Original Article
OBESITY BIOLOGY AND INTEGRATED PHYSIOLOGY

Obesity

Effects of Glucose and Insulin on Secretion of Amyloid-β by Human Adipose Tissue Cells

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Objective: Obesity and type 2 diabetes mellitus are risk factors for developing Alzheimer disease. Overlapping patterns of metabolic dysfunction may be common molecular links between these complex diseases. Amyloid-β (Aβ) precursor protein and associated β- and γ-secretases are expressed in adipose tissue. Aβ precursor protein is up-regulated with obesity and correlated to insulin resistance. Aβ may be secreted by adipose tissue, its production may be regulated through metabolic pathways, and Aβ may exert effects on adipose tissue insulin receptor signaling.
Methods: Human stromal-vascular cells and differentiated adipocytes were cultured with different combinations of glucose and insulin and then assayed for Aβ in conditioned media. Aβ was measured *in vivo* using adipose tissue microdialysis.
Results: Aβ secretion was increased by glucose and insulin *in vitro*. Adipose tissue microdialysates contained Aβ. Adipocytes treated with Aβ had decreased expression of insulin receptor substrate-2 and reduced Akt-1 phosphorylation.
Conclusions: Aβ was made by adipose tissue cells *in vitro* at concentrations similar to *in vivo* measurements. Regulation of Aβ production by glucose and insulin and effects of Aβ on the insulin receptor pathway suggest similar cellular mechanisms may exist between neuronal dysfunction in Alzheimer disease and adipose dysfunction in type 2 diabetes.

Obesity (2016) **24**, 1471–1479. doi:10.1002/oby.21494

Introduction

Patients with obesity or type 2 diabetes have increased risk of developing Alzheimer disease (1-3). Obesity and type 2 diabetes are epidemic, and Alzheimer disease accounts for more than 90% of diagnosed dementias (4-6). Alterations of glucose and insulin homeostasis are seen in regions of the brain with high amyloid-β (Aβ) plaque burden (7,8). Aβ is a 37 to 43 amino acid fragment of Aβ precursor protein (AβPP) produced by membrane-bound proteases that forms soluble oligomers and insoluble fibrillar polymers (9-11). AβPP is ubiquitously expressed in eukaryotic cells, and variable proteolytic processing of AβPP produces a variety of peptides in addition to Aβ (12). Murine AβPP gene knock-outs have improved metabolic function, and transgenic mouse models of Alzheimer disease with chronically elevated plasma Aβ develop glucose intolerance, insulin resistance, and inflammation (13-15). Combined models of Alzheimer disease and obesity or type 2 diabetes demonstrate accelerated Aβ deposition and neurological dysfunction (16-19). Insulin increases Aβ secretion in neuronal culture, and cerebrospinal Aβ rises following hyperinsulinemic-euglycemic clamp (20,21). Aβ competitively binds the insulin receptor and insulin degrading enzyme (IDE) decreasing insulin receptor signaling, slowing Aβ and insulin degradation (22-24). These studies suggest a relationship between AβPP and metabolic dysfunction.

Recently, we found adipocytes express AβPP and the secretases required to make Aβ (25). AβPP transcription was increased in subjects with obesity and correlated with insulin resistance and inflammatory cytokine expression. We hypothesized adipose tissue secretes Aβ and insulin and glucose may regulate this process. We also hypothesized Aβ induces local effects on adipose insulin receptor signaling. To test these questions, we performed *in vivo* studies

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Funding agencies: WGT was supported by an individual fellowship award from the National Institute of Diabetes and Digestive and Kidney Diseases (F30 DK084605).
Disclosure: The authors declared no conflict of interest.
Author contributions: REP and WGT designed the study, analyzed the data, and wrote the manuscript. DG conducted the immunoprecipitation and Western blotting experiments. JS processed blood samples and adipose tissue cells. KPJ and AMJ assisted in conducting all clinical research procedures. All authors take responsibility for the contents of the article. WGT and REP are the guarantors of this work, had full access to all the data in the study, and take responsibility for the integrity of the data and the accuracy of the data analysis.
Additional Supporting Information may be found in the online version of this article.
Received: 9 December 2015; **Accepted:** 5 February 2016; **Published online 13 May 2016. doi:10.1002/oby.21494**

TABLE 1 Microdialysis cohort clinical data

	Lean	With obesity	With obesity and type 2 diabetes	*P*
Subjects (*n*)	6	3	4	
Sex (M/F)	3/3	2/1	2/2	
Age (years)	46.7 ± 15.7	39.7 ± 6.4	46.3 ± 10.5	0.7
Body mass index (kg/m^2)	21.4 ± 2.6	47.5 ± 8.8	40.2 ± 6.2	<0.001
Percent body fat	25.0 ± 7.5	53.8 ± 1.1	47.9 ± 5.2	<0.001
Hemoglobin A1c % (mmol/mol)	5.6 ± 0.5 (38.0 ± 5.5)	5.9 ± 0.4 (41.0 ± 4.4)	6.9 ± 0.5 (52.0 ± 5.5)	0.005
Fasting blood glucose (mg/dL)	89.1 ± 7.8	90.6 ± 9.6	124.9 ± 24.1	0.009
Fasting blood insulin (μIU/mL)	3.3 ± 1.5	22.5 ± 14.1	12.3 ± 4.0	0.008

Data are presented as mean ± standard deviation.

measuring Aβ production in human subcutaneous adipose tissue by microdialysis and *in vitro* studies using cultured human stromal-vascular cells (SVF) and differentiated adipocytes.

Methods

Study design

This study was approved by University of Vermont and Florida Hospital Institutional Review Boards. All subjects provided written informed consent. Subjects were lean (body mass index; BMI $\leq$ 25 kg/m^2), had obesity without type 2 diabetes (BMI $\geq$ 30 kg/m^2; hemoglobin A1c (HbA1c) < 6.5%; 48 mmol/mol), or obesity with type 2 diabetes (BMI $\geq$ 30 kg/m^2, HbA1c > 6.5%; 48 mmol/mol). Subjects with type 2 diabetes were excluded if taking thiazolidinediones, long-acting incretin agonists, or more than two of the following: metformin, sulfonylurea, short-acting GLP-1analog, or DPP-IV inhibitor. Subjects were excluded if they had recent weight changes or chronic medical conditions or took medications affecting glucose metabolism.

Adipose tissue biopsy, fractionation, and culture

Subcutaneous abdominal adipose tissue was obtained by percutaneous needle biopsy. SVCs were isolated by collagenase digestion and stored in liquid nitrogen (25). Cultures were established using standard protocols with modified glucose and insulin concentrations (26). SVC were passaged twice and then grown with 5.5 mM or 25 mM glucose as indicated. Adipocyte cultures were grown from SVC expanded in 5.5 mM glucose for 10 days, triggered to differentiate for 4 days, then matured for another 12 days. Maturation media contained 5.5 mM or 25 mM glucose plus 1, 10, or 500 nM insulin and was changed every 72 h. In certain experiments, media was supplemented with Aβ40 or Aβ42 (MesoScale Diagnostics) for the final 6 days of maturation or β-secretase inhibitor IV (EMD-Calbiochem) for the final 72 h of culture. Each condition was tested in three to six wells per subject. Media from similar conditions from the same subject were pooled and treated with protease inhibitor (Roche), one to two wells were fixed for cell counting and lipid quantification, one to two wells lysed with QIAzol, and one to two wells lysed with protein extraction buffer.

Microdialysis

Subjects were recruited from an ongoing study (see Table 1). After overnight fasting, microdialysis catheters (CMA63; Harvard Apparatus) were inserted into the subcutaneous abdominal adipose tissue and perfused with saline plus 4% human albumin for 45 min. A plasma sample was drawn into K + ethylenediaminetetra-acetic acid with 10 nM AEBSF (Sigma). After equilibration, the catheters were perfused at 0.5 μL/min for 240 min. Recovery of Aβ40 and Aβ42 estimated by the zero net flow was calibrated to dialyzed plasma (27).

Measurement of Aβ

Aβ40 and Aβ42 were measured using multiplex 4G8 immunoassay kits (Meso-Scale Discovery; Rockville, MD). Intra-assay percent coefficients of variation averaged 3.37 ± 3.1% for Aβ40 and 3.37 ± 2.8% for Aβ42. Inter-assay percent coefficients of variation averaged 5.8% for Aβ40 and 26.0% for Aβ42.

Immunoprecipitation

Conditioned adipocyte culture media was precipitated with acetone and resuspended in PBS. Aβ was immunoprecipitated using Dynabeads Protein G kit (Life Technologies). Beads were conjugated to anti-Aβ monoclonal mouse antibody 4G8 (Covance) or IgG isotype control, incubated with media precipitate or recombinant Aβ40 (5 ng/mL), eluted per kit instructions, prepared with Lamelli buffer, and separated with 18% Tris-sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Separated samples were transferred to polyvinylidene fluoride (PVDF) membrane, which was incubated with 4G8 followed by anti-mouse-horseradish peroxidase (HRP)-conjugated secondary antibody, treated with PicoWest luminol reagent (Pierce Biosciences), and exposed to film.

Western blotting

Adipocytes were lysed in 50 mM HEPES, 150 mM sucrose, 2 mM sodium orthovanadate, 80 mM ß-glycerophosphate, 10 mM sodium fluoride, 10 mM sodium pyrophosphate, 2 mM sodium ethylene glycol tetra-acetic acid, 2 mM sodium ethylenediaminetetra-acetic acid, 1% Triton X-100, 0.1% SDS, protease inhibitor cocktail (Sigma-Aldrich), and 1 mM phenyl-methyl-sulfonyl-fluoride. Samples were separated on 10% SDS-PAGE, transferred to PVDF, probed with rabbit monoclonal Akt-1 antibody (Cell Signaling), followed by goat anti-rabbit-HRP-conjugated antibody (Bio-Rad), and bands detected using ECL reagent (Amersham). Membranes were stripped and incubated with rabbit monoclonal phospho-Akt (Ser473) (Cell Signaling) with the same secondary detection. Bands were quantitated using NIH-ImageJ software.

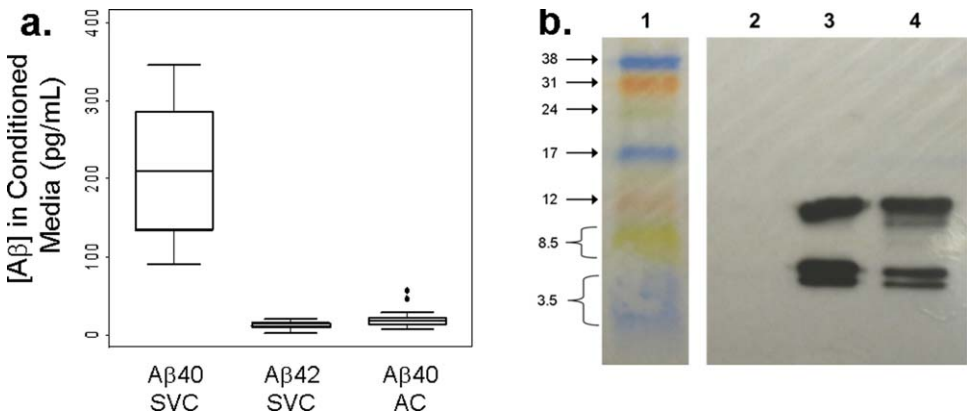

Figure 1 (**a**) Amyloid-β (Aβ) in 72-h conditioned media from stromal-vascular cell (SVC) and adipocyte (AC) cultures ($n = 14$ each). (**b**) Western blot of immunoprecipitated Aβ from concentrated conditioned media. Lane 1: ladder (in kDa), lane 2: IgG precipitation of conditioned media – negative control, lane 3: 4G8 precipitation of conditioned media, lane 4: 4G8 precipitation of recombinant amyloid-β40 – positive control. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Gene expression

Sample RNA was extracted using RNeasy Lipid Tissue Mini Kits (Qiagen; Valencia, CA) with RNase-Free DNase treatment (Qiagen). A cDNA library was made using 1 μg RNA template and oligo-dT primers (Clontech; Mountain View, CA). Gene expression was measured with Applied Biosystems 7300 (Life Technologies Inc.; Carlsbad, CA) using TaqMan primers (see Supporting Information Table 1) normalized to GAPDH.

Cell counting and lipid quantification

Cells were fixed in 4% paraformaldehyde and stained with DAPI (Roche, IN) and Nile Red (Sigma). A 4 cm^2 tiled-image was taken using a Nikon TE2000 microscope using Nikon Elements (Nikon, USA). Cell counts were generated by counting all individual nuclei (diameter <30 microns and circularity index >0.8), dividing the entire DAPI binary mask by the average individual nuclear area, and extrapolating to the entire well. Lipid content (μm^2/cell) was generated by dividing the Nile Red binary mask by the cell count and is an index of differentiation (26).

Immunofluorescence microscopy

Cultures from three subjects were fixed with 4% paraformaldehyde and 0.1% Triton-X in PBS, blocked with 1% BSA in PBS, stained with antibodies against Aβ (monoclonal 6E10, Covance; polyclonal anti-oligomeric Aβ, EMD-Millipore), AβPP (monoclonal 22C11, Abcam; San Francisco, CA), CD68 (monoclonal PG-M1, DakoCytomation; Carpinteria, CA), CD45 (monoclonal F10-89-4, Abcam), or βIII tubulin (monoclonal 2G10, Abcam), incubated with secondary antibodies, counterstained with DAPI and Nile Red, then imaged. Antibodies were used at recommended concentrations, with dilutions between 1:200 and 1:1,000 in PBS. All image channels were adjusted based on no-primary negative control images (see Supporting Information Figures 1–3).

Statistical analyses

Data were analyzed using STATA v11.2 (StataCorp LP; College Station, TX). Differences among cultures were assessed by repeated measures of ANOVA with linear Wald tests for multiple testing correction. Differences among microdialysis groups were assessed by ANOVA. All appropriate statistical assumptions were met. Non-normal data were log-transformed as indicated. A P value <0.05 was considered significant. All values are presented as mean $\pm$ SEM unless noted.

Results

Human adipocytes and SVC secrete Aβ *in vitro*

SVC and adipocytes were grown from abdominal adipose biopsies of 16 subjects (four lean, six with obesity, and six with obesity and type 2 diabetes). Cultures were grown with 5.5 mM glucose and 10 nM insulin. Media from the last 72 h of culture were assayed for Aβ by electrochemiluminescence assay. SVC basal media contained 2.5% FBS and 23.0 $\pm$ 4.1 pg/mL Aβ40, but no detectable Aβ42. Basal adipocyte trigger and maintenance media were serum free and no Aβ species were detectable before culture. Aβ40 and Aβ42 were detected in all SVC conditioned media at 214.6 $\pm$ 21.1 pg/mL and 10.8 $\pm$ 1.4 pg/mL, respectively (corrected for media base content) (Figure 1a). Aβ40 was detected in all adipocyte conditioned media at 21.0 $\pm$ 3.7 pg/mL (Figure 1a), but Aβ42 was not (data not shown).

We also used immunoprecipitation and treatment with a β-secretase inhibitor to assure that we were measuring Aβ and not a cross-reactive product. Aβ peptides were immunoprecipitated from concentrated adipocyte media and recombinant Aβ40 solution using 4G8 monoclonal antibody (Figure 1b, lanes 3, 4). Bands were nearly identical between media and Aβ40 solution. No peptides were detected using IgG control (Figure 1b, lane 2). Treatment of adipocyte cell cultures with a β-secretase inhibitor for 72 h showed a dose-dependent reduction in Aβ concentrations (Supporting Information Figure 4). These data show human SVC and adipocytes make and secrete soluble Aβ peptides *in vitro*.

Microanatomy and immunofluorescence

Cultures derived from primary adipose biopsies initially contain multiple cell types. After several passages, these cultures are mainly

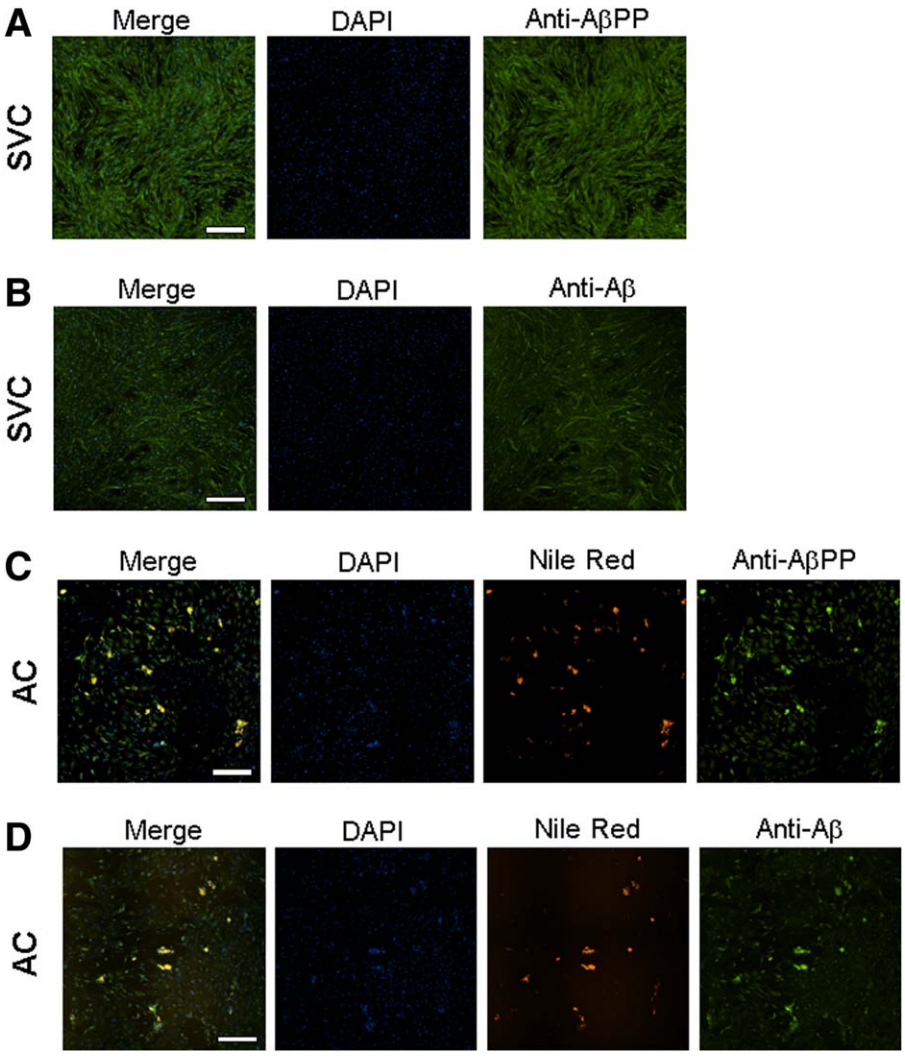

Figure 2 Imaging of amyloid-β precursor protein (AβPP) and amyloid-β (Aβ) *in vitro*. Representative images from (**A,B**) stromal-vascular cell (SVC) and (**C,D**) adipocyte (AC) cultures ($n = 3$ each). AβPP was visualized with murine monoclonal antibody 22C11 and Aβ with murine monoclonal antibody 6E10. Donkey anti-mouse Cy2 antibody was used for secondary detection. Cultures were counterstained with DAPI. Lipid in AC cultures was visualized using Nile Red. Each channel was individually adjusted to primary antibody control images; see Supporting Information figures. Scale bar = 400 μm.

adipose stromal cells; however, we could not initially exclude contaminating leukocytes or neurons as the source of Aβ (26). From our prior studies, we know Aβ can be found in adipose tissue macrophages, but whether this reflects production or phagocytosis by these cells is unknown (25). Using immunofluorescence microscopy we examined the distribution of AβPP and Aβ *in vitro* and probed for macrophage, leukocyte, and neuronal markers in our cultures.

AβPP was homogenously expressed in SVC cultures (Figure 2a). In adipocyte cultures, AβPP was expressed in both stromal cells and differentiating adipocytes (Figure 2a, c). Using monoclonal and polyclonal anti-Aβ antibodies we found widespread presence of Aβ in SVC and adipocyte cultures (Figure 2b, d; Supporting Information Figure 1). We did not find punctate or cell-specific restriction of Aβ staining. There was no immunoreactivity to the macrophage marker CD68 or the leukocyte antigen CD45 in our cultures (Supporting Information Figures 2 and 3). There was no immunostaining of neuronal-specific βIII tubulin (Supporting Information Figures 2 and 3). Taken together with our secretion data, these results confirmed Aβ production by human adipose tissue stromal cells and adipocytes *in vitro*.

Aβ is present in human adipose tissue *in vivo*

In vivo adipose cell-derived Aβ constitutes a fraction of local concentrations in balance with degradation, production by other cells within the adipose niche, and flux to or from the plasma. With this in mind we sought to determine if Aβ was measureable in adipose tissue *in vivo* using microdialysis.

Aβ recovery was calibrated by dialyzing plasma *in vitro* ($n = 8$; Figure 3a) (27). We recruited 13 subjects with a range of body composition and metabolic function (six lean, three with obesity, four with

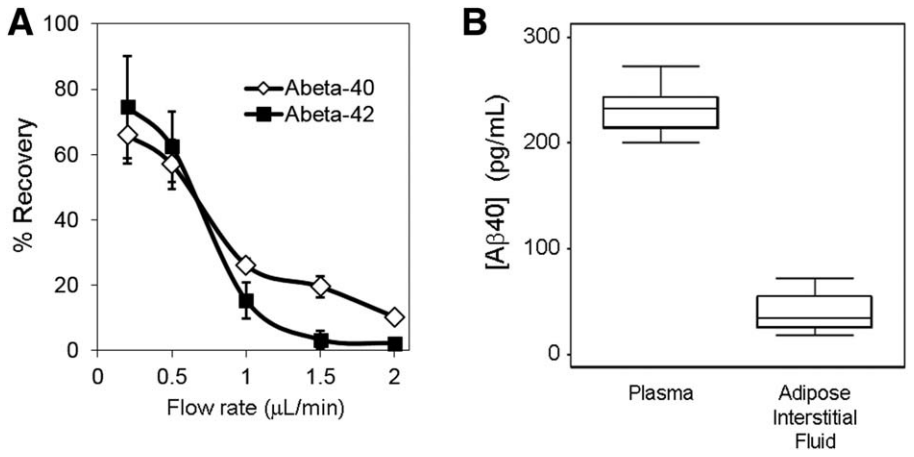

Figure 3 Measurement of amyloid-β in subcutaneous adipose tissue *in vivo* by microdialysis. (**A**) Zero net flow recovery of amyloid-β; $n = 8$ per data point. (**B**) Measured plasma [amyloid-β40] (pg/mL) and estimated adipose interstitial [amyloid-β40] from subcutaneous abdominal adipose microdialysates from human subjects ($n = 13$).

obesity and type 2 diabetes; Table 1). Circulating Aβ40 averaged 247.4 ± 17.1 pg/mL and Aβ42 31.6 ± 16.9 pg/mL. Microdialysates from subcutaneous abdominal adipose tissue contained an average 20.3 ± 2.2 pg/mL Aβ40. With 60% recovery at the flow rate used the estimated interstitial adipose Aβ40 concentration was 39.3 ± 4.9 pg/mL, which was 16.4 ± 2.2% of the circulating plasma concentrations (Figure 3b). Aβ42 was not robustly detected in all the microdialysates. These results show soluble Aβ is present in the subcutaneous adipose interstitial fluid. The relative contribution of adipose cells to the local concentrations or circulating levels is yet to be determined.

Effects of glucose and insulin on Aβ secretion *in vitro*

Next we asked if alterations in glucose or insulin concentrations altered Aβ production similar to neuronal cultures (20,21). SVC cultures from 14 subjects (four lean, six with obesity, and four with obesity and type 2 diabetes) were grown at 5.5 mM or 25 mM glucose. The cells in each well were counted (Supporting Information Figure 5a). As SVC in 25 mM glucose had reduced cell counts compared with 5.5 mM ($P \ll 0.0001$; Supporting Information Figure 5b), secretion data were normalized to cell count. SVC secreted more Aβ40 and Aβ42 when cultured in 25 mM compared with 5.5 mM glucose ($P = 0.003$ and 0.031, respectively; Figure 4a, b), without differences among donors ($P > 0.3$). AβPP and presenilin-1 (PSEN1) expression trended down in 25 mM compared with 5.5 mM glucose ($P = 0.11$ and 0.08, respectively) without differences in β-secretase-1 (BACE1; $P = 0.6$) or presenilin-2 (PSEN2; $P = 0.3$; Supporting Information Figure 6a–d). No differences in insulin receptor (INS-R), insulin receptor substrate-1 (IRS1), insulin receptor substrate-2 (IRS2), or IDE expression were observed between conditions ($P = 0.13$–0.55; data not shown). Glucose transporter-4 (GLUT4) was undetectable ($C_t > 35$ for all). These results show glucose increased SVC Aβ secretion without changing AβPP system transcription.

SVC from 14 subjects (four lean, six with obesity, and four with obesity and type 2 diabetes) were grown at 5.5 mM glucose, differentiated, and matured with 1, 10, or 500 nM insulin and 5.5 mM or 25 mM glucose. Proliferation or differentiation of the cells did not differ among conditions ($P > 0.18$ for both; Supporting Information Figure 5c–e). Adipocytes secreted more Aβ40 in 500 nM insulin compared with 1 or 10 nM insulin at both glucose concentrations ($P \ll 0.001$; Figure 4c). Secretion of Aβ40 was not different with 1 or 10 nM insulin or with either glucose concentration ($P = 0.11$–0.63). Aβ40 secretion was not different among donors phenotypes ($P > 0.15$).

At 5.5 mM glucose, increasing insulin reduced AβPP and secretase genes transcription ($P < 0.01$ for all; Figure 5a–d). Adipocytes in 25 mM glucose demonstrated elevated AβPP transcription with increasing insulin ($P < 0.05$ for all) without differences in secretase transcription. At 1 nM insulin, high glucose reduced transcription of AβPP, BACE1, and PSEN2 compared with low glucose ($P < 0.04$ for all) without altering PSEN1. Conversely, adipocytes in high glucose and insulin had elevated AβPP and secretase expression compared with those in low glucose and high insulin ($P \leq 0.008$ for all).

Insulin decreased adipocyte INS-R, IRS1, IRS2, and IDE expression at 5.5 mM glucose ($P \leq 0.0004$ for all; Supporting Information Figure 7). At 1 nM insulin, high glucose reduced IRS1 and IRS2 expression compared with low ($P < 0.01$ for both). At 500 nM insulin, high glucose increased IRS1 and IDE compared with low ($P < 0.001$ for both). There was no change in GLUT4 expression (Supporting Information Figure 7b). Together, these data show that glucose increases Aβ secretion by SVCs without alteration of AβPP system gene expression, insulin increases Aβ secretion by adipocytes, and both glucose and insulin alter the expression of AβPP system genes in adipocyte cultures.

Incubation with Aβ reduces expression of IRS2 and activation of Akt-1

Last we asked whether Aβ could have local effects on adipose tissue function. Adipocyte cultures from 11 subjects (four lean, four with obesity, three with obesity and type 2 diabetes) were treated with Aβ40 or Aβ42 for 6 days. Media contained 10 nM insulin and 5.5 mM glucose. Aβ40 and Aβ42 did not change cell numbers or lipid content (Supporting Information Figure 8a–d). Neither Aβ

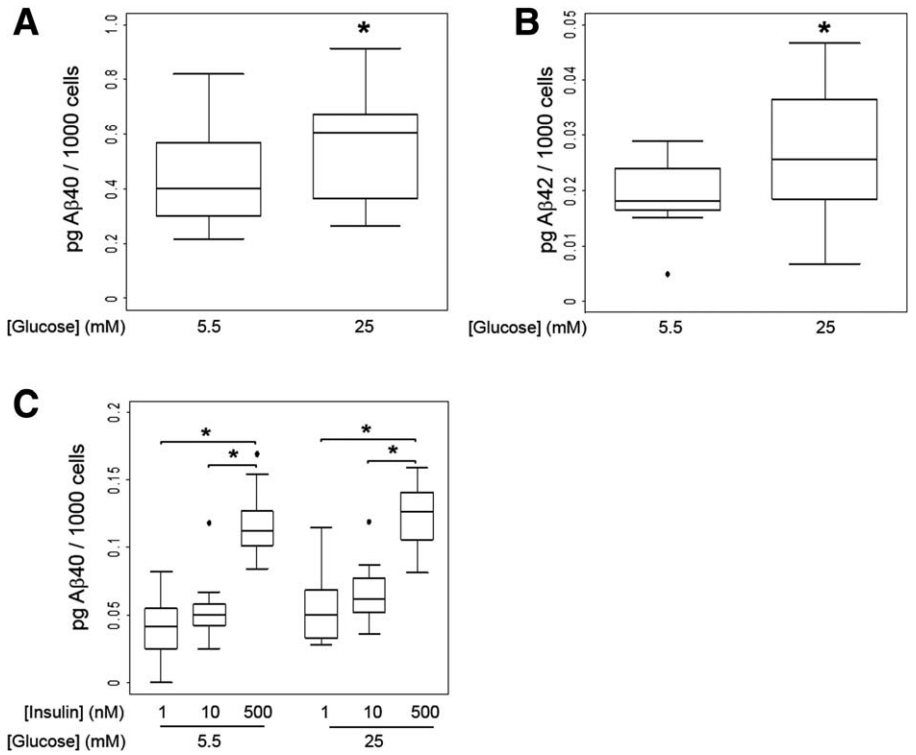

Figure 4 Secretion of amyloid-β in response to glucose and insulin *in vitro*. Stromal-vascular cell (SVC) secretion of (**A**) amyloid-β40 and (**B**) amyloid-β42 in 5.5 mM and 25 mM glucose. (**C**) Adipocyte secretion of amyloid-β40 in 1, 10, and 500 nM insulin. $n = 14$ for each condition. *$P < 0.05$ by RM-ANOVA.

species altered AβPP, BACE1, PSEN2, INS-R, GLUT4, IRS1, or IDE transcription ($P > 0.09$ for all; data not shown).

Adipocytes cultured with Aβ40 or high concentrations of Aβ42 had reduced IRS2 transcription ($P < 0.023$; Figure 6a, b). Total Akt-1 levels were not altered by Aβ ($P > 0.6$ for log-transformed data; Figure 6c). Akt-1 serine-472 phosphorylation was reduced with high Aβ40 ($P = 0.04$, log-transformed data; Figure 6d) and the ratio of phosphorylated to total Akt-1tended to decrease with high Aβ ($P = 0.07$, Figure 6e). These results show that treatment of adipocytes with physiological concentrations of Aβ induced changes in the insulin receptor signaling pathway.

Discussion

Obesity and type 2 diabetes are risk factors for developing Alzheimer disease in later life. While the role of Aβ in Alzheimer disease is debated, a growing body of data shows the AβPP system may be involved in peripheral glucose and insulin metabolism. Previously, we found AβPP expression in isolated adipocytes was increased in obesity and correlated with measures of insulin resistance and cytokine expression (25). Here we show that human adipocytes and SVC cultures secrete Aβ peptides and that Aβ is present in the adipose interstitium *in vivo*. Aβ secretion *in vitro* was increased by insulin in adipocytes independent of AβPP system transcription. SVC secreted more Aβ when cultured with high glucose concentrations. Incubation of adipocytes with Aβ altered expression and activation within the insulin receptor signaling pathway. These results show Aβ formation in adipose tissue may be linked to altered metabolic dysfunction and elevated Aβ concentrations in the adipose tissue interstitial fluid could alter insulin receptor signaling.

SVC secreted Aβ at ~1/10th the neuronal rate, but at similar levels to cultured fibroblasts (27,28). Differentiated adipocytes secreted Aβ at ~1/100th the neuronal rate. The rates were not affected by the donor's metabolic phenotype, consistent with a prior study showing *in vitro* conditions can normalize adipocytes cultured from subjects with type 2 diabetes (29). We found increased Aβ secretion in SVC cultured at high glucose and in adipocytes cultured with high insulin without correlation to AβPP system transcription. Our cultures were derived from primary SVC which contain multiple cell types when collected, but become homogenous following multiple passages. We did not find contaminating leukocytes or neurons in our cultures using microscopy.

Using microdialysis we measured Aβ in adipose tissue *in situ*. Measured concentrations were consistent with those found in culture and represented a fraction (~16%) of the circulating plasma levels. There are several considerations for interpreting these data. This is a small, exploratory cohort and it is not powered to determine differences between metabolic phenotypes of the subjects. The study cohort included subjects with obesity without diabetes. This group of three subjects had much higher insulin levels than the control group and the obesity with diabetes group. It is important to note the small size of these groups precludes extrapolation about the

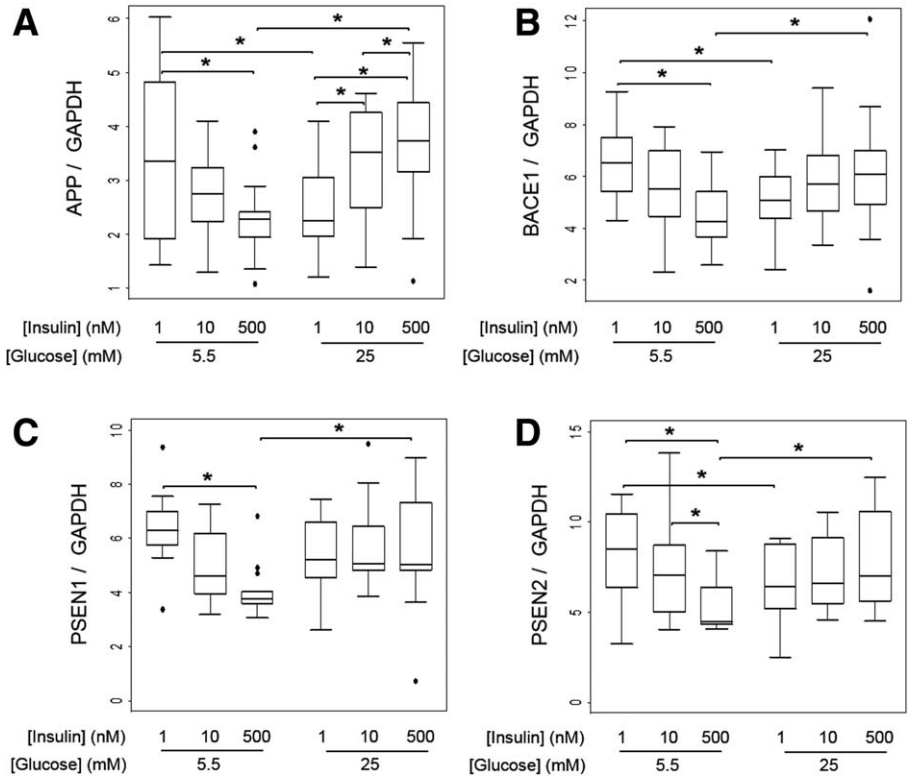

Figure 5 Transcription of amyloid-β precursor protein (AβPP) system genes in cultured adipocytes. (**A**) AβPP, (**B**) β-secretase-1 (BACE1), (**C**) presenilin-1 (PSEN1), and (**D**) presenilin-2 (PSEN2) (**D**) expression in adipocytes cultured with 5.5 mM or 25 mM glucose and 1, 10, or 500 nM insulin. $n = 14$ for each condition. *$P < 0.05$ by RM-ANOVA.

association of insulin or glucose levels with the measured Aβ concentrations. Further, these measurements represent the steady-state production, destruction, and transport of Aβ between the adipose tissue and vascular compartment. In this study, we did not measure differences in adipose tissue blood flow which has been shown to vary with obesity (30). The kinetics and mechanisms of Aβ distribution among peripheral tissues has not yet been described. Further experiments incorporating measurements of flux between the vascular compartment and the adipose interstitium are needed to more precisely measure the secretion rate and dynamics of Aβ in human adipose tissue *in vivo*. These foundational experiments are necessary for examination of Aβ secretion differences among metabolic phenotypes.

Cultured neurons secrete Aβ when treated with insulin (0.1 nM–100 μM), in part, due to increased transport of AβPP to the cell surface and exocytosis of intracellular Aβ (21,28). Endogenous Aβ in CSF and blood can fluctuate acutely in response to glucose and insulin (14,20,25,31). Secretion of Aβ by adipose tissue cells could result from direct effects of glucose and insulin on secretase activity and exocytosis pathways, or through secondary effects like induction of inflammation, mitochondrial dysfunction, or the unfolded protein response. Aβ competes with insulin for the insulin receptor and for degradation by IDE (22,23). This competition reduces signal transduction through the insulin receptor signaling pathway and prolongs the half-lives of both Aβ and insulin (32,33). Chronic *in vitro* treatment of adipocytes with Aβ species at 100 to 1,000 pg/mL reduced IRS2 expression. Interestingly, insulin resistance in human adipocytes is generally associated with alterations in IRS1, but loss of negative feedback on the insulin signaling pathway reduces expression and activation of both IRS1 and IRS2 (30,34-36). Impaired IRS2 activation in brains from patients with Alzheimer disease is associated with insulin-like growth factor-1 (IGF-1) resistance, but human adipocytes have low expression of IGF-1 receptors (8,37). In addition, we found treatment with high levels of Aβ reduced phosphorylation of Akt-1 at serine 473. This site is phosphorylated during insulin receptor signaling and reductions are associated with insulin and IGF-1 resistance (38-40). These observations merit deeper investigation, but suggest chronic elevations in Aβ could induce insulin receptor pathway dysfunction in adipocytes.

Beyond those noted above, there are several important limitations to these data. We have not delineated the exact mechanisms involved in Aβ secretion. With the exception of Aβ and Akt-1, we have not measured levels of other proteins, nor have we measured enzymatic activity of the secretases or IDE. It is quite possible that subtleties among transcription, translation, and function exist which may refine our understanding of Aβ regulation in adipose tissue. We have not measured an acute effect of Aβ on insulin-stimulated glucose uptake. Whether the changes observed in our culture experiments

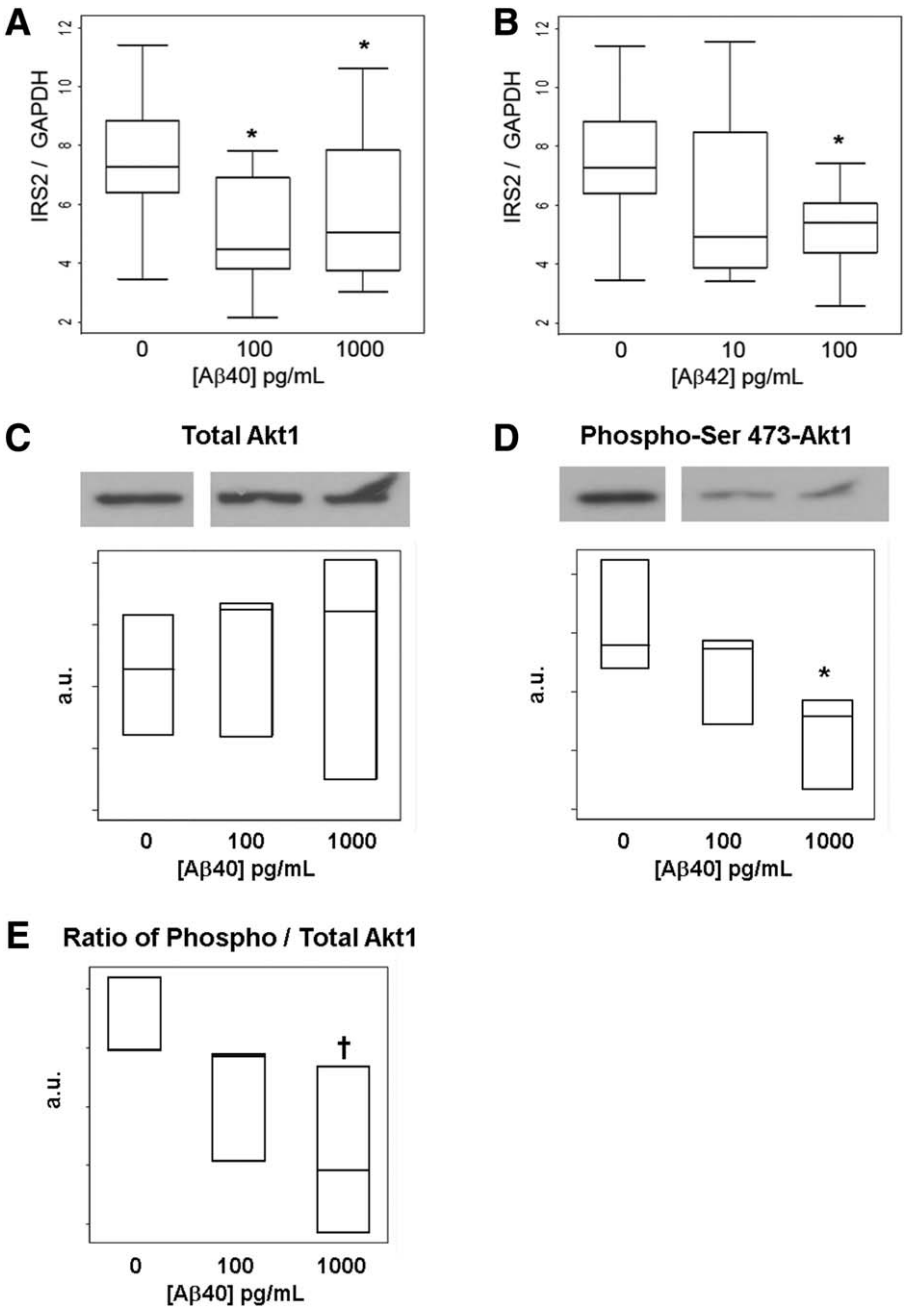

Figure 6 Alteration of the insulin receptor signaling pathway in adipocytes cultured with amyloid-β. (**A**) Insulin receptor substrate-2 (*IRS2*) gene expression in differentiated adipocytes incubated for 6 days with 100 or 1,000 pg/mL amyloid-β40 ($n = 10$–11 per condition). (**B**) IRS2 transcription in cultures treated with amyloid-β42 at 10 or 100 pg/mL for 6 days ($n = 10$–11 per condition). Representative immunoblot and quantification of (**C**) total Akt-1 and (**D**), phosphor-serine 473 Akt-1 and (**E**) ratio of phospho/total Akt-1 in adipocyte cultures grown at 5.5 mM glucose with 10 nM insulin with 100 pg/mL or 1,000 pg/mL amyloid-β40; $n = 3$ per condition, log-transformed data. $^*P < 0.05$ and $\dagger P = 0.07$ by RM-ANOVA. Arbitrary units (a.u.).

correspond to functional impairments in cellular metabolism need to be addressed in future experiments. In addition, our microdialysis cohort is small and further experiments will be needed to assess the adipose cell contribution to interstitial Aβ, the local kinetics of adipose Aβ production and clearance, and associations with metabolic dysfunction.

In summary, here we show that human adipose tissue cells secrete Aβ *in vitro* at concentrations similar to interstitial adipose Aβ *in vivo*, adipose Aβ secretion may be regulated by insulin or glucose, and incubation of adipose cells with Aβ alters the insulin receptor signaling pathway. These data suggest a direct relationship between adipose Aβ and metabolic function and highlight dysfunction in

cellular pathways that could link obesity, type 2 diabetes, and Alzheimer disease. O

Acknowledgments

We gratefully acknowledge Dr. Russell P. Tracy, PhD, from the University of Vermont for helpful discussions about study design and technical assistance with measurement of amyloid peptides.

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