Hyperinsulinemia Provokes Synchronous Increases in Central Inflammation and β-Amyloid in Normal Adults

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Background: Inflammation has been implicated as a pathogenetic factor in Alzheimer disease, possibly via effects on β-amyloid (Aβ). Hyperinsulinemia induces inflammation and is a risk factor for Alzheimer disease. Thus, insulin abnormalities may contribute to Alzheimer disease pathophysiology through effects on the inflammatory network.

Objectives: To determine the effects of induced hyperinsulinemia with euglycemia on Aβ, transthyretin, and inflammatory markers and modulators in plasma and cerebrospinal fluid (CSF).

Design: Randomized crossover trial.

Setting: Veterans Affairs hospital clinical research unit.

Participants: Sixteen healthy adults ranging from 55 to 81 years of age (mean age, 68.2 years).

Interventions: On separate mornings, fasting participants received randomized infusions of saline or insulin ($1.0\ mU \cdot kg^{-1} \cdot min^{-1}$) with variable dextrose levels to maintain euglycemia, achieving plasma insulin levels typical of insulin resistance. Plasma and CSF were collected after an approximately 105-minute infusion.

Main Outcome Measures: Plasma and CSF levels of interleukin 1α, interleukin 1β, interleukin 6, tumor necrosis factor α, F_2-isoprostane (CSF only), Aβ, norepinephrine, transthyretin, and apolipoprotein E.

Results: Insulin increased CSF levels of F_2-isoprostane and cytokines (both $P<.01$), as well as plasma and CSF levels of Aβ42 (both $P<.05$). The changes in CSF levels of Aβ42 were predicted by increased F_2-isoprostane and cytokine levels (both $P<.01$) and reduced transthyretin levels ($P=.02$). Increased inflammation was modulated by insulin-induced changes in CSF levels of norepinephrine and apolipoprotein E (both $P<.05$).

Conclusion: Moderate hyperinsulinemia can elevate inflammatory markers and Aβ42 in the periphery and the brain, thereby potentially increasing the risk of Alzheimer disease.

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Conditions of insulin resistance and hyperinsulinemia are associated with elevated levels of inflammatory markers and increase the risk for Alzheimer disease (AD).[1-3] Inflammation has been proposed as a key pathogenetic factor for AD.[4] Patients with AD have elevated cerebrospinal fluid (CSF) concentrations of the inflammatory cytokine interleukin (IL) 6 and the lipid peroxidation marker F_2-isoprostane.[5,6] Furthermore, in vitro and animal studies suggest that inflammation interacts with processing and deposition of β-amyloid (Aβ), a key peptide linked to AD pathogenesis.[7]

In the periphery, insulin modulates many aspects of the inflammatory network. Low doses of insulin exert anti-inflammatory effects[8]; however, excessive hyperinsulinemia exacerbates inflammation and increases the levels of markers of oxidative stress.[9] In humans, plasma concentrations of the proinflammatory cytokines IL-6 and tumor necrosis factor α (TNF-α) increase synergistically when insulin is administered with the endotoxin lipopolysaccharide.[10] Insulin may also contribute to inflammation in the central nervous system (CNS), partially through effects on Aβ. Insulin promotes the release of Aβ from intracellular neuronal compartments and inhibits its degradation by the metalloprotease insulin-degrading enzyme.[11,12] Insulin-like peptides may also regulate carrier proteins that transport Aβ between the CNS and the periphery.[13]

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Table. Subject Characteristics

Characteristic	Mean (SD)
Age, y	68.2 (7.3)
Sex, men/women	6/10
Education, y	16.0 (2.6)
Body mass index, kg/m^2	25.9 (3.4)
Fasting plasma glucose, mmol/L	6.0 (1.1)
Mini-Mental State Examination score	29.1 (1.4)

Intervening factors may modify insulin's proinflammatory effects. In the brain, insulin regulates levels of norepinephrine,[14] an endogenous, anti-inflammatory neuromodulator that attenuates Aβ42-provoked increases in IL-1β levels in rats and potentially modulates the effects of IL-1β on Aβ processing.[15] Thus, noradrenergic depletion in AD may increase vulnerability to Aβ-provoked inflammation. Additionally, insulin regulates apolipoprotein E (apoE) levels, in part though interactions with low-density lipoprotein receptor–related protein.[16] The apoE protein down-regulates the inflammatory cascade, lowering IL-6 and TNF-α levels in animals following inflammatory stimulation.[17]

In the present study, we tested the hypothesis that peripheral insulin administration would modulate CSF inflammatory markers. We raised plasma insulin levels (while maintaining euglycemia) in 16 healthy older adults, achieving moderately high physiological elevations typical of postprandial levels in patients with insulin resistance. We then measured changes in plasma and CSF levels of inflammatory markers (IL-1α, IL-1β, IL-6, TNF-α, and F_2-isoprostane), modulators (transthyretin, apoE, and norepinephrine), and Aβ.

METHODS

SUBJECTS

This study was approved by the University of Washington institutional review board. All of the subjects gave written informed consent. Participants included 16 cognitively normal adults ranging in age from 55 to 81 years (**Table**) in good health who received extensive medical and cognitive screening as previously described.[18] None were receiving medications with known CNS or glucoregulatory effects.

PROCEDURE

On separate mornings at least 1 week apart, fasting participants received 2 randomized infusions: (1) saline (baseline) and (2) insulin (1.0 mU·kg^{-1}·min^{-1}), yielding corresponding plasma insulin levels of approximately 10 µU/mL and 85 µU/mL, with variable dextrose levels (20%) to maintain plasma glucose levels of approximately 100 mg/dL. Intravenous catheters were inserted for infusions and blood sampling. Following a 30-minute habituation period, infusions were begun; target plasma levels were attained in approximately 90 minutes. Afterward, subjects completed a 15-minute cognitive protocol and then underwent lumbar puncture to collect CSF (both procedures are described in a separate article[18]). Blood samples were obtained prior to beginning infusions and prior to CSF acquisition.

ASSAYS

Insulin and norepinephrine levels were determined by radioenzymatic assay or radioimmunoassay.[19] The F_2-isoprostane levels were quantified using gas chromatography with negative-ion chemical-ionization mass spectrometry and selective ion monitoring.[5] The IL-1α, IL-1β, IL-6, TNF-α, and transthyretin levels (plasma and CSF) and Aβ40 and Aβ42 levels (plasma) were determined using enzyme-linked immunosorbent assays.[20,21] The CSF Aβ42 levels were determined with enzyme-linked immunosorbent assays (Athena Diagnostics, Worcester, Mass).

STATISTICAL ANALYSIS

Biomarker values were subjected to repeated-measures analysis of covariance with infusion condition (saline or insulin) as the within-subjects factor. Age and body mass index (BMI), which are associated with insulin resistance, were used as covariates. When they did not contribute significantly to analyses, they were deleted from the model. Relationships among biomarker changes due to induced hyperinsulinemia were examined by correlating difference scores (value in the insulin condition minus the corresponding value in the saline condition). Higher scores reflected greater increases due to insulin infusion. The relationship between changes in inflammatory markers and Aβ levels in response to insulin was examined using stepwise multiple regression analysis. Dependent variables were difference scores for plasma and CSF Aβ40 and Aβ42 levels (separate analyses). Predictors were age, BMI, and plasma or CSF inflammatory reactants.

RESULTS

INSULIN, CYTOKINES, AND F_2-ISOPROSTANE

Intravenous insulin administration produced reliable elevations in CSF insulin levels, which is consistent with animal models showing insulin transport into the brain and subsequent egress into CSF[22] (mean [SEM] saline and insulin infusions were 1.44 [0.20] µU/mL and 2.22 [0.35] µU/mL, respectively; $P=.02$).[18]

We then examined changes in cytokine and F_2-isoprostane levels during hyperinsulinemia. Insulin increased CSF levels of all 4 cytokines (**Figure 1**A-D; IL-1α [$P<.001$], IL-1β [$P<.001$], IL-6 [$P=.007$], and TNF-α [$P=.002$]) and F_2-isoprostane (Figure 1E; $P=.01$). Adults with greater BMIs tended to have higher CSF TNF-α levels in response to insulin ($r=0.49$, $P=.06$). In contrast, plasma cytokine levels did not change reliably in response to insulin. Plasma and CSF cytokine levels were uncorrelated, as were insulin-induced changes. Insulin did not affect CSF protein, suggesting that changes in inflammatory reactants were not due to nonspecific effects on CSF turnover ($P=.33$).

INSULIN AND Aβ

Plasma Aβ42 increased with insulin, an effect that was associated with BMI (**Figure 2**A; $P=.046$). Adults with greater BMIs showed greater plasma Aβ42 elevations with insulin ($r=0.49$, $P=.047$) (Figure 2B). Consistent with the observation that TNF-α modulates Aβ transport between the CNS and the periphery,[13] insulin-induced

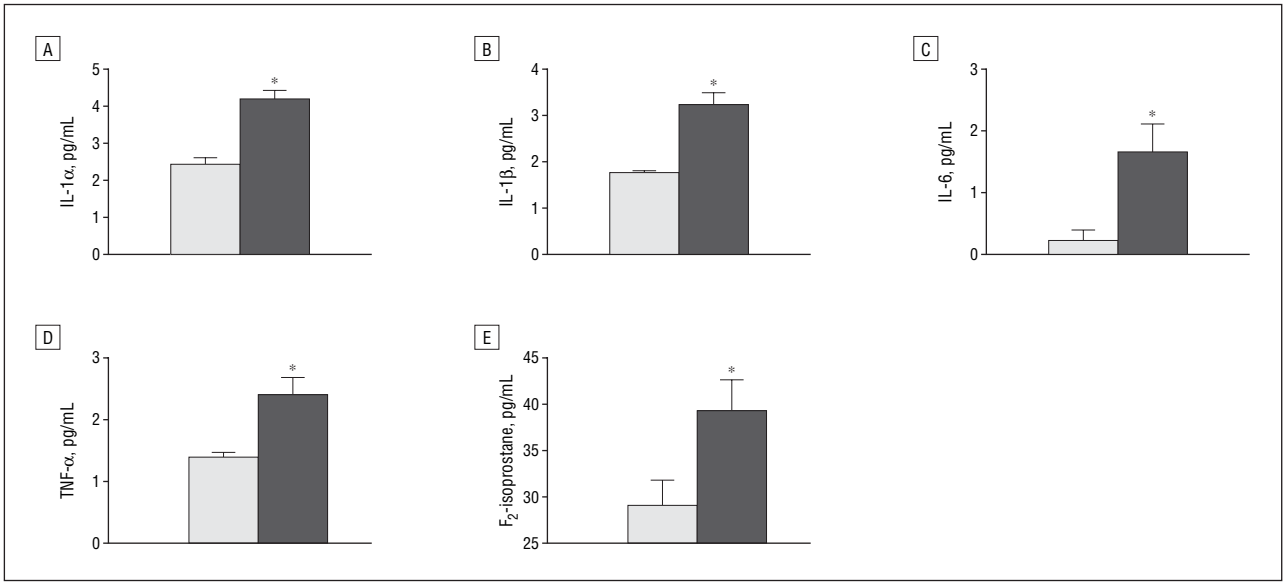

Figure 1. Cerebrospinal fluid cytokines and F_2-isoprostane (mean±SEM) for study participants (n=15). Hyperinsulinemia increased cerebrospinal fluid levels of interleukin (IL) 1α ($F_{1,14}$=61.46, P<.001) (A), IL-1β ($F_{1,14}$=33.14, P<.001) (B), IL-6 ($F_{1,14}$=9.90, P=.007) (C), tumor necrosis factor α (TNF-α) ($F_{1,14}$=14.15, P=.002) (D), and F_2-isoprostane ($F_{1,14}$=8.07, P=.01) (E). Shaded bars indicate saline condition; solid black bars, insulin condition; asterisks, significant change with insulin infusion relative to saline infusion.

changes in CSF TNF-α levels predicted changes in plasma Aβ42 levels (R^2=0.44, P=.007); subjects with higher TNF-α levels during insulin infusion had greater increases in plasma Aβ42 levels (r=0.64, P=.01). Higher plasma Aβ42 levels were also associated with increased CSF transthyretin levels (Figure 2C; r=0.63, P=.02), which binds Aβ and facilitates its transport from the brain to the periphery. Interestingly, insulin infusion did not affect plasma Aβ40 levels (mean [SEM] plasma Aβ40 level was 224.7 [26.2] pg/mL for saline conditions and 221.6 [26.4] pg/mL for insulin conditions). Insulin-induced changes in plasma Aβ40 or Aβ42 levels were unrelated to changes in plasma inflammatory markers.

We previously reported that insulin provoked an age-dependent increase in CSF Aβ42 levels for this group of normal adults.[18] We have now determined that transthyretin and inflammatory marker levels strongly predict insulin-induced changes in CSF Aβ42 levels (omnibus $F_{4,9}$=11.14, P=.002). The best predictors were age (P=.003) and difference scores for IL-6 (P=.003), F_2-isoprostane (P=.002), and transthyretin (P=.01). Older age and greater increases in IL-6 and F_2-isoprostane levels were associated with greater increases in CSF Aβ42 levels following insulin infusion. In contrast, increased transthyretin levels predicted lowering of CSF Aβ42 levels, which is consistent with enhanced transport from the CNS to the periphery (Figure 2D; r=–0.59, P=.03).

CSF NOREPINEPHRINE, IL-1β, AND Aβ42

Since norepinephrine attenuates Aβ42-provoked increases in IL-1β levels in rodents,[15] we examined whether insulin-induced increases in CSF norepinephrine levels attenuate increases in CSF Aβ42 and IL-1β levels. Subjects with higher CSF norepinephrine levels during insulin infusion had lower levels of Aβ42 (**Figure 3**A; r=–0.51, P=.04) and IL-1β (Figure 3B; r=–0.60, P=.02).

CSF APOE AND CYTOKINES

Insulin regulates apoE levels,[16] and apoE moderates the inflammatory cascade.[17] Hyperinsulinemia provoked age-related changes in CSF apoE levels (P=.04). Insulin raised apoE levels for most subjects, which was an effect that increased with age (**Figure 4**B; r=0.46, P=.08). Higher CSF apoE levels with insulin infusion were associated with smaller increases in CSF IL-6 (r=–0.54, P=.04) and TNF-α (r=–0.42, P=.12) levels, and they were associated with greater CSF IL-1α levels (r=0.60, P=.02). Plasma and CSF apoE levels were uncorrelated.

COMMENT

Moderate peripheral hyperinsulinemia provoked striking increases in CNS inflammatory markers. Our findings suggest that insulin-resistant conditions such as diabetes mellitus and hypertension may increase the risk for AD, in part through insulin-induced inflammation. Although our study cannot determine the precise mechanisms through which insulin increases CSF inflammatory marker levels, the results suggest several possibilities. We observed neither insulin-induced changes in plasma cytokines nor correlations between CSF and plasma cytokines. Thus, elevated CSF cytokine levels are likely not due to peripheral cytokine transport into the CNS, but may instead reflect insulin's effects on blood-brain barrier endothelial cells, brain glia, or neurons, all of which express insulin receptors.[23]

Insulin may also have indirectly affected CSF cytokine levels through modulation of CSF and plasma Aβ42 levels. Our data provide, to our knowledge, the first demonstration of acute manipulation of peripheral Aβ42 in vivo in humans. The role of plasma Aβ42 in AD pathogenesis is uncertain; however, elevations have been docu-

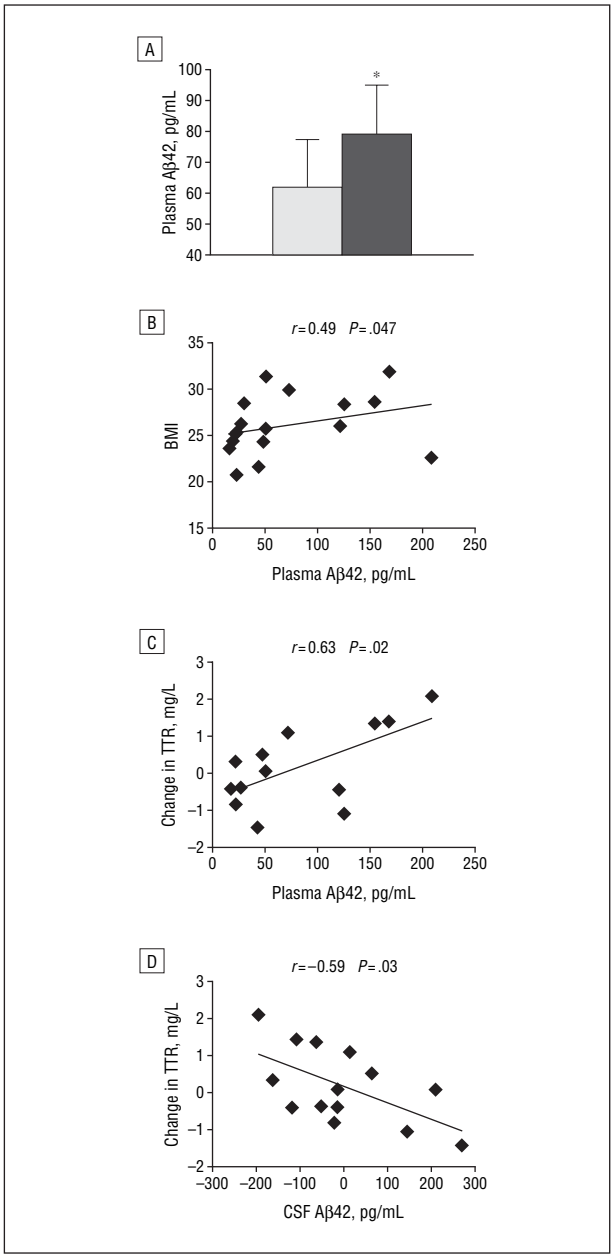

Figure 2. A, Hyperinsulinemia increased plasma β-amyloid 42 (Aβ42) levels ($F_{1,13}$=4.86, P=.046). Open bar indicates saline condition; filled bar, insulin condition. B, Participants with greater body mass index (BMI) showed greater increases in plasma Aβ42 levels with hyperinsulinemia. C, Insulin-induced elevations of cerebrospinal fluid (CSF) transthyretin (TTR) levels were associated with higher plasma Aβ42 levels. D, Insulin-induced elevations of CSF TTR levels were associated with lower CSF Aβ42 levels. Mean±SEM CSF TTR levels were 4.75±0.48 mg/L and 4.91±0.52 mg/L during saline and insulin infusions, respectively. Mean±SEM CSF Aβ42 levels were 1113.45±117.95 pg/mL and 1159.65±115.14 pg/mL during saline and insulin infusions, respectively.

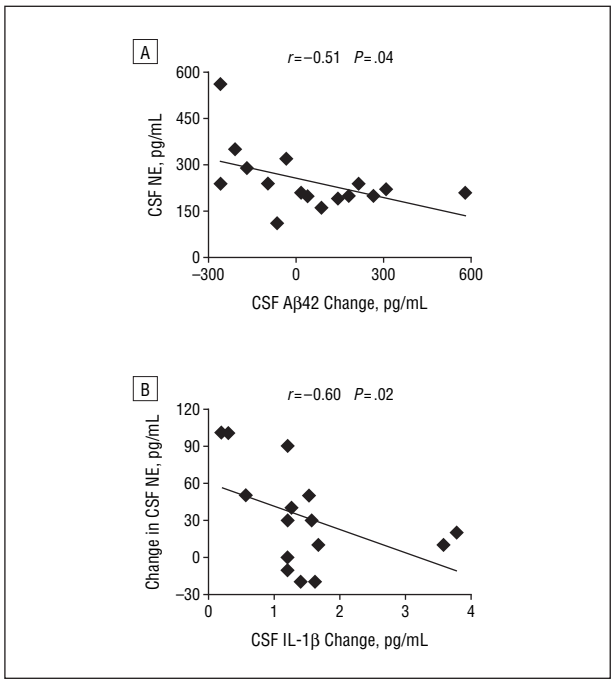

Figure 3. Inverse relationship between cerebrospinal fluid (CSF) norepinephrine (NE) and insulin-induced changes in CSF β-amyloid 42 (Aβ42) (A) and interleukin 1β (IL-1β) (B).

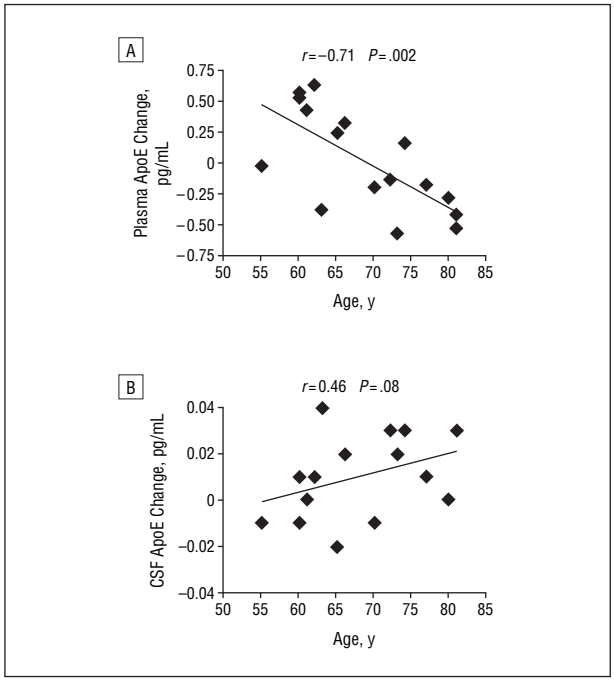

Figure 4. Insulin infusion caused greater increases in plasma apolipoprotein E (apoE) levels for younger subjects (<70 years) than for older subjects (≥70 years) (A), but greater increases in cerebrospinal fluid (CSF) apoE levels for older subjects than for younger subjects (B). Mean±SEM plasma apoE levels with saline and insulin infusions were 3.68±0.37 pg/mL and 4.00±0.36 pg/mL, respectively, for younger subjects, and 4.49±0.37 pg/mL and 4.22±0.36 pg/mL, respectively, for older subjects. Mean±SEM CSF apoE levels with saline and insulin infusions were 0.46±0.05 pg/mL and 0.47±0.05 pg/mL, respectively, for younger subjects, and 0.53±0.05 pg/mL and 0.54±0.06 pg/mL, respectively, for older subjects.

mented in patients with AD and in adults who later develop AD.[24] Notably, insulin's effect on plasma Aβ42 levels was enhanced in subjects with greater BMIs, a characteristic associated with both insulin resistance and AD risk.[25] Thus, the interactive effects of hyperinsulinemia and BMI on plasma Aβ42 levels may contribute to this increased risk. It has been hypothesized that prolonged elevations of plasma Aβ levels obstruct a peripheral sink through which CNS Aβ is cleared, leading to increased accumulation in the brain.[26] High insulin levels may inhibit peripheral clearance of Aβ42 by insulin-degrading

enzyme in the liver or other tissues. The selective effects of insulin on Aβ42 levels but not on Aβ40 levels are puzzling. Such effects may reflect the increased tendency of Aβ42 to oligomerize, rendering it impervious to degradation by insulin-degrading enzyme, or insulin-induced changes in lipids that differentially bind and enhance clearance of Aβ species.

Alternatively, insulin may have increased Aβ42 efflux from the brain to the plasma. Levels of transthyretin, a protein that can bind Aβ and facilitate transport from the CNS to the periphery, are reduced in patients with AD.[13] We found that insulin-induced elevations of CSF transthyretin levels were associated with increased plasma Aβ42 levels and decreased CSF Aβ42 levels. This inverse relationship suggests that insulin-induced transthyretin changes facilitated Aβ clearance from the CNS to the periphery for some participants. Transthyretin is synthesized in the liver and the choroid plexus, sites rich with insulin receptors, and its synthesis is increased by insulin-like growth factor I, a peptide closely related to insulin. An insulin-responsive element has recently been identified in the promoter region of the transthyretin gene (D.G., unpublished data, 2004). Transthyretin is also regulated by IL-6 and TNF-α.[27,28] Thus, insulin-induced increases of cytokine levels may have reduced transthyretin levels for some participants.

The Aβ42 peptide interacts with inflammatory agents in a cyclically reinforcing manner, such that elevations in Aβ levels increase proinflammatory cytokine levels.[29] In vitro, soluble Aβ oligomers rapidly increase IL-1β and TNF-α levels.[30] Conversely, several cytokines affect Aβ production or clearance. Both IL-6 and IL-1β can regulate processing of the amyloid precursor protein from which Aβ is derived and can increase production of Aβ42.[31,32] The mutually reinforcing effects of Aβ, TNF-α, IL-1β, and IL-6 may, therefore, create a "cytokine cycle."[29] Aspects of our results support this model. Changes in CSF Aβ42 levels were predicted by increases in the levels of these 3 cytokines, but these changes were unrelated to changes in the levels of IL-1α. Also, levels of CSF F_2-isoprostane, a lipid peroxidation marker produced by neurons and glia, increased with insulin infusion, and the magnitude of this effect was directly related to elevations of CSF Aβ42 levels. In contrast, elevations of plasma Aβ42 levels following insulin infusion were associated solely with increased CSF TNF-α levels. This pattern contradicts a rodent study[13] showing that TNF-α inhibits Aβ42 clearance from the brain, although effects of TNF-α only on CSF Aβ and not on plasma Aβ were reported. It is possible that in humans, the insulin-induced rise in plasma Aβ42 levels is multifactorial, reflecting Aβ transport from the CNS, effects on peripheral clearance, or Aβ release from peripheral sources such as platelets.

Norepinephrine may also mediate insulin's effects on Aβ and inflammatory reactants. Insulin can regulate CNS norepinephrine,[14] an endogenous, anti-inflammatory neuromodulator that blocks IL-1β expression.[15] Increased Aβ plaque load in AD has been linked to neuronal loss in the locus coeruleus, the primary source of brain norepinephrine.[33] Thus, decreased norepinephrine activity in AD may potentiate the deleterious inflammatory effects of Aβ. Consistent with this notion, higher CSF norepinephrine levels with insulin infusion were associated with selective attenuation in elevated IL-1β levels and reduced CSF Aβ42 levels.

Insulin produced age-dependent effects on CSF levels of apoE, a lipoprotein that plays a critical role in cholesterol metabolism and injury repair and that downregulates TNF-α and IL-6 production in animal models.[17] In the periphery, insulin reduces hepatic production of apoE and regulates its uptake by low-density lipoprotein receptor–related protein.[16] We found that insulin reduced plasma apoE levels, an effect that increased with age. In contrast, insulin increased CSF apoE concentrations for older subjects. Increased brain apoE levels have been reported in AD in association with polymorphisms in the promoter region of the *APOE* gene that influence protein expression.[34] Insulin may influence CNS apoE expression through interactions with these polymorphisms or through other factors, such as low-density lipoprotein receptor–related protein. We observed that insulin-induced elevations of CSF apoE levels were associated with attenuated increases in levels of proinflammatory cytokines IL-6 and TNF-α and with higher levels of IL-1α, an anti-inflammatory cytokine. This selective pattern suggests multiple insulin effects that modulate the role of apoE in response to inflammation.

Our results can be integrated into a model describing the role of peripheral insulin resistance and hyperinsulinemia in AD pathogenesis. During early pathogenesis, high plasma insulin levels raise plasma Aβ42 levels by promoting Aβ release and inhibiting its clearance by insulin-degrading enzyme. As a result, more Aβ42 may be transported from the periphery into the brain, or the transport of Aβ42 from the brain to the periphery may be obstructed. Failure of insulin to appropriately regulate transthyretin may also interfere with clearance of Aβ42 from the brain. Concomitantly, peripheral hyperinsulinemia increases CNS levels of IL-1β, IL-6, TNF-α, and F_2-isoprostane, agents that interact synergistically to promote Aβ synthesis (IL-6 and IL-1β) and reduce its clearance (TNF-α). The resulting elevations of Aβ levels provoke a correspondingly greater inflammatory response. Prolonged inflammation also likely exerts deleterious effects independent of Aβ that contribute to AD pathogenesis. For example, noradrenergic dysfunction that characterizes patients with insulin resistance may reduce norepinephrine's anti-inflammatory influence. Reduced availability or efficacy of apoE may affect its ability to inhibit IL-1β expression and thereby to modulate the inflammatory response.

Although this model has obvious relevance for diabetes mellitus, hyperinsulinemia and insulin resistance are widespread conditions that affect many nondiabetic adults with obesity, impaired glucose tolerance, cardiovascular disease, and hypertension. Our results provide a cautionary note for the current epidemic of such conditions, which, in the context of an aging population, may provoke a dramatic increase in the prevalence of AD. More encouragingly, greater understanding of insulin's role in AD pathogenesis may lead to novel and more effective strategies for treating, delaying, or even preventing this challenging disease.

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