Amyloid-β Induces Hepatic Insulin Resistance In Vivo via JAK2

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Amyloid-β (Aβ), a natural product of cell metabolism, plays a key role in the pathogenesis of Alzheimer's disease (AD). Epidemiological studies indicate patients with AD have an increased risk of developing type 2 diabetes mellitus (T2DM). Aβ can induce insulin resistance in cultured hepatocytes by activating the JAK2/STAT3/SOCS-1 signaling pathway. Amyloid precursor protein and presenilin 1 double-transgenic AD mouse models with increased circulating Aβ level show impaired glucose/insulin tolerance and hepatic insulin resistance. However, whether Aβ induces hepatic insulin resistance in vivo is still unclear. Here we show C57BL/6J mice intraperitoneally injected with Aβ42 exhibit increased fasting blood glucose level, impaired insulin tolerance, and hepatic insulin signaling. Moreover, the APPswe/PSEN1dE9 AD model mice intraperitoneally injected with anti-Aβ neutralizing antibodies show decreased fasting blood glucose level and improved insulin sensitivity. Injection of Aβ42 activates hepatic JAK2/STAT3/SOCS-1 signaling, and neutralization of Aβ in APPswe/PSEN1dE9 mice inhibits liver JAK2/STAT3/SOCS-1 signaling. Furthermore, knockdown of hepatic JAK2 by tail vein injection of adenovirus inhibits JAK2/STAT3/SOCS-1 signaling and improves glucose/insulin tolerance and hepatic insulin sensitivity in APPswe/PSEN1dE9 mice. Our results demonstrate that Aβ induces hepatic insulin resistance in vivo via JAK2, suggesting that inhibition of Aβ signaling is a new strategy toward resolving insulin resistance and T2DM. ***Diabetes*** **62:1159–1166, 2013**

More than 250 million people worldwide were diagnosed with type 2 diabetes mellitus (T2DM) in 2011, and this number is expected to double within the next 20 years (1). Insulin resistance is a key element in the pathogenesis of T2DM, defined as a state of reduced responsiveness to normal circulating levels of insulin in insulin-target liver, skeletal muscle, and adipose tissues (2). Many states give rise to insulin resistance, and all are explained by numerous mechanisms in which insulin signaling is decreased (3). Interestingly, it has been reported that brains from Alzheimer's disease (AD) patients display impaired insulin signaling (4,5), and some AD patients exhibit impaired glucose metabolism and hyperinsulinemia (6,7). Furthermore, an epidemiological study indicates patients with AD have an increased risk of developing T2DM (8), and experimental studies demonstrate AD model mice also exhibit diabetic phenotype (9,10). These studies together reveal a strong correlation between AD and insulin resistance/T2DM.

Amyloid-β (Aβ) is a natural product during cell metabolism and originates from proteolysis of the amyloid precursor protein (APP) by the sequential enzymatic actions of β-site amyloid precursor protein–cleaving enzyme 1 (BACE-1) and γ-secretase (11). According to the amyloid cascade hypothesis, Aβ has an early and vital role in the pathogenesis of AD (11,12). In the central nervous system, Aβ has been reported to impair neuronal synaptic function in early AD by compromising insulin signaling (13–16). Interestingly, Aβ can be detected in the peripheral circulation and tissues (17–19).

We have previously reported that Aβ induces insulin resistance in cultured hepatocytes mainly through the JAK2/STAT3/SOCS-1 signaling pathway (10), indicating that Aβ is an inducer of insulin resistance in vitro. On the other hand, animal studies demonstrate that the crossbred mice of APP23 transgenic AD model mice and *ob/ob* mice showed an accelerated diabetic phenotype (20). Moreover, APPswe/PS1$^{(A246E)}$ transgenic AD model mice with increased plasma Aβ42 level exhibit impaired glucose tolerance when fed a chow diet (9,21). Consistently, we have recently reported that APPswe/PSEN1dE9 (APP/PS1) transgenic AD model mice with increased plasma Aβ40/42 levels show impaired glucose/insulin tolerance and hepatic insulin signaling, hyperinsulinemia, and upregulation of SOCS-1 and phosphorylated JAK2 and STAT3 in the liver (10). However, it is still possible that the insulin resistance in AD model mice might be due to the overexpression of presenilin 1, APP, and/or APP cleavage products except Aβ. Thus, whether Aβ itself can induce insulin resistance in vivo is yet to be elucidated. In addition, we previously showed that Aβ induces insulin resistance by activating the JAK2/STAT3/SOCS-1 signaling pathway in cultured hepatocytes (10). Whether Aβ also induces hepatic insulin resistance in vivo by activating the JAK2/STAT3/SOCS-1 signaling pathway is still unclear.

In this study, we investigated the effect of Aβ on insulin sensitivity in vivo by injection of Aβ42 into wild-type mice and injection of Aβ-neutralizing antibodies or adenovirus expressing JAK2 small interfering (si)RNA into APP/PS1 AD model mice. We found that injection of Aβ42 into C57BL/6J mice induces insulin resistance and activates hepatic JAK2/STAT3/SOCS-1 signaling. Moreover, APP/PS1 mice treated with anti–Aβ-neutralizing antibodies show improved insulin sensitivity and attenuated hepatic JAK2/STAT3/SOCS-1 signaling. Furthermore, knockdown of hepatic JAK2 by adenovirus inhibited JAK2/STAT3/SOCS-1 signaling and improved insulin sensitivity in APP/PS1 mice.

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See accompanying commentary, p. 1005.

RESEARCH DESIGN AND METHODS

Reagents. Aβ42 and the reverse peptide of Aβ42 named Aβ(42-1) were from Apeptide (Jiangsu, China). Control IgG, 1H3, and 6C8 mouse anti-human Aβ monoclonal antibodies were from Anogen-Yes Biotech Laboratories (Canada). Antibodies against Tyr1150/1151-phosphorylated insulin receptor (InsR), Thr308-phosphorylated Akt, Ser473-phosphorylated Akt, SOCS-1, SOCS-3, Tyr705-phosphorylated STAT3, Tyr1007/1008-phosphorylated JAK2, InsR, Akt, STAT3, and JAK2 were from Cell Signaling. Antibody against α-tubulin was from Sigma.

Aβ42 and Aβ(42-1) preparation and injection. Aβ42 was prepared as described previously (22). Briefly, Aβ42 was dissolved in sterile water at ~6 μg/μL, and the solution was brought to neutral pH with NaOH. Then, 10× PBS and water were added to reach a final peptide concentration of 5 μg/μL in 1× PBS, and the peptide solution was incubated at 37°C for 5 days. Aβ(42-1) was prepared as Aβ42. The prepared Aβ42 or Aβ(42-1) solution was diluted with 1× PBS to 0.5 and 0.125 μg/μL, and 100 μL of 0.5 μg/μL (high-dose) or 0.125 μg/μL (low-dose) Aβ42, Aβ(42-1), or 100 μL of 1× PBS was intraperitoneally injected into 10-week-old C57BL/6J male mice twice daily at ZT2 and ZT10.

Animals. All animals were maintained and used in accordance with the guidelines of the Institute for Nutritional Sciences Institutional Animal Care and Use Committee. C57BL/6J mice were from Slac (Shanghai, China). APPswe/PSEN1dE9 (APP/PS1) mice and their wild-type littermates were from Jackson Laboratory (Stock No. 004462). The mice were genotyped by PCR analysis as described by Jackson Laboratory. Mice were maintained on a 12-h light/dark cycle with free access to water and standard rodent chow. Tissues of interest were snap-frozen in liquid nitrogen immediately after resection and stored at −80°C.

Antibody neutralization. Control IgG, 1H3, and 6C8 anti-Aβ antibodies were dissolved in PBS at 1 mg/mL. Three-month-old male APP/PS1 mice were injected intraperitoneally with control IgG, 1H3, or 6C8 anti-Aβ antibody at a dose of 10 mg/kg body weight once weekly for 1 week or 4 or 9 months. Blood glucose and insulin levels were measured in mice after 4-h fasting.

Metabolic parameters measurements. Glucose tolerance tests were performed on mice fasted for 14 h, and the mice were intraperitoneally injected with 2 g/kg body weight of glucose. Insulin tolerance tests were performed on mice fasted for 4 h, and 0.75 and 1.5 units/kg body weight of human insulin (Novo Nordisk) were injected intraperitoneally for C57BL/6J and APP/PS1 mice, respectively. Glucose levels were measured from tail vein blood using the FreeStyle blood glucose monitoring system (TheraSense) at the indicated time points. Plasma insulin levels were measured by an ELISA kit from Shibayagi (Japan). Homeostasis model assessment-insulin resistance (HOMA-IR) was obtained using the HOMA Calculator v2.2 (23). Alanine transaminase and aspartate transaminase activity in plasma were determined by assay kits from Shensuo Unf Medical Diagnostics (Shanghai, China).

Insulin signaling in mouse liver. After fasting for 16 h, the mice were injected with PBS or human insulin (Novo Nordisk) intraperitoneally at a dose of 5 units/kg body weight. Mice were killed 10 min later, and liver samples were collected to detect the indicated proteins in insulin signaling by immunoblot.

Immunoblot and ELISA. Immunoblot was performed with antibodies against the indicated protein and quantified as previously described (10). Plasma Aβ42 levels were measured by an ELISA kit from Covance.

Adenovirus preparation and injection. We designed the JAK2-specific RNA interference (RNAi) by targeting 5′-GCAAACCAGGAATGCTCA-3′ in the JAK2 coding sequence, which has been shown to efficiently knock down the JAK2 protein level (10,24). The oligonucleotides 5′-CACCGCAAACCAGGA ATGCTCAACGAATTGAGCATTCCTGGTTTGC-3′ and 5′-AAAAGCAAACCAGG AATGCTCAATTCGTTGAGCATTCCTGGTTTGC-3′ were synthesized, annealed, and inserted into the U6 entry vector (Invitrogen). The plasmid expressing small interfering RNA (siRNA) for LacZ (LacZ RNAi) from Invitrogen was used as a control. The JAK2 RNAi and LacZ RNAi recombinant adenoviral plasmids were generated by homologous recombination with the pAd promoterless vector (Invitrogen). The recombinant adenoviruses were produced in 293A cells and purified as previously described (25). After several rounds of propagation, the recombinant adenoviruses were purified by CsCl gradient centrifugation. The LacZ RNAi or JAK2 RNAi adenoviruses were injected into mice through the tail vein at a dose of 1×10^9 plaque-forming units per mouse.

Statistical analyses. Data are expressed as mean ± SD of at least three independent experiments. Statistical significance was assessed by two-tailed unpaired Student t test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Injection of Aβ42 upregulates fasting blood glucose level and impairs insulin tolerance and hepatic insulin signaling in mice. To investigate whether Aβ itself can induce insulin resistance in vivo, we directly injected Aβ42, the most toxic form of Aβ in the brain (26), into normal male C57BL/6J mice intraperitoneally at a relatively high dose of 50 μg per mouse twice daily. After injection for 3 days, plasma Aβ42 levels increased to about 1,450 pg/mL (Fig. 1*A*), which is about 6 times of that in hyperglycemic patients and about 2 to 10 times of that in AD model mice (10,21). Injection of high-dose Aβ42 for 9 days resulted in increased overnight fasting blood glucose level (Fig. 1*B*). Injection of high-dose Aβ42 for 19 days reduced insulin sensitivity markedly, as measured by the insulin tolerance test (Fig. 1*C*). The area under the curve of plasma glucose abundance during the insulin tolerance test was significantly increased after injection of Aβ42 (Fig. 1*D*).

Because circulating peripheral Aβ is taken up and catabolized mainly by the liver and Aβ can impair insulin signaling in cultured hepatocytes (10,27), we studied the effect of Aβ42 injection on hepatic insulin signaling. Injection of insulin induced phosphorylation of the insulin receptor (InsR) at Tyr1150/1151 as well as Akt at Thr308 and Ser473 in the liver (Fig. 1*E* and *F*). As expected, injection of high-dose Aβ42 significantly inhibited insulin-induced phosphorylation of InsR and Akt in the mouse livers (Fig. 1*E* and *F*). The mice injected with high-dose Aβ42 displayed normal body weight, food intake, and plasma alanine transaminase and aspartate transaminase activity compared with those injected with PBS (Supplementary Fig. 1*A*–*C*), indicating that Aβ42 induces insulin resistance without affecting body weight, food intake, and liver function. To ensure the insulin resistance–inducing effect of Aβ42 in vivo, we injected Aβ(42-1), the reverse peptide of Aβ42, into male C57BL/6J mice intraperitoneally at a dose of 50 μg per mouse twice daily as well. Injection of Aβ (42-1) did not significantly affect the fasting blood glucose and insulin sensitivity (Supplementary Fig. 2*A*–*C*). The mice injected with Aβ(42-1) also displayed normal body weight, food intake, and plasma alanine transaminase and aspartate transaminase activity compared with those injected with PBS (Supplementary Fig. 2*D*–*F*). Together, these results demonstrate that high-dose Aβ can independently induce insulin resistance in vivo.

We then assessed whether injection with a relatively low dose of Aβ could induce insulin resistance as well. We injected Aβ42 into C57BL/6J mice intraperitoneally at a dose of 12.5 μg per mouse twice daily. After injection for 3 days, plasma Aβ42 levels reached to ~500 pg/mL (Fig. 1*G*), which is close to the reported Aβ42 levels in hyperglycemic patients or AD model mice (10,21). Although injection of low-dose Aβ42 for 9 days did not affect the fasting blood glucose, the injection of low-dose Aβ42 for 17 days resulted in increased fasting blood glucose level (Fig. 1*H*). Consistently, injection of low-dose Aβ42 for 25 days reduced insulin sensitivity significantly (Fig. 1*I* and *J*) and impaired insulin signaling in liver (Fig. 1*K* and *L*). The mice injected with low-dose Aβ42 also displayed normal body weight, food intake, and plasma alanine transaminase and aspartate transaminase activity compared with those injected with PBS (Supplementary Fig. 3*A*–*C*). In addition, we also injected Aβ(42-1) into C57BL/6J mice intraperitoneally at a low dose of 12.5 μg per mouse twice daily. These mice showed similar fasting blood glucose, insulin sensitivity, body weight, food intake, and plasma alanine transaminase and aspartate transaminase activity compared with those injected with PBS (Supplementary Fig. 4*A*–*F*). Taken together, these data indicate that Aβ at a relatively low dose can induce insulin resistance in vivo as well.

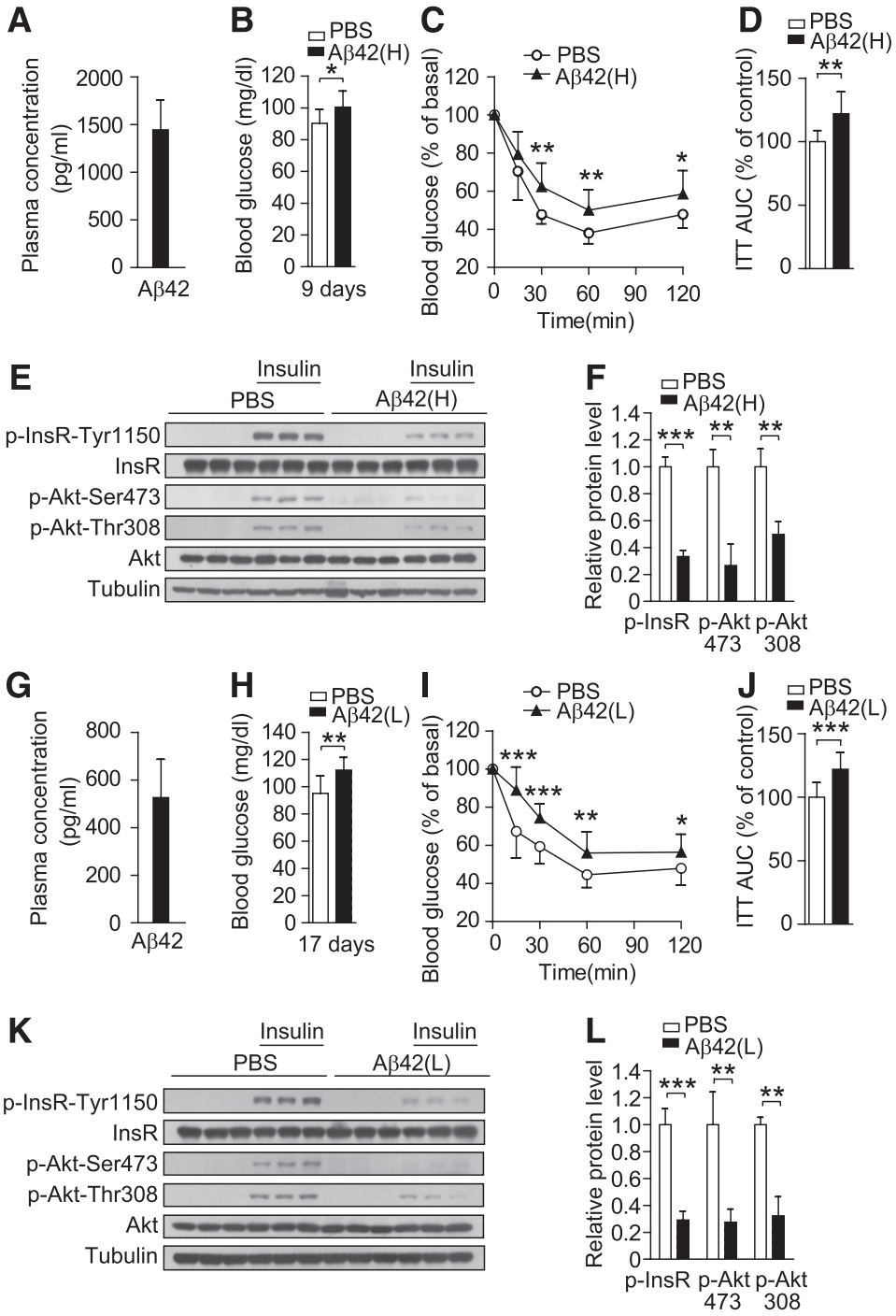

FIG. 1. Aβ42 upregulates fasting glucose level and impairs insulin tolerance and hepatic insulin signaling. *A*: Plasma Aβ concentration of 10-week-old C57BL/6J mice intraperitoneally injected with high-dose Aβ42 (50 μg/mice, twice daily) for 3 days (n = 9). *B*: Fasting blood glucose levels of 10-week-old C57BL/6J mice injected with PBS or high-dose Aβ42 for 9 days (n = 12 for each group). Fasting blood glucose levels were measured in mice fasted overnight for 14 h. *C*: Insulin tolerance test of 10-week-old C57BL/6J mice injected with PBS or high-dose Aβ42 for 19 days (n = 11–12 for each group). *D*: Area under the curve (AUC) of insulin tolerance tests (ITT) in panel *C*. *E*: Insulin-stimulated phosphorylation of InsR and Akt in the livers of 10-week-old C57BL/6J mice injected with PBS or high-dose Aβ42 for 23 days was measured by immunoblot. *F*: Quantification of phosphorylated InsR and Akt levels in panel *E*. *G*: Plasma Aβ concentration of 10-week-old C57BL/6J mice injected with low-dose Aβ42 (12.5 μg/mice, twice daily) for 3 days (n = 4). *H*: Fasting blood glucose levels of 10-week-old C57BL/6J mice injected with PBS or low-dose Aβ42 for 17 days (n = 12 for each group). *I*: Insulin tolerance test of 10-week-old C57BL/6J mice injected with PBS or low-dose Aβ42 for 25 days (n = 12 for each group). *J*: AUC of insulin tolerance tests in panel *I*. *K*: Insulin-stimulated phosphorylation of InsR and Akt in the liver of 10-week-old C57BL/6J mice injected with PBS or low-dose Aβ42 for 29 days. *L*: Quantification of phosphorylated InsR and Akt levels in panel *K*. The protein levels were normalized to tubulin. Aβ42(H), high-dose Aβ42; Aβ42(L), low-dose Aβ42. Data are presented as mean and SD. *P < 0.05, **P < 0.01, ***P < 0.001.

Neutralization of Aβ downregulates fasting blood glucose level and improves insulin sensitivity in APP/PS1 AD model mice. We recently reported that APP/PS1 AD model mice with increased plasma Aβ40/42 levels show insulin resistance (10). However, whether Aβ itself induces insulin resistance in APP/PS1 AD model mice is still unclear. To further confirm the role of Aβ in the development of insulin resistance in vivo, we treated the

APP/PS1 mice with control IgG, 6C8, or 1H3 anti–Aβ-neutralizing antibodies. Intraperitoneal injection of 1H3 and 6C8 for 1 week did not significantly affect fasting blood glucose levels (Supplementary Fig. 5*A*). After intraperitoneal injection for 4 months, 1H3 and 6C8 anti–Aβ-neutralizing antibodies both significantly reduced fasting blood glucose levels compared with control IgG, and the effect of the 6C8 anti-Aβ antibody on blood glucose level might be a little bit better than that of the 1H3 anti-Aβ antibody (Fig. 2*A*).

We then chose the 6C8 anti-Aβ antibody for a long-term treatment. After treatment for 9 months, the 6C8 anti-Aβ antibody not only reduced fasting glucose levels more significantly but also markedly alleviated hyperinsulinemia and improved insulin sensitivity as evaluated by the HOMA-IR index (Fig. 2*B–D*). These results demonstrate that neutralization of Aβ can attenuate hyperglycemia and insulin resistance in vivo.

Aβ42 induces SOCS-1 upregulation and JAK2/STAT3 activation in mouse liver. We then investigated the mechanisms underlying the insulin resistance induced by Aβ in vivo. We previously found that Aβ induces insulin resistance in cultured hepatocytes mainly by upregulating SOCS-1, a well-known inhibitor of insulin signaling, and that SOCS-1 expression was significantly increased in the livers of APP/PS1 and APPswe/PS1$^{(A246E)}$ mice (10). Therefore, we explored the SOCS-1 expression in the livers of mice injected with Aβ42. Immunoblot showed that the protein level of SOCS-1 was markedly elevated in the livers of mice injected with high-dose Aβ42 compared with those injected with PBS, whereas the SOCS-3 protein level remained unchanged (Fig. 3*A* and *B*). This finding is consistent with the increased SOCS-1 and unchanged SOCS-3 protein levels in the livers of APP/PS1 mice and primary cultured hepatocytes treated with Aβ (10). SOCS-1 expression can be induced by activation of the JAK/STAT pathway (28), and Aβ can regulate JAK2 and STAT3 in neurons and hepatocytes (10,29,30). Hepatic JAK2/STAT3 is in a more active state in APP/PS1 and APPswe/PS1$^{(A246E)}$ mice (10).

We then investigated phosphorylation levels of JAK2 and STAT3 in mouse liver. Immunoblot showed that the tyrosine phosphorylation levels of JAK2 and STAT3 were also significantly increased in the livers of mice injected with high-dose Aβ42 compared with those injected with PBS (Fig. 3*C* and *D*). Furthermore, immunoblot showed that the protein level of SOCS-1 and the tyrosine phosphorylation levels of JAK2 and STAT3 were markedly elevated in the livers of mice injected with low-dose Aβ42 as well (Fig. 3*E–H*). Together, these results indicate that Aβ42 can upregulate SOCS-1 and activate JAK2/STAT3 signaling in the mouse liver.

Neutralization of Aβ inhibits hepatic JAK2/STAT3/SOCS1 signaling in APP/PS1 AD model mice. Then we investigated the effect of anti–Aβ-neutralizing antibodies on JAK2/STAT3/SOCS-1 signaling. Immunoblot showed that injection with 1H3 and 6C8 for 1 week had no significant effect on hepatic tyrosine phosphorylation levels of JAK2 and STAT3 and the protein level of SOCS-1 compared with control IgG injection (Supplementary Fig. 5*B* and *C*). However, immunoblot showed that injection with 1H3 anti-Aβ antibody for 4 months significantly reduced the hepatic tyrosine phosphorylation levels of JAK2 and STAT3 and the protein level of SOCS-1 compared with control IgG injection (Fig. 4*A* and *B*). Moreover, immunoblot showed that injection with 6C8 anti-Aβ antibody for 9 months reduced the hepatic tyrosine phosphorylation levels of JAK2 and STAT3 and the protein level of SOCS-1 significantly as well (Fig. 4*C* and *D*). These results suggested that JAK2/STAT3/SOCS-1 signaling is involved in the insulin resistance induced by Aβ in vivo.

Knockdown of hepatic JAK2 inhibits liver JAK2/STAT3/SOCS1 signaling and improves insulin sensitivity in APP/PS1 mice. To evaluate the role of JAK2/STAT3/SOCS-1 signaling in Aβ-induced insulin resistance in vivo, we used adenoviral RNAi to reduce hepatic JAK2 expression and decrease JAK2/STAT3/SOCS-1 signaling. Immunoblot showed that the JAK2 RNAi significantly suppressed hepatic JAK2 expression in APP/PS1 mice compared with LacZ RNAi, and STAT3 expression was not affected (Fig. 5*A* and *B*). As expected, knockdown of JAK2 markedly reduced tyrosine phosphorylation levels of JAK2 and STAT3 and the protein level of SOCS-1 in the livers of APP/PS1 mice compared with treatment with LacZ RNAi (Fig. 5*A* and *B*). These results indicate that knockdown of JAK2 can inhibit JAK2/STAT3/SOCS-1 signaling in vivo.

We then investigated whole-body insulin action and glucose metabolism in APP/PS1 mice injected with adenovirus expressing JAK2 siRNA. The glucose tolerance test revealed that knockdown of JAK2 significantly improved glucose metabolism in APP/PS1 mice compared with treatment with LacZ RNAi (Fig. 6*A* and *B*). The insulin tolerance test showed that knockdown of JAK2 markedly enhanced insulin sensitivity, and the area

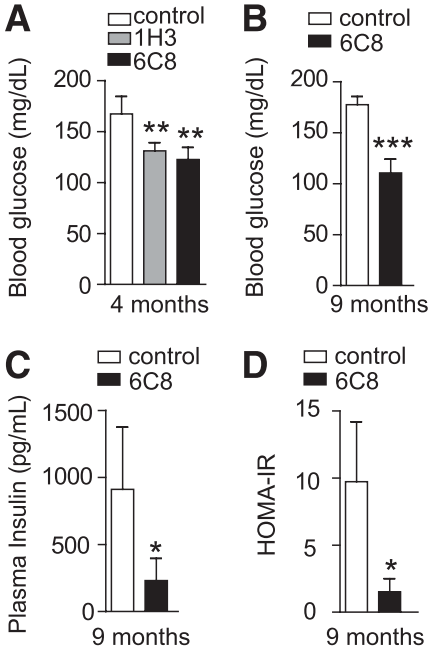

FIG. 2. Neutralization of Aβ downregulates fasting blood glucose and improves insulin sensitivity in APP/PS1 AD model mice. *A*: Fasting blood glucose levels of APP/PS1 mice intraperitoneally injected once weekly with control IgG, 1H3, or 6C8 anti–Aβ-neutralizing antibody for 4 months (n = 4 for each group). *B* and *C*: Fasting blood glucose levels were measured after fasting for 4 h. Fasting blood glucose (*B*) and plasma insulin (*C*) of APP/PS1 mice intraperitoneally injected once weekly with control IgG (n = 3) or 6C8 anti-Aβ antibody (n = 4) for 9 months. Blood glucose and insulin levels were measured after fasting for 4 h. *D*: HOMA-IR was calculated from fasting glucose and insulin levels in panels *B* and *C*. Data are presented as mean and SD. *P < 0.05, **P < 0.01, ***P < 0.001.

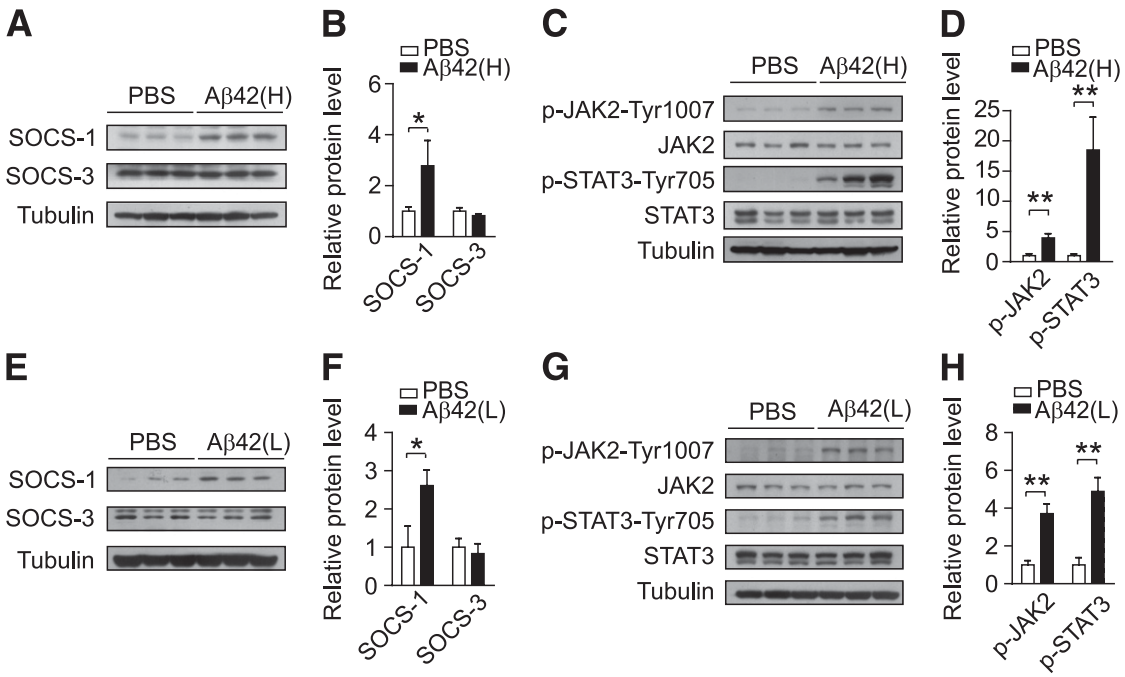

FIG. 3. Aβ42 induces SOCS-1 upregulation and JAK2/STAT3 activation in mouse liver. *A*: Immunoblot analysis of liver SOCS-1 and SOCS-3 protein levels in C57BL/6J mice injected with PBS or high-dose Aβ42 (50 μg per mouse, twice daily) for 23 days (n = 3 per group). *B*: Quantification of SOCS-1 and SOCS-3 protein levels in panel *A*. *C*: Immunoblot analysis of liver JAK2 and STAT3 phosphorylation states in C57BL/6J mice injected with PBS or high-dose Aβ42 for 23 days (n = 3 per group). D: Quantification of phosphorylated JAK2 and STAT3 levels in panel *C*. *E*: Immunoblot analysis of liver SOCS-1 and SOCS-3 protein levels in C57BL/6J mice injected with PBS or low-dose Aβ42 (12.5 μg per mouse, twice daily) for 29 days (n = 3 per group). *F*: Quantification of SOCS-1 and SOCS-3 protein levels in panel *E*. *G*: Immunoblot analysis of liver JAK2 and STAT3 phosphorylation states in C57BL/6J mice injected with PBS or low-dose Aβ42 for 29 days (n = 3 per group). *H*: Quantification of phosphorylated JAK2 and STAT3 levels in panel *G*. All protein levels were normalized to tubulin. Aβ42(H), high-dose Aβ42; Aβ42(L), low-dose Aβ42. Data are presented as mean and SD. *P < 0.05, **P < 0.01.

under the curve of plasma glucose abundance during the insulin tolerance test was significantly decreased (Fig. 6*C* and *D*). To further confirm the effect of JAK2 RNAi on insulin sensitivity, we investigated insulin signaling in mouse liver. Immunoblot showed that insulin-stimulated phosphorylation of InsR at Tyr1150/1151 and Akt at Thr308 and Ser473 was markedly increased in APP/PS1 mouse liver with JAK2 knockdown (Fig. 6*E* and *F*). These results together demonstrate that knockdown of hepatic JAK2 improves insulin sensitivity in APP/PS1 mice, suggesting JAK2 mediates Aβ-induced insulin resistance in vivo.

DISCUSSION

We have previously reported that APP/PS1 AD model mice with increased plasma Aβ40/42 levels show impaired glucose/insulin tolerance and hepatic insulin signaling (10). Another AD mouse model, APPswe/PS1$^{(A246E)}$, which also overexpresses Aβ, exhibits impaired glucose tolerance on chow diet as well (9). Furthermore, APP23 AD model mice, which also have elevated plasma Aβ, crossed with *ob/ob* or NSY diabetic model mice, could deteriorate their diabetic phenotype (20). These studies implicate that Aβ might contribute to the development of insulin resistance in vivo. In this study, we provide the first evidence that Aβ can directly induce insulin resistance in vivo by injection of synthetic Aβ42 (Fig. 1).

Mice deficient in BACE-1 with a reduced Aβ level show improved glucose disposal and insulin sensitivity on a chow and high-fat diet (31), suggesting that blocking Aβ is beneficial for improving glucose homeostasis and insulin sensitivity. Consistently, when APP/PS1 mice were treated with two different anti–Aβ-neutralizing antibodies to block the effect of Aβ, we found that the mice injected with anti-Aβ antibodies showed improved fasting blood glucose and insulin sensitivity (Fig. 2). These results suggest that Aβ is required for the development of hyperglycemia and insulin resistance in APP/PS1 AD model mice. Collectively, these data clearly indicate that Aβ can induce insulin resistance in vivo and suggest the protective role of lowering peripheral Aβ in insulin sensitivity.

JAK2 signaling has been implied in insulin resistance induced by cytokines (28). Circulating retinol binds to retinol-binding protein and then activates JAK2/STAT5/SOCS-3 signaling to inhibit insulin signaling through the cell-surface protein STRA6 (32). Moreover, knockdown of JAK2 in muscle cells partially restores insulin sensitivity in insulin-resistant states (33), and hepatocyte-specific deletion of JAK2 protects against diet-induced glucose intolerance (34). In addition, Aβ induces insulin resistance in cultured hepatocytes mainly by activating JAK2/STAT3/SOCS-1 signaling (10), and the JAK2/STAT3/SOCS-1 signaling is in a more active state in the APP/PS1 and APPswe/PS1$^{(A246E)}$ mouse liver (10). Similarly, we show here that injection of Aβ activates hepatic JAK2 signaling in wild-type mice (Fig. 3) and that neutralization of Aβ inhibits JAK2 signaling in livers of APP/PS1 mice (Fig. 4). Taken together, these studies suggest that JAK2 signaling mediates the effect of Aβ on insulin sensitivity in vivo. Consistently, we show that knockdown of hepatic JAK2 in APP/PS1 mice inhibits liver JAK2/STAT3/SOCS-1 signaling and improves insulin sensitivity in APP/PS1 mice (Fig. 5 and Fig. 6). These results raise the possibility that targeting

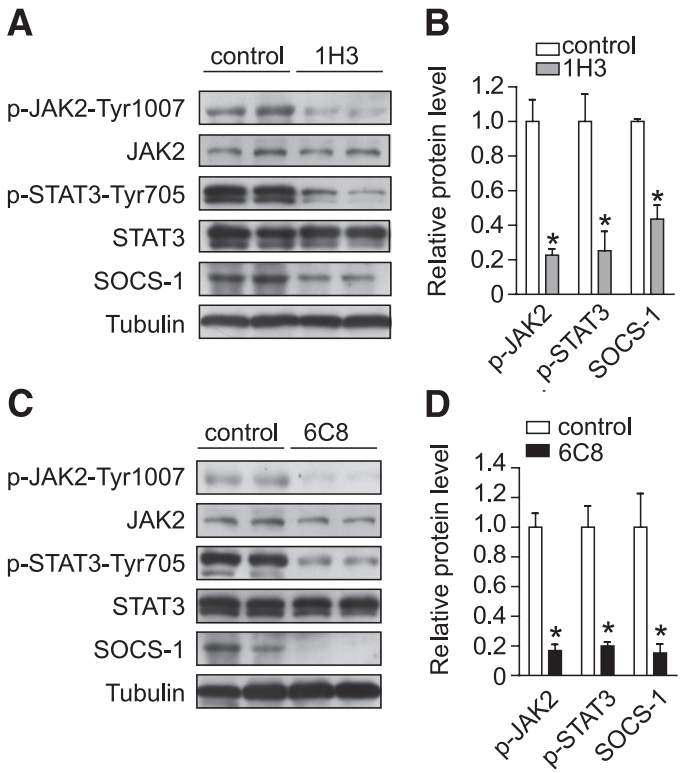

FIG. 4. Neutralization of Aβ inhibits hepatic JAK2/STAT3/SOCS1 signaling in APP/PS1 AD model mice. *A*: Immunoblot analysis of liver JAK2 and STAT3 phosphorylation states and SOCS-1 protein levels in APP/PS1 mice injected with control IgG or anti-Aβ 1H3 antibody for 4 months. *B*: Quantification of phosphorylated JAK2 and STAT3 levels and SOCS-1 protein levels in panel *A*. *C*: Immunoblot analysis of liver JAK2 and STAT3 phosphorylation states and SOCS-1 protein levels in APP/PS1 mice injected with control IgG or anti-Aβ 6C8 antibody for 9 months. *D*: Quantification of phosphorylated JAK2 and STAT3 levels and SOCS-1 protein levels in panel *C*. All protein levels were normalized to tubulin. Data are presented as mean and SD. *$P < 0.05$.

JAK2 may be an effective strategy for treating Aβ-induced insulin resistance.

Numerous epidemiological and experimental studies have examined the possibility of a relationship between T2DM and AD. Patients with T2DM were demonstrated to have an increased risk of developing AD compared with healthy individuals (35). Insulin-resistant or diabetic conditions can exacerbate the AD phenotype in mouse models resulting from the increase of Aβ generation or aggregation (20,36,37). Indeed, insulin can increase the Aβ level by promoting its production/secretion and inhibiting its degradation via insulin-degrading enzyme (38,39). On the other hand, patients with AD have an increased risk of developing T2DM (6,8). Of note, plasma Aβ levels in AD patients have been reported to be increased or unchanged compared with controls (40,41), which may explain why only a small portion of AD patients develop impaired fasting glucose and T2DM. Animal studies demonstrate AD mouse models exhibit impaired glucose/insulin tolerance (9,10) and can accelerate the diabetic phenotype when crossbred with genetic diabetic mouse models (20). Taken together, these findings combined with findings that Aβ itself can induce insulin resistance in vivo support that Aβ signaling might be the common mechanism for the pathogenesis of both T2DM and AD.

Elevation of plasma Aβ might be a partial result from obesity, because it has been reported that APP expression is upregulated in subcutaneous abdominal adipocytes from obese subjects (42) and that the plasma Aβ level is positively correlated with body fat in healthy individuals (43). Elevation of plasma Aβ also might be a consequence of increased age, because plasma Aβ40/42 levels have been reported to increase with age in humans (41). Obesity and age are both associated with increased risks of developing insulin resistance and T2DM (44,45), thus the elevation of plasma Aβ caused by obesity and age is likely to be involved in the pathogenesis of insulin resistance and T2DM. Future studies on how obesity and age induce the production of Aβ would deepen our understanding of the pathogenesis of insulin resistance and T2DM.

T2DM is a chronic and age-related disease (44). Commonly used diet-induced or genetic diabetic mouse models usually develop T2DM at a relative younger age compared with the onset of T2DM in humans, which is usually older than age 45 years (46). The report that plasma Aβ40/42 levels increase with age in humans (41) along with our observation for the contribution of Aβ to insulin resistance and T2DM provides a possible mechanism for the age-related onset of T2DM in humans. Future studies focused on the underlying mechanisms of the age-related onset of T2DM, especially peripheral Aβ signaling, will shed new light on the diagnosis and treatment of T2DM.

In summary, we show here that Aβ can cause insulin resistance in vivo through activation of JAK2 signaling and that neutralization of Aβ or knockdown of JAK2 attenuates insulin resistance in APP/PS1 mice. These results therefore provide new insights into the role of Aβ, a major pathogenic factor of AD, in regulating insulin action. Given the observation that Aβ is increased in obese or hyperglycemic patients (10,43), therapeutic strategies to inhibit Aβ signaling might provide novel approaches to ameliorate insulin resistance and related diseases.

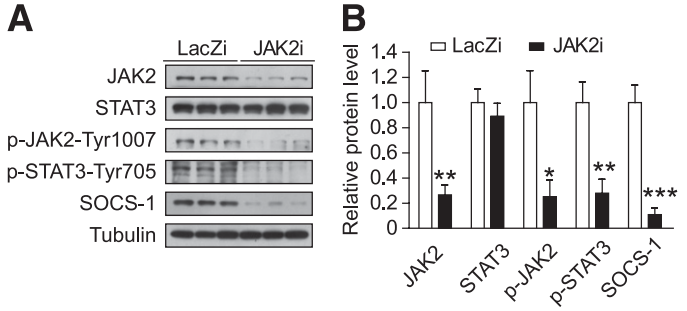

FIG. 5. Knockdown of hepatic JAK2 inhibits JAK2/STAT3/SOCS1 signaling in the livers of APP/PS1 mice. *A*: Immunoblot analysis of liver JAK2, STAT3, and SOCS-1 protein levels and phosphorylated JAK2 and STAT3 levels in 7-month-old APP/PS1 mice injected with adenovirus encoding siRNA targeting JAK2 (JAK2i) or LacZ (LacZi) via tail vein for 15 days ($n = 3$ per group). *B*: Quantification of JAK2, STAT3, and SOCS-1 protein levels and phosphorylated JAK2 and STAT3 levels in panel *A*. All protein levels were normalized to tubulin. Data are presented as mean and SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

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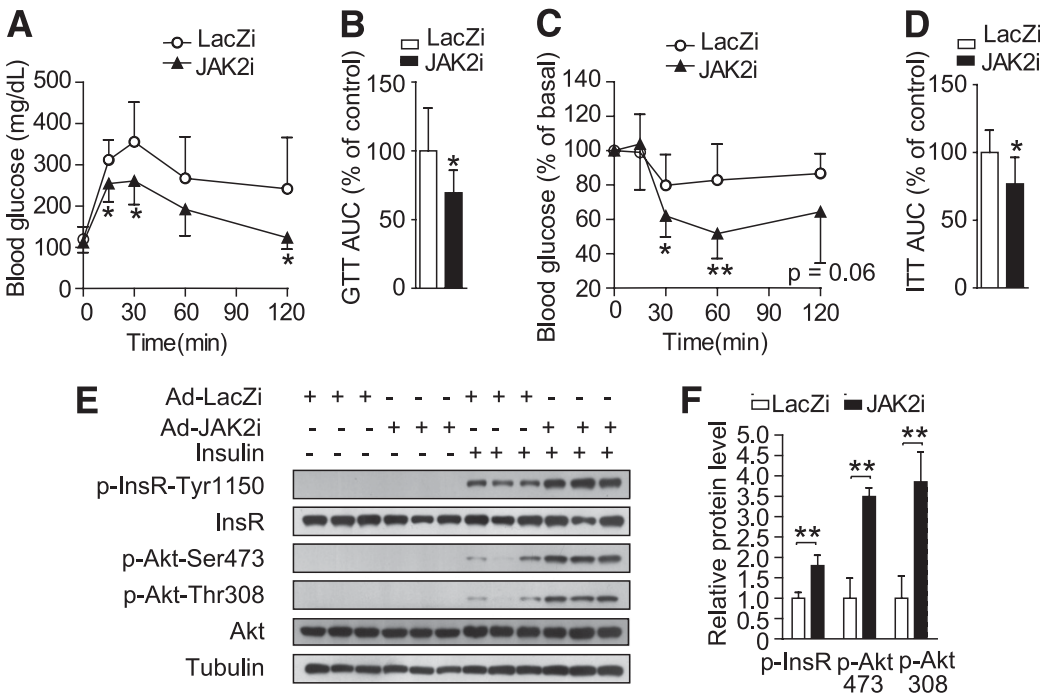

FIG. 6. Knockdown of hepatic JAK2 improves insulin sensitivity in APP/PS1 mice. *A*: Glucose tolerance test of APP/PS1 mice injected with LacZi or JAK2i adenovirus via tail vein for 6 days (n = 8 per group). *B*: Area under the curve (AUC) of glucose tolerance tests (GTT) in panel *A*. *C*: Insulin tolerance test of APP/PS1 mice injected with LacZi or JAK2i adenovirus for 12 days (n = 8 per group). *D*: AUC of insulin tolerance tests (ITT) in panel *C*. *E*: Insulin-stimulated phosphorylation of InsR and Akt in the livers of APP/PS1 mice injected with LacZi or JAK2i adenovirus for 15 days was measured by immunoblot. *F*: Quantification of phosphorylated InsR and Akt levels in panel *E*. All the protein levels were normalized to tubulin. Data are presented as mean and SD. *$P < 0.05$, **$P < 0.01$.

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Y.Z. designed the study, performed experiments, analyzed data, and wrote the paper. B.Z. designed the study and performed experiments. B.D., F.Z., J.W., and Y.W. performed experiments. Y.L. analyzed data. Q.Z. designed the study, analyzed data, supervised the project, and wrote the paper. Q.Z. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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