

Leptin: A Novel Therapeutic Strategy for Alzheimer's Disease

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Abstract. Adipocyte-derived leptin appears to regulate a number of features defining Alzheimer's disease (AD) at the molecular and physiological level. Leptin has been shown to reduce the amount of extracellular amyloid beta, both in cell culture and animal models, as well as to reduce tau phosphorylation in neuronal cells. Importantly, chronic administration of leptin resulted in a significant improvement in the cognitive performance of transgenic animal models.

In AD, weight loss often precedes the onset of dementia and the level of circulating leptin is inversely proportional to the severity of cognitive decline. It is speculated that a deficiency in leptin levels or function may contribute to systemic and CNS abnormalities leading to disease progression. Furthermore, a leptin deficiency may aggravate insulin-controlled pathways, known to be aberrant in AD. These observations suggest that a leptin replacement therapy may be beneficial for these patients.

Keywords: AICAR, AMP-activated kinase, amyloid- β , glycogen synthase kinase-3, leptin, tau

INTRODUCTION

Leptin is a protein that was originally discovered as an adipocyte-derived hormone controlling feeding behavior through receptors in the hypothalamus [1]. Since its discovery, it has been shown that leptin has other important physiological roles in the control of fat storage or mobilization, the reproductive system, the immune system, bone homeostasis, insulin sensitivity [2–4], and neuronal activity and protection [5]. Lep-

tin receptors have been identified in peripheral tissues, and of importance to this review, in neurons in the brain, including the hippocampus [5–7], which is particularly vulnerable in Alzheimer's disease (AD) [8].

While application of leptin agonists can reduce body weight and glucose levels in obese animals, such molecules have only met with limited success in clinical trials for obesity and diabetes as monotherapies [9]. However, greater success has recently been reported from rat and human studies when used in combination with pramlintide, an analogue of amylin [10]. The lack of efficacy with leptin monotherapy has been suggested to result from the development of leptin resistance, either due to the saturation of the transporter responsible for the movement of leptin from the periphery across

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the blood brain barrier or because of the desensitization of post-receptor signaling pathways in obese patients. Nonetheless, because of its pleiotropic nature, leptin or leptin agonists/antagonists hold promise as therapeutics in a variety of conditions. Leptin directly stimulates bone growth by inducing osteoblasts to make insulin-like growth factor-I, providing a basis for osteoporosis treatments [11]. The role of leptin in inflammation, may lead to therapies in inflammatory bowel disease, multiple sclerosis, sepsis, and arthritis [12]. In this review, the involvement of leptin in pathological pathways relevant to AD is examined. Specifically, *in vitro* and *in vivo* studies have shown leptin to positively impact amyloid- β ($A\beta$) homeostasis and tau phosphorylation and to improve, following chronic supplementation, the cognitive ability of animal models of AD. Interestingly, the observations from mechanistic studies suggest that an upstream component possibly linked to metabolic pathways and modulated by leptin precedes amyloid plaque and neurofibrillary tangle (NFT) development. Indeed, evidence is provided to suggest that neuronal AMP-activated kinase (AMPK) may fulfill that role.

An abnormal AMPK activity could potentially translate to abnormal lipid levels and membrane composition in neurons that would affect membrane fluidity and the physiology of lipid rafts, which accommodate many components of the machinery leading to $A\beta$ production. Leptin has been shown to maintain balanced AMPK activity. Also, AMPK is known to regulate glycogen synthase kinase-3 β (GSK-3 β), a central tau kinase [13], and leptin, like the AMPK activator AICAR, can reduce tau phosphorylation at several sites [14,95]. Taken together, the mechanistic pathways that are influenced by leptin and that occur prior to amyloid deposition or tau phosphorylation, as well as potential cognitive improvements, highlight the importance of further development of therapies that increase leptin availability in the central nervous system (CNS). These therapies may include leptin supplementation, leptin receptor activation, or controlling the development of leptin resistance, all with the goal of alleviating AD etiology and pathobiology.

LEPTIN IN OBESITY AND LEPTIN-DEFICIENCY SYNDROMES

Plasma leptin concentrations are highly correlated with amount of body fat, with women having higher concentrations at every level of relative or absolute adi-

posity [15,16]. This gender dimorphism is maintained even in postmenopausal women, despite a drop in leptin levels (such that levels in premenopausal females > postmenopausal females > males) [16]. The range of leptin serum levels in humans range from 0 ng/ml leptin (in congenital leptin deficiency cases) to more than 100 ng/ml, representing morbidly obese cases. Normally, plasma leptin levels are highest late at night and lowest in the morning.

Leptin has been extensively used in trials for treatment of obesity, but its relatively low therapeutic index as a monotherapy, despite an overall well-tolerated profile, limits its use. The rather disappointing results, although associated with a clinically significant moderate weight loss, are attributed partially to the fact that obese individuals have hyperleptinemia [15,16] and are generally resistant to further increments of leptin [17]. The dosages of leptin utilized in obesity trials were high, up to 0.4 mg/kg daily. In contrast, in replacement therapies, addressing a number of leptin-deficiency syndromes, clinical efficacy was achieved with doses as low as 0.02 mg/kg [18]. The most dramatic results using leptin as a therapy were obtained with rare genetic cases of obesity with complete leptin deficiency, where patients responded to leptin replacement therapy with reduced appetite and food intake as well as body fat loss [19].

Human testing of leptin as a monotherapy for partially leptin-deficient states has also met with success, among which are trials for lipodystrophy, increasingly common in HIV-infected individuals undergoing highly active anti-retroviral therapy. In addition, trials for hypothalamic amenorrhea, usually reported in women athletes, and for anorexia nervosa, have shown positive results. Leptin treatment of patients with these conditions improved metabolic parameters, reduced insulin resistance, and restored reproductive function respectively [20]. From these trials, only mild side effects from injection site complications were reported.

LEPTIN (OB), LEPTIN RECEPTOR (OB-R) AND SIGNALING

The wild-type leptin gene (OB) encodes a 16kDa polypeptide [1]. The primary amino acid sequence of leptin and crystallographic data [21] indicate that leptin adopts a three-dimensional helical structure similar to that of certain cytokines, such as interleukin-2. The human leptin receptor (OB-R) [22] is a member of the class I cytokine receptor (gp130) superfamily [23]. It

is present as five alternatively spliced isoforms, among which the expressed OB-Rb is the longest form and the only one responsible for ligand binding-induced signaling. Within the brain, the expression of OB-Rb is highest in the arcuate nucleus and median eminence of the hypothalamus, but is also abundantly expressed in the hippocampus, primarily in the dentate gyrus and CA1 [23,24], areas heavily affected in AD.

Activation of the OB-R triggers the JAK/STAT pathway to induce gene transcriptional changes via activation of Janus tyrosine kinase 2 (JAK2), the signal transducer and activator 3 (STAT3), and the suppressor of cytokine signaling 3 (SOCS3). In addition, leptin can evoke an increased response via activation of the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) [25,26] pathways, which in turn reduce GSK-3 activity and consequently may decrease tau phosphorylation [27].

AMPK may also mediate leptin signaling post OB-Rb binding [28]. Upon phosphorylation, AMPK can be activated when cellular AMP/ATP ratio is high leading to an increase in ATP through a number of pathways that increase glucose uptake, facilitate lipolysis, and inhibit lipogenesis [29]. As such, AMPK may act as an energy management switch, manipulated by leptin levels.

Blood brain barrier

Although there is some evidence that leptin could be synthesized within the brain [30], it is believed that the majority of leptin in the CNS is derived from peripheral white adipose tissue [31]. Evidence has been provided for a specific transport system for leptin to cross the blood brain barrier and enter the brain of mice, rats, and humans. The rate of transport can be decreased by high plasma concentrations of leptin. Thus, reduced entry of leptin to the brain may be one of the mechanisms of reduced sensitivity of the leptin pathway in obese individuals [31]. Nonetheless, accumulating data suggest that AD patients bear low plasma leptin levels, similarly to other patients with lipodystrophies, making them good candidates for a leptin replacement therapy.

LEPTIN AND THE NEUROBIOLOGY OF ALZHEIMER'S DISEASE

Several studies to date have addressed the correlation between reduced levels of circulating leptin and risk for AD. In the initial reports, only a limited number of cases

were examined [32,33] but have since been corroborated with larger patient populations. More interestingly, a negative correlation between leptin levels and severity of dementia has been observed [34]. Furthermore, a large prospective study involving about 3,000 older persons followed over more than four years showed that those with the lowest leptin levels had a greater decline in their cognitive ability than those with the highest levels [35,36]. Additionally, a large longitudinal analysis showed that central obesity in midlife increases the risk of dementia independent of diabetes and cardiovascular co-morbidities later in life [37]. These observations indicate that leptin deficiency is common in AD and is somehow associated with mid-life obesity, characterized by leptin "resistance" or "inefficiency".

A high concentration of leptin receptors in the hippocampus [24] (central for cognition and memory) underscores the possibility of a multifaceted role for leptin. Early work by Harvey's group demonstrated that leptin was capable of inhibiting hippocampal neurons' excitability via activation of large conductance calcium-activated K⁺ channels [38]. More recently it was shown that direct injection of leptin into the hippocampus of rodents can improve memory processing and modulate long term potentiation and synaptic plasticity [39]. Improved memory following leptin administration was also found in SAMP-8 mice, an accelerated senescence rodent model that develops amyloid plaques [40].

Amyloid

Although historically the "amyloid cascade" and "tau and tangle" hypotheses have largely been considered distinct proposals to explain AD pathobiology, accumulating data indicate multiple factors, many of which interconnect between the A β and tau molecular pathways, must come into play to exacerbate the progression of pathogenesis and cognitive decline. Exposure of neurons to A β can promote tau phosphorylation at sites identical to those found in paired helical filaments [41,42].

The extracellular accumulation of A β is a hallmark pathological feature of AD and the amount clearly depends on the rates of its production, secretion, and clearance. Based on our previous data [43], neurons depend on the interaction between presenilin-1 (PS1) and Cytoplasmic-Linker Protein 170 (CLIP-170) to generate A β . Further to this requirement, formation of A β depends on the assembly of key proteins in lipid rafts (LRs) [44]. LR are mem-

brane microdomains enriched in cholesterol, glycosphingolipids, and glucosylphosphatidyl-inositol-(GPI)-tagged proteins, implicated in signal transduction, protein trafficking, and proteolysis. Within the LRs, it is believed that the amyloid- β protein precursor (A β PP) is cleaved by β -secretase (BACE) to generate the intermediate fragment, CAPP β . The latter is subsequently processed by γ -secretase, a high molecular weight multi-protein complex containing PS1 fragments [45]. Once outside the neuron, A β can: 1) exert a biological activity by binding and activating specific receptors, including the receptor for advanced glycation-end products (RAGE) [46] and scavenger receptors [47]; 2) be removed by mechanisms of endocytosis involving apolipoprotein E (ApoE) and lipoprotein receptor-like protein (LRP) or scavenger receptors; and 3) be degraded by extracellular proteases including insulin-degrading enzyme and neprilysin [48].

BACE is known to be imbedded in membranes where it targets A β PP, the precursor of A β within lipid rafts. Our previous *in vitro* studies have demonstrated that leptin treatment of neuronal cells reduces the amount of A β secreted into the medium in a time- and dose-dependent fashion [49]. This was coincident with the subcellular redistribution of membrane lipids, BACE, and A β PP, and may be attributed to the lipolytic action of leptin. In accord, inhibitors of lipogenesis (i.e., TOFA, targeting acetyl coenzyme A carboxylase, and Cerulinin, targeting fatty-acid synthase) also inhibited A β production, whereas inhibitors of lipolysis (i.e., Etomoxir, targeting carnithine palmitoyl transferase-1) had the opposite effect of leptin, increasing A β production [49]. Overall, an abnormal accumulation of lipids in non-adipocytes (neurons specifically) may favor amyloidogenic pathways, which can be prevented if sufficient leptin is present.

The uptake of lipoprotein-like particles by neural cells [50] may be a mechanism by which neurons acquire lipids for membrane remodeling as well as an avenue that supplies neurons with precursors for other lipid metabolites and second messengers essential for function. Finally, the uptake of ApoE/A β complexes may serve as a mechanism for clearing A β from the brain interstitium. Interestingly, the uptake of the ApoE/A β complexes by neurons appears to be allele specific and a more efficient uptake of A β was achieved with ApoE3 compared to ApoE4 [49]. Leptin is one compound that facilitates the uptake of ApoE/A β complexes via LRP [49].

Tau phosphorylation

NFTs are intraneuronal aggregates of highly phosphorylated tau protein that correlate closely with cognitive loss in AD [51]. Tau is expressed predominantly in axons where it acts to stabilize microtubules, a function that is regulated by phosphorylation. Highly phosphorylated tau shows decreased binding to microtubules [52,53]. The abnormal phosphorylation of tau protein leads to disrupted microtubule function, abnormal protein trafficking, the formation of NFTs, and eventual neuronal death. Furthermore, increased phosphorylation of tau promotes self-aggregation suggesting that in AD a misregulation of tau phosphorylation would result in loss of microtubule stability and loss of function, and aggregation of tau into the paired helical filaments that form NFT [54]. Since NFT density correlates reasonably well with the clinical scale of dementia severity [55], and is increased in subjects with dementia and psychosis [56], therapies blocking this cascade may have therapeutic potential in AD.

Considerable evidence points to GSK-3 β as a predominant tau kinase in brain. Overexpression of GSK-3 β in animal models induces neurodegeneration [57, 58] and overexpression of GSK-3 β in *Drosophila* induces aggregation of tau into tangles similar to those of AD [59]. Although both known mammalian GSK-3 isotypes (α and β) can induce paired helical filament-like phosphorylation of tau, active GSK-3 β is co-expressed in tangle-bearing neurons [60], suggesting that GSK-3 β activity is likely to contribute to tau abnormalities in AD.

Recently, it was demonstrated that leptin can phosphorylate GSK-3 β at Ser-9 and deactivate it, contributing to the development of mouse cortical neurons [61]. Our laboratory confirmed those observations [95] and has further demonstrated that leptin treatment can lead to a reduction in tau phosphorylation through the modulation of GSK-3 β [95]. Specifically, leptin, in a time- and concentration-dependent fashion, reduced the amount of phosphorylation of tau at Ser²⁰², Ser³⁹⁶, and Ser⁴⁰⁴ [14], all sites which are phosphorylated in NFTs [62,63]. A similar activity has been reported for insulin [64–66]. Most interestingly, as determined by IC50 values for the Ser³⁹⁶ phosphorylation reaction of tau, leptin was two orders of magnitude more potent than insulin [14]. This finding is particularly important because brain insulin resistance has long been thought as a possible cause for sporadic AD [67].

Thus leptin is capable of modulating both the production of A β and phosphorylation of tau [14], the

building blocks of two pathological hallmarks of AD. Further, leptin-deficient mice have distinctly different synaptic profiles from wild-type mice [68], documented by electron microscopy and electrophysiology. Administration of leptin rapidly, within six hours, normalized synaptic function, a time well ahead of appetite, food intake, or weight changes. Loss of synapse function is indeed another feature related to the cognitive decline in AD.

METABOLIC PATHWAYS AND ALZHEIMER'S DISEASE

There is overwhelming evidence underlying the importance of metabolic pathways in the course of AD. First, from several genetic studies, it has been established that carriers of the ApoE4 allele are an order of magnitude more likely to get AD [69]. Second, the effect of diet and nutrition on the prevalence of AD has been documented [70–72] and weight loss is frequently observed in AD patients prior to the onset of dementia [73,74]. Further, central obesity is associated with an increased risk for dementia [37]. Third, in cell culture and animal models it has been demonstrated that lipids play an important role in amyloidogenic pathways [75]. Fourth, the majority of AD patients have some form of insulin resistance or hyperinsulinemia or type II diabetes [76].

Thus, it is not surprising that modulators of cholesterol (i.e., statins) and glucose (i.e., rosiglitazone) [77] are being developed as potential AD therapeutics. In fact, cholesterol-reducing therapies such as statins have been shown to reduce A β deposition both *in vivo* and *in vitro* [78]. These are in agreement with epidemiological studies documenting a decreased prevalence of AD with the use of statins [79]. Interestingly, the rosiglitazone studies have revealed an important association between the drug's efficacy as a cognitive enhancer in AD patients and their ApoE genotype [80]. As the mechanism of action for leptin appears to be substantially unique compared to any of the approved drugs and any of those under development, increasing the availability of leptin in the CNS holds promise as an AD therapy (Fig. 1).

AMPK is an important enzyme for regulation of cellular metabolic activity. AMPK acts as a master switch regulating several intracellular systems including the cellular uptake of glucose, the β -oxidation of fatty acids and the biogenesis of glucose transporter 4 (GLUT4) and mitochondria [81]. AMPK may be important for

CNS development as supported by the phenotype of the *Drosophila* mutant *l6chrig* which has a defective AMPK [82] and abnormal cholesterol levels that results in extensive neurodegeneration.

It has been shown that leptin directly activates AMPK [83] and our laboratory has demonstrated that the ability of leptin to modulate both tau phosphorylation and A β production are mediated through AMPK [14,95]. By utilizing a panel of known inhibitors and activators of candidate leptin signaling molecules, in addition to the employment of recombinant expression and siRNA technology, it became apparent that a number of targets upstream, but not downstream, of AMPK could simultaneously modulate A β and tau phosphorylation.

Thus based on the available data, perhaps in AD, select neuronal populations may have a deficient/defective AMPK system for one or more of the following reasons: low leptin/insulin levels, low leptin/insulin sensitivity, hyperactive GSK-3 β , abnormal cholesterol and fatty acid membrane composition, and low glucose uptake. This can then lead to changes in synaptic properties, an increase in amyloid deposition, an increase in tau phosphorylation, and finally neuronal death.

Other pharmacological agents known to modulate AMPK, such as metformin, an insulin-sensitizing antidiabetic drug [28], and 5-aminoimidazole-4-carboxamide ribonucleoside, an adenosine analogue (AICAR) could in principal at least partially replace the biological activity of leptin in pathways leading to AD pathology. To this end, rosiglitazone, a thiozolidazone, and PPAR γ agonist (see above), may also act through AMPK [84].

IMMUNE SYSTEM AND ALZHEIMER'S DISEASE

The immune system has been implicated in the pathobiology of neurodegeneration in AD. Amyloid plaques accumulate proteins of the complement system, eicosanoids and cytokines, which are integral components of ongoing inflammatory processes that augment the harmful effects of A β [85]. Important regulators of the immune system include the cytokines and chemokines, which are secreted by leukocytes (B or T cells, normally scarce in the brain) or antigen presenting cells (APC) (microglia, perivascular macrophages, astrocytes in the brain). In the AD brain, both pro-inflammatory cytokines (IFN- γ , TNF- α , IL-12, IL-1, IL-2, IL-15, IL-16, IL-17, IL-18) and anti-inflammatory cytokines (IL-4, IL-5, IL-10, IL-13, IL-

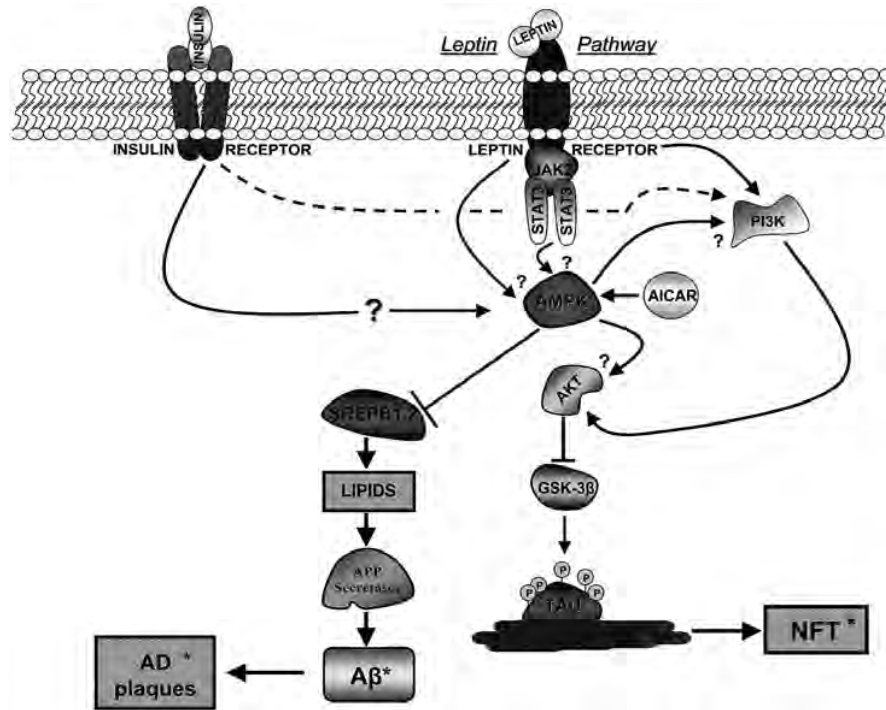


Fig. 1. Leptin can modulate AMPK activity following binding to the leptin receptor. The precise mechanism is currently unknown but there is some evidence that this may involve STAT3. There is considerable information regarding events downstream of AMPK leading to tau phosphorylation, which involves modulation of GSK-3 β via Akt. In contrast, the cascade from AMPK to A β homeostasis is less defined. Activated AMPK may turn-off the transcriptional factors SREBP1,2, known to regulate lipid metabolism (fatty acid synthase, palmitoyl transferase etc.), previously shown to be downregulated by leptin [40]. There is some evidence that insulin is also capable of modulating AMPK. In addition to AMPK, insulin and leptin may share another common target, PI3K, which also regulates GSK-3 β through Akt. Activation of Akt by AMPK leads to the phosphorylation of GSK-3 β at Ser-9 which deactivates it. GSK-3 β is the major kinase for tau.

Based exclusively on our studies, AMPK is emerging as a central modulator of major pathological pathways in AD. We propose that leptin deficiency in AD contributes to the downregulation of the AMPK system. This in turn causes increases in A β and phosphorylated tau. A lower AMPK activity may also be associated with a general low metabolic activity within neurons, an overall neuronal "fatigue". Infusing leptin in the AD brain may improve the outlook, boosting metabolic pathways and reducing A β and phospho-tau. A better understanding of the AMPK system as it relates to AD pathways can provide more targets for future drug discovery efforts.

14, TGF- β) are expressed [86]. In addition to participating in immune response, cytokines may also directly affect the processing of A β PP [87].

Leptin has similar structural and functional characteristics to the cytokines [23], sharing post-receptor pathways and participating in the immune response to pathogens and infections. Leptin deficiency is associated with impaired T cell immunity [88] and increased sensitivity to the lethal effects of bacterial endotoxin and TNF- α . Importantly, these effects can be reversed by leptin administration which attenuates inflammatory cytokine and neuroendocrine responses to infection [89]. Further, in critically ill septic patients, higher leptin levels are positively correlated with survival [90]. Although, previous trials have not reported any inflam-

matory side-effects from leptin treatments, monitoring of inflammatory markers in leptin trials is important.

BEHAVIORAL STUDIES

Mouse models of A β PP overexpression (Tg2576) have proven to be valid models for testing these leptin hypotheses. Chronic leptin supplementation to the Tg2576, using implanted miniosmotic pumps, significantly reduced brain amyloid levels [49]. From this study and others [91], leptin is known to cross the blood brain barrier. However, it is currently unknown how leptin passage to the CNS in AD patients is affected, ultimately affecting its bioavailability.

More recently, studies were conducted using the CRND8 mice which overexpress an A β PP gene containing the Swedish (K670N and M671L) and Indiana (V717F) FAD mutations. These mice show an age-related increase in A β production, as well as an early onset of plaque deposition in the cortex and hippocampus [92] and develop pathology at a younger age (15 weeks old) [93] compared to the Tg2576 mouse (10 months old) [94]. Our data suggest that chronic leptin supplementation, starting at 4 months of age and continuing through to post-plaque age for 2 months, can significantly improve the performance of these mice in cognitive and memory tests (Tezapsidis et al., unpublished results). Leptin treatment was associated, as determined by pathological examination, with reduced brain amyloid burden, particularly in the hippocampus, known to abundantly express the long isoform of leptin receptor, OB-R [23,24]. Furthermore, biochemical analysis showed that leptin treatment reduced levels of solubilized A β and phospho-tau in brain, and reduced levels of plasma A β . Significantly, no changes in the levels of plasma inflammatory markers (CRP, TNF- α , cortisol) were recorded.

CLINICAL TRIALS FOR ALZHEIMER'S DISEASE

The preclinical data obtained from our and other laboratories, the ample safety data from leptin human trials, and the epidemiological data, favorably support a clinical trial for leptin as a novel therapy for AD. However, we lack adequate evidence of a biological signal indicative of a therapeutic effect in patients. Some concerns are related to side effects, along with uncertainty about dosing. A pilot study in patients with AD that is sufficient in scope to assess the impact of leptin on biomarkers selected for their relevance to the mechanism of action of leptin and the pathobiology of AD (i.e., in CSF: A β , tau, phospho-tau, leptin; in plasma: A β , leptin, insulin) may be a prudent first step. Such a pilot trial will also help to clarify tolerability, safety, and feasibility issues relevant for consideration of a larger subsequent trial.

CONCLUSION

The mechanistic and animal study data presented, as well as the ample clinical data using leptin, suggest that increasing leptin availability is a valid target for a

novel treatment for AD. While the potential side effects of increased leptin to an already susceptible population are currently unknown, the intriguing biological activities of leptin, especially inhibition of A β production, upregulation of A β uptake, and inhibition of GSK-3 β , hold promise for long-term therapeutic benefit for AD patients. Its unique mode of action in the CNS can positively sway disease pathways and may be utilized as a desirable complementation to other strategies. Future projects involving the evaluation of a number of other products capable of modulating the AMPK system, including novel leptin and AICAR products, in combination with various formulations allowing alternative deliveries could provide further opportunities to widen the portfolio of products addressing AD.

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REFERENCES

- [1] Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. *Nature* **372**, 425-432.
- [2] Leibel RL, Chung WK, Chua SC, Jr. (1997) The molecular genetics of rodent single gene obesities. *J Biol Chem* **272**, 31937-31940.
- [3] Schwartz MW, Woods SC, Porte D, Jr., Seeley RJ, Baskin DG (2000) Central nervous system control of food intake. *Nature* **404**, 661-671.
- [4] Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL (1999) Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature* **401**, 73-76.

- [5] Harvey J (2007) Leptin: a diverse regulator of neuronal function. *J Neurochem* **100**, 307-313.
- [6] Garza JC, Guo M, Zhang W, Lu XY (2008) Leptin promotes adult hippocampal neurogenesis *in vivo* and *in vitro*. *J Biol Chem* **283**, 18238-18247.
- [7] Paulus K, Schulz C, Lehnert H (2005) Central nervous effects of leptin and insulin on hippocampal leptin and insulin receptor expression following a learning task in wistar rats. *Neuropsychobiology* **51**, 100-106.
- [8] Terry RD, Davies P (1980) Dementia of the Alzheimer type. *Annu Rev Neurosci* **3**, 77-95.
- [9] Foster-Schubert KE, Cummings DE (2006) Emerging therapeutic strategies for obesity. *Endocr Rev* **27**, 779-793.
- [10] Roth JD, Roland BL, Cole RL, Trevaskis JL, Weyer C, Koda JE, Anderson CM, Parkes DG, Baron AD (2008) Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies. *Proc Natl Acad Sci U S A* **105**, 7257-7262.
- [11] Hamrick MW, Ferrari SL (2008) Leptin and the sympathetic connection of fat to bone. *Osteoporos Int* **19**, 905-912.
- [12] Lago R, Gomez R, Lago F, Gomez-Reino J, Gualillo O (2008) Leptin beyond body weight regulation-Current concepts concerning its role in immune function and inflammation. *Cell Immunol* **252**, 139-145.
- [13] Rankin CA, Sun Q, Gambin TC (2007) Tau phosphorylation by GSK-3 β promotes tangle-like filament morphology. *Mol Neurodegener* **2**, 12.
- [14] Greco SJ, Sarkar S, Johnston JM, Zhu X, Su B, Casadesus G, Ashford JW, Smith MA, Tezapsidis N (2008) Leptin reduces Alzheimer's disease-related tau phosphorylation in neuronal cells. *Biochem Biophys Res Commun* **376**, 536-541.
- [15] Prins JB, O'Rahilly S (1997) Regulation of adipose cell number in man. *Clin Sci (Lond)* **92**, 3-11.
- [16] Rosenbaum M, Nicolson M, Hirsch J, Heymsfield SB, Gallagher D, Chu F, Leibel RL (1996) Effects of gender, body composition, and menopause on plasma concentrations of leptin. *J Clin Endocrinol Metab* **81**, 3424-3427.
- [17] Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, Lubina JA, Patane J, Self B, Hunt P, McCamish M (1999) Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA* **282**, 1568-1575.
- [18] Lee JH, Chan JL, Sourlas E, Raptopoulos V, Mantzoros CS (2006) Recombinant methionyl human leptin therapy in replacement doses improves insulin resistance and metabolic profile in patients with lipoatrophy and metabolic syndrome induced by the highly active antiretroviral therapy. *J Clin Endocrinol Metab* **91**, 2605-2611.
- [19] Licinio J, Caglayan S, Ozata M, Yildiz BO, de Miranda PB, O'Kirwan F, Whitby R, Liang L, Cohen P, Bhasin S, Krauss RM, Veldhuis JD, Wagner AJ, DePaoli AM, McCann SM, Wong ML (2004) Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults. *Proc Natl Acad Sci U S A* **101**, 4531-4536.
- [20] Brennan AM, Mantzoros CS (2006) Drug Insight: the role of leptin in human physiology and pathophysiology-emerging clinical applications. *Nat Clin Pract Endocrinol Metab* **2**, 318-327.
- [21] Zhang F, Basinski MB, Beals JM, Briggs SL, Churgay LM, Clawson DK, DiMarchi RD, Furman TC, Hale JE, Hsiung HM, Schonher BE, Smith DP, Zhang XY, Wery JP, Schevitz RW (1997) Crystal structure of the obese protein leptin-E100. *Nature* **387**, 206-209.
- [22] Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Wool EA, Monroe CA, Tepper RI (1995) Identification and expression cloning of a leptin receptor, OB-R. *Cell* **83**, 1263-1271.
- [23] Heshka JT, Jones PJ (2001) A role for dietary fat in leptin receptor, OB-Rb, function. *Life Sci* **69**, 987-1003.
- [24] Huang XF, Koutcherov I, Lin S, Wang HQ, Storlien L (1996) Localization of leptin receptor mRNA expression in mouse brain. *Neuroreport* **7**, 2635-2638.
- [25] Harvey J (2003) Leptin: a multifaceted hormone in the central nervous system. *Mol Neurobiol* **28**, 245-258.
- [26] Sanchez-Margalet V, Martin-Romero C, Santos-Alvarez J, Goberna R, Najib S, Gonzalez-Yanes C (2003) Role of leptin as an immunomodulator of blood mononuclear cells: mechanisms of action. *Clin Exp Immunol* **133**, 11-19.
- [27] Li X, Lu F, Tian Q, Yang Y, Wang Q, Wang JZ (2006) Activation of glycogen synthase kinase-3 induces Alzheimer-like tau hyperphosphorylation in rat hippocampus slices in culture. *J Neural Transm* **113**, 93-102.
- [28] Kola B, Boscaro M, Rutter GA, Grossman AB, Korbonits M (2006) Expanding role of AMPK in endocrinology. *Trends Endocrinol Metab* **17**, 205-215.
- [29] Long YC, Zierath JR (2006) AMP-activated protein kinase signaling in metabolic regulation. *J Clin Invest* **116**, 1776-1783.
- [30] Ur E, Wilkinson DA, Morash BA, Wilkinson M (2002) Leptin immunoreactivity is localized to neurons in rat brain. *Neuroendocrinology* **75**, 264-272.
- [31] Campfield LA, Smith FJ, Burn P (1996) The OB protein (leptin) pathway—a link between adipose tissue mass and central neural networks. *Horm Metab Res* **28**, 619-632.
- [32] Olsson T, Nasman B, Rasmuson S, Ahren B (1998) Dual relation between leptin and cortisol in humans is disturbed in Alzheimer's disease. *Biol Psychiatry* **44**, 374-376.
- [33] Power DA, Noel J, Collins R, O'Neill D (2001) Circulating leptin levels and weight loss in Alzheimer's disease patients. *Dement Geriatr Cogn Disord* **12**, 167-170.
- [34] Ray S, Wyss-Coray A (2005) Methods for diagnosis, stratification, and monitoring of Alzheimer's disease. *World Intellectual Property Organization*, WO 2005/052592 A2 (Satoris, Inc.).
- [35] Holden KF, Lindquist K, Rosano C, Tylavsky FA, Harris TB, Yaffe K (2006) Low serum leptin is associated with poor cognitive performance in the elderly. *American Academy of Neurology, 58th Annual Meeting* (San Diego), p. S41.006.
- [36] Holden KF, Lindquist K, Tylavsky FA, Rosano C, Harris TB, Yaffe K (2008) Serum leptin level and cognition in the elderly: Findings from the Health ABC Study. *Neurobiol Aging*, in press.
- [37] Whitmer RA, Gustafson DR, Barrett-Connor E, Haan MN, Gunderson EP, Yaffe K (2008) Central obesity and increased risk of dementia more than three decades later. *Neurology* **71**, 1057-1064.
- [38] Shanley LJ, Irving AJ, Rae MG, Ashford ML, Harvey J (2002) Leptin inhibits rat hippocampal neurons via activation of large conductance calcium-activated K⁺ channels. *Nat Neurosci* **5**, 299-300.
- [39] Harvey J, Shanley LJ, O'Malley D, Irving AJ (2005) Leptin: a potential cognitive enhancer? *Biochem Soc Trans* **33**, 1029-1032.
- [40] Farr SA, Banks WA, Morley JE (2006) Effects of leptin on memory processing. *Peptides* **27**, 1420-1425.

- [41] Busciglio J, Lorenzo A, Yeh J, Yankner BA (1995) beta-amyloid fibrils induce tau phosphorylation and loss of microtubule binding. *Neuron* **14**, 879-888.
- [42] Lee VM, Goedert M, Trojanowski JQ (2001) Neurodegenerative tauopathies. *Annu Rev Neurosci* **24**, 1121-1159.
- [43] Tezapsidis N, Merz PA, Merz G, Hong H (2003) Microtubular interactions of presenilin direct kinesis of Abeta peptide and its precursors. *FASEB J* **17**, 1322-1324.
- [44] Simons M, Keller P, De Strooper B, Beyreuther K, Dotti CG, Simons K (1998) Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. *Proc Natl Acad Sci U S A* **95**, 6460-6464.
- [45] De Strooper B (2003) Aph-1, Pen-2, and Nicastrin with Presenilin generate an active gamma-Secretase complex. *Neuron* **38**, 9-12.
- [46] Yan SD, Chen X, Fu J, Chen M, Zhu H, Roher A, Slattery T, Zhao L, Nagashima M, Morser J, Migheli A, Nawroth P, Stern D, Schmidt AM (1996) RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. *Nature* **382**, 685-691.
- [47] Husemann J, Loike JD, Anankov R, Febbraio M, Silverstein SC (2002) Scavenger receptors in neurobiology and neuropathology: their role on microglia and other cells of the nervous system. *Glia* **40**, 195-205.
- [48] Farris W, Mansourian S, Chang Y, Lindsley L, Eckman EA, Frosch MP, Eckman CB, Tanzi RE, Selkoe DJ, Guenette S (2003) Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain *in vivo*. *Proc Natl Acad Sci U S A* **100**, 4162-4167.
- [49] Fewlass DC, Noboa K, Pi-Sunyer FX, Johnston JM, Yan SD, Tezapsidis N (2004) Obesity-related leptin regulates Alzheimer's Abeta. *FASEB J* **18**, 1870-1878.
- [50] Van Uden E, Kang DE, Koo EH, Masliah E (2000) LDL receptor-related protein (LRP) in Alzheimer's disease: towards a unified theory of pathogenesis. *Microsc Res Tech* **50**, 268-272.
- [51] Nagy Z, Esiri MM, Jobst KA, Morris JH, King EM, McDonald B, Litchfield S, Smith A, Barnettson L, Smith AD (1995) Relative roles of plaques and tangles in the dementia of Alzheimer's disease: correlations using three sets of neuropathological criteria. *Dementia* **6**, 21-31.
- [52] Billingsley ML, Kincaid RL (1997) Regulated phosphorylation and dephosphorylation of tau protein: effects on microtubule interaction, intracellular trafficking and neurodegeneration. *Biochem J* **323** (Pt 3), 577-591.
- [53] Lovestone S, Hartley CL, Pearce J, Anderton BH (1996) Phosphorylation of tau by glycogen synthase kinase-3 beta in intact mammalian cells: the effects on the organization and stability of microtubules. *Neuroscience* **73**, 1145-1157.
- [54] Alonso A, Zaidi T, Novak M, Grundke-Iqbal I, Iqbal K (2001) Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. *Proc Natl Acad Sci U S A* **98**, 6923-6928.
- [55] Thal DR, Holzer M, Rub U, Waldmann G, Gunzel S, Zedlick D, Schober R (2000) Alzheimer-related tau-pathology in the perforant path target zone and in the hippocampal stratum oriens and radiatum correlates with onset and degree of dementia. *Exp Neurol* **163**, 98-110.
- [56] Farber NB, Rubin EH, Newcomer JW, Kinscherf DA, Miller JP, Morris JC, Olney JW, McKeel DW, Jr. (2000) Increased neocortical neurofibrillary tangle density in subjects with Alzheimer disease and psychosis. *Arch Gen Psychiatry* **57**, 1165-1173.
- [57] Lucas JJ, Hernandez F, Gomez-Ramos P, Moran MA, Hen R, Avila J (2001) Decreased nuclear beta-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3beta conditional transgenic mice. *EMBO J* **20**, 27-39.
- [58] Munoz-Montano JR, Moreno FJ, Avila J, Diaz-Nido J (1997) Lithium inhibits Alzheimer's disease-like tau protein phosphorylation in neurons. *FEBS Lett* **411**, 183-188.
- [59] Jackson GR, Wiedau-Pazos M, Sang TK, Wagle N, Brown CA, Massachi S, Geschwind DH (2002) Human wild-type tau interacts with wingless pathway components and produces neurofibrillary pathology in Drosophila. *Neuron* **34**, 509-519.
- [60] Pei JJ, Braak E, Braak H, Grundke-Iqbal I, Iqbal K, Winblad B, Cowburn RF (1999) Distribution of active glycogen synthase kinase 3beta (GSK-3beta) in brains staged for Alzheimer disease neurofibrillary changes. *J Neuropathol Exp Neurol* **58**, 1010-1019.
- [61] Valerio A, Ghisi V, Dossena M, Tonello C, Giordano A, Frontini A, Ferrario M, Pizzi M, Spano P, Carruba MO, Nisoli E (2006) Leptin increases axonal growth cone size in developing mouse cortical neurons by convergent signals inactivating glycogen synthase kinase-3beta. *J Biol Chem* **281**, 12950-12958.
- [62] Trojanowski JQ, Schmidt ML, Shin RW, Bramblett GT, Rao D, Lee VM (1993) Altered tau and neurofilament proteins in neuro-degenerative diseases: diagnostic implications for Alzheimer's disease and Lewy body dementias. *Brain Pathol* **3**, 45-54.
- [63] Su JH, Cummings BJ, Cotman CW (1994) Early phosphorylation of tau in Alzheimer's disease occurs at Ser-202 and is preferentially located within neurites. *Neuroreport* **5**, 2358-2362.
- [64] Hong M, Lee VM (1997) Insulin and insulin-like growth factor-1 regulate tau phosphorylation in cultured human neurons. *J Biol Chem* **272**, 19547-19553.
- [65] Planel E, Tatebayashi Y, Miyasaka T, Liu L, Wang L, Herman M, Yu WH, Luchsinger JA, Wadzinski B, Duff KE, Takashima A (2007) Insulin dysfunction induces *in vivo* tau hyperphosphorylation through distinct mechanisms. *J Neurosci* **27**, 13635-13648.
- [66] Lesort M, Jope RS, Johnson GV (1999) Insulin transiently increases tau phosphorylation: involvement of glycogen synthase kinase-3beta and Fyn tyrosine kinase. *J Neurochem* **72**, 576-584.
- [67] Hoyer S (1998) Is sporadic Alzheimer disease the brain type of non-insulin dependent diabetes mellitus? A challenging hypothesis. *J Neural Transm* **105**, 415-422.
- [68] Pinto S, Roseberry AG, Liu H, Diano S, Shanabrough M, Cai X, Friedman JM, Horvath TL (2004) Rapid rewiring of arcuate nucleus feeding circuits by leptin. *Science* **304**, 110-115.
- [69] Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD (1993) Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A* **90**, 1977-1981.
- [70] Grant WB (1999) Dietary links to Alzheimer's disease: 1999 update. *J Alzheimers Dis* **1**, 197-201.
- [71] Grant WB (2004) Obesity and Alzheimer disease: roles of diet and genetics. *Arch Intern Med* **164**, 109-110; author reply 110.
- [72] Smith MA, Petot GJ, Perry G (1999) Diet and oxidative stress: a novel synthesis of epidemiological data on Alzheimer's disease. *J Alzheimers Dis* **1**, 203-206.

- [73] Barrett-Connor E, Edelman SL, Corey-Bloom J, Wiederholt WC (1996) Weight loss precedes dementia in community-dwelling older adults. *J Am Geriatr Soc* **44**, 1147-1152.
- [74] Mazzali G, Bissoli L, Gambina S, Residori L, Pagliari P, Guariento S, Sun M, Broglio E, Bosello O, Zamboni M (2002) Energy balance in Alzheimer's disease. *J Nutr Health Aging* **6**, 247-253.
- [75] Puglielli L, Tanzi RE, Kovacs DM (2003) Alzheimer's disease: the cholesterol connection. *Nat Neurosci* **6**, 345-351.
- [76] Fishel MA, Watson GS, Montine TJ, Wang Q, Green PS, Kulstad JJ, Cook DG, Peskind ER, Baker LD, Goldgaber D, Nie W, Asthana S, Plymate SR, Schwartz MW, Craft S (2005) Hyperinsulinemia provokes synchronous increases in central inflammation and beta-amyloid in normal adults. *Arch Neurol* **62**, 1539-1544.
- [77] Risner ME, Saunders AM, Altman JF, Ormandy GC, Craft S, Foley IM, Zvartau-Hind ME, Hosford DA, Roses AD (2006) Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease. *Pharmacogenomics J* **6**, 246-254.
- [78] Sparks DL, Sabbagh M, Connor D, Soares H, Lopez J, Stankovic G, Johnson-Traver S, Ziolkowski C, Browne P (2006) Statin therapy in Alzheimer's disease. *Acta Neurol Scand Suppl* **185**, 78-86.
- [79] Simons M, Keller P, Dichgans J, Schulz JB (2001) Cholesterol and Alzheimer's disease: is there a link? *Neurology* **57**, 1089-1093.
- [80] Jiang Q, Heneka M, Landreth GE (2008) The role of peroxisome proliferator-activated receptor-gamma (PPARgamma) in Alzheimer's disease: therapeutic implications. *CNS Drugs* **22**, 1-14.
- [81] Winder WW, Hardie DG (1999) AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. *Am J Physiol* **277**, E1-10.
- [82] Tschape JA, Hammerschmid C, Muhlig-Versen M, Athentstaedt K, Daum G, Kretschmar D (2002) The neurodegeneration mutant lochrig interferes with cholesterol homeostasis and Appl processing. *EMBO J* **21**, 6367-6376.
- [83] Yu X, McCorkle S, Wang M, Lee Y, Li J, Saha AK, Unger RH, Ruderman NB (2004) Leptinomimetic effects of the AMP kinase activator AICAR in leptin-resistant rats: prevention of diabetes and ectopic lipid deposition. *Diabetologia* **47**, 2012-2021.
- [84] Fryer LG, Parbu-Patel A, Carling D (2002) The Anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. *J Biol Chem* **277**, 25226-25232.
- [85] Emmerling MR, Watson MD, Raby CA, Spiegel K (2000) The role of complement in Alzheimer's disease pathology. *Biochim Biophys Acta* **1502**, 158-171.
- [86] Benveniste EN, Nguyen VT, O'Keefe GM (2001) Immunological aspects of microglia: relevance to Alzheimer's disease. *Neurochem Int* **39**, 381-391.
- [87] Blasko I, Marx F, Steiner E, Hartmann T, Grubeck-Loebenstien B (1999) TNFalpha plus IFNgamma induce the production of Alzheimer beta-amyloid peptides and decrease the secretion of APPs. *FASEB J* **13**, 63-68.
- [88] Faggioni R, Feingold KR, Grunfeld C (2001) Leptin regulation of the immune response and the immunodeficiency of malnutrition. *FASEB J* **15**, 2565-2571.
- [89] Xiao E, Xia-Zhang L, Vulliamoz NR, Ferin M, Wardlaw SL (2003) Leptin modulates inflammatory cytokine and neuroendocrine responses to endotoxin in the primate. *Endocrinology* **144**, 4350-4353.
- [90] Arnalich F, Lopez J, Codoceo R, Jim nez M, Madero R, Montiel C (1999) Relationship of plasma leptin to plasma cytokines and human survival in sepsis and septic shock. *J Infect Dis* **180**, 908-911.
- [91] Suzuki M, Ding Q, Muranaka S, Kigure M, Kojima M, Terada M, Kataoka N, Hagiwara A, Kromkhun P, Moritani N, Hashimoto H, Yokosuka M, Sakurai F, Tanaka M, Saito TR (2008) Correlation between body weight (epididymal fat) and permeation rate of serum leptin through the blood-brain barrier (BBB) in male rats aged 8 months. *Exp Anim* **57**, 485-488.
- [92] Chishti MA, Yang DS, Janus C, Phinney AL, Horne P, Pearson J, Strome R, Zuker N, Loukides J, French J, Turner S, Lozza G, Grilli M, Kunicki S, Morissette C, Paquette J, Gervais F, Bergeron C, Fraser PE, Carlson GA, George-Hyslop PS, Westaway D (2001) Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. *J Biol Chem* **276**, 21562-21570.
- [93] Hyde LA, Kazdoba TM, Grilli M, Lozza G, Brusa R, Zhang Q, Wong GT, McCool MF, Zhang L, Parker EM, Higgins GA (2005) Age-progressing cognitive impairments and neuropathology in transgenic CRND8 mice. *Behav Brain Res* **160**, 344-355.
- [94] Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G (1996) Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science* **274**, 99-102.
- [95] Greco, SJ, Sarkar, S, Johnston, JM, Tezapsidis, N (2009) Leptin regulates tau phosphorylation and amyloid through AMPK in neuronal cells. *Biochem Biophys Res Commun* **380**, 98-104.

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