Amyloid Precursor Protein and Proinflammatory Changes Are Regulated in Brain and Adipose Tissue in a Murine Model of High Fat Diet-Induced Obesity

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Abstract

Background: Middle age obesity is recognized as a risk factor for Alzheimer’s disease (AD) although a mechanistic linkage remains unclear. Based upon the fact that obese adipose tissue and AD brains are both areas of proinflammatory change, a possible common event is chronic inflammation. Since an autosomal dominant form of AD is associated with mutations in the gene coding for the ubiquitously expressed transmembrane protein, amyloid precursor protein (APP) and recent evidence demonstrates increased APP levels in adipose tissue during obesity it is feasible that APP serves some function in both disease conditions.

Methodology/Principal Findings: To determine whether diet-induced obesity produced proinflammatory changes and altered APP expression in brain versus adipose tissue, 6 week old C57BL6/J mice were maintained on a control or high fat diet for 22 weeks. Protein levels and cell-specific APP expression along with markers of inflammation and immune cell activation were compared between hippocampus, abdominal subcutaneous fat and visceral pericardial fat. APP stimulation-dependent changes in macrophage and adipocyte culture phenotype were examined for comparison to the in vivo changes.

Conclusions/Significance: Adipose tissue and brain from high fat diet fed animals demonstrated increased TNF-α and microglial and macrophage activation. Both brains and adipose tissue also had elevated APP levels localizing to neurons and macrophage/adipocytes, respectively. APP agonist antibody stimulation of macrophage cultures increased specific cytokine secretion with no obvious effects on adipocyte culture phenotype. These data support the hypothesis that high fat diet-dependent obesity results in concomitant pro-inflammatory changes in brain and adipose tissue that is characterized, in part, by increased levels of APP that may be contributing specifically to inflammatory changes that occur.

Introduction

Obesity, particularly in mid-life, is an increased risk factor for AD independent of other conditions [1,2,3,4,5,6,7]. Particular saturated versus unsaturated fat ingestion at midlife also increases the risk of developing AD [8,9]. In addition, metabolic syndrome and diabetes, often comorbid with obesity, are factors of increased risk for AD in some [6,7,10,11] but not all studies [12]. Interestingly, late life obesity and metabolic syndrome are either not risk factors or actually decrease the risk of AD in several studies [3,13,14]. Others have reported that obesity itself is associated with poorer cognitive performance in humans [15,16,17] as well as decreased brain volumes [18] independent of age or disease. In spite of this abundance of correlational data, a particular mechanism linking the pathophysiology of obesity to the brain changes of AD remains unclear.

One possibility of linking the conditions focuses on the biology of amyloid precursor protein, APP. It is expressed in the brain primarily by neurons [19] where it can be metabolized to Aβ1-40 and 1-42 peptides which aggregate to form amyloid plaques characteristic of AD [20]. Moreover, mutations in the gene coding for APP [21] or its protease presenilins [22,23,24] are responsible for a rare autosomal dominant form of disease. Therefore, APP and its proteolytic fragments are likely to play a central role in the pathophysiology of AD.

Recent data suggests that APP expression or function may also be involved in the pathophysiology of obesity. It is known that adipose tissue [25,26,27] and adipocyte cell lines [27] express APP. More importantly, adipose APP and Aβ1-40 plasma levels increase in obese individuals [23,26] and plasma Aβ1-42 and 1-40 levels correlate with increased body fat in humans [28,29]. Rodent studies have examined the brain in a variety of diet-induced obesity paradigms confirming that brain changes leading to increased Aβ levels occur in both AD transgenic [30,31] and wild type mice [32]. These findings indicate that changes in APP expression or function may be coordinated across diverse tissue types.
In this study a high fat diet-induced model of obesity was used with C57BL6/J mice to determine whether changes in APP expression occurred similarly in brain versus visceral and subcutaneous fat depots in correlation with simultaneous proinflammatory changes in each tissue.

**Results**

High fat diet feeding increased brain levels of APP and multiple pro-inflammatory proteins compared to control diet fed mice

In order to establish the system for comparing changes in adipose tissue to brain, a standard high fat diet feeding paradigm was used. 24 six week old weight matched male C57BL6/J mice were placed on either a 21.2% by weight high fat diet or a 5.5% by weight control diet, *ad libitum*, beginning at six weeks of age (Table 1). Twelve animals in each group were weighed weekly for 22 weeks and mean (+/− SD) weight gain per group was graphed versus time (Fig. 1). By week five the high fat diet fed mice demonstrated a statistically significant increase in weight gain over the control diet fed mice (Fig. 1). After 22 weeks, the high fat diet fed mice had on average a 217% total weight gain, whereas control diet fed mice had on average only a 158% total weight gain. To examine whether proinflammatory or degenerative changes were occurring in the brains of high fat diet fed animals, Western blot analysis of hippocampi from both groups was performed. As expected, high fat diet fed mice demonstrated a significant increase in expression of APP when compared to control diet fed mice (Figs. 2, 3). This did not correlate with any change in protein levels of the postsynaptic protein marker, PSD95, or the presynaptic marker, synaptophysin (Figs. 2, 3). However, there was a significant increase in astrocyte GFAP protein levels, but no change in microglia CD68 protein levels associated with the high fat diet fed mice. Interestingly, two markers of inflammatory change, iNOS and Cox-2 were not altered in hippocampi of high fat diet versus control diet fed animals (Figs. 2, 3). Although Cox-2 protein levels were not altered, we further examined enzyme activity and total brain prostaglandin (PG) levels (a sum of PGE2, PGD2, 6-koPGF1a, PGF2a, and thromboxane B2) were quantitated from animals in each diet group. Interestingly, high fat diet fed animals demonstrated a significant increase in brain prostaglandin levels compared to control fed animals indicating elevated arachidonic acid metabolism in spite of no significant change in protein levels of Cox-2 (Fig. 3). Additionally, high fat diet feeding did not significantly alter phosphorylation levels of tau protein [33] as assessed using the PHF-1 antibody (Figs. 2, 3). These data demonstrate that high fat diet feeding stimulates an increase in APP protein levels in the brain which correlates with an increased level of gliosis and elevated prostaglandin levels. This supports the notion that the chronic inflammatory changes associated with

**Table 1. Commercial diet formulations for control and high fat diet.**

|            | Control Diet | High Fat Diet |
|------------|--------------|---------------|
| protein (% by weight) | 22.0         | 17.3          |
| carbohydrate (% by weight) | 40.6         | 48.5          |
| fat (% by weight) | 5.5          | 21.2          |
| energy density (Kcal/g) | 3.0          | 4.5           |

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Figure 1. Average weight gain per week for mice fed a high fat versus control diet. C57BL6/J mice at 6 weeks of age and weight matched were fed, *ad libitum*, a control (5.5% fat/weight) or high fat (21.2% fat/weight) diet for 22 weeks. 12 animals in each group were weighed weekly and mean (+/− SD) weight gain per group was graphed versus time. *p = 0.001.

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Peripheral tissue during diet-induced obesity extend into the brain. Moreover, these changes are consistent to some degree with those that are observed during Alzheimer’s disease.

**Proinflammatory protein immunoreactivity increased in neurons in brains from high fat diet versus control fed mice**

Although the Western analysis demonstrated significant protein changes in high fat diet fed mice compared to controls, the cellular identity of those proteins was unknown. Understanding the cellular contribution was of particular interest since APP is expressed by several cells in the brain. In order to determine which cells may be responsible for the changes in protein levels, immunohistochemistry was performed from the collected hippocampi. In agreement with the fact that the majority of APP is expressed by neurons in the brain [34] both high fat and control diet fed mice displayed neuronal immunoreactivity for APP with a diet-induced increase in immunoreactivity in the high fat diet fed mice (Fig. 4). To examine whether APP was increasingly processed or deposited as the proteolytic fragment, beta amyloid (Aβ), brain sections were also immunostained using an anti-mouse Aβ antibody. However, there was no obvious change in intensity or distribution of Aβ immunoreactivity in the different diet fed animals (Fig. 4). Cox-2, iNOS, and CD68 histologic analysis demonstrated what appeared to be elevated immunoreactivity for each (Fig. 4) although this did not reach the level of statistical significance via Western blot analysis (Fig. 3). Interestingly, the immunoreactivity for both Cox-2 and iNOS in high fat diet fed animals appeared within neurons as opposed to glia (Fig. 4). Although, the difference in immunoreactivity for reactive microglia (CD68) was modest, there was a clear increase in astrocyte GFAP immunoreactivity in the high fat diet fed animals compared to controls demonstrating that at least an astrogliosis was occurring (Fig. 4). These data demonstrated that high fat diet
feeding induced a reactive gliosis in the brain. However, since the Western blot analysis detected increased protein levels of APP within neurons, the proinflammatory changes were not limited to any particular cell type in the brain but instead appeared to include a multi-cellular response.

In spite of the fact that microglia and astrocytes demonstrated immunoreactivity for typical activation markers, CD68 and GFAP respectively, this was not necessarily a reflection of a contribution to any proinflammatory changes that were occurring. As a means of assessing this, microglia were further examined based upon our prior experience with isolating these cells from adult animals [35]. For comparison, microglial secreted TNF-α levels were compared to values derived from resting peritoneal macrophage as a positive control. In general microglia isolated from the 22 week old control fed animals secreted far less TNF-α when compared to their peripheral cell counterparts (Fig. 5). However, microglia from high fat diet fed mice secreted significantly more TNF-α than the control diet fed animals (Fig. 5). This was entirely consistent with the subtle increase in CD68 immunoreactivity observed even though quantified Western blot analysis revealed no significant difference in CD68 protein levels in the brains of high fat diet fed mice compared to controls. Taken together, these data support the idea that proinflammatory changes occur in brains of high fat diet fed animals. These involved not only neuronal upregulation of proteins but also increased cytokine secretion from reactive glia.

High fat diet feeding increased APP and TNF-α protein levels compared to control diet fed mice in both subcutaneous and visceral fat depots

Based upon the changes observed in the brain, adipose tissue was next examined to determine whether similar changes in proinflammatory protein expression occurred in the periphery. Because visceral and subcutaneous fat depots can have altered protein expression changes during diet-induced obesity [36,37,38,39] both types of adipose reservoirs were assessed. To begin comparing protein changes between brain and adipose tissue, Western blot analysis was again performed. Subcutaneous abdominal fat and visceral pericardial fat were examined as representative samples of two distinct fat depots. Precisely as observed in the brain, high fat diet fed mice demonstrated a significant increase in APP protein levels in both fat depots over control diet fed mice (Figs. 6, 7). To again assess if there was a proinflammatory change, the two proinflammatory protein markers quantified from brain, iNOS and Cox-2, were next examined in the fat depots. Consistently, the diets demonstrated no difference in either iNOS or Cox-2 protein levels in either type of adipose tissue (Figs. 6, 7). However, based upon the fact that microglial-secreted TNF-α levels were increased in high fat diet fed mice and TNF-α elevations are a well characterized change in adipose tissue from obese individuals [40,41] or animals [42], we next quantified TNF-α protein levels. Similar to the changes observed from brain microglia, both visceral and subcutaneous fat depots demonstrated increased TNF-α levels compared to pair fed controls (Figs. 6, 7). These data demonstrate that although there were no significant differences between visceral and subcutaneous fat depots, the overall proinflammatory changes were consistent between adipose tissue and brain during high fat diet feeding. In particular, the proinflammatory proteins, Cox-2 and iNOS, were not significantly increased in either the brain or adipose tissue in spite of the observed elevation in TNF-α from both tissue types. Perhaps the most interesting observation was that APP levels increased in both brain and adipose tissue. This demonstrated that a coordinated, increased expression of APP occurred in the brain and adipose tissue upon diet-induced obesity along with acquisition of proinflammatory tissue phenotypes.

Adipose tissue APP immunoreactivity from high fat diet fed animals localized to adipocytes and macrophage

Although brain changes in APP were determined to be neuronally localized it was not likely that the increased APP levels in adipose tissue were also neuronal. Therefore, to identify the cellular origin of the increased APP levels in high fat diet fed adipose tissue immunohistochemistry was again performed. In both subcutaneous (Fig. 8) and visceral (Fig. 9) adipose tissue increased APP immunoreactivity was observed in high fat diet fed...
Figure 3. APP and GFAP protein and total prostaglandin levels were increased in the hippocampi of high fat versus control diet fed mice. Optical densities of the Western blotted hippocampal proteins (APP, CD68, iNOS, Cox-2, GFAP, synaptophysin, PSD95, and phospho-tau) from the control and high fat diet Western blots were normalized against their respective averaged O.D. values of combined α-tubulin + βIII tubulin + actin.
versus control diet fed animals. Similar to the observations found during brain analysis, immunostaining of the two different adipose depots with anti-mouse Aβ antibody did not demonstrate a robust increase in immunoreactivity in high fat diet fed animals nor any plaque-type patterns (Figs. 8, 9). Although prior work has demonstrated increased adipocyte APP immunoreactivity in samples from obese humans, it was possible that the increased APP immunoreactivity observed in our animal paradigm was also localized to macrophage. Our prior work has shown that monocyte lineage cells express APP and levels increase upon differentiation and activation [43,44,45]. Moreover, a variety of studies have demonstrated that increased macrophage infiltration into adipose tissue occurs during diet-induced obesity [16,47,40].

Interestingly, both subcutaneous and visceral adipose tissue demonstrated increased immunoreactivity for CD68 to identify macrophage (Figs. 8, 9). Importantly, this increase in CD68 positive adipose tissue macrophage correlated precisely with the slight increase in CD68 positive microglia observed in the brains of high fat diet fed mice compared to controls (Fig. 4). Double labeling immunohistochemistry was performed to determine whether a portion of the increased APP immunoreactivity observed was within adipose tissue infiltrated macrophage. In either type of adipose tissue a population of the APP immunoreactive cells also co-localized with CD68 immunoreactivity indicating that both adipocytes and macrophage may be responsible for the upregulation of APP observed in adipose tissue of high fat diet fed animals (Fig. 10). Indeed, visceral adipose tissue demonstrated nearly complete co-localization of APP with CD68 immunoreactivity. These results confirm that increased APP levels observed in adipose tissue from high fat diet fed animals is multicellular with a significant portion likely from the increasing numbers of tissue infiltrating macrophages. This correlates with the increased levels of brain APP observed in high fat diet fed mice as well as the increased gliosis that occurs.

The APP agonist antibody, 22C11, increased macrophage cytokine production but had no effect on viability, lipid storage/accumulation, or TNF-α secretion in adipocytes

To begin examining whether increased expression of APP had any role in altering the phenotype of macrophage or adipocytes, primary murine cultures were generated from non-elicited peritoneal macrophage and subcutaneous adipocytes and then stimulated using an agonist antibody for APP [44]. We first stimulated peritoneal macrophage with 1 μg/mL IgG1 (isotype control) or 22C11 (APP agonist antibody) and measured cytokine secretion (Fig. 11). The APP agonist, 22C11, stimulated a significant increase in secretion of granulocyte-macrophage colony stimulating factor (GM-CSF) which reportedly increases the production of macrophages [49]. This was consistent with the increase in immunoreactivity for CD68 (macrophages) in both adipose tissue depots during high fat diet-induced obesity. Stimulation with 22C11 also significantly increased secreted levels of IFNγ, a macrophage-activating factor, that plays a critical role in immunostimulatory and immunomodulatory effects [30,51]. In contrast, 22C11 stimulation significantly increased secreted IL-13 levels which is reportedly responsible for down-regulation of macrophage activity and thereby inhibits the production of pro-inflammatory cytokines and chemokines [52]. Although these findings do not demonstrate precisely what secretory changes APP-overexpressing macrophage may be exhibiting in situ during diet-induced obesity, they do provide clear evidence that APP stimulated changes in macrophage phenotype are complex with alterations in both proinflammatory and anti-inflammatory secretion that will need to be further resolved in vivo in the diet-induced obesity model.

To assess any effect of APP on adipocyte phenotype, we next stimulated primary murine abdominal subcutaneous fat derived-adipocytes with 1 μg/mL IgG1 or 22C11 antibodies. Unlike the macrophage studies, agonist antibody stimulation of adipocytes did not produce any obvious change in phenotype. There was no significant toxicity of the 22C11 or IgG1 stimulated adipocytes as assessed by LDH release (Fig. 12A). Since TNFα levels were increased under diet-induced obesity conditions in both adipose tissue depots, TNFα secretion was measured from APP stimulated adipocytes (Fig. 12B). However, the APP agonist, 22C11, did not stimulate a significant change in TNFα secretion. To assess a differentiative phenotype, stimulated adipocytes were stained and quantified using Oil Red O (Fig. 12C and 12D) to examine lipid storage/accumulation. APP stimulation with 22C11 produced no changes in Oil Red O staining. Although a minor subset of all the possible changes in adipocyte phenotype was examined, the data thus far indicates that viability, proinflammatory secretion, and differentiation state are not affected by APP signaling. This suggests that further investigation is needed to fully understand the role of APP in adipocyte biology both basally and during instances of increased APP expression such as that which occurs during diet-induced obesity.

Discussion

We demonstrate that a high fat diet feeding paradigm in C57BL6/J mice drives a concomitant increase in protein levels of APP in brain and visceral and subcutaneous adipose tissue correlating with proinflammatory changes in a cell specific fashion. APP protein levels increased within primarily neurons in the brain and macrophage and adipocytes in adipose tissue of high fat diet fed mice. This correlated with reactive gliosis and elevated microglial TNFα production. This elevated proinflammatory, neurodegenerative phenotype of the high fat diet fed brain correlated with a similar increase in APP and TNFα levels in high fat diet fed adipose tissue. This supports the idea that a particular function of APP may contribute to the metabolic changes that occur in each tissue during diet-induced obesity. At the very least, the APP changes observed may serve as one of the growing list of bio-markers that identify obesity-related changes.

In particular, we provide a crucial data set of comparison changes in brain versus adipose tissue that was lacking from earlier studies which have focused on a particular organ. Prior work from human subjects has demonstrated increased adipose tissue APP and plasma Aβ levels in obese subjects [25,26,27,28,53] although comparable brain data was not reported. Similarly, brain but not adipose data from Alzheimer’s disease transgenic rodent studies using diet-induced obesity paradigms have indicated that obesity correlates with increased levels of brain Aβ [30,31,54] although not necessarily an increase in full length APP as we observed. Our findings are in greater agreement with those of Thrumanaga and colleagues who found using wild type C57BL6/J mice that a high fat/high cholesterol diet induced increased brain changes in APP, Aβ, and diverse proinflammatory proteins [32].
Figure 4. Hippocampi of high fat diet fed mice demonstrated microglial (CD68), astrocytic (GFAP), and neuronal (Cox-2, APP and iNOS) immunoreactivity versus control diet mice. C57BL6/J mice at 6 weeks of age and weight matched were fed, ad libitum, a control (5.5% fat/weight) or high fat (21.2% fat/weight) diet for 22 weeks. Tissue samples were collected, fixed in 4% paraformaldehyde, serially sectioned, and immunostained. Tissue sections were immunostained using anti-APP, Aβ, GFAP, CD68, Cox-2, and iNOS antibodies and antibody binding was visualized using Vector VIP as the chromagen. Arrows indicate the location of APP immunoreactivity shown as an enlarged inset in upper right corner of each panel. Representative images from 12 animals per condition are shown.

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although a related study in C57BL6/J mice reported no change in brain APP or Aβ levels [55]. To link these diverse data sets we suggest, that based upon our findings, a high fat feeding paradigm producing a diet-induced obesity in animals expressing APP under the control of its endogenous promoter is sufficient to increase APP expression in both adipose and brain tissue simultaneous with similar proinflammatory changes that occur in each tissue.

It is not clear what the exact stimulus is to increase APP levels in the cell types in our paradigm although we speculate that proinflammatory stimuli are responsible. It is well established that obesity correlates with a host of increased circulating proinflammatory molecules [56,57,58,59] released by both adipocytes and adipose macrophage [58,60,61]. Prior work from the 3T3-L1 adipocyte cell line has demonstrated that stimulation with TNF-α is sufficient to increase APP expression [27]. We [43,44,45] as well as others [62,63,64,65,66,67,68,69,70,71,72,73] have demonstrated that APP expression and plasmalemmal localization in monocytic lineage cells, including macrophage and microglia, increases during proinflammatory or degenerative conditions. Finally, neurons themselves have a well established ability to increase APP expression during diverse degenerative and inflammatory stimulations [74,75,76,77,78,79,80].

It is important to point out that focus of this work was on the particular cellular changes in full-length APP rather than assessment of APP processing to Aβ. However, we did assess potential Aβ generation and deposition in both brain and visceral and subcutaneous adipose tissue via immunostaining. Although the mouse Aβ-specific antibody detected no robust changes in either tissue from control or high-fat diet fed mice it is possible that Aβ production was increased in parallel with the increased APP protein levels observed in high fat diet-fed mice but was simply not detectable via immunostaining. We have not ruled out the possibility that a longer feeding paradigm and more sensitive detection method such as an Aβ ELISA might demonstrate a significant difference in APP processing to increased levels of Aβ between diets. Indeed, prior work has already demonstrated that high fat/cholesterol feeding increases Aβ levels in the murine brain [32]. This indicates, perhaps not surprisingly, that although...
APP levels were increased in brain and adipose tissue, its processing and perhaps function(s) is unique based upon cell type expression and requires future study. Regardless of what cell type is potentially producing the Aβ in brain and adipose tissue, the peptide in either its oligomeric or fibrillar form has been shown in numerous studies to be a potent stimulus for activating microglia and monocyte/macrophage cells to acquire a reactive phenotype [81,82,83,84,85,86,87,88]. Again, although processing of APP to Aβ was not the focus of this work, it is intriguing to consider that in addition to proinflammatory stimuli potentially driving increased APP expression, increased Aβ production may act in a feedforward fashion to increase proinflammatory secretions in brain and adipose tissue by directly stimulating microglia and macrophage, respectively. These data support the idea that limiting inflammatory changes during diet induced weight gain may not only attenuate pathologic events in peripheral organs but also those in the brain. Indeed, it appears that use of non-steroidal anti-inflammatory drugs during mid-life, in particular, may offer some protective benefit against developing AD [89]. Although our study has focused specifically on changes related to diet induced obesity it is difficult not to speculate that the changes observed may be directly relevant to the mechanism of Alzheimer’s disease. As already mentioned, mid-life obesity is a well-recognized increased risk factor for developing AD [1,2,3,4,6,7] and several rodent studies using transgenic mouse models of AD have demonstrated that diet-induced obesity paradigms increase Aβ levels in the brain [30,31,54]. More importantly, caloric restriction of these transgenic models is

Figure 7. APP and TNF-α protein levels increased in subcutaneous abdominal and visceral pericardial fat in high versus control diet fed mice. Optical densities of the adipose protein Western blots from the control and high fat diet subcutaneous and visceral samples were normalized against their respective GAPDH loading controls and averaged (+/− SD) from 12 animals per each condition. *p<0.05, **p<0.01.
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Figure 8. APP, CD45, and CD68 immunoreactivity increased in the subcutaneous (abdominal) fat from high fat versus control diet fed mice with no robust change in Aβ immunoreactivity. C57BL6/J mice at 6 weeks of age and weight matched were fed, *ad libitum*, a control (5.5% fat/weight) or high fat (21.2% fat/weight) diet for 22 weeks. Subcutaneous abdominal adipose tissue was collected, immersion fixed in 4% paraformaldehyde, sectioned, and immunostained using anti-APP, Aβ, CD45 and CD68 antibodies and antibody binding visualized using Vector VIP as the chromogen. Representative images from 12 animals per condition are shown.

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Figure 8. APP, CD45, and CD68 immunoreactivity increased in the subcutaneous (abdominal) fat from high fat versus control diet fed mice with no robust change in Aβ immunoreactivity. C57BL6/J mice at 6 weeks of age and weight matched were fed, *ad libitum*, a control (5.5% fat/weight) or high fat (21.2% fat/weight) diet for 22 weeks. Subcutaneous abdominal adipose tissue was collected, immersion fixed in 4% paraformaldehyde, sectioned, and immunostained using anti-APP, Aβ, CD45 and CD68 antibodies and antibody binding visualized using Vector VIP as the chromogen. Representative images from 12 animals per condition are shown.

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sufficient to decrease brain Aβ levels and plaque load [90]. It was particularly interesting that microglia isolated from high fat diet fed mice basally secreted elevated levels of TNFα compared to microglia from control diet fed mice. The ability to isolate these cells acutely from adult mouse brains without the confound of prolonged in vitro culturing in serum containing conditions allows us to quantify with confidence the basal microglial secretory phenotype in the brain during either diet paradigm. The elevated

Figure 9. APP, CD45, and CD68 immunoreactivity increased in the visceral (pericardial) fat from high fat versus control diet fed mice with no robust change in Aβ immunoreactivity. C57BL6/J mice at 6 weeks of age and weight matched were fed, ad libitum, a control (5.5% fat/weight) or high fat (21.2% fat/weight) diet for 22 weeks. Visceral pericardial adipose tissue was collected, immersion fixed, sectioned, and immunostained using anti-APP, Aβ, CD45 and CD68 antibodies and antibody binding visualized using Vector VIP as the chromogen. Representative images from 12 animals per condition are shown.

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proinflammatory state suggested by the glia was supported by elevated levels of total prostaglandins in the high-fat-fed mice. Although we did not attempt to determine effects of APP stimulation on neuronal phenotype in this study it is interesting to speculate that APP-dependent stimulation of neurons may lead directly to increased neuronal prostaglandin production as well as generation of Aβ that may be direct stimuli for the increased microglial TNFα secretion that occurred in high-fat-fed brains. This APP-dependent mechanism linking generation of these proinflammatory mediators with gliosis would certainly be reasonable to consider during similar degeneration events in AD.

Perhaps even more interesting is the possibility that APP dependent proinflammatory events contribute to the classic inflammatory changes commonly observed in peripheral adipose tissue during diet-induced obesity. For instance, based upon the increased APP levels observed in macrophage and adipocytes, we examined a role for APP in regulating the phenotype of these cells. Although we were unable to determine any phenotype change in adipocyte downstream of APP stimulation, macrophage exhibited a significant increase in secretion of three particular cytokines out of the 40 analyzed that may be relevant to adipose changes observed during high fat diet feeding. APP stimulation increased macrophage secretion of GM-CSF, IFNγ, and IL-13. GM-CSF has a well characterized role in regulating infiltration of macrophage into adipose tissue [91]. An APP-dependent increase in GM-CSF secretion would certainly help to explain some of the observed increased in reactive macrophage in the high fat diet adipose tissue. IFNγ has an increasingly apparent role in regulating not only adipocyte cytokine secretion including TNFα but also insulin resistance and infiltration of T cells into obese adipose tissue [92,93,94]. Elevated IL-13 expression is a hallmark of recently defined alternative M2 phenotype macrophage in obese adipose tissue [95]. Although APP stimulation did not alter adipocyte phenotype in our hands a more extensive assessment would likely identify APP-dependent changes in adipocytes relevant to obesity. One interesting possibility is that an APP-APP dependent interaction between adipocytes and macrophage is involved in activating both cell types. That is, APP on macrophage may interact with APP on adipocytes in a complex trans APP-APP interaction as APP can act as a receptor for monocytic lineage cells [43]. It is known that although adipocytes are quite capable of secreting a range of inflammatory molecules [96] that macrophage-adipocyte interaction can potentiate inflammatory changes that can occur during obesity and metabolic disorder [97]. Determination of specific roles for APP in macrophage and adipocyte changes during high fat diet feeding might be addressed in future work through the use of APP knockout mice or cell specific APP deletion or even expression of mutant forms of APP such as those associated with AD.

A final interesting speculation is that if APP and/or Aβ levels increase in adipose tissue during obesity and AD and coordinated expression is tightly regulated between brain and fat then it is not unreasonable to predict that monitoring APP expression and metabolism in adipose tissue could serve as a surrogate for brain with regard to assessing efficacy of particular drug interventions or monitoring disease pathophysiology of obesity or AD. For instance, it is possible that a portion of any generated Aβ in adipose tissue could accumulate as amyloid deposits in either obese or AD individuals. It is well established that adipose tissue can accumulate amyloid proteins, for example, in individuals with rheumatoid arthritis which is often assessed via needle biopsy [98,99].

Materials and Methods

Ethics Statement

The investigation conforms to the National Research Council of the National Academies Guide for the Care and Use of Laboratory Animals (8th edition). Animal use was approved by the University of North Dakota IACUC, protocol #1003-1.

Materials

Anti-β-Amyloid Precursor Protein (APP) antibody was purchased from Invitrogen (Carlsbad, CA, USA). Anti-mouse IgM (goat), anti-rabbit (goat), anti-goat (bovine), anti-rat (goat), and anti-mouse (bovine) horseradish peroxidase-conjugated secondary antibodies, Cox-2, GAPDH, α-tubulin, and β actin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Elite Vectastain ABC Avidin and Biotin, Vector VIP, Vector DAB, biotinylated anti-rabbit, anti-mouse, and anti-rat antibodies were purchased from Vector Laboratories Inc (Burlington, CA, USA). CD45 antibody was purchased from BD Biosciences Pharmingen (San Jose, CA, USA). TNFα antibody was purchased from Abcam Inc (Cambridge, MA, USA). CD68 antibody was purchased from AbD Serotec (Oxford, UK). iNOS antibody was purchased from Chemicon international, Inc (Temecula, CA, USA). CD45 antibody was purchased from BD Biosciences Pharmingen (San Jose, CA, USA). TNFα antibody was purchased from Abcam Inc (Cambridge, MA, USA). GPAP and PSD95 antibody was purchased from Cell Signaling Technology Inc (Danvers, MA, USA). PHF-1 antibody was a gift from Dr. Peter Davies (Albert Einstein College of Medicine, NY). Cox-2 and Prostaglandin (PG) E₂d4 were purchased from Cayman Chemical (Ann Arbor, MI, USA). β-Amyloid (Aβ), rodent specific polyclonal antibody (SIG-39151) was purchased from Covance (Emeryville, CA, USA).
Mice

APP<sup>minTgb/J</sup> homozygous (APP<sup>-/-</sup>) mice and wild type (C57BL6/J) mice were purchased from Jackson Laboratory. Mice were provided food and water ad libitum and housed in a 12 hour light/dark cycle.

High Fat vs. Control Diet Feeding

At six weeks of age, 34 male weight matched C57BL6/J wild type mice were placed on either a 21.2% by weight high fat diet (Harlan Teklad TD.88137) or a 5.5% by weight regular fat diet (Harland Teklad 8640), ad libitum. 12 animals in each group were weighed each week for 22 weeks.

Western Blotting

After 22 weeks the animals were perfused with PBS containing CaCl<sub>2</sub> and brain, hippocampus, visceral (pericardial or heart) fat and subcutaneous (abdominal or stomach) fat were collected and lysed using ice cold RIPA buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 1 mM Na<sub>2</sub>VO<sub>4</sub>, 10 mM NaF, 1 mM EDTA, 1 mM EGTA, 0.2 mM phenylmethylsulfonyl fluoride, 1% Triton, 0.1% SDS, and 0.5% deoxycholate) with protease inhibitors (AEBSF 1 mM, Aprotinin 0.8 M, Leupeptin 21 M, Bestatin 36 M, Pepstatin A 15 M, E-64 14 M). To remove insoluble material cell lysates were sonicated and centrifuged (14,000 rpm, 4°C, 10 min). The Bradford method [100] was used to quantify protein concentrations. Proteins were resolved by 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes for Western blotting using anti-APP, iNOS, Cox-2, synaptophysin, PSD95, phospho-tau (PHF-1), TNF-α, GFAP, CD68, βIII tubulin (loading control), α-tubulin (loading control), GAPDH (loading control). Antibody binding was detected with enhanced chemiluminescence (GE Healthcare, Piscataway, NJ, USA). In some instances, antibodies were stripped from blots with 0.2 NaOH, 10 min, 25°C, for antibody reprobing. Western blots were quantified using Adobe Photoshop software. In order to be able to compare all the samples per dietary condition for each antibody it was necessary to run two gels per antibody probe. To minimize any variability across gels, control and high fat diet samples were resolved and transferred for individual antibody comparisons at the same time in the same gel running/transfer apparatus. The blots were incubated in the same antibody solution and visualized and quantified at the same time. This minimized the variability of across-gel comparisons. Optical density of bands were normalized against their respective positive controls per blot and averaged (+/−SD).

Immunohistochemistry

After 22 weeks the animals were perfused with PBS containing CaCl<sub>2</sub> and brain, hippocampus, visceral (pericardial/heart) fat and subcutaneous (abdominal/ stomach) fat was collected and immersion fixed for 24 hrs in 4% paraformaldehyde, cryoprotected through two successive 30% sucrose changes. Brains were embedded in gelatin and serially sectioned (40 μm) via freezing microtome and immunostained using anti-APP, β-amyloid, CD68, iNOS, Cox-2, or GFAP antibodies or respective secondary only antibodies. Subcutaneous and visceral fat tissue samples were serially cryosectioned (10 μm) or paraffin embedded and sectioned (7 μm) for immunostaining with anti-APP, β-amyloid, CD45 or CD68 antibodies or respective secondary only antibodies. Antibody binding in brain or fat was visualized using Vector

|                | IgG   | 22C11 |
|----------------|-------|-------|
| BLC            | 0.89±0.12 | 1.25±0.48 |
| CD30L          | 0.82±0.08 | 0.86±0.09 |
| Eotaxin        | 0.88±0.09 | 0.90±0.1  |
| Eotaxin-2      | 1.11±0.13 | 1.17±0.13 |
| Fas Ligand     | 0.83±0.11 | 0.86±0.11 |
| Fractalkine    | 0.92±0.12 | 0.91±0.1  |
| GCSF           | 1.50±0.28 | 1.38±0.21 |
| GM-CSF         | 0.78±0.05 | 0.86±0.06* |
| IFNy           | 0.86±0.05 | 0.96±0.06* |
| IL-1α          | 0.88±0.14 | 0.95±0.10 |
| IL-1β          | 0.80±0.08 | 0.87±0.09 |
| IL-2           | 0.85±0.09 | 0.92±0.10 |
| IL-3           | 0.73±0.09 | 0.79±0.08 |
| IL-4           | 1.19±0.14 | 1.23±0.16 |
| IL-6           | 0.97±0.28 | 0.74±0.08 |
| IL-9           | 0.88±0.11 | 0.92±0.11 |
| IL-10          | 1.04±0.31 | 1.02±0.25 |
| IL-12p40p70    | 1.10±0.39 | 0.97±0.10 |
| IL-12p70       | 1.19±0.18 | 1.15±0.08 |
| IL-13          | 0.83±0.04 | 0.96±0.05** |
| IL-17          | 0.73±0.06 | 0.81±0.09 |
| I-TAC          | 0.76±0.08 | 0.84±0.09 |
| KC             | 1.10±0.27 | 1.03±0.18 |
| Leptin         | 0.87±0.09 | 0.93±0.11 |
| LIX            | 1.18±0.11 | 1.18±0.20 |
| Lymphotactin   | 1.01±0.12 | 1.06±0.13 |
| MCP-1α         | 0.88±0.17 | 0.91±0.12 |
| M-CSF          | 1.45±0.16 | 1.50±0.22 |
| MIF            | 0.85±0.08 | 0.89±0.11 |
| MIP-1α         | 0.96±0.09 | 0.98±0.10 |
| MIP-1γ         | 1.62±0.27 | 2.00±0.56 |
| RANTES         | 0.76±0.06 | 0.85±0.08 |
| SDF-1          | 0.97±0.11 | 1.09±0.09 |
| TCA-3          | 1.49±0.11 | 1.64±0.15 |
| TECK           | 0.76±0.06 | 0.82±0.06 |
| TIMP-1         | 1.53±0.10 | 1.49±0.11 |
| TIMP-2         | 0.95±0.08 | 1.01±0.09 |
| TNFa           | 0.72±0.06 | 0.77±0.06 |
| sTNFRII        | 1.53±0.15 | 1.66±0.15 |
| sTNFRII        | 1.22±0.16 | 1.11±0.23 |

**Figure 11. Secreted levels of GM-CSF, IFNγ, and IL-13 increased in media from peritoneal macrophage stimulated with APP agonist antibody, 22C11, compared to IgG, isotype control. Non-elicited peritoneal macrophage were stimulated with 1 μg/mL IgG<sub>1</sub> or 1 μg/mL 22C11 APP agonist antibody overnight and the media was removed and used according in a commercial antibody-based 40 cytokine antibody array. A representative dot blot per each condition is shown. The optical densities of individual cytokine detection spots were normalized against their respective positive controls per blot and averaged (+/−SD) and graphed from 5 animals in each group. Data were analyzed via unpaired two-tailed t-test. *p<0.05; **p<0.01. doi:10.1371/journal.pone.0030378.g011**

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VIP or DAB as chromogens (Vector Laboratories, Burlingame, CA, USA). For double labeling, immunostained tissue was stripped of antibodies with 0.2N HCl for 10 min prior to processing with the second primary antibody incubation and visualization steps. Images were taken using an upright Leica DM1000 microscope and Leica DF320 digital camera system. Figures were made using Adobe Photoshop 7.0 software.

**Macrophage Isolation and Stimulation**

At 22 weeks non-elicited peritoneal macrophage were rinsed from the peritoneal cavity with sterile PBS and allowed to adhere to tissue culture wells for 2–3 hours in DMEM/F12 with 10% FBS and 5% horse serum. Nonadherent and non-macrophage cells were rinsed from the wells using ice cold DMEM/F12. The remaining macrophage were scraped from the wells and replated for stimulations.

**Microglia Isolation and Stimulation**

Acutely isolated microglia were collected at 22 weeks as previously described [35]. Briefly, cortices were isolated, finely minced and filtered through 140 and 70 μm filters then gently digested with DNAse I and collagenase before being separated on a Percoll gradient. The microglial layer was collected, counted and used for stimulations.

**Enzyme-linked Immunosorbent Assay**

Isolated microglia and macrophage were plated (40,000 cell/150 μL) in DMEM/F12 overnight then media was collected for quantifying secreted TNF-α according to the manufacturer’s protocol (R&D Systems, Minneapolis, MN, USA).

**Prostaglandin Analysis**

PG were analyzed as previously described [101]. Brain samples were homogenized in 1 mL of saline and extracted with 2 mL of...
Acetone containing 0.005% butylated hydroxytoluene (BHT) following with 2 mL of chloroform with 0.005% BHT. PG₆₋ₓ₋₁ was used as internal standard. After extraction, the samples were dried under a stream of nitrogen and then redissolved in 30 mL of acetonitrile/water (1:2 by volume). Reverse-phase LC electrospray ionization mass spectrometry was used for PG analysis. The PG were separated on a Luna C-18(2) (3 μm column, 100 A pore diameter, 150 x 2.0 mm) (Phenomenex, Torrance, CA, USA) with security guard cartridge system (Phenomenex, Torrance, CA, USA). The LC system consisted of an Agilent 1100 series LC pump with a wellplate autosampler (Agilent Technologies, Santa Clara, CA). The solvent system was composed of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The flow rate was 0.2 mL/min. The separation program started with 10% of solvent B. At 2 min, the percentage of B was increased to 65% over 8 min, at 15 min the percentage of B was increased to 90% over 5 min, and at 35 min it was reduced to 10% over 2 min. Equilibration time between runs was 13 min. Mass spectrometry analysis was performed using a quadrupole mass spectrometer (API3000, Applied Biosystem, Foster City, CA, USA) equipped with a TurboIonSpray ionization source. Analyst software version 1.5.1 (Applied Biosystem, Foster City, CA, USA) was used for instrument control, data acquisition, and data analysis. The mass spectrometer was optimized in the multiple reaction-monitoring mode. The source was operated in negative ion electrospray mode at 450°C, electrospray voltage was −4250 V, nebulizer gas was 8 L/min and curtain gas was 11 L/min. Declustering potential, focusing potential, and entrance potential were optimized individually for each analyte. The quadrupole mass spectrometer was operated at unit resolution.

**Antibody-based Cytokine Array**

At 7 months of age non-elicited peritoneal macrophage were rinsed from the peritoneal cavity with sterile PBS, counted, plated at 1.5 x 10⁴ cells/well/6well dish and allowed to adhere to the tissue culture wells for 2–3 hours in DMEM/F12. Nonadherent and non-macrophage cells were rinsed from the wells using ice-cold DMEM/F12. Media containing isotype negative control 1 antibody 1 (Millipore, Billerica, Massachusetts USA) or 1 IgG1 (isotype negative control) or 1 APP agonist antibody 1 (Millipore, Billerica, Massachusetts USA) was added to the wells overnight for stimulation. The cells were treated with serum free DMEM/F12 and unstimulated (control) or stimulated for 24 hr with 1 μg/mL IgG1 (isotype negative control) or 1 μg/mL 22C11 (APP agonist antibody). After 24 hours, secreted LDH was measured according to the manufacturer’s protocol for both the media and the cell lysates using commercial reagents (Promega, Madison, WI). Secreted media LDH values were normalized against cellular LDH values. Media was also collected for ELISA analysis quantifying secreted TNF-a according to the manufacturer’s protocol (R&D Systems, Minneapolis, MN). Cells were also fixed, stained with Oil Red O (Alfa Aesar, Ward Hill, MN, USA) and DAPI (4',6-diamidino-2-phenylindole) (Sigma Aldrich, St. Louis, MO, USA) counterstained and changes in Oil Red O absorbance (358/461 nm) were quantified via plate reader. Experiments were repeated three independent times.

**Statistical Analysis**

The data were analyzed by unpaired two-tailed t-test with or without Welch correction for unequal variance as required. When three groups were analyzed one-way ANOVA with Holm-Sidak post hoc test or a Kruskal-Wallis nonparametric ANOVA with Dunn’s post hoc test were utilized for analysis.

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**Author Contributions**

Conceived and designed the experiments: KP MG CC. Performed the experiments: KP AF RA MG. Analyzed the data: KP AF RA MG CC. Contributed reagents/materials/analysis tools: MG CC. Wrote the paper: KP MG CC.

**References**

1. Kivipelto M, Ngandu T, Fratiglioni L, Viitanen M, Kareholt I, et al. (2005) Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. Archives of Neurology 62: 1556–1560.

2. Beydoun MA, Beydoun HA, Wang Y (2008) Obesity and central obesity as risk factors for incident dementia and its subtypes: a systematic review and meta-analysis. Obesity Reviews 9: 204–218.

3. Fitzpatrick AL, Keller LH, Lopez OL, Diehr P, O’Meara ES, et al. (2009) Midlife and late-life obesity and the risk of dementia: cardiovascular health study. Archives of Neurology 66: 336–342.

4. Whitmer RA (2007) The epidemiology of adiposity and dementia. Current Alzheimer research 4: 117–122.

5. Whitmer RA, Guderson EP, Quesenberry CP Jr., Zhou J, Yaffe K (2007) Body mass index in midlife and risk of Alzheimer disease and vascular dementia. Current Alzheimer research 4: 103–109.

6. Profenno LA, Faraone SV (2008) Diabetes and overweight associate with non-APOE4 genotype in an Alzheimer’s disease population. Am J Med Genet B Neuropsychiatr Genet 147B: 822–829.

7. Profenno LA, Faraone SV (2010) Meta-analysis of Alzheimer’s disease risk with obesity, diabetes, and related disorders. Biol Psychiatry 67: 505–512.

8. Lahtinen MH, Ngandu T, Rovio S, Helkala EL, Uutela A, et al. (2006) Fat intake at midlife and risk of dementia and Alzheimer’s disease: a population-based study. Dement Geriatr Cogn Disord 22: 99–107.

9. Eskelinen MH, Ngandu T, Helkala EL, Tuomilehto J, Nissinen A, et al. (2008) Fat intake at midlife and cognitive impairment later in life: a population-based CAIDE study. Int J Geriatr Psychiatry 23: 741–747.

10. Razeny G, Vreugdenhil A, Wilcock G (2006) Obesity, abdominal obesity and Alzheimer disease. Dementia and geriatric cognitive disorders 22: 173–176.
38. Matsubara Y, Kano K, Kondo D, Mugishima H, Matsumoto T (2009)

37. Ibrahim MM (2010) Subcutaneous and visceral adipose tissue: structural and functional differences. Obesity reviews: an official journal of the International Association for the Study of Obesity 11: 11–18.

36. Borst SE, Conover CF (2005) High-fat diet increases insulin resistance in the beta A4-amyloid precursor protein promoter. Science (New York, NY) 311: 511–514.

35. Sondag CM, Combs GK (2006) Amyloid precursor protein cross-linking stimulates beta amyloid production and pro-inflammatory cytokine release in monocyte lineage cells. The Journal of Immunology 176: 983–992.

34. Fain JN (2006) Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat mass. Proceedings of the National Academy of Sciences of the United States of America 103: 12363–12368.

33. Lang E, Szendrei GI, Lee VM Jr, Otvos L Jr. (1992) Immunological and pathological characterization of a phosphorylated immunodominant epitope of Alzheimer's disease amyloid A4 protein resembles a cell surface receptor. Life Sciences 77: 2156–2165.

32. Schroder K, Sweet MJ, Hume DA (2006) Signal integration between transcription factors regulates constitutive secretion. Journal of Neuroscience 11: 3783–3793.

31. Kohjima M, Sun Y, Chan L (2010) Increased food intake leads to obesity and Alzheimer-type neurodegeneration in experimental obesity and type 2 diabetes. The American journal of pathology 176: 1749–1766.

30. Barnati RB, Gehrmann J, Czech C, Monning U, Jones LL, et al. (1993) Early-onset familial Alzheimer disease. Science (New York, NY) 269: 973–977.

29. Sondag CM, Combs GK (2006) Adhesion of monocytes to type I collagen is associated with increased concentrations of inflammatory markers in morbidly obese individuals. International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity 30: 785–790.

28. Hotamisligil GS, Shargill NS, Spiegelman BM (1993) Adipose expression of tumor necrosis factor-alpha: role in obesity-linked insulin resistance. Science (New York, NY) 259: 87–91.

27. Sommer G, Kralisch S, Lipfert J, Weise S, Krause K, et al. (2009) Amyloid precursor protein in brain and fat.

26. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, et al. (2003) Obesity markedly increases MCP-1 expression in adipose tissue. The Journal of clinical investigation 112: 1796–1808.

25. Lee YH, Martin JM, Maple RL, Nair S, Permana PA, et al. (2008) Amyloid precursor protein mediates proinflammatory activation of monocyte lineage cells. The Journal of Immunology 180: 564–571.

24. Poitou C, Coussieu C, Rouault C, Coupaye M, Cancello R, et al. (2006) Serum leptin concentrations correlate with increased concentrations of inflammatory markers in morbidly obese individuals. International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity 30: 96–111.

23. Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, et al. (1995) Segregation of a missense mutation in the amyloid precursor protein gene with Alzheimer disease. Science (New York, NY) 269: 973–977.

22. Kern PA, Sagizbailez M, Ong JM, Banati RB, Gehrmann J, et al. (1993) Tumor necrosis factor-alpha: a marker of adiposity-induced low-grade inflammation but not of metabolic status. Obesity (Silver Spring) 1: 211–216.

21. Kern PA, Sagizbailez M, Ong JM, Banati RB, Gehrmann J, et al. (1993) Tumor necrosis factor-alpha: a marker of adiposity-induced low-grade inflammation but not of metabolic status. Obesity (Silver Spring) 1: 211–216.

20. Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, et al. (1987) The precursor of Alzheimer's disease amyloid A4 protein is associated with cell surface receptor. Nature 325: 733–736.

19. LeBlanc AC, Chen HY, Autilio-Gambetti L, Gambetti P (1991) Differential expression of TNF-alpha. Life Sciences 77: 2156–2165.

18. Jeong SK, Nam HS, Park JH, Park SY, Park TH, et al. (2005) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 349: 704–706.

17. Jeong SK, Nam HS, Son MH, Son EJ, Cho KH (2005) Interactive effect of interleukin-1beta on adipocyte amyloid precursor protein gene expression in human adipose tissue. The Journal of biological chemistry 280: 14356–14363.

16. Leppänen L, Wasco W, Poirier P, Romano DM, Oshima J, et al. (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science (New York, NY) 269: 973–977.

15. Gunstad J, Paul RH, Brickman AM, Cohen RA, Arns M, et al. (2006) Patterns of change in cognitive function among older adults with obesity and type 2 diabetes. The Journal of clinical investigation 112: 1796–1808.

14. Fain JN (2006) Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat mass. Proceedings of the National Academy of Sciences of the United States of America 103: 12363–12368.

13. Floden AM, Combs GK (2006) Beta-amyloid stimulates murine postnatal and adult microglia cultures in a unique manner. Journal of neurochemistry 101: 4644–4648.

12. Raffaitin C, Gin H, Empana JP, Helmer C, Berr C, et al. (2009) Metabolic syndrome and risk for incident Alzheimer's disease or vascular dementia: the Thrombosis in sealed arteries of the 55-year-old French population (TinaF55) study. Stroke; a journal of cerebrovascular diseases 42: 169–174.

11. Razay G, Vreugdenhil A, Wilcock G (2007) The metabolic syndrome and risk for incident Alzheimer's disease or vascular dementia: the Three-City Study. Neurology 66: 59–66.

10. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM (1995) Inhibition of tumor necrosis factor-alpha in human adipose tissue and insulin resistance. The Journal of clinical investigation 95: 2409–2415.

9. Kern PA, Sagizbailez M, Ong JM, Bosk JC, Deem R, et al. (1993) Expression of the tumor necrosis factor alpha promoter in adipose tissue: Regulation by obesity, weight loss, and relationship to lipoprotein lipase. The Journal of clinical investigation 95: 2111–2119.

8. Leppänen L, Wasco W, Poirier P, Romano DM, Oshima J, et al. (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science (New York, NY) 269: 973–977.

7. Sommer G, Kralisch S, Lipfert J, Weise S, Krause K, et al. (2009) Amyloid precursor protein is induced by tumor necrosis factor alpha in 3T3-L1 adipocytes. J Cell Biochem 108: 1418–1422.

6. Levy-Lahad E, Wasco W, Poirier P, Romano DM, Oshima J, et al. (1995) Increased body mass index is associated with increased concentrations of inflammatory markers in morbidly obese individuals. Compr Psychiatry 48: 57–61.

5. Sondag CM, Combs GK (2004) Amyloid precursor protein mediates proinflammatory activation of monocyte lineage cells. The Journal of Immunology 173: 3890–3897.

4. Kern PA, Sagizbailez M, Ong JM, Banati RB, Gehrmann J, et al. (1993) Tumor necrosis factor-alpha: a marker of adiposity-induced low-grade inflammation but not of metabolic status. Obesity (Silver Spring) 1: 211–216.

3. Hotamisligil GS, Shargill NS, Spiegelman BM (1993) Adipose expression of tumor necrosis factor-alpha: role in obesity-linked insulin resistance. Science (New York, NY) 259: 87–91.

2. Hotamisligil GS, Shargill NS, Spiegelman BM (1993) Adipose expression of tumor necrosis factor-alpha: role in obesity-linked insulin resistance. Science (New York, NY) 259: 87–91.

1. Hotamisligil GS, Shargill NS, Spiegelman BM (1993) Adipose expression of tumor necrosis factor-alpha: role in obesity-linked insulin resistance. Science (New York, NY) 259: 87–91.
Amyloid Precursor Protein in Brain and Fat

66. Banati RB, Gehrmann J, Kreutzberg GW (1994) GIal beta-amyloid precursor protein expression in the dentate gyrus after entorhinal cortical lesion. Neuroreport 5: 1359–1361.

67. Banati RB, Gehrmann J, Monning U, Czech C, Beyreuther K, et al. (1994) Amyloid precursor protein (APP), as a microglial acute phase protein. Neurupathol Appl Neurobiol 20: 194–195.

68. Banati RB, Gehrmann J, Lannes-Vieira J, Wekerle H, Kreutzberg GW (1995) Inflammatory reaction in experimental autoimmune encephalomyelitis (EAE) is accompanied by a microglial expression of the beta A4-amyloid precursor protein (APP). Glia 14: 209–215.

69. Banati RB, Gehrmann J, Wiesner C, Hossmann KA, Kreutzberg GW (1995) Gland expression of the beta-amyloid precursor protein (APP) in global ischemia. J Cereb Blood Flow Metab 15: 647–654.

70. Gehrmann J, Banati RB, Cunner ML, Kreutzberg GW, Newcombe J (1995) Amyloid precursor protein (APP) expression in multiple sclerosis lesions. Glia 15: 141–151.

71. Gehrmann J, Banati RB, Wiesner C, Hossmann KA, Kreutzberg GW (1995) Reactive microglia in cerebral ischaemia: an early mediator of tissue damage? Neurupathol Appl Neurobiol 21: 277–289.

72. Monning U, Sandbrink R, Weidemann A, Banati RB, Masters CL, Beyreuther K (1994) Transforming growth factor beta mediates increase of mature transmembrane amyloid precursor protein in microglial cells. FEBS Lett 334: 267–272.

73. Monning U, Sandbrink R, Weidemann A, Banati RB, Masters CL, et al. (1995) Extracellular matrix influences the biogenesis of amyloid precursor protein in microglial cells. J Biol Chem 270: 7104–7110.

74. Blame AJ, Vitek MP (1989) Focusing on IL-1-promotion of beta-amyloid precursor protein synthesis as an early event in Alzheimer’s disease. Neurobiol Aging 10: 406–410; discussion 412–414.

75. Forlou G, Demichel F, Giorgi S, Bendotti C, Angeretti N (1992) Expression of amyloid precursor protein mRNAs in endothelial, neuronal and glial cells: modulation by interleukin-1. Brain Res Mol Brain Res 16: 128–134.

76. Hung AK, Koo EH, Haass C, Selkoe DJ (1992) Increased expression of beta-amyloid precursor protein during neuronal differentiation is not accompanied by secretory cleavage. Proc Natl Acad Sci U S A 89: 9439–9443.

77. Ohya Y, Tadiba T (1995) Effect of growth factors and cytokines on expression of amyloid beta protein precursor mRNAs in cultured neural cells. Brain Res Mol Brain Res 18: 127–132.

78. Smith CJ, Johnson EM Jr., Osborne P, Freeman RS, Neveu I, et al. (1993) NGF deprivation and neuronal degeneration trigger altered beta-amyloid precursor protein gene expression in the rat superior cervical ganglia in vivo and in vitro. Brain Res Mol Brain Res 17: 328–334.

79. Sola C, Garcia-Ladona FJ, Menged G, Probst A, Frey P, et al. (1995) Increased levels of the Kunitz protease inhibitor-containing beta APP mRNAs in rat brain following neurotoxic damage. Brain Res Mol Brain Res 17: 41–52.

80. Willoughby DA, Johnson SA, Pasinetti GM, Tocco G, Najm I, et al. (1992) Amyloid precursor protein mRNA encoding the Kunitz protease inhibitor domain is increased by kainic acid-induced seizures in rat hippocampus. Exp Neurol 110: 332–339.

81. Medi L, Cassadella MA, Szendrei GI, Otvos L Jr., Baron P, et al. (1995) Activation of microglial cells by beta-amyloid protein and interferon-gamma. Nature 374: 647–650.

82. Bitting L, Naidu A, Cordei B, Murphy GM Jr. (1996) Beta-amyloid peptide secretion by a microglial cell line is induced by beta-amyloid-(25-35) and lipopolysaccharide. J Biol Chem 271: 16084–16089.

83. Lorton D, Kocsis JM, King L, Malden K, Bruned KR (1996) Beta-Amyloid induces increased release of interleukin-1 beta from lipopolysaccharide-activated human monocytes. J Neuroimmunol 67: 21–29.

84. Lorton D, Schaller J, Lala A, De Nardin E (2000) Chemotactic-like receptors and Alpha peptide induced responses in Alzheimer’s disease. Neurobiol Aging 21: 463–473.

85. Lue LF, Walker DG, Brachova L, Beach TG, Rogers J, et al. (2001) Involvement of microglial receptor for advanced glycation endproducts (RAGE) in Alzheimer’s disease: identification of a cellular activation mechanism. Exp Neurol 171: 29–45.

86. Combs CK, Johnson DE, Cannady SB, Lehman TM, Landreth GE (1999) Identification of microglial signal transduction pathways mediating a neurotoxic response to amyloidogenic fragments of beta-amyloid and prion proteins. J Neurosci 19: 928–939.

87. Sundag CM, Dhawan G, Combs CK (2009) Beta amyloid oligomers and fibrils stimulate differential activation of primary microglia. J Neuroinflammation 6: 1.

88. Wu SZ, Bodles AM, Porter MM, Grillin WS, Basile AS, et al. (2004) Induction of serine racemase expression and D-serine release from microglia by amyloid beta-peptide. J Neuroinflammation 1: 2.

89. Hayden KM, Zandi PP, Khachaturian AS, Szekely CA, Fotuhi M, et al. (2007) Does NSAID use modulate cognitive trajectories in the elderly? The Cache County study. Neurology 69: 275–282.

90. Wang J, Ho L, Qin W, Rocher AB, Seror I, et al. (2005) Caloric restriction attenuates beta-amyloid neuropathology in a mouse model of Alzheimer’s disease. Faseb J 19: 659–661.

91. Kim DH, Sandoval D, Reed JA, Matter EK, Tolod EG, et al. (2008) The role of GM-CSF in adipose tissue inflammation. Am J Physiol Endocrinol Metab 295: E1038–E1046.

92. McGillivray FC, Chiquoine EH, Hinkle CC, Kim RJ, Shah R, et al. (2009) Interferon gamma attenuates insulin signaling, lipid storage, and differentiation in human adipocytes via activation of the JAK/STAT pathway. J Biol Chem 284: 31936–31944.

93. O’Rourke RW, Metcalf MD, White AE, Madala A, Winters BR, et al. (2009) Depot-specific differences in inflammatory mediators and a role for NK cells and IFN-gamma in inflammation in human adipose tissue. Int J Obes (Lond) 33: 978–980.

94. Rocha VZ, Folco EJ, Sukhova G, Shimizu K, Gottron I, et al. (2008) Interferon-gamma, a Th1 cytokine, regulates fat inflammation: a role for adaptive immunity in obesity. Cire Res 103: 467–476.

95. Shaul ME, Bennett G, Strisfeld KJ, Greenberg AS, Obin MS (2010) Dynamic, M2-like remodeling phenotypes of CD11c+ adipose tissue macrophages during high-fat diet-induced obesity in mice. Diabetes 59: 1171–1181.

96. Meijer K, de Vries M, Al-Lahham S, Bruneberg M, Weening D, et al. (2011) Human primary adipocytes exhibit immune cell function: adipocytes prime inflammation independent of macrophages. PLoS One 6: e17134.

97. Nakarai H, Yamashita A, Nagayasu S, Inagaki H, Kumamoto S, et al. (2011) Adipocyte-macrophage interaction may mediate LPS-induced low-grade inflammation: potential link with metabolic complications. Innate Immun. 15: 141–151.

98. Gomez-Casanovas E, Sanmarti R, Sole M, Caneve JD, Munoz-Gomez J (2001) The clinical significance of amyloid fat deposits in rheumatoid arthritis: a systematic long-term followup study using abdominal fat aspiration. Arthritis Rheum 44: 66–72.

99. Barile L, Ariza R, Muci H, Pizarro S, Fraza A, et al. (1993) Tru-cut needle biopsy of subcutaneous fat in the diagnosis of secondary amyloidosis in rheumatoid arthritis. Arch Med Res 24: 189–192.

100. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248–254.

101. Golovko MY, Murphy EF (2008) An improved LC-MS/MS procedure for brain prostanooid analysis using brain fixation with head-focused microwave irradiation and liquid/liquid extraction. J Lipid Res 49: 893–902.

102. Fernyhough ME, Vierck JL, Hausman GJ, Mir PS, Okine EK, et al. (2004) Inflammatory reaction in experimental autoimmune encephalomyelitis (EAE) is accompanied by a microglial expression of the beta A4-amyloid precursor protein (APP). Glia 14: 209–215.

103. Monning U, Sandbrink R, Weidemann A, Banati RB, Masters CL, Beyreuther K (1994) Transforming growth factor beta mediates increase of mature transmembrane amyloid precursor protein in microglial cells. FEBS Lett 334: 267–272.

104. Monning U, Sandbrink R, Weidemann A, Banati RB, Masters CL, et al. (1995) Extracellular matrix influences the biogenesis of amyloid precursor protein in microglial cells. J Biol Chem 270: 7104–7110.