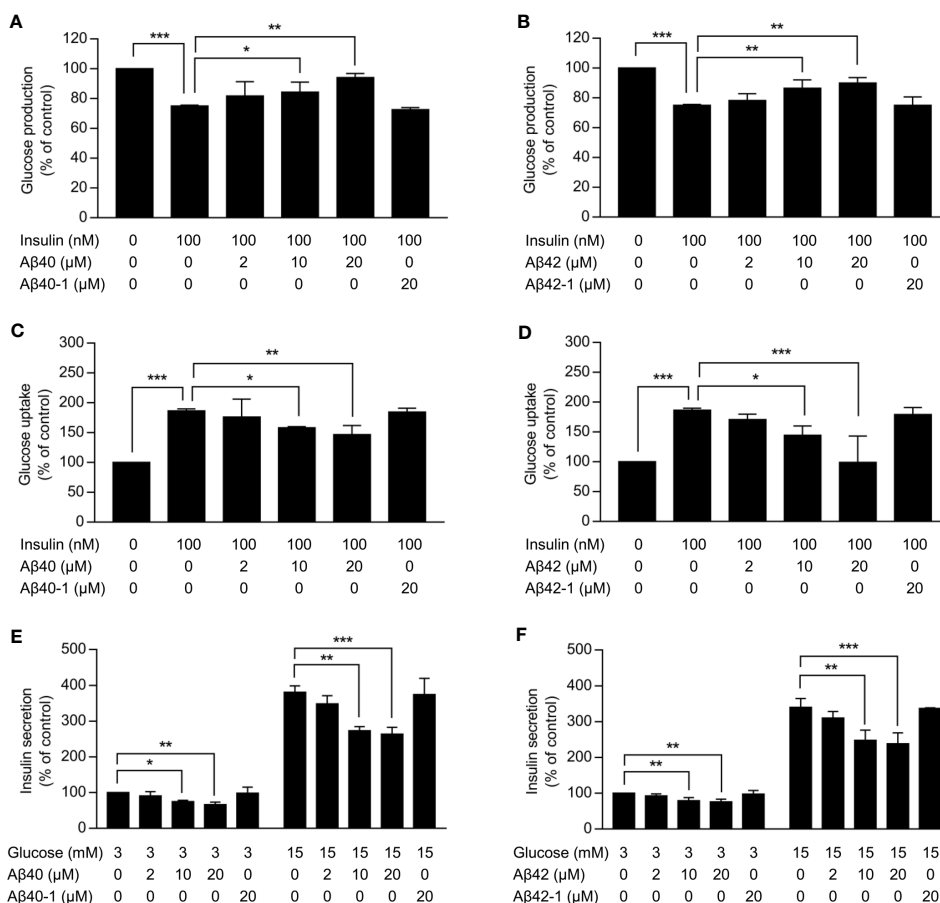


FIGURE 1 Effects of Aβ on insulin sensitivity in hepatocytes and myotubes and insulin secretion in pancreatic β-cells. (A,B) Glucose production in HepG2 cells treated with the indicated concentrations of Aβ40 or Aβ40-1 (A) or Aβ42 or Aβ42-1(b) for 48 h and then incubated without or with 100 nmol/l insulin for 3 h (n = 3). (C, D) Glucose uptake in C2C12 myotubes treated with the indicated concentrations of Aβ40 or Aβ40-1 (C) or Aβ42 or Aβ42-1(F) for 48 h and glucose starved for 1 h, and then incubated without or with 100 nmol/l insulin for 2 h (n = 3). (E, F) Insulin secretion in INS-1 cells treated with the indicated concentrations of Aβ40 or Aβ40-1 (E) or Aβ42 or Aβ42-1(F) for 24 h and glucose starved for 2 h, then incubated with 3 mmol/l or 15 mmol/l glucose for 2 h (n = 3). Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001 for indicated comparisons.

Table 2 presents odds of newly-diagnosed prediabetes associated with plasma Aβ40 and Aβ42 levels. In multivariate-adjusted analyses, the ORs of prediabetes were 2.00 (95% CI: 1.34, 3.01) for plasma Aβ40 and 1.94 (95% CI: 1.33, 2.83) for plasma Aβ42 when comparing the extreme quartiles. The adjusted ORs for prediabetes were 1.19 (95% CI: 1.03, 1.38) for every 30 ng/l higher plasma Aβ40 and 1.41 (95% CI: 1.20, 1.65) for every 5 ng/l higher plasma Aβ42.

3.3 Nested case-control study

The baseline characteristics of all subjects, including 100 incident prediabetes cases and 200 matched controls, are described in Table 3. Individuals who subsequently developed prediabetes had higher FPG, FPI, triglyceride, HOMA-IR, TyG index, and plasma Aβ40 and Aβ42 levels compared to control subjects.

Table 4 presents odds of incident prediabetes associated with plasma Aβ40 and Aβ42 levels. In multivariable analyses, compared

to the lowest quartile, the highest quartile of plasma Aβ40 and Aβ42 were associated with 3.51-fold (95% CI: 1.61, 7.62) and 2.75-fold (95% CI: 1.21, 6.22) greater odds of prediabetes, respectively. Every 30 ng/l of plasma Aβ40 and every 5 ng/l of plasma Aβ42 were associated with 56% (95% CI: 24%, 96%) and 60% (95% CI: 17%, 119%) increased odds of prediabetes, respectively.

The AUC for prediction of incident prediabetes was 0.69 (95% CI: 0.62, 0.75) by Model 1, including age, gender, BMI, family history of diabetes, alcohol drinking habit, smoking habit, physical activity, CVD, hypertension, FPG, and FPI (Figure 2). The AUC was markedly improved to 0.74 (95% CI: 0.69, 0.80; p = 0.018 for the comparison) when plasma Aβ40 and Aβ42 were added to the model (Model 2).

4 Discussion

In the *in vitro* studies, Aβ40 and Aβ42 dose-dependently attenuated insulin-inhibited glucose production in HepG2 cells

TABLE 1 Characteristics of the newly diagnosed prediabetes cases and matched controls in the case-control study.

Characteristics	Prediabetes (n = 571)	Control (n = 571)	p
Age (years)	51.46 (11.18)	51.87 (11.08)	0.525
Gender (male), n (%)	376 (65.85)	376 (65.85)	1.000
BMI (kg/m ²)	24.85 (3.30)	23.63 (3.10)	<0.001
Family history of diabetes, n (%)	78 (13.66)	42 (7.36)	<0.001
Current drinker, n (%)	172 (30.12)	177 (30.40)	0.748
Current smoker, n (%)	176 (30.82)	206 (36.08)	0.060
Regular physical activity, n (%)	214 (37.48)	233 (40.81)	0.249
CVD, n (%)	55 (9.63)	32 (5.60)	0.010
Hypertension, n (%)	178 (31.17)	115 (20.14)	<0.001
FPG (mmol/l)	6.33 (6.14–6.61)	5.49 (5.18–5.78)	<0.001
FPI (pmol/l)	64.91 (46.18–92.33)	50.57 (34.76–72.99)	<0.001
HOMA-IR	2.63 (1.86–3.67)	1.75 (1.21–2.58)	<0.001
HOMA-β	67.27 (46.97–101.05)	77.36 (52.12–110.44)	0.011
Triglyceride (mmol/l)	1.49 (1.00–2.39)	1.29 (0.91–1.70)	<0.001
Total cholesterol (mmol/l)	4.76 (4.12–5.46)	4.62 (4.10–5.19)	0.054
LDL-C (mmol/l)	2.57 (1.85–3.25)	2.44 (1.78–3.03)	0.014
HDL-C (mmol/l)	1.29 (1.03–1.51)	1.34 (1.19–1.51)	<0.001
TyG index	8.93 (8.53–9.37)	8.63 (8.26–8.91)	<0.001
Aβ40 (ng/l)	134.09 (118.72–153.59)	127.68 (114.58–145.10)	<0.001
Aβ42 (ng/l)	13.25 (10.78–16.58)	12.27 (10.19–14.92)	<0.001

Values are means (standard deviations) for parametrically distributed data, medians (interquartile ranges) for nonparametrically distributed data, or n (%) for categorical data.

and insulin-promoted glucose uptake in C2C12 myotubes. Meanwhile, Aβ40 and Aβ42 dose-dependently inhibited the basal and glucose-stimulated insulin secretion in INS-1 cells. In the cross-sectional and prospective population-based studies, we consistently found that elevated plasma Aβ40 and Aβ42 levels were associated with higher odds of prediabetes. Additionally, plasma Aβ40 and Aβ42 significantly improved the predictive value for prediabetes.

It has been reported that *APP* transgenic mice with high plasma Aβ40 and Aβ42 concentrations exhibited peripheral insulin resistance (17, 26). Peripheral insulin resistance in the mice was ameliorated by the active immunity and passive immunity for Aβ (26, 27). Similarly, mice deficient in *BACE1*, a hydrolase for APP, showed decreased Aβ production and improved insulin resistance when fed a high-fat diet (28). These experimental studies suggest Aβ as a negative regulator of peripheral insulin sensitivity. The current study found positive relevance of plasma Aβ40 and Aβ42 to HOMA-IR and TyG index in healthy subjects. Meanwhile, we observed that both Aβ40 and Aβ42 attenuated insulin action on hepatocytes and myotubes. Our findings indicate that Aβ may promote peripheral insulin resistance in human by directly impairing insulin signaling in liver and skeletal muscle. Supporting our findings, Zhang and colleagues discovered that Aβ could impair hepatic insulin signaling via activating Janus kinase 2 (17, 27). In addition, Aβ has also been reported to

impair neuronal insulin signaling through competing for insulin binding to the insulin receptor (29), removing insulin receptors from neuronal surface (30, 31) and activating c-Jun N-terminal kinase to trigger insulin receptor substrate-1 inhibition (32, 33). But whether these mechanisms are involved in Aβ-induced insulin resistance in peripheral tissues needs to be further explored.

Previous study, using gene regulation network analyses, identified *APP* as a top candidate gene for the regulation of insulin secretion from pancreatic islets (34). Meanwhile, the study found that knockout of *APP* in mice enhanced insulin secretion from pancreatic islets through an unclear mechanism. Our *in vitro* study revealed the negative effect of Aβ on insulin secretion, suggesting APP-derived Aβ may mediate the role of *APP* in pancreatic islets. Supporting this hypothesis, mice overexpressing APP exhibited Aβ accumulation in the pancreatic islets and decreased insulin levels in Aβ-positive regions (7). Additionally, Aβ deposition has been detected in the pancreas of subjects with T2D, leading us to speculate that peripheral Aβ might be a biomarker for β-cell dysfunction in human (8, 9). However, we did not find significant correlations between plasma Aβ40 and Aβ42 and HOMA-β among controls. This may be explained by the normal β-cell function in healthy controls, and we found significant and negative association of plasma Aβ40 with HOMA-β when combining both cases and controls (data not shown). Further

TABLE 2 ORs (95% CIs) for newly diagnosed prediabetes associated with plasma Aβ40 and Aβ42 concentrations in the case-control study.

Variable	Quartiles of plasma Aβ concentrations				p for trend	Continuous ^b
	Q1 (lowest)	Q2	Q3	Q4 (highest)		
Plasma Aβ40						
Range (ng/l)	<114.52	114.52–<127.69	127.69–<145.13	≥145.13		
Median (ng/l)	106.30	121.14	134.54	164.86		
Case/control, n	105/143	115/143	159/143	192/142		
Model ^a						
Crude	1.00 (ref.)	1.13 (0.78, 1.62)	1.55 (1.10, 2.19)	2.07 (1.43, 2.99)	<0.001	1.21 (1.06, 1.37)
Model 1	1.00 (ref.)	1.13 (0.77, 1.66)	1.57 (1.09, 2.24)	2.10 (1.43, 3.09)	<0.001	1.22 (1.06, 1.40)
Model 2	1.00 (ref.)	1.10 (0.74, 1.63)	1.53 (1.05, 2.24)	2.00 (1.34, 3.01)	<0.001	1.19 (1.03, 1.38)
Plasma Aβ42						
Range (ng/l)	<10.19	10.19–<12.28	12.28–<14.95	≥14.95		
Median (ng/l)	8.70	11.28	13.44	17.69		
Case/control, n	116/143	109/142	140/144	206/142		
Model ^a						
Crude	1.00 (ref.)	0.96 (0.68, 1.36)	1.25 (0.89, 1.76)	1.91 (1.35, 2.69)	<0.001	1.41 (1.22, 1.63)
Model 1	1.00 (ref.)	1.01 (0.70, 1.46)	1.37 (0.96, 1.97)	2.00 (1.40, 2.87)	<0.001	1.42 (1.22, 1.65)
Model 2	1.00 (ref.)	0.93 (0.64, 1.37)	1.26 (0.86, 1.85)	1.94 (1.33, 2.83)	<0.001	1.41 (1.20, 1.65)

^aModel 1 was adjusted for age, gender, and BMI. Model 2 was additionally adjusted for family history of diabetes, alcohol drinking habit, smoking habit, physical activity, CVD, and hypertension.
^bORs (95% CIs) of newly diagnosed prediabetes associated with each 30 ng/l increase in plasma Aβ40 and each 5 ng/l increase in plasma Aβ42.
 Q, quartile; ref., reference.

epidemiological researches are needed to evaluate the forecast value of plasma Aβ for β-cell dysfunction.

Prediabetes is a metabolic intermediate state between normoglycemia and T2D, with 5–10% of prediabetic people progressing to T2D each year (15). Prediabetes is considered as a critical stage in preventing or delaying the onset of T2D due to its reversibility (15), therefore, it is important to understand the underlying mechanisms of prediabetes and to develop corresponding human biomarkers. This study, for the first time, reveals that plasma Aβ40 and Aβ42 are positively associated with prediabetes in human subjects. Similarly, our previous report demonstrated that higher plasma Aβ40 and Aβ42 levels were associated with increased risk of T2D (35). Previous case-control study also observed higher serum Aβ-autoantibody concentration, a biomarker reflecting Aβ level, in subjects with T2D (36). Yet, another case-control study reported that T2D cases exhibited lower plasma Aβ40 and Aβ42 levels than the controls (37). A plausible explanation for the inconsistency might be that the aforementioned study included cases with using hypoglycemic agents (37), which have been reported to affect plasma Aβ40 and Aβ42 levels (38–40). Therefore, the use of hypoglycemic agents should be considered when plasma Aβ is used as a biomarker for prediabetes and T2D.

Our study is the first that has combined *in vitro* studies and population-based studies to systematically explore the role of

peripheral Aβ in insulin resistance, insulin secretion, and prediabetes. However, our study also has several limitations. Firstly, we were unable to determine the causality between plasma Aβ and prediabetes due to the observational nature of population-based studies. Secondly, the findings from population-based studies might be affected by residual confounding of other unmeasured factors, although we controlled for multiple risk factors of prediabetes. Thirdly, new-onset prediabetes was diagnosed only based on FPG in the Tongji-Ezhou cohort, and subjects who developed T2D might be misdiagnosed as prediabetes. Taken together with our previous study (35), plasma Aβ levels were not significantly different between prediabetes and T2D individuals. Hence, the positive association between plasma Aβ and new-onset prediabetes could persist if misdiagnosed subjects were excluded. Fourthly, glycated hemoglobin, an important parameter for glucose homeostasis, was not determined in our population-based studies. Fifthly, cognitive function was not evaluated in our population-based studies. Considering the positive associations of prediabetes and T2D with cognitive impairment and Alzheimer’s disease (41, 42), further studies are needed to explore the role of plasma Aβ in linking these conditions.

In conclusion, elevated plasma Aβ40 and Aβ42 levels were associated with increased risk of prediabetes in human subjects, which may be through impairing insulin sensitivity in hepatocytes and myotubes and insulin secretion in pancreatic β-cells. Plasma

TABLE 3 Baseline characteristic of the incident prediabetes cases and matched controls in the nested case-control study.

Characteristics	Prediabetes (<i>n</i> = 100)	Control (<i>n</i> = 200)	<i>p</i>
Age (years)	62.40 (5.94)	62.39 (5.80)	0.983
Gender (male), <i>n</i> (%)	63 (63.00)	126 (63.00)	1.000
BMI (kg/m ²)	24.11 (2.93)	23.59 (2.76)	0.128
Family history of diabetes, <i>n</i> (%)	7 (7.00)	8 (4.00)	0.261
Current drinker, <i>n</i> (%)	31 (31.00)	59 (29.50)	0.789
Current smoker, <i>n</i> (%)	25 (25.00)	66 (33.00)	0.155
Regular physical activity, <i>n</i> (%)	57 (57.00)	106 (53.00)	0.512
Hypertension, <i>n</i> (%)	31 (31.00)	54 (27.00)	0.469
CVD, <i>n</i> (%)	10 (10.00)	20 (10.00)	1.000
FPG (mmol/l)	5.54 (5.15–5.78)	5.31 (4.99–5.62)	0.003
FPI (pmol/l)	56.95 (40.42–80.16)	48.75 (33.70–67.64)	0.022
HOMA-IR	2.05 (1.37–2.78)	1.69 (1.09–2.37)	0.008
HOMA-β	87.17 (62.23–121.00)	81.33 (56.84–115.78)	0.416
Triglyceride (mmol/l)	1.40 (0.98–1.84)	1.15 (0.82–1.58)	0.004
Total cholesterol (mmol/l)	4.72 (4.30–5.23)	4.85 (4.10–5.40)	0.912
LDL-C (mmol/l)	2.79 (2.38–3.44)	2.86 (2.33–3.37)	0.849
HDL-C (mmol/l)	1.37 (1.16–1.61)	1.38 (1.19–1.60)	0.611
TyG index	8.70 (8.35–8.96)	8.46 (8.13–8.85)	0.001
Aβ40 (ng/l)	143.98 (124.22–174.89)	130.36 (113.03–150.65)	<0.001
Aβ42 (ng/l)	13.96 (12.12–17.31)	12.42 (10.81–14.88)	<0.001

Values are means (standard deviations) for parametrically distributed data, medians (interquartile ranges) for nonparametrically distributed data, or *n* (%) for categorical data.

TABLE 4 ORs (95% CIs) for incident prediabetes associated with plasma A β 40 and A β 42 concentrations in the nested case-control study.

Variable	Quartiles of plasma A β concentrations				<i>p</i> for trend	Continuous ^b
	Q1 (lowest)	Q2	Q3	Q4 (highest)		
Plasma A β 40						
Range (ng/l)	<113.02	113.02–<130.37	130.37–<150.68	≥150.68		
Median (ng/l)	101.06	121.63	140.96	175.97		
Case/control, n	13/50	20/50	23/50	44/50		
Modela						
Crude	1.00 (ref.)	1.51 (0.67, 3.38)	1.78 (0.78, 4.03)	3.25 (1.56, 6.79)	0.001	1.50 (1.21, 1.87)
Model 1	1.00 (ref.)	1.47 (0.65, 3.33)	1.71 (0.75, 3.94)	3.37 (1.60, 7.08)	<0.001	1.53 (1.23, 1.92)
Model 2	1.00 (ref.)	1.49 (0.64, 3.49)	1.92 (0.81, 4.54)	3.51 (1.61, 7.62)	0.001	1.56 (1.24, 1.96)
Plasma A β 42						
Range (ng/l)	<10.82	10.82–<12.42	12.42–<14.91	≥14.91		
Median (ng/l)	9.24	11.71	13.49	17.77		
Case/control, n	14/50	16/50	34/50	36/50		
Modela						
Crude	1.00 (ref.)	1.24 (0.53, 2.93)	2.74 (1.25, 6.01)	2.85 (1.32, 6.15)	0.003	1.58 (1.17, 2.12)
Model 1	1.00 (ref.)	1.19 (0.49, 2.86)	2.73 (1.24, 6.00)	2.84 (1.30, 6.21)	0.003	1.57 (1.16, 2.13)
Model 2	1.00 (ref.)	1.17 (0.47, 2.90)	2.61 (1.16, 5.90)	2.75 (1.21, 6.22)	0.006	1.60 (1.17, 2.19)

^aModel 1 was adjusted for age, gender, and BMI. Model 2 was additionally adjusted for family history of diabetes, alcohol drinking habit, smoking habit, physical activity, CVD, and hypertension.

^bORs (95% CIs) of incident prediabetes associated with each 30 ng/l increase in plasma A β 40 and each 5 ng/l increase in plasma A β 42.

Q, quartile; ref., reference.

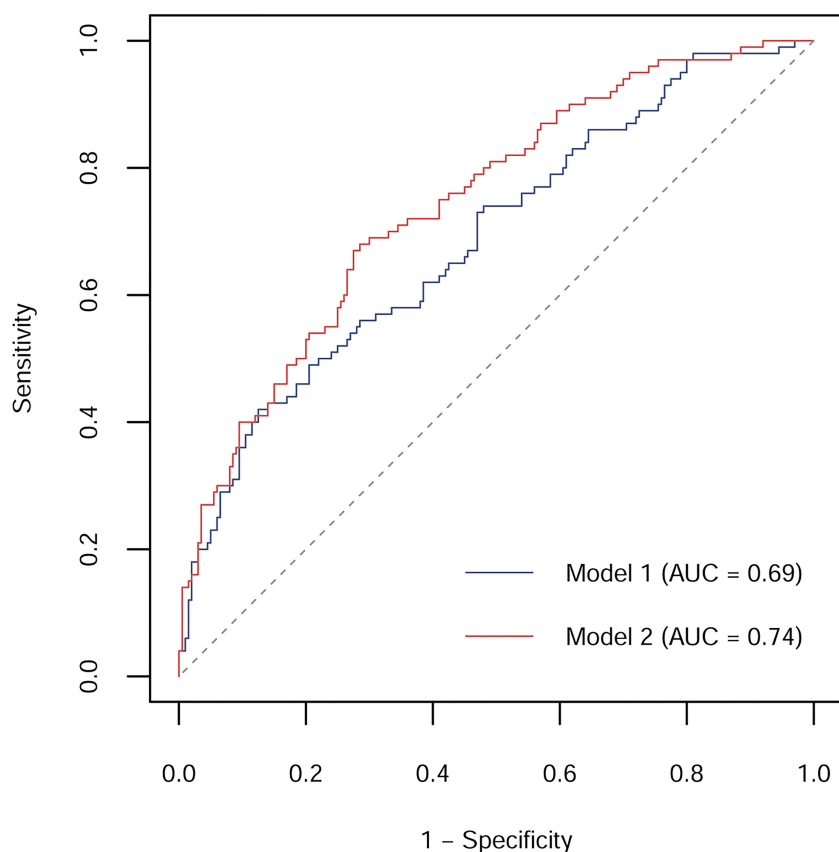


FIGURE 2

ROC curves and corresponding AUCs for prediction of incident prediabetes by models without or with plasma A β 40 and A β 42 in the nested case-control study. Model 1 included age, gender, BMI, family history of diabetes, alcohol drinking habit, smoking habit, physical activity, CVD, hypertension, FPG, and FPI; Model 2 included Model 1 plus plasma A β 40 and A β 42.

A β could be used as a predictor for prediabetes, and reducing plasma A β level may be a novel therapy for prediabetes. The molecular mechanisms of A β affecting peripheral insulin sensitivity and insulin secretion need to be further clarified.

analysis, or interpretation of data. XP wrote the drafter of the paper. ZX, JC, PW, LYL, SH, HL, YH, XM, HY, ZS, DW, JX, and LGL contributed to reviewing and revising the paper. All authors read and approved the final manuscript.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Tongji Medical College. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZX, LGL, and XP designed the study. ZX, JC, PW, LYL, SH, HL, YH, XM, HY, ZS, DW, JX, and XP contributed to the acquisition,

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fendo.2023.1195658/full#supplementary-material>

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