Effect of Obesity and Diabetes on Alzheimer’s APP Gene Expression in Mouse Adipose Tissues

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The aim of this study was to determine whether Alzheimer’s amyloid precursor protein (APP) is dysregulated in adipose tissues of C57BL/6 male mice by high-fat diet (HFD) induced obesity, aging, or streptozotocin (STZ)-induced diabetes. APP mRNA expression was examined by quantitative real-time PCR (QPCR) in subcutaneous (SAT) and epididymal adipose tissues (EAT) from mice in 8 different condition groups. By combining conditions of age (16 weeks/26 weeks of age), diet (normal diet (ND)/high-fat diet), and induction of diabetes (non-diabetic/diabetic), 88 mice were divided into 8 different groups. QPCR demonstrated that APP expression in SAT was significantly increased by about two-fold in HFD-induced obese mice compared to both 16 week-old and 26 week-old mice in the ND group (16 weeks p=0.001; 26 weeks p<0.0001), but no changes in EAT was found. Particular effects of aging on APP gene expression were not observed in either adipose tissue depot. Significantly decreased APP expression was found in SAT in STZ-induced diabetic mice fed on ND or HFD at 16 weeks of age (ND p<0.05; HFD p<0.01). Linear regression analysis demonstrated that APP expression levels correlated with body weight in both the non-diabetic group (R=0.687, p<0.0001, n=39) and the diabetic group (R=0.508, p<0.0001, n=49), but did not correlate with plasma glucose levels, which suggests that decreased APP expression in STZ-induced diabetic mice is most likely due to weight loss rather than hyperglycemia. These data confirm APP dysregulation by weight changes in humans and suggest a possible role linking midlife obesity with the later development of amyloidogenesis in the brain of older patients with Alzheimer’s disease.

Key words: Amyloid precursor protein, adipose tissue, obesity, diabetes, Alzheimer’s disease

Introduction

Alzheimer’s disease (AD) is the most common cause of dementia in the elderly [6,29]. The deposition of amyloid beta (Aβ) peptides, including Aβ40 and Aβ42, is an early and consistent finding in AD [8,11]. Both over-expression of amyloid precursor protein (APP) and abnormal APP enzymatic processing play key roles, resulting in the production of Aβ fragments, that are neurotoxic and proinflammatory. These peptides aggregate to form an insoluble extracellular deposit constituting the core of the neuritic plaques pathognomonic of AD [2,3,22,30].

APP is a type 1 transmembrane protein normally present in wide variety of cell types, including neurons, adrenal, liver, spleen, heart, fibroblasts, intestine, and adipose cells [7,14,16,28]. The human APP gene, located on the long arm of chromosome 21 (21q21), spans approximately 240 kb [34], while the mouse App gene has been mapped to a syntenic region of mouse chromosome 16 spanning 220 kb [19]. Three major mRNA isoforms of APP are generated by alternative splicing. In non-neuronal tissues, the APP751 and APP770 variants predominate in human and mouse [24,32,33]. These two variants contain a Kunitz proteinase inhibitor domain in the extracellular portion of the protein. In neuronal tissue, the APP695 variant, which lacks a Kunitz domain, predominates [24].

While the link between obesity and insulin resistance, metabolic syndrome, and T2DM is widely appreciated, emerging data indicate that mid-life obesity also increases risk for late-life dementia and AD [9,10,12,20,26,27,31]. Abdominal obesity, indexed as the waist-hip ratio, has also been associated with an increased risk for AD [26]. In addition, recent studies demonstrated that plasma Aβ levels were directly related to BMI in a small group of young, non-demented individuals [1] and adipocyte APP expression levels [14]. It is possible that up-regulated expression of APP and production of Aβ peptides in adipose cells of obese individuals during adulthood could alter circulating levels and CNS clearance of Aβ peptides and thereby directly enhance
risk for AD in later life [14].

Patients with AD, like patients with T2DM, have been reported to be insulin resistant and hyperinsulinemic compared to non-demented controls [5]. Several studies have also demonstrated that hyperinsulinemia and T2DM appear to be risk factors for developing AD [4,13,21,25]. Thus, insulin resistance in obesity and T2DM may predispose to AD.

In previous studies [15-17,23], we have demonstrated that APP mRNA was highly expressed in adipocytes, up-regulated in obesity and correlated to insulin resistance, hyperinsulinemia and a pro-inflammatory gene expression profile in adipocytes.

In a recent study, we have shown that APP gene expression levels in subcutaneous adipocytes in obese individuals are correlated with plasma Aβ40 levels and significantly decreased with weight loss [14]. These novel observations suggest that plasma concentrations of Aβ40 may be derived, at least in part, from adipose tissue and that APP expression in adipocytes may be related to insulin resistance [14].

In this study using male C57BL/6 mice, we aimed to confirm the overexpression of APP with obesity as we previously demonstrated in human subjects and further investigate APP regulation by a couple of treatments which can not be easily applied to human subjects, such as HFD-induced obesity, STZ-induced diabetes, and weight loss by diabetes.

Materials and Methods

Animals

The protocols used in this study were approved by the Animal Experimentation and Ethics Committee of Catholic University of Daegu. Male C57BL/6 mice were obtained from Haehan Biolink (Fumseong, Korea) at 4 weeks of age. They were housed four per cage in a temperature-controlled room with a 12 hr light/12 hr darkness cycle. Six-week-old male C57BL/6 mice were divided into a normal diet group or a high-fat diet group. Mice in normal diet group were given chow containing 4.0% (wt/wt) total fat (Rodent NIH-31 Open Formula Auto, Zeigler Bros., Inc., Gardners, PA, USA). Mice on the high-fat diet were given chow containing 45% fat on a caloric basis (Feedlab Korea Co., Ltd, Korea). Mice were given ad libitum access to food and water and weighed on an electronic balance once weekly. After 10 and 20 weeks of feeding (at 16 and 26 weeks of age), mice were anesthetized with sodium pentobarbital and exsanguinated. The adipose tissues (subcutaneous abdominal and epididymal depots) were isolated, flushed with phosphate-buffered saline and then quickly frozen and stored in -80°C.

Induction of diabetes mellitus

Diabetic mice were generated according to the multiple low-dose streptozotocin (STZ) induction protocol of Animal Models of Diabetic Complications Consortium. STZ (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.1M sodium citrate buffer (pH 4.5) at a concentration of 0.75%. C57BL/6 male mice of 11 and 21 weeks of age were given intraperitoneal injections of STZ at a dose of 50 mg/kg for five consecutive days. Diabetes induction was confirmed by the presence of hyperglycemia at four weeks after STZ injection. Blood glucose levels were measured by a glucose meter. The minimum blood glucose level accepted for a diabetic mouse was higher than 250 mg/dl. Mice that did not exhibit hyperglycemia at this time were given an additional one injection of STZ, as necessary for hyperglycemia to develop. Adipose tissue depots were isolated from injected mice week 3 after the first injection (mice at 16 or 26 weeks of age).

Total RNA extraction and cDNA synthesis

Total RNA was extracted from isolated adipose tissues using an RNeasy Lipid Tissue Mini Kit from Qiagen (Valencia, CA). During the extraction, RNA was treated with DNase I using the RNase free DNase Set (Qiagen) according to the manufacturer’s instructions to minimize potentially contaminating genomic DNA. The analyzed total RNA samples were reverse-transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA).

Quantitative real-time PCR

Quantitative real-time PCR was performed using cDNA samples from adipose tissues to assess APP gene expression levels. Real-time PCR was carried out as previously described [18], using the TaqMan Gene Expression Assay (assay ID: APP_Mm00431827_m1, Applied Biosystems), which can detect all 3 major APP mRNA variants, on a 7500 Real-Time PCR System (Applied Biosystems). The transcript level of APP was normalized to that of GAPDH (TaqMan Mouse Endogenous Control, Applied Biosystems).

The 20 μl reaction mixture included a cDNA template cor-
responding to 10 ng of the original total RNA. The PCR conditions were as follows: 1 cycle at 50°C for 2 min, 1 cycle at 95°C for 10 min, and 40 cycles at 95°C for 15 sec and 60°C for 1 min. Standard curves for each gene were generated by serial dilution of a cDNA mixture, which was made using equal amounts of cDNA from subcutaneous and epididymal adipose tissue samples.

All results are expressed as means±SD unless otherwise specified. Real-time PCR expression data for APP mRNA were analyzed using a student’s t-test. General linear regression models were used to assess the relationships between APP gene expression levels and plasma glucose levels and body weight. P values less than 0.05 were considered significant.

Results

Weight change by high-fat diet and induction of diabetes

Six-week-old male C57BL/6j mice were divided into a normal diet (ND) group or a high-fat diet (HFD) group. Mice eating the HFD exhibited a significant increase in body weight gain one week after dividing (p<0.05), and ~1.5 fold body weight at 26 weeks of age at the end of the experimental trial as compared with normal-diet control animals (HFD 47.7±1.5 g, n=10 vs. ND 31.9±2.0 g, n=10, p<0.0001).

Induction of diabetes with STZ was associated with the characteristic development of hyperglycemia and loss of body weight. All STZ-induced diabetic mice in groups of 16-week-old mice and 26-week-old mice fed on normal or high-fat diet had significantly lower body weight compared to non-diabetic controls (all 4 groups, p<0.0001).

Effect of HFD-induced obesity on APP gene expression in adipose tissue

The expression levels of APP mRNA transcripts from adipose tissue depots were compared between high-fat diet group and normal diet group. APP gene expression in subcutaneous abdominal adipose tissue was significantly increased by about 2.4 fold in both 16 and 26-week-old HFD-induced obese mice compared to mice in normal diet group (16 weeks 125.0±13.9 vs. 63.5±9.9, arbitrary number, p=0.001; 26 weeks 120.2±6.0 vs. 51.8±6.3, p<0.0001, Fig. 1). Linear regression analysis demonstrated that APP expression in subcutaneous abdominal adipose tissue from euglycemic mice without STZ treatment used in this study significantly correlated with body weight (R=0.657, p<0.0001, Fig. 2). However, APP gene expression in epididymal adipose tissue was not changed by high-fat diet feeding (16 weeks p=0.46; 26 weeks p=0.52, Fig. 1).

In addition, APP gene expression levels in subcutaneous and epididymal adipose tissues were not significantly changed by aging between 16- and 26-week-old mice in both HFD group and ND group (p>0.4, Fig. 1)

Effect of hyperglycemia on APP gene expression in adipose tissue

Induction of diabetes by STZ led to significantly de-

![Fig. 1. Effect of diet induced obesity on APP gene expression](image1)

![Fig. 2. Relation of APP gene expression to body weight](image2)
creased APP gene expression in subcutaneous adipose tissue from ND or HFD fed mice at 16 weeks of age (ND16 diabetic 63.5±9.9 vs. non-diabetic 40.2±5.0, p=0.05; HFD16 125.0±13.9 vs. 67.0±9.0, p<0.01, Fig. 3). However, APP gene expression in subcutaneous adipose tissue of 26-week-old mice was not significantly changed by STZ-induced diabetes (ND26 51.8±6.3 vs. 57.4±8.8, p=0.6; HFD26 120.2±6.0 vs. 104.8±10.3, p=0.3, Fig. 3).

Linear regression analysis also demonstrated APP in subcutaneous adipose tissue from diabetic mice correlated with body weight (R=0.508, p<0.0001, n=49, Fig. 4), but not with plasma glucose levels (R=0.095, p=0.52, data not shown).

APP gene expression in epididymal adipose tissue did not tend to be affected by induction of hyperglycemia in most conditions investigated (HFD16 p=0.34; ND26 p=0.36; HFD26 p=0.33), except 16-week-old mice fed on normal diet (102.6±5.5 vs. 85.8±6.6, p<0.05, data not shown).

**Discussion**

This study was aimed to elucidate the effects of HFD-induced obesity, aging, hyperglycemia on APP gene regulation in subcutaneous and epididymal adipose tissue in mice. This study demonstrates, for the first time, that APP mRNA expression is up-regulated by HFD-induced obesity and down-regulated by diabetes related weight loss in mouse subcutaneous abdominal adipose tissues. These findings were consistent with the previously published data describing the APP regulation associated with obesity or weight loss in human subjects.

We have previously demonstrated that APP mRNA was highly expressed in subcutaneous abdominal and visceral adipose tissues and levels in subcutaneous adipocytes were upregulated in obesity and related to in vivo measures of insulin resistance [16]. In a gene-chip microarray expression profiling study, APP was expressed approximately 2.5 fold (p<0.0001) in subcutaneous adipocytes from 19 obese compared to 20 nonobese human subjects. We also found that the APP gene-chip signal was highly correlated to insulin concentration and HOMA-IR [15,16]. In a recent study, we have shown that APP gene expression levels in subcutaneous adipocytes in obese individuals are correlated with plasma Aβ40 levels and significantly decreased with weight loss and changes in APP expression levels of subcutaneous adipocyte with weight loss were related to changes in plasma Aβ40 levels and changes in the 2-hour insulin levels [14]. These novel observations suggest that plasma concentrations of Aβ40 may be derived, at least in part, from adipose tissue and that APP expression in adipocytes may be related to insulin resistance [14].

Increased APP mRNA expression in subcutaneous adipocytes in obesity demonstrated in our previous studies [16] was corroborated by the current study in mice which confirmed that APP mRNA expression increased by about 2-fold in subcutaneous abdominal adipose tissue from HFD-induced obese mice compared to control mice fed on normal chow (Fig. 1). These data suggest that APP expression in mice may be regulated in a similar way to hu-
man beings. However, APP expression in epididymal adipose tissue was not affected by HFD-induced obesity, which suggests a depot specific pattern of APP expression.

In the present study, we demonstrated that induction of diabetes by STZ led to significantly decreased APP gene expression in subcutaneous adipose tissue (Fig. 3). With regression analysis, we also found correlation between APP expression levels in subcutaneous adipose tissue and body weight of mice in both euglycemic (without STZ treatment, Fig. 2) and diabetic (with STZ treatment, Fig. 4) groups, which is similar to our previous observation that APP expression in human subcutaneous adipocytes was significantly decreased after 6 months weight loss by diet and exercise intervention [14]. However, a relation between APP expression and plasma glucose levels was not observed (p=0.52). STZ-induced diabetes is an animal model for type II diabetes mellitus without accompanying insulin resistance or hyperinsulinemia. In this study, diabetes by STZ treatment was induced to investigate the effects of hyperglycemia and consequent weight loss without insulin resistance. These data imply that APP down-regulation in STZ-induced diabetic mice is due to weight loss or attenuated weight gain rather than hyperglycemia.

Aging between the weeks 16 to weeks 26 did not appear to change APP mRNA expression in mouse adipose tissues (Fig. 1). AD is usually associated with older age and some studies have indicated that global APP gene expression is increased with aging. No relation between age and the level of APP gene expression in adipose tissue was found in this study. This is most likely due to the fact that animals were mainly young adults and the range of ages was not broad enough to detect significant changes in APP expression levels.

Previous studies, together with present results, support the potential importance of peripheral tissues including adipose tissues in the regulation of APP and Aβ in individuals at risk for AD, which will have to be ascertained in future studies of the physiological significance of adipose tissue APP.

In summary, this study indicates that APP expression is significantly up-regulated in adipose tissue from diet induced obese compared with non-obese control mice. In addition, APP gene expression in adipose tissue was significantly down-regulated by induction of diabetes by STZ treatment. This down-regulation is most likely due to weight loss. The mechanism by which adipose tissue from obese animals increases APP gene expression needs further investigation. In addition, it will be important to determine whether up-regulated adipose tissue APP expression contributes to higher circulating Aβ peptides in obesity. Finally, the relationship between adipose tissue expression of APP and Aβ peptides and amyloidogenesis in brain risk for AD are not known and will require long-term studies to address.

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References


초록: 비만 및 당뇨가 생쥐 지방조직에서의 Alzheimer's APP 유전자 발현에 미치는 영향

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본 연구에서, 고지방식이에 의한 비만, streptozotocin 처리에 의한 당뇨 및 노화에 의해 알츠하이머병의 원인 유전자가 APP의 발현이 상승하는데 영향을 미치는지를 조사하였다. 일반식이(ND)/고지방식이(HFD), 16주령/26주령, 및 정상/ 당뇨 등의 조건별 8가지 조합에 88마리의 C57BL/6 쥐를 각 그룹에 최소 10마리씩 나누어 키웠으며, 각 실험 쥐로부터 추출한 부고피지방 및 부고환지방조직에서의 APP mRNA 발현양을 quantitative real-time PCR로 측정하였다. 그 결과, 피하지방조직에서의 APP 유전자는 16주령과 26주령에서 고지방식이로 키워 비만을 유도한 동물에서 2배정도 높게 발현되었으나(16주령 125.0±13.9 vs. 63.5±9.9, p=0.001; 26주령 120.2±6.0 vs. 51.8±6.3, p<0.0001), 부고환지방조직에서는 APP 발현양의 차이가 나타나지 않았다. 16주령과 26주령 사이에서의 노화에 따른 APP 발현양의 차이도 나타나지 않았다. 또한, 일반 및 고지방식이로 키운 16주령 생쥐의 피하지방조직 APP 유전자 발현은 STZ에 의해 유도된 당뇨병 생쥐에서 크게 감소되었다(ND non-diabetic 63.5±9.9 vs. diabetic 40.2±5.0, p<0.05; HFD 125.0±13.9 vs. 67.0±9.0, p<0.01). APP mRNA 발현양의 선형회귀분석에 의하면, 당뇨병이 유발되지 않은 일반 생쥐(R=0.657, p<0.0001, n=39)와 당뇨병 생쥐(R=0.508, p<0.0001, n=49) 모두에서의 APP mRNA 발현양이 체중과 깊은 연관성이 있음이 확인되었으나, APP mRNA 양과 혈당의 연관성은 나타나지 않았다. 이는 STZ에 의한 당뇨병 생쥐에서 판찰한 APP mRNA 발현의 감소는 고혈당증에 의한 것이 아닌 체중감소의 영향인 것으로 추측된다. 본 연구의 결과로 APP mRNA는 생쥐의 피하지방 및 부고환지방에서 발현되고, 비만의 영향에 의해 증가하며, 체중감소에 의해 감소한다는 것을 확인하였다. 이는 결과는 사람에게서 확인된 체중변화에 의한 APP 이상발현 현상을 더욱 분명히 보여주었으며, 이런 APP 이상발현이 중년기 비만과 노령기의 알츠하이머병 환자에서의 amyloidogenesis 증가 메개자로서의 역할 가능성을 시사한다.