Role of amyloid β in the induction of lipolysis and secretion of adipokines from human adipose tissue

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Introduction

Amyloid-β (Aβ) peptide, which is generated from proteolytic cleavage of amyloid precursor protein (APP), is a key molecule involved in the pathology of Alzheimer disease. Both APP and Aβ peptides are expressed in adipose tissue, however it is currently unclear whether Aβ can affect the key functions of adipose tissue. We aimed to explore whether Aβ affected lipolysis and adipokine secretion in cultured human adipose tissue. We found that Aβ25–35, which contains the main functional domain of the Aβ, stimulated lipolysis via PKA and ERK1/2-dependent pathways and that Aβ25–35 induced leptin and IL-6 secretion. It is concluded that Aβ peptide exerts functional effects on adipose tissue that may lead to increased release of free fatty acids and pro-inflammatory adipokines.

APP and several Aβ cleavage peptides have also been reported in adipose tissue of obese subjects. At the cellular level, treatment of 3T3-L1 adipocytes with tumor necrosis factor (TNF)-α resulted in a significant increase in in vitro APP protein levels in a dose-dependent manner. This is consistent with the findings from a study in diet-induced obese mice where TNF-α and APP expression were increased in adipose tissue. Studies exploring how Aβ may influence adipose tissue function are limited; however, treatment of isolated murine subcutaneous fat with an APP agonist antibody in vivo had no effect on lipid storage or TNF-α secretion. Collectively, it is of interest to further explore how Aβ impacts adipose tissue function.

Lipolysis is a unique function of adipose tissue under energy demanding conditions such as exercise and fasting. Excessive lipolysis also contributes to increased release of free fatty acids, and subsequent ectopic lipid storage, in conditions of insulin resistance. Two key signaling pathways involved in the lipolytic pathways are the protein kinase A (PKA) and extracellular-signal-regulated kinase 1/2 (ERK1/2) signaling pathways. The activation of PKA and ERK1/2 results in the phosphorylation of hormone sensitive lipase (HSL) at multiple sites, leading to the breakdown of stored triglycerides and release of glycerol and free fatty acids from adipose tissue.

In addition to releasing glycerol and fatty acids, adipose tissue is also an active endocrine organ secreting a variety of substances known as adipokines. Accumulating evidence suggests that altered adipokine secretion is involved in promoting chronic low-grade inflammation and insulin resistance in obesity. Leptin was the first adipokine described in 1994 and
traditionally acts as a hormone regulating fat storage. Human obesity is characterized by high circulating leptin levels and higher levels of leptin are associated with a pro-inflammatory response. Adiponectin is one of the most abundant adipokines secreted from adipose tissue and is reported to have insulin-sensitizing, anti-inflammatory and cardioprotective effects. IL-6 is classically recognized as a pro-inflammatory cytokine that can induce insulin resistance in adipocytes by reducing adiponectin expression and secretion or decreasing insulin-stimulated glucose uptake and lipogenesis. Collectively, increased secretion of leptin and IL-6 and reduced secretion of adiponectin from adipose tissue in obesity have been linked with systemic inflammation, insulin resistance, and appetite dysregulation.

The aim of this study was to: 1) Determine the effects of Aβ on lipolysis, and associated signaling pathways, in human adipose tissue; and 2) Explore whether Aβ can affect secretion of the key adipokines adiponectin, leptin and IL-6 from human adipose tissue. We hypothesized that 1) Aβ would induce lipolysis in cultured human adipose tissue via PKA- and ERK1/2-dependent signaling pathways and that 2) Aβ would reduce adiponectin secretion while increasing leptin and IL-6 secretion.

As shown in Figure 1, Aβ25–35 resulted in significant induction of glycerol release at 24 h (4.66 ± 1.05 vs. 8.24 ± 2.00 mmol/L/g tissue, P < 0.05 vs. vehicle control). Aβ25–35 has no effect on glycerol release at 2, 6, 12 h (data not shown). H89, a well-recognized inhibitor of PKA partially inhibited Aβ25–35 induced glycerol release (P < 0.05 vs. Aβ25–35 alone). Similarly, PD98059, an inhibitor of ERK1/2 also partially inhibited Aβ25–35 induced glycerol release (P < 0.05 vs. Aβ25–35 alone).

As shown in Figure 2, treatment of cultured human adipose tissue with Aβ25–35 for 24 h resulted in significant induction of leptin and IL-6 secretion (P < 0.05 vs. vehicle control), while there was no detectable effect on adiponectin secretion.

Discussion

While traditionally viewed as a molecule involved in AD pathology, an accumulating body of evidence suggests that Aβ, and its precursor protein APP, could also be involved in physiological and pathological processes in adipose tissue. However, currently there is no research examining whether Aβ can affect the key functions of human adipose tissue.

In this study, we aimed to explore how Aβ affects lipolysis and secretion of major adipokines from human adipose tissue. Aβ25–35 is the main functional domain of the Aβ molecule that is required for neurotoxic effects. We found that Aβ25–35 can induce lipolysis in cultured adipose tissue in a manner that appeared partially dependent on PKA and ERK1/2-dependent signaling pathways. Lipolysis for the provisions of fatty acids as a fuel for other tissues is a unique function of adipose. However, chronically elevated circulating levels of fatty acids, commonly observed in obesity, are associated with negative metabolic consequences such as ectopic lipid deposition and insulin resistance. Our results may suggest that over-accumulation of Aβ in adipose tissues might promote lipolysis, which could increase availability of fatty acids and glycerol and contribute to lipotoxicity in other organs. However, we were unable to measure mRNA expression of key lipolytic molecules so we cannot ascertain if changes in gene expression are required for the functional responses seen in human adipose after 24 h treatment with Aβ. Future research is also required to determine whether and how Aβ may directly activate ERK1/2 or PKA signaling pathways in adipose.

We also found that Aβ25–35 can induce leptin and IL-6 secretion in cultured adipose
tissue while there was no detectable influence on adiponectin secretion. Serum leptin concentration and adipose leptin mRNA level are positively associated with BMI. Similarly, chronic low-grade elevations of IL-6 in adipose tissue are posited to directly contribute to insulin resistance. Given the previous associations between adipose tissue APP and Aβ in rodent models of obesity, the induction of leptin and IL-6 secretion by Aβ25–35 may indicate that Aβ might contribute to adipose tissue inflammation and promote insulin resistance. It should be noted that in the present study we assessed the impact of aggregated Aβ on cultured human adipose tissue. Although overproduction of Aβ leads to aggregation formation in vivo, future research is needed to determine whether Aβ peptides or other forms of this diverse molecule also affect adipose tissue function.

In conclusion, we revealed that Aβ25–35 stimulated lipolysis and induced leptin and IL-6 secretion in cultured human adipose tissue. The effects of Aβ25–35 on lipolysis appeared to be partially dependent on PKA and ERK1/2-dependent signaling pathways but future research is warranted to fully characterize how Aβ influences adipose tissue function. Our current findings implicate Aβ in the regulation of lipolysis and adipokine secretion in human adipose tissue and suggest that the previously observed increase in adipose tissue Aβ in obesity might have functional effects in this tissue.

Materials and Methods

Aβ25–35 (cat#A-1060–1) was purchased from R-Peptide (GA, USA). Human enzyme-linked immunosorbent assay (ELISA) Duoset kits for adiponectin (cat#DY1065), leptin (cat#DY398) and IL-6 (cat#DY206) were from R&D systems (NE, USA). Specific chemical inhibitors PD98059 (cat# 10006726) and H89 (cat#10010556) were obtained from Cayman Chemicals (KS, USA). Medium 199 was from Life Technologies (NY, USA). Glycerol assay kit (cat# F6428) was from Sigma (MO, USA). Fatty acid-free bovine serum albumin (FA-free BSA) (cat#152401) was from MP Biomedical (OH, USA). All other chemicals were purchased from Sigma (MO, USA).

Aβ25–35 was dissolved in endotoxin-free deionized distilled water at a concentration of 2 mM and incubated at 37°C for 7 d to induce aggregation as described by Xian et al. After aggregation, the solution was stored at −20°C until experimentation.

Preperitoneal adipose tissue samples were obtained from 10 non-obese male subjects; age = 56 ± 4 yr, body mass index (BMI) = 25.2 ± 1.0 kg/m² undergoing abdominal surgeries. The samples of adipose tissue were put in sterile PBS and immediately transported to the laboratory for experiments. Patients were free of acute or chronic disease and were not currently taking any medications known to affect lipid metabolism or inflammation, with the exception of one participant who had previous history of colon cancer. The present study was conducted according to the guideline laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by
University of British Columbia Clinical Research Ethics Board (H12-02330). Written informed consent was obtained from all subjects. Adipose tissue was sectioned into ~100 mg pieces and weighed on an analytical balance (Mettler Toledo MS204S) before being minced into ~5–10 mg pieces and placed into culture dishes containing 3 ml of M199 supplemented with 1% antibiotic/antimycotic, 50 µU insulin and 2.5 nM dexamethasone, as described previously.9 The cultures were placed in a humidified 5% CO2 incubator at 37°C to equilibrate for 24 h. On the morning of the experiment, media was replaced with fresh M199 supplemented with 2.5% fatty Acid-free BSA and treated with Aβ25–35 (40 µM), Aβ25–35 + t-H89 (1 µM, an inhibitor of PKA), Aβ25–35 +PD98059 (25 µM, an inhibitor of ERK1/2). Media were collected at 24 h for further analysis of glycerol, adiponectin, IL-6 and leptin levels. We chose these concentrations of Aβ25–35 and signal inhibitors based on published literature3-34 and pilot experiments from our laboratory.

Culture media was analyzed for glycerol concentrations using colorimetric assays according to the manufacturer’s instructions. Glycerol concentrations were corrected for tissue weight and reported as mmol/L released per g tissue. The coefficient of variation for these assays in our laboratory based on duplicate measurements is <10%.

The concentrations of adiponectin, IL-6 and leptin were measured by ELISA according to the manufacturer’s instruction.

Adiponectin, IL-6 and leptin concentrations were corrected for tissue weight and reported as ng/mL per g tissue. The coefficient of variation for these assays in our laboratory based on duplicates is <10%.

All values are expressed as the means ± standard error of the mean (SEM). Effects of Aβ25–35 and inhibitors on Aβ25–35 induced glycerol release versus vehicle control were compared by one-way ANOVA with LSD post-hoc comparisons. Effects of Aβ25–35 vs. vehicle control on adiponectin, IL-6 and leptin secretion were compared by Student’s t-test. All statistics were performed using GraphPad Prism v6.0. Statistical significance was set at P < 0.05.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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