Clusterin as a Potential Biomarker of Obesity-Related Alzheimer's Disease Risk

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ABSTRACT: Over 35% of the adult US population is obese. In turn, excess adiposity increases the risk of multiple complications including type 2 diabetes (T2D), insulin resistance, and cardiovascular disease; yet, obesity also independently heightens risk of Alzheimer's Disease (AD), even after adjusting for other important confounding risk factors including blood pressure, sociodemographics, cholesterol levels, smoking status, and Apolipoprotein E (ApoE) genotype. Among patients over the age of 65 with dementia, 37% have coexisting diabetes, and an estimated 7.3% of cases of AD are directly attributable to midlife obesity. Clusterin, also known as apolipoprotein J (ApoJ), is a multifunctional glycoprotein that acts as a molecular chaperone, assisting folding of secreted proteins. Clusterin has been implicated in several physiological and pathological states, including AD, metabolic disease, and cardiovascular disease. Despite long-standing interest in elucidating clusterin's relationship with amyloid beta (Aβ) aggregation/clearance and toxicity, significant knowledge gaps still exist. Altered clusterin expression and protein levels have been linked with cognitive and memory function, disrupted central nervous system lipid flux, as well as pathogenic brain structure; and its role in cardiometabolic disease suggests that it may be a link between insulin resistance, dyslipidemia, and AD. Here, we briefly highlight clusterin's relevance to AD by presenting existing evidence linking clusterin to AD and cardiometabolic disease, and discussing its potential utility as a biomarker for AD in the presence of obesity-related metabolic disease.

KEYWORDS: Alzheimer's Disease, amyloid beta, adipose tissue, clusterin

Introduction

Alzheimer's disease (AD) is the most prevalent form of dementia, and is expected to rise precipitously with an increasingly aging population.[1,2] Unfortunately, treatments that prevent or slow the progression of AD are lacking, likely secondary to an incomplete understanding of AD pathogenesis. The etiology of AD is multifactorial, with contributions including genetic risk polymorphisms,[3] lifestyle and dietary factors,[4] and advancing age, among others.[5] Although the hallmark neuropathological changes in AD include intracellular accumulation of hyperphosphorylated tau (p-tau), all forms of AD also exhibit overproduction and/or reduced clearance of amyloid-beta (Aβ).[6] Therefore, one of the prominent signs of AD is the excess formation of diffuse senile plaques, composed of both aggregating and non-aggregating Aβ derived from endoproteolytic cleavage of the Aβ precursor protein (APP) by beta- and gamma-secretase.

Interestingly, acquired risk factors for AD include numerous factors associated with the metabolic syndrome (hypertension, dyslipidemia, cerebro- and cardiovascular disease, and insulin resistance and Type 2 diabetes [T2D]).[7,8] Patients with ⩾ 2 vascular risk factors in midlife have a threefold higher risk of brain Aβ deposition later in life[9]; and T2D, which is closely linked with obesity,[10] has long been associated with a higher risk of cognitive decline and AD.[11–21] Obesity-related comorbidities have important effects on Aβ production and deposition, underscoring the importance of better elucidating their role in AD pathogenesis.

Yet, despite the large impact that AD has on individuals, families, society, and the health care system,[1] the underlying mechanisms connecting obesity and AD remain largely unknown.

Potential mechanism(s) linking excess adiposity, insulin resistance, and AD risk

The role of insulin and insulin signaling in AD

One potential reason for the connection between dysregulated metabolism and AD is that insulin has direct effects on neurotransmission and neuropathology in the brain,[22–25] including alterations in the production, degradation and clearance of Aβ that subsequently lead to plaque deposition.[26] Raising peripheral insulin levels acutely elevates brain and cerebrospinal fluid (CSF) insulin levels,[27] as illustrated by the finding that peripheral intravenous infusion of different concentrations of insulin in 8 normal, lean subjects over 4.5 hours not only increased mean plasma concentrations of insulin (12 ± 1.2 to 268 ± 35 μU/ml), but CSF insulin levels as well (0.9 ± 0.1 to 2.8 ± 0.4 μU/ml) ($P < .006$). In contrast, prolonged peripheral hyperinsulinemia (as seen in obesity and T2D) down-regulates blood-brain barrier (BBB) insulin receptors and reduces insulin transport into the brain.[28] In obese Zucker rats, Stanley et al observed a 65% reduction in brain capillary insulin binding sites compared to controls, with the degree of insulin binding negatively correlating with circulating plasma insulin levels ($P < .05$).[29] However, data supporting this

contention in humans is limited and oftentimes conflicting.[29] In the sole study reporting brain insulin protein levels in AD patients, there was an equivalent decrease in both AD patients and age-matched controls, suggesting that the lower insulin protein levels may be related to aging, and not AD pathology per se.[30] Yet, 2 other studies reported significant reductions in insulin mRNA *gene expression* in age-matched AD patients.[31,32]

Patients with mild cognitive impairment (MCI) and AD also have documented dysregulation in central nervous system (CNS) insulin signaling. This is evident in postmortem brain samples, even in the absence of diabetes, including decreased phosphorylation of protein kinase B (Akt) and reduced activation of phosphoinositide 3-kinase (PI3K),[32–34] key factors in the canonical insulin signaling pathway.[35] Inhibiting PI3K activity increases Aβ production, and restoring proper signaling, even peripherally, leads to reduced amyloid deposition.[36] As a result of these conflicting findings, a better understanding of insulin's effects in the human brain is needed, particularly accounting for the severity of AD and relative to age-matched controls.

There have been numerous studies suggesting that peripheral hyperinsulinemia also alters the risk of AD via its effects on degradation and/or clearance of Aβ. In the CNS, insulin and Aβ are degraded by insulin degrading enzymes (IDEs); and with elevated insulin levels, IDEs preferentially degrade insulin in favor of Aβ, which could lead to Aβ deposition.[37] Aβ clearance is also significantly reduced in the setting of elevated insulin levels.[38,39] In fact, small interventional trials with intravenous insulin infusion,[26,40,41] inhaled insulin,[42,43] the insulin-sensitizing agent pioglitazone,[44,45] metformin,[46,47] and incretin-based therapies[48] have shown beneficial effects on memory.

Brain activity may be affected by obesity and peripheral insulin resistance. In various murine models of obesity and diabetes (including after high-fat diet feeding),[49–52] there exists a strong relationship between peripheral and "brain" insulin resistance; and in humans, altered metabolic brain activity occurs in peripherally insulin-resistant subjects.[53–55] In a study of lean vs. obese humans (scanned by functional magnetic resonance imaging (fMRI) while simultaneously completing memory testing),[56] regions of the brain known to be important for recollecting episodic memories (ie, the hippocampus, angular gyrus, and dorsolateral prefrontal cortex) had significantly impaired functional activity in the obese subjects.

The role of CNS lipid flux in AD

Distinct from insulin resistance and insulin signaling defects, lipid flux and cholesterol metabolism in the CNS are also involved in the pathogenesis of AD and Aβ pathology. Apolipoprotein E (ApoE) is the principal carrier of cholesterol in the CNS, the ApoE epsilon 4 (ε4) genetic polymorphism is the most prominent genetic determinant of AD risk,[57] and ApoE from human stem-cell derived neurons is directly related

to increased Aβ production and secretion.[58,59] In both mice and human stem-cell derived astrocytes and microglia, ApoE isoforms alter Aβ clearance from the brain.[59–61] Although early, more limited studies suggested that polymorphisms in apolipoprotein A1 (ApoA1), a major component of protective high density lipoprotein (HDL) cholesterol in the CNS, were related to early onset and late onset AD,[62–64] this finding has not been replicated in larger genome wide association (GWAS) studies. Nevertheless, low plasma and CSF levels of ApoA1 have been observed in AD patients[65] and are connected to earlier AD onset in non-demented elderly patients.[65,66] To further illustrate clinically that Aβ may be affected by the amount and type of cholesterol in the CNS, its production in hippocampal neurons can be inhibited by the lipid lowering medication lovastatin.[67]

Clinical implications of obesity on AD risk

Despite the above mechanistic findings, the relationship between obesity, insulin resistance, T2D and pathological markers of AD, such as Aβ and p-tau, remains controversial. Studies assessing the relationship between T2D and accumulation of Aβ, either by PET imaging or by histology, have been mixed. While one study showed a close association between insulin resistance and Aβ by imaging in middle-age subjects,[68] others have found no relationship between glucose tolerance and Aβ in diabetic vs. nondiabetic elderly patients with normal cognition, MCI, or AD,[42,43,69] and no association with systemic insulin resistance and CSF levels of Aβ[69] or histological Aβ burden.[70,71] However, these cross-sectional studies included only elderly patients as controls, and after the onset of clinical dementia symptoms. As Aβ deposition begins up to 20 years before clinical symptomatology,[72] the negative findings may not accurately reflect the typical time course of AD pathophysiologic progression. In fact, brain insulin levels and insulin receptor density are reduced in older patients with AD compared to middle-aged controls even without clinical symptomatology,[30] suggesting the need for further human studies that compare insulin resistance-associated Aβ pathology in younger high risk, potentially "preclinical" patients to older patients who have already developed clinically-evident disease.

Clusterin as a potential biomarker for Alzheimer's disease

Clusterin *(encoded by the gene CLU)* was originally isolated from ram testis fluid in 1983,[73] and has since been identified as a molecular chaperone expressed by a wide-ranging number of tissues.[74,75] Its traditionally identified function has been to assist folding of secreted proteins; as such, clusterin overexpression protects cells from apoptosis induced by chemotherapy, radiotherapy, and androgen/estrogen depletion.[65,66] Its relationship with Aβ has been well studied (summarized in Foster et al[76]), but the precise contribution(s) of clusterin to AD pathology remains confounded by complexities involving its

biogenesis, the role of extra- vs. intracellular clusterin, and its vast number of attributable functions. In addition to its direct role in Aβ pathology, clusterin appears to play a role in neurodegeneration. The ApoE-ε4 allele exacerbates synapse degeneration and leads to accumulation of toxic oligomeric Aβ. Interestingly, synapses containing higher amounts of clusterin are seen in APOE-ε4 carriers compared to ApoE-ε3 carriers, with correspondingly higher oligomeric Aβ burden.[77] This finding potentially explains the synergistic effect of 2 prominent genetic risk factors on synapse degeneration in AD.

The role of clusterin genetic variants on AD

Clusterin is highly expressed in the brain by both astrocytes and neurons,[78] and has been linked with an increased risk of AD.[79] In large-scale GWAS studies, polymorphisms in the *CLU* gene strongly associate with late-onset AD (LOAD),[3,80] although this finding has since been questioned when analyzed specifically in different ethnic/racial groups.[81–83] In turn, polymorphisms in *CLU* may have critical implications on brain structure. An MRI-based study of nearly 400 young healthy carriers of the *CLU* (rs11136000) allele showed a distinct deterioration in white matter integrity, suggesting increased vulnerability to developing AD later in life.[84]

Few studies have evaluated the relationship between *CLU* genetic changes and coexisting metabolic disease. In 550 women with a history of gestational diabetes mellitus (GDM), T2D, or impaired glucose tolerance (IGT) compared to controls, no significant association with *CLU* rs11136000 was observed in any of the groups.[85] Another study in 418 individuals (236 with MCI and 192 control subjects), however, did report a relationship between *CLU* and metabolic disease, with T2D prevalence higher in individuals carrying the *CLU* variant, and rs11136000 specifically associated with elevated MCI risk (OR 1.79, *P* = .019).[86] In patients with clinical AD, the relationship between dysregulated metabolism and CLU genetic variations is largely unknown and requires further investigation.

Clusterin levels in the pathogenesis of AD

In patients with both MCI and AD, a majority of studies have documented elevated brain, CSF and circulating clusterin levels.[87–90] In the study by Nilselid et. al., involving CSF analyses from Alzheimer patients (n = 99) and controls (n = 39), clusterin was significantly higher in AD patients, quantified both before and after deglycosylation using sandwich enzyme-linked immunosorbent assay (ELISA) (Before deglycosylation: 7.17 ± 2.43 versus 5.73 ± 2.09 AU; p=0.002; After deglycosylation: 12.19 ± 5.00 vs 9.68 ± 4.38 AU; *P* = .004).[87] In a separate cohort of 44 subjects representing a continuum of disease (27 with mild to moderate AD and 17 with MCI) plasma clusterin by ELISA (coefficient of variation 3.5%) was associated with entorhinal cortex atrophy, baseline disease severity,

and quicker clinical progression.[88] In addition, plasma clusterin levels predicted higher Aβ burden in the medial temporal lobe. These findings were confirmed in a meta-analysis of 28 studies that demonstrated higher clusterin concentration both in plasma (SDM = 0.73, *P* = .002) and brain tissue (SDM = 0.71, *P* = .022) compared to normal controls.[89] Yet interestingly, CSF clusterin was not different by patient group. In a separate study of 231 T2D patients, including 126 with MCI and 105 cognitively healthy controls, plasma clusterin was significantly higher in MCI patients vs. controls (*P* = .007), and negatively correlated with the Montreal cognitive assessment and auditory verbal learning test, and delayed recall scores (*P* = .027 and *P* = .020, respectively).[91] Multivariable regression modeling showed that educational attainment, duration of diabetes, high-density lipoprotein cholesterol (HDL-c), and plasma clusterin levels were all associated with MCI in T2D patients.

As a result of these and other reports, clusterin has been proposed as a potential biomarker of AD.[79] Yet from a physiologic perspective, the existing evidence supports both neurotoxic and neuroprotective effects, and may be dependent on the balance of clusterin and Aβ in the CNS,[92] and, importantly, its cell distribution.[76] Clusterin is capable of binding to Aβ, preventing aggregation,[93] and enabling LRP2 (megalin)-mediated Aβ removal.[94] In the physiologic state, it therefore exhibits neuroprotective properties.[95–97] In contrast, a decreased clusterin/Aβ ratio, observed in AD patients, appears to be neurotoxic[92] by raising soluble oligomeric Aβ peptides.[98]

Adipocyte clusterin as a potential novel player in human AD (Figure 1)

The adipose tissue (AT) microenvironment is increasingly recognized as a determinant of systemic insulin action and inflammation, and is to some extent determined by the adipocyte.[99] Recent studies indicate that adaptive and innate immune functions of the adipocyte regulate the overall immune cell composition of AT, which is enhanced during the transition from a lean to obese state.[100] As the major storage depot for excess calories, and comprised of a variety of metabolically-active immune cells, AT is uniquely poised to influence systemic inflammation as well as key systemic metabolic pathways such as insulin action. Several adipokines, comprised of a number of cell signaling proteins secreted by AT,[101] have been proposed as biomarkers of AD.[102,103]

Clusterin is not only expressed by astrocytes and neurons, but by a large number of peripheral tissues, including AT[104] and it readily crosses the BBB.[105] Indeed, the cognitive decline in MCI and AD has been related to circulating, and not CNS clusterin,[106] and the meta-analysis of 12 studies comparing patients with AD to controls showed that plasma clusterin was increased, but not CSF clusterin.[89] A recent study expanded on these findings by classifying 59 total participants by cognitive status (normal cognition, MCI, or AD) and by degree of metabolic impairment (healthy, prediabetes, or T2D) in order to determine associations with circulating clusterin levels.[107] They
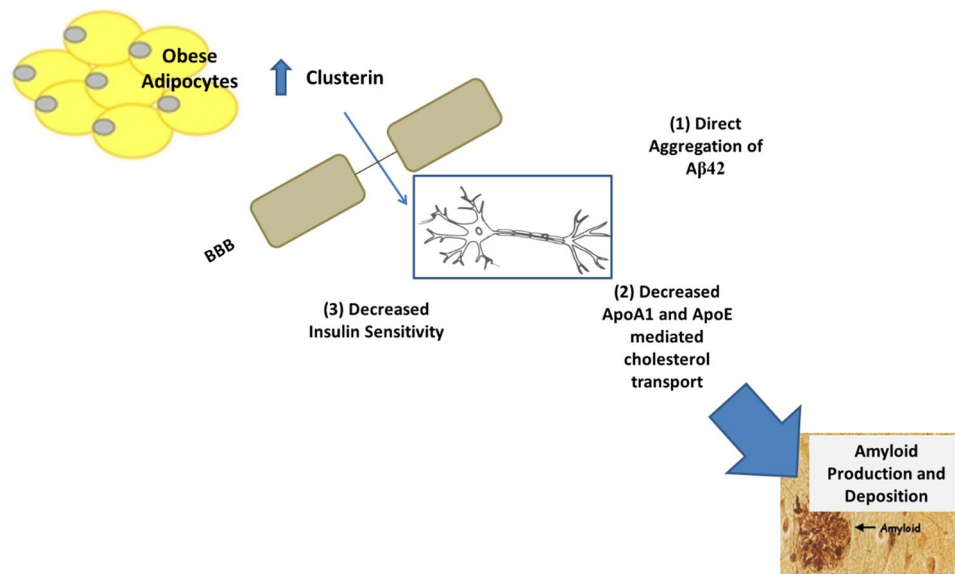
Figure 1. Summary of hypothesized mechanisms (labeled 1-3) responsible for clusterin-mediated amyloid beta (Aβ) deposition.
Abbreviations: ApoA1, apolipoprotein A1; ApoE, apolipoprotein E; BBB, blood brain barrier; CSF, cerebrospinal fluid; HDL, high density lipoprotein.

found that plasma clusterin levels were not only higher in participants with both AD and prediabetes/T2D compared to those without metabolic impairment, but that clusterin was negatively associated with cognitive scores, and positively with worsening metabolic parameters (hemoglobin A1c, insulin resistance by HOMA-IR, and fasting C-peptide levels) and brain pathology by MRI (medial temporal atrophy and white matter lesions). Although the study involved a cross-sectional, observational design, in mediation analysis plasma clusterin was determined to be a direct mediator of these important associations. Mechanistically, substantial weight loss induced by gastric bypass surgery reduces clusterin expression in peripheral blood mononuclear cells (PBMCs) in association with attenuated expression of amyloid precursor protein (APP) and presenilin-2.[108] These studies suggest that peripherally-derived clusterin may have a unique role in CNS AD pathology, particularly with coexistent metabolic impairment.

In fact, metabolic effects of clusterin have recently emerged. In obese patients, plasma clusterin levels are elevated and associate with BMI, waist circumference, markers of inflammation (hsCRP and retinol-binding protein-4), and insulin resistance.[109] In mice, skeletal muscle and hepatic gene expression of *CLU* is increased after high-fat diet feeding, and whole-body clusterin knockout (KO) mice are insulin sensitive compared to wild-type (WT) mice.[110] On array analysis, we have previously identified subcutaneous adipocyte *CLU* as one of the top 15 extracellular matrix-related genes overexpressed in human obesity.[104] In addition, we found in 54 obese patients compared to 18 lean patients that human adipocyte expression, protein levels and serum concentrations of clusterin were higher in obesity and directly associated with multiple sequelae of obesity-related cardiometabolic disease: systemic insulin resistance, dyslipidemia, elevated blood pressure, hepatic steatosis/steatohepatitis, key biomarkers of cardiovascular disease and

risk, and atherosclerotic lesions.[104] We also demonstrated that clusterin in vivo has a progressive abrogating effect on hepatic *ApoA1* expression (HepG2 cells cultured with increasing levels of recombinant clusterin). ApoA1 a major component of HDL cholesterol and a biomarker of reduced myocardial infarction risk,[111] through binding to low density lipoprotein-related protein 2 (LRP2/Megalin).[104] Similar to the liver, LRP2 is the main clusterin receptor found in the brain and high concentrations of CNS clusterin are internalized and degraded via LRP2.[94] Although far from conclusive, these results open the possibility that clusterin derived from obese adipocytes may play a role in the heightened risk of AD in the setting of obesity.

Conclusion

The development of AD is complex and multifactorial. However, one of the prominent risk factors includes obesity-related cardiometabolic disease, particularly when these comorbidities develop in midlife. Therefore, factors such as adipokines from adipose tissue, linking obesity and AD, may prove useful as biomarkers of AD risk and development. One of these potential biomarkers is clusterin, which is elevated in the blood and CNS of patients with both MCI and AD. However, studies have attributed both a neuroprotective and neurotoxic role to clusterin. This discrepancy may be due to the myriad of functions that have been ascribed to clusterin and its cellular distribution.

Despite its known association with AD, and findings that clusterin is increased in AD, T2D, and obesity, the role of clusterin as a biomarker in AD pathophysiology remains an enigma, especially its relationship to metabolic disease. In particular, a better understanding of insulin's effects in the human brain is needed, accounting for both the severity of AD and relative to age-matched controls. In addition, well-designed

studies that compare insulin resistance-associated Aβ pathology in younger patients at risk (potentially in "preclinical" stages) to older patients with clinically-evident disease will shed more light on the contribution of clusterin to AD pathogenesis. Although adipocyte-derived clusterin may play a role in Aβ pathology, future studies are needed to determine if is a viable biomarker for AD, and if it offers a key link between obesity, metabolic disease, and AD.

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