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Natural Compounds Modulate Apolipoprotein E Gene and Protein Expression in Fibroblasts Derived from Young and Old Female Alzheimer's Patients

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Abstract Alzheimer's disease is the most common cause of dementia, accounting for approximately 60–80% of all dementia cases. Among the genetic risk factors identified, the apolipoprotein E (*APOE*) gene remains the strongest and most prevalent, impacting more than half of all Alzheimer's disease cases. Targeting the *APOE* gene with nutrients and natural compounds could potentially benefit Alzheimer's disease patients. In the search for such natural-derived compounds, we evaluated the effects of soy-derived estrogenic compounds such as genistein and daidzein, phospholipid precursors such as inositol and choline, phospholipid phosphatidylserine, and vitamins C and E as potent antioxidants on *APOE* gene transcription as well as APOE, APOE4, and Tau proteins expression in cells cultured in non-inflammatory and pro-inflammatory conditions. The study was conducted on fibroblasts derived from young and old female Alzheimer's disease patients and normal human dermal fibroblasts. Depending on culturing conditions, we observed very distinct patterns of changes of the *APO*E gene as well as APOE, APOE4, and Tau protein status, which differed significantly upon treatment with test compounds in the studied cell lines. To our knowledge, this study is recognizing for the first time, the link between natural compounds and important biomarkers of Alzheimer's disease identifying that selective estrogen receptor modulator, daidzein, and carbocyclic sugar, inositol, as compounds, which might have importance in Alzheimer's disease, and other dementia or other brain pathologies. Further in vivo and clinical studies are however warranted to support these findings.

Keywords: *APOE, Alzheimer's disease, phytoestrogens, phospholipid precursors, inflammation*

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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most frequent cause of memory loss, cognitive deficits, and behavioral changes accounting for up to 70% of all dementia cases. More than five million people in the United States are currently diagnosed with AD, with the estimated cases reaching up to 16 million by 2050, unless new treatments or interventions to prevent or delay its onset are identified. [1] Risk factors of AD and its progression include sex and aging. Nearly two-thirds of AD cases in the USA are assigned to post-menopausal women and those with rapid loss of estrogen. However, the more gradual decrease of androgens in aging men may run the risk of AD development as well. Other factors such as increased plasma triglycerides and low levels of high-density lipids (HDL) have been linked to increased risk of cognitive decline and AD as well. [2]

Human apolipoprotein E (APOE, APOE, APOE) is a ~ 34 kDa glycoprotein composed of 299 amino acids. [3] Its gene is polymorphic and consists of three alleles: *APOEe2*, *APOEe3*, and *APOEe4*, respectively, codifying different protein isoforms such as APOE2, APOE3 and APOE4. [4] These three APOE isoforms have a high level of homology and differ with only one or two amino acids: cysteine (C) or arginine (R) within their protein sequence in positions 112 and/or 158. Thus, APOE2 contains C112/C158, APOE3 contains C112/R158, and APOE4 contains R112/R158. [5,6] APOE isoforms APOE2, APOE3, and APOE4 are secreted primarily by the liver, brain astrocytes, skin, and to a lesser extent by the adrenal glands and macrophages. [7,8] They play a major and differential role in the absorption and transfer of dietary lipids between peripheral tissues to the brain. [9] However, they not only maintain lipid homeostasis by affecting cholesterol, triglycerides, and phospholipid metabolism, but are also involved in other biological processes such as mitochondrial metabolism, and inflammatory and immune responses. Also, their expression differs by cell type and

depends on dietary factors, which underscore their diverse pleotropic effects. [10,11,12,13,14,15]

Neuropathology studies have revealed that *APOEe4* homozygosity inheritance is the major risk in developing Late Onset Alzheimer's Disease (LOAD) and other neurodegenerative diseases. Its protein product, APOE4, has been associated with neuropathological features such as the presence of amyloid-β (Aβ) plaque in the extracellular space and the neurofibrillary tangles (NTF) in the intracellular space. [16,17] The main components of NFT are paired helical filaments (PHF) of hyperphosphorylated, microtubule associated protein Tau. Tau is a 50 kDa phosphoprotein that in normal conditions exists in a dynamic equilibrium between microtubule bound and free state controlled by the extent of its phosphorylation. It is differentially phosphorylated at over 30 sites in AD brains compared to normal brain tissue and is a well-established substrate of several kinases, including GSK-3β, P35, CDK5 and SAPKs (i.e., p38 and JNK). [18] *In vitro* experiments demonstrated that APOE3 binds with unphosphorylated Tau with stronger affinity than APOE4. [19] Interestingly, none of these isoforms are bound to phosphorylated Tau. The interaction occurs between the APOE3 lipid binding domain and the repeat domain (R) of Tau, which is also its microtubule binding domain. Also, hyperphosphorylation of this domain allows the PHF and thus the NFT formation. This would imply an isoform-specific interaction between APOE and Tau and the affinity of these interactions play a protective role from hyperphosphorylation and self-assembly of Tau. [20] APOE4 is found in the nucleus and functions as a transcription factor in the brain cells, binds to promoter of genes linked to microtubule disassembly, programed cell death, synaptic function, and aging. [21] It has been demonstrated that carrying one *APOEe4* allele increases AD risk by 2-3 times, while carrying two *APOEe4* alleles increases this risk 10-12 times, indicating that gene dose is sufficient to cause AD by age 80. [22,23] Therefore, efforts to reduce APOE4 protein expression or to change its function through small molecules structural correctors are therapeutic strategies for AD. [24,25]

However, it is worth noticing that although APOE4 protein is a risk factor for AD, its pathogenic role is still unclear. In addition, since *APOE4* allele expression is observed before the clinical onset of dementia, it is thought that age-related cognitive disorders are associated with earlier activation of the integrated stress response signals, most notably oxidative stress, and stressors such as environmental, metal and/or air pollution. [26,27,28] In addition, it has been shown that pro-inflammatory cytokines IL1β and TNFα, secreted by reactive microglia, are present at the highest levels in patients with a higher risk of developing AD. [29,30,31] These cytokines are also involved in activating stress activated protein kinases (SAPK) such as JNK and MAPK p38 signaling pathways, which can also be triggered in response to cellular genotoxic, osmotic, hypoxic, or oxidative stresses. [32,33,34] Moreover, IL1β and TNFα cytokines signaling pathways converge on the activation of promoters of genes related to innate immune inflammatory response such as granulocyte-macrophage colony stimulating factor-2 (GM-CSF, CSF2), cyclooxygenase-2 (COX2), and nitric oxide synthase. [35]

Nutrients and micronutrients affect gene expression in several ways. They may directly function as ligands for transcription factors such as vitamin A and vitamin D, which bind to their cognate receptors, translocate to the nucleus, and regulate transcription of their target genes. Dietary phytoestrogens such as the polyphenols genistein and daidzein that are known as selective estrogen receptor modulators (SERM), interact with estrogen receptor (ER) alpha and/or beta (ERα, ERβ) promoting conformational change on the coactivator or corepressor complexes, and selectively modulate estrogenic responses. [36,37,38] These interactions are dependent on ER subtype, their distribution across tissues, and cell type. [39,40] In addition, other nutrients such as vitamin C and vitamin E, metabolized in different pathways can alter concentration, stability, or OX-REDOX state of substrates. [41] Even moderate micronutrient deficiency, nutrient deprivation, and redox imbalances stresses can cause genome damage. [42] In addition, metabolic impact on gene expression of many natural compounds expressing either pro- or anti-inflammatory properties should be explored further regarding their clinical applications in AD. [43] Expanding research on natural compounds in modulating gene expression through epigenetic programming undoubtedly opened a new field of nutrigenomics. [44,45]

In this work we investigated the effects of a different class of natural compounds including vitamins C and E, which are potent antioxidants that have been investigated in relation to neuroinflammatory and neurodegenerative diseases, [46,47] nutrients involved in lipid metabolism (choline, inositol) and phosphatidylserine (the major component of cellular membranes), as well as soy-derived estrogenic polyphenols genistein and daidzein, and various plant extracts on expression of *APOE* at the mRNA. We also took into consideration the status of distinguished AD biomarkers at protein levels, such as APOE, its isoform APOE4, and microtubule associated protein Tau. We used young and old female dermal fibroblasts from AD patients as our research model which, due to their metabolic and biochemical relationships with neurons and their capacity to be reprogrammed into neuronal cells, have been used in studying various neurodegenerative aspects. [48] We compared them to human normal dermal fibroblasts (HNDF) from a healthy donor. The effects of test agents were evaluated under non-inflammatory and pro-inflammatory conditions (i.e., in the absence of and in the presence of IL1β, respectively).

2. Materials and Methods

Cell lines, test agents, and inhibitors. AG07887 cells were obtained from Coriell Institute for Medical Research (Camden, New Jersey, USA), which are skin-derived fibroblasts from an 18-year-old (at the time of sampling) female AD donor, with presenile dementia in large kindred (the donor is 50% at risk for Alzheimer's disease, the cell morphology is fibroblast-like). [49,50] The AG08269 cells were also obtained from Coriell Institute for Medical Research (Camden, New Jersey, USA), which are skin-derived fibroblasts from an 82-year-old (at the time of sampling) female (patient was a clinically

unaffected member of an Alzheimer's disease family; donor's diseased son and granddaughter were affected; the cell morphology is fibroblast-like). [51,52] Primary dermal normal fibroblasts were obtained from ATCC (Manassas, VA, USA). All cell types were grown in DMEM/F12 without phenol red (Gibco, USA) medium supplemented with charcoal stripped, sterile-filtered 10% fetal bovine serum (Sigma-Millipore, Burlington, MA, USA), and 1% penicillin-streptomycin at 37°C and 5% CO_2. Chaste tree (*Vitex agnus-castus*) extract was purchased from Monterey Bay Spice Company (Watsonville, CA, USA). Rosemary extract, inositol hexa-phosphate (food grade), and choline bitartrate (food grade) were obtained from Powder City (York, PA, USA). Phosphatidylserine (food grade) and soybean isoflavones (food grade) powder (containing 40% of isoflavones) were purchased from Bulk Supplements (Henderson, NV, USA). Genistein and daidzein (pharmaceutical grade) were from Sigma-Millipore (Burlington, MA, USA). Human recombinant interleukin 1β (IL1β) was obtained from R&D Systems (Minneapolis, MN, USA). The stock solutions at 1.0 mg/ml concentrations for all test compounds were done in DMSO.

Cell Culture Conditions and Treatments for RNA purification. The cells were trypsinized from the original culture flask, centrifuged at 1200 rpm at 4°C, seeded at $1.0x10^6$ in 35 mm cell culture dishes, and allowed to attach for 16h. Next, cells were treated for an additional 12h with either 0.001% DMSO (serving as a control) or with the test compounds (i.e., 1.0 µg/ml vitamin C, 1.0 µg/ml vitamin E, 1.0 µg/ml genistein, 1.0 µg/ml daidzein, 1.0 µg/ml soybean isoflavones, 1.0 µg/ml rosemary extract, 1.0 µg/ml chaste tree extracts, 1.0 µg/ml phosphatidylserine, 1.0 µg/ml inositol-hexa-phosphate, 1.0 µg/ml choline-bitartrate), respectively. In pro-inflammatory conditions, cells additionally were exposed to 50 pg/ml IL1β. Next, the cells were either lysed with RIPA buffer (Cell Signaling Danvers MA, USA) supplemented with 1mM PMSF immediately before and cell extracts used for western blot or were subjected to total RNA isolation and purification for RT-PCR analysis, as described below.

Real-time polymerase chain reaction (RT-PCR). Total RNA was obtained by standard method using a RNeasy Plus kit (Qiagen, Germantown, MD, USA) according to the manufacturer's instructions. The concentration of purified RNA was obtained by recording the absorbance at 260 nm using a Nano-drop 2000c spectrophotometer (Thermo Fisher, Wilmington, DE, USE) and the purity and integrity of the isolated RNA was checked by detecting the A260/A280 ratio (>1.8) and A260/A230 ratio (>1,5). Equal amounts of isolated RNA for each set of cells treatments, were reverse transcribed in total 20 µl reactions with an RT-QuantiTech kit (Qiagen, Germantown, MD, USA), and 2.0 µl of the resulting cDNA was amplified in 20 µl of final volume using QuantiNova SYBR-Green master mix for qRT-PCR in the Bio-Rad CFX96 thermocycler (Bio-Rad, Hercules, USA). The PCR program was started by denaturation step at 95°C for 5 min., followed by 40 cycles of denaturation (95°C for 10s) and combined annealing/extension (60°C for 30s). A final melting step was performed at 95°C for 10s. Relative expression of the target gene and/or reference gene obtained from individual treatments quantification data (Ct) was compared to the quantification data of non-treated cells (dCt). Relative fold expression of each sample was calculated with comparative threshold cycle (2^ddCt) LIVAK method. [53] Specific primers for target gene *APOE* and *CSF2* as well as reference (housekeeping) gene *GADPH* were obtained from Qiagen (Germantown, MD, USA). Each primer set was verified by the manufacturer to ensure amplification of a sole product and high PCR efficiency and their sequence is proprietary information, but they are commercially available from Qiagen (Germantown, MD, USA).

Western Blot (WB). Cells were grown in 10 cm plates, treated for 48h, and then collected, rinsed with 1 x PBS, and lysed with RIPA buffer (Cell Signaling, Danvers, MA, USA) supplemented with 1.0 mM PMSF immediately before use. Cells lysates were next centrifuged at 14,000 rpm at 4°C for 15 min., and protein concentration was determined with DC Protein Assay (Bio-Rad, Hercules, CA, USA). Next 15-30 µg/ml of total protein from the cell extract was resolved on 4-15% gradient polyacrylamide gel electrophoresis, transferred to a polyvinylidene difluoride 0.2 µm membrane (PVDF membrane, Bio-Rad Laboratories, Hercules, CA, USA), and probed with monoclonal antibody against APOE4 conjugated with HRP at 1:5000 dilution (Novus Biologicals, Co, USA), monoclonal antibody against APOE at 1:1000 dilution (Abcam, Cambridge, UK), monoclonal antibody against Tau at 1:1000 dilution (Novus Biologicals, Co, USA), and monoclonal anti-GADPH antibodies at 1:1500 dilution. Target proteins, if necessary, were detected with a polyclonal HRP-conjugated secondary antibody at 1:1500 dilution (Cell Signaling, Danvers, MA, USA). Signals were visualized by ECL detection kit (Azure Biosystems, Dublin, CA, USA) and the images were captured with Azure Series 600 system and auto-exposure settings.

Statistical analysis. All data are presented as means ± SD (n = 3). All experiments were performed at least three times each, at least in triplicates. The Student's two-tailed t test was used to determine statistically significant differences set at 0.05 levels. Statistical analysis was performed using GraphPad software.

3. Results

Considering increased risk of AD in females, we used dermal fibroblasts from female AD patients of different ages (i.e., 18 and 82 years old) and HNDF as suitable model to study an important metabolic aspect of AD, which includes expression of APOE (Figure 1A) and Tau proteins. The results presented in Figure 1B show that the expression level of endogenous *APOE* transcript differed among studied cell lines. By comparing percentage of *APOE* of gene transcripts in non-treated cells of a young AD donor, an old AD donor, and normal HNDF (arbitrarily set at 100%), we noticed that the cells from a young AD donor contained highest percentage of *APOE* transcripts (2500%) per equal amount of total RNA than non-treated cells from an old AD donor (140%) (Figure 1B). However, cellular level of APOE4 isoform under non-inflammatory cell culture conditions in all these cell types did not differ significantly (Figure 1C).

Effects of individual test agents on APOE expression in fibroblasts derived from young and old AD individuals and HNDF cultured under non-inflammatory conditions.

Subsequently, we tested the effects of the selection of natural compounds on *APOE* gene expression in skin fibroblasts from young and old AD donors as well as HNDF under regular (i.e., non-inflammatory) cell culture conditions. Figure 2A shows relative expression of *APOE* gene transcripts in treated samples compared to non-treated cell (set up as 100% control). Interestingly, test compounds differently affected *APOE* gene expression in fibroblasts derived from young and old AD donors. Most of the test compounds had significant inhibitory effects on *APOE* gene expression in AD cells ranging between 15% and 98% compared to control with a few increasing *APOE* gene expression by about 46-157%. As such, in cells derived from a young AD donor, all compounds significantly down-regulated *APOE* gene expression, except for choline, which stimulated *APOE* gene expression by 67% compared to control. In cells derived from an old AD donor, all compounds significantly down-regulated *APOE* gene expression, except for daidzein and chaste tree, and choline, which stimulated *APOE* gene expression by 167.5%, 50.5%, and 129%, respectively. Inositol showed no effect. The inhibitory effects of the natural compounds on *APOE* gene expression in HNDF were observed but less pronounced compared to young and old AD cells with only rosemary extract showing 68.6% stimulation of *APOE* gene expression.

The impact of treatments with these compounds on APOE, APOE4, and Tau protein levels was evaluated in the extracts of tested cell lines, as presented on Figure 2B. The results show that all these cell types expressed APOE, APOE4 and Tau in an amount detectable by Western blot. Interestingly however, cells derived from both young and old AD donors expressed less amount of APOE molecule when compared to HNDF. The test compounds had differential and cell specific effects on the expression of APOE and Tau protein, and they did not affect expression level of APOE4 molecule.

In cells from a young AD donor, daidzein and inositol showed inhibitory effect, while genistein and phosphatidylserine increased APOE molecule levels. The correlation between *APOE* gene expression and APOE protein expression was noticed with daidzein and inositol only. In old AD cells, all compounds increased APOE protein level except for vitamin C, which seemed to have no effect. Interestingly, in HNDF we observed the opposite, vitamin C showed no effect on APOE protein level, contrary to its effect on the expression of *APOE* gene, and all other test compounds noticeably reduced APOE protein cellular content.

We also checked the status of Tau protein. The 50 kDa band on Western blots corresponding to Tau protein was detected in all cell extracts (Figure 2B). Interestingly, its level in young AD cells was lower in the presence of daidzein and phosphatidylserine, and to a lesser degree with inositol and genistein. Vitamin C and choline showed no effect. The level of Tau protein in old AD cells was barely detectable upon treatment with daidzein, phosphatidylserine, inositol, and choline, but increased upon treatment with vitamin C and to a lesser degree with genistein. In HNDF, an opposite effect was observed. Here, vitamin C had no effect on Tau protein level in contrast to genistein, daidzein, phosphatidylserine, inositol, and choline, which increased its level.

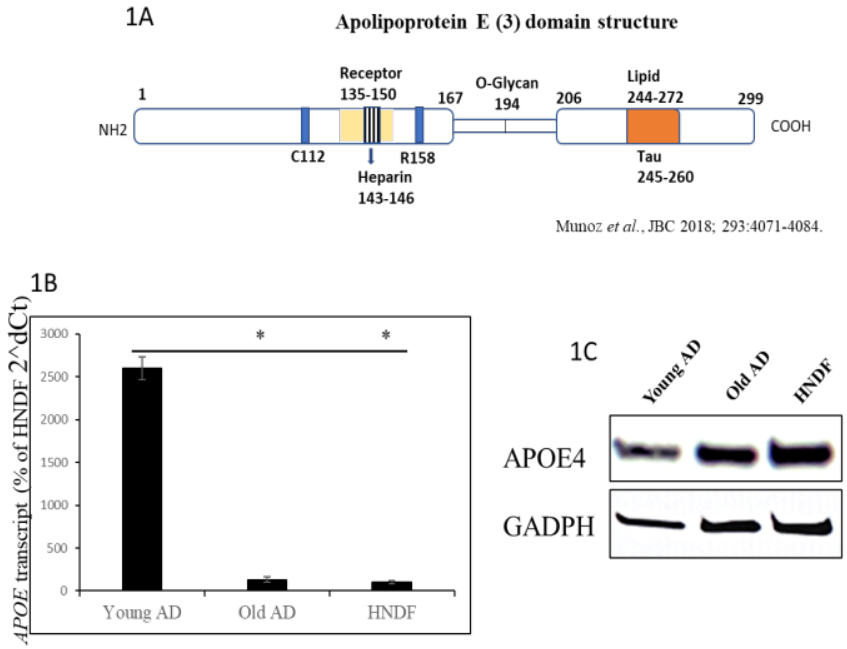

Figure 1. (1A) Rendered image of the APOE protein structural domains as reported by Munoz et al [68]. (1B) Expression status of *APOE* gene in non-treated AD fibroblasts and HNDF cultured in non-inflammatory conditions. (1C) Western blot analysis of APOE4 molecule levels in HNDF and AD fibroblasts cultured in non-inflammatory conditions as described in Materials and Methods section. Significant differences between HNDF and AD fibroblasts are presented as * $p \leqslant 0.001$

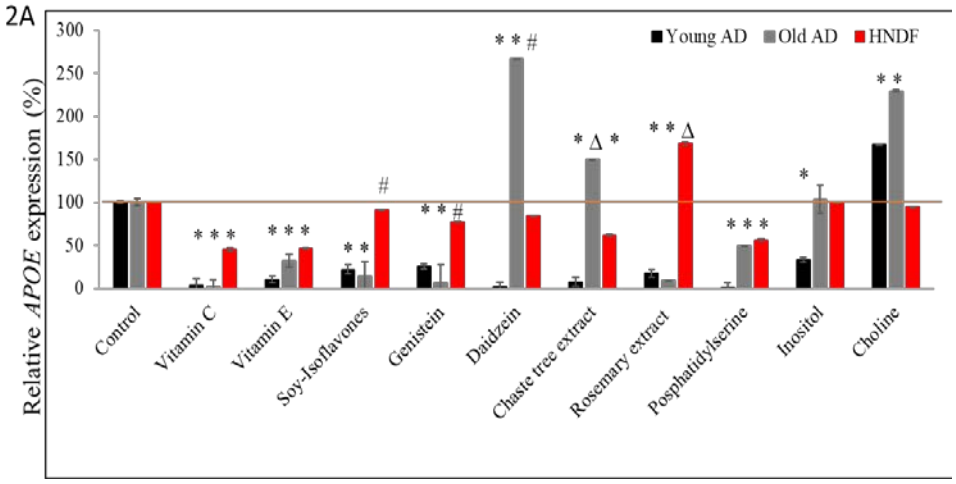

Figure 2. *APOE* expression status in AD fibroblasts and HNDF cultured in non-inflammatory conditions. (2A) Effect of different natural compounds on *APOE* gene expression at mRNA level. The status of *APOE* gene upon treatment of cells with 1.0 μg/ml of selected agents, respectively, for 12h was assessed with 0.7 μg of mRNA by RT-qPCR as described in Materials and Methods section. Significant differences between treatment and control are presented as # $p \leq 0.05$, Δ $p \leq 0.01$, * $p \leq 0.001$; control - 0.01% DMSO

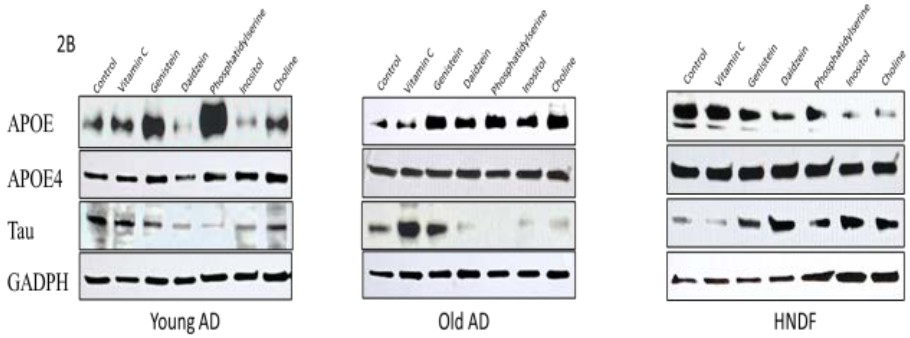

Figure 2. B. Western blot analysis of APOE, APOE4, and Tau molecules upon treatment of cells with 1.0 μg/ml of selected agents for 48h, respectively, as described in Materials and Methods section

Effects of individual nutrients on APOE expression in HNDF and in fibroblasts derived from young and old AD individuals cultured under pro-inflammatory conditions.

Considering that AD pathology includes increased inflammatory microenvironment, we first confirmed that *CSF2* gene expression increases upon treatment with IL1β, since it was reported that *CSF2* promoter is robustly induced by IL1β. [35,54] As presented in Figure 3A, the inflammatory cytokine IL1β induced *CSF2* gene transcription in young, old, and normal fibroblasts, but by different orders of magnitude compared to non-treated cells. The strongest induction was observed in fibroblasts from old AD cells reaching ~16,000-fold increase, followed with normal HNDF with ~7800-fold increase, and young AD cells with less than ~ 4000-fold increase in *CSF2* gene expression. Thus, we concluded that all cell types tested were highly responsive to inflammatory cytokine IL1β, although to a different degree.

Subsequently we evaluated the effects of test compounds on the *APOE* gene expression under inflammatory conditions (Figure 3B), and towards this, we exposed the cells simultaneously to IL1β and our test compounds. We observed that IL1β differently affects *APOE* gene expression in the studied cell lines. Although IL1β increased *APOE* gene expression in HNDF by about 154.3% compared to unstimulated cells, it did not significantly affect its expression in the cells from an old AD donor and had an inhibitory effect in the cells from a young AD donor (50.3% decrease in *APOE* gene expression). Interestingly, at protein level no differences in APOE4 molecule upon co-treatment with IL1β and respective test compound was observed in an old AD cell and HNDF, although in young AD cells, treatment with IL1β mildly increased APOE4 cellular level, and daidzein blandly reduced its expression but all the rest of the test compound, respectively, showed to have no effect (Figure 3C).

The individual test compounds differently affected *APOE* gene expression in HNDF and fibroblasts from young and old AD donors, when cultured under inflammatory conditions (i.e., in the presence of 50 pg/ml IL1β) compared to IL1β treated cells control (set up at 100%). As such, in HNDF most of the test compounds stimulated *APOE* gene expression (i.e., 47%-424%), except for vitamin E and inositol, which inhibited *APOE* gene expression by about 23% and 83%, respectively, and phosphatidylserine, which showed no effect. Also, in cells derived from a young AD donor, most of the compounds up-regulated *APOE* gene expression, except for-daidzein, which inhibited *APOE* gene expression by about 68%, respectively. Phosphatidylserine, inositol, and choline had no effect when compared to IL1β treated control. Yet, the same test compounds displayed different effects in the

cells derived from an old AD donor. Here, interestingly, the compounds that inhibited *APOE* gene expression were soybean isoflavones, genistein, and inositol (i.e., 64%, 20%, and 30%, respectively), whereas vitamin C, chaste tree extract, rosemary extract, and choline up-regulated *APOE* gene expression (i.e., 56%, 41%, 106%, and 46%, respectively). The rest of the test compounds (i.e., daidzein and phosphatidylserine) did not show any effect.

Western blot revealed that the level of APOE protein in young AD cells co-treated with IL1β and the test compound, respectively, did not always correlate with results obtained from RT-qPCR (compare Figure 3B and Figure 3C). As such, genistein that strongly activated *APOE* gene expression, did not show a difference with APOE protein expression level from IL1β induced control. Similarly, vitamin C, which up-regulated *APOE* gene expression, showed no effect on APOE protein level. Daidzein markedly inhibited *APOE* gene expression and reduced APOE protein to the level corresponding to untreated control (Figure 3C). Moreover, phosphatidylserine, inositol, and choline, that showed no effect on *APOE* gene, also did not show any effect on APOE protein cellular content. Co-treatments of cells from an old AD donor with IL1β and test compounds, revealed *APOE* gene expression and APOE protein cellular levels with all these compounds, except for choline–showed to be indifferent. Interestingly, similarly, in HNDF, vitamin C, genistein, which up-regulated *APOE* gene expression, and phosphatidylserine that demonstrated no effect, have shown to inhibit APOE protein level in cells. Inositol, which inhibited *APOE* gene expression, visibly increased APOE protein level. Choline mildly augmented *APOE* gene expression and caused the APOE molecule level to strongly increase, while daidzein up-regulated *APOE* gene expression and showed to have no significant effect on APOE protein level.

Consistently, we also checked the status of Tau protein level in the cells (Figure 3C). Here, we noticed two bands corresponding to ~ 50 kDa and ~ 48 kDa upon treatment with IL1β in a young AD cell line, but not in an old AD cell or HNDF. Tau protein level in young and old AD cells was higher than in HNDF upon treatment with IL1β as well. In addition, with IL1β treatment, the Tau protein level was elevated in young AD cells and more profoundly in cells from an old AD donor, but not in HNDF where Tau protein detection was barely achievable. Moreover, all test compounds, except phosphatidylserine decreased its cellular level in young AD cells. Compounds such as genistein and daidzein showed to have the same decreasing effect on Tau level in old AD cells, but vitamin C, phosphatidylserine, inositol, showed to have no effect and choline increased Tau expression. However, in HNDF, daidzein and phosphatidylserine together with inositol and choline increased Tau protein level, while vitamin C and genistein had no effect.

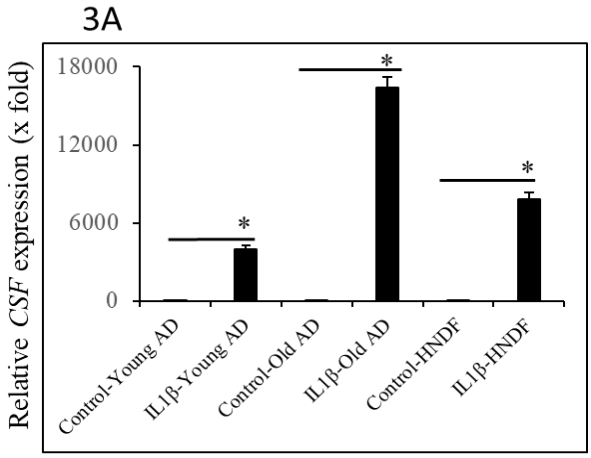

Figure 3. A: Status of *CSF* gene expression upon treatment with 50 pg/ml of IL1β only in AD fibroblasts and HNDF after 12h incubation. Significant differences between treatment and control are presented as * $p \leqslant 0.001$; control - 0.01% DMSO

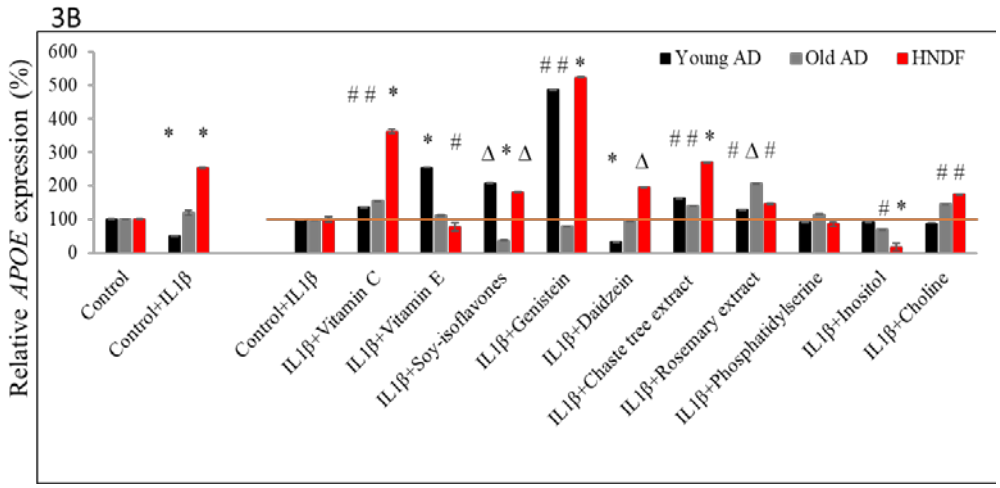

Figure 3. B: *APOE* expression status in AD fibroblasts and HNDF cultured in inflammatory conditions. Effect of different natural compounds on *APOE* gene expression at mRNA level. Status of *APOE* gene upon co-treatment of cells with 50 pg/ml of IL1β and 1.0 μg/ml of selected agents, respectively, for 12h and was assessed with 0.7 μg of mRNA by RT-qPCR as described in Materials and Methods section. Significant differences between treatment and control are presented as # $p \leqslant 0.05$, Δ $p \leqslant 0.01$, * $p \leqslant 0.001$

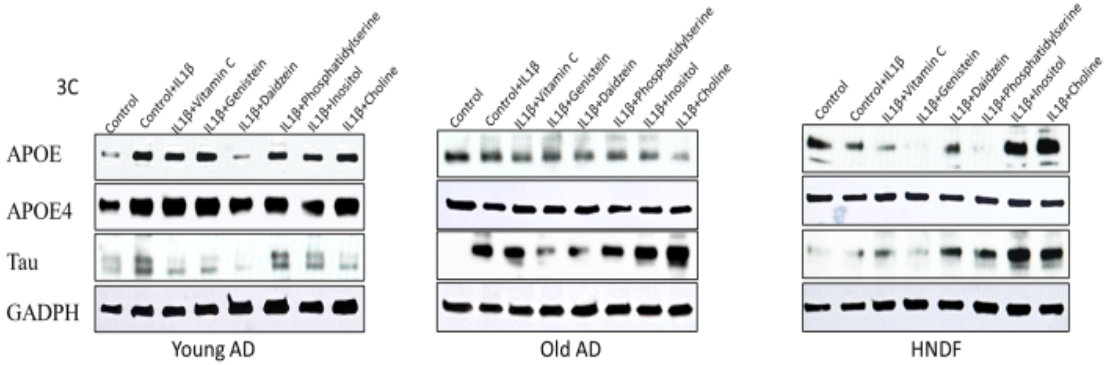

Figure 3. C: Western blot analysis of APOE, APOE4, and Tau proteins upon co-treatment of cells with 50 pg/ml of IL1β and 1.0 μg/ml of selected agents for 48h, respectively, as described in Materials and Methods section

4. Discussion

We evaluated the effects of selected natural compounds on *APOE* gene and protein expression in relation to AD using skin fibroblasts derived from young and old female AD patients in parallel with normal HNDF, under regular and pro-inflammatory conditions, and evaluated the outcome relevant to this pathology. In general, we observed that the profiles of *APOE* gene expression differed and were cell type specific, as well as dependent on the applied test compound and the regular or pro-inflammatory conditions. For example, vitamins C and E had strong inhibitory effect on the relative amount of *APOE* gene transcripts in all studied cell lines cultured in normal conditions, whereas the same vitamins have differential effect on the cell lines under inflammatory conditions, e.g., vitamin C up-regulated *APOE* gene in all tested fibroblasts, whereas vitamin E up-regulated it in fibroblasts from a young AD donor, but not in cells from an old AD donor or HNDF.

Interestingly, in some instances we observed that changes of *APOE* at mRNA level upon treatment with individual test compounds, did not always positively correlate with APOE protein levels. As an example, upon treatment with vitamin C, a compound widely investigated in relation to its role as an antioxidant and endogenous neuromodulator, [46,55] no significant differences were observed in APOE protein levels in all tested cell lines cultured in normal conditions (compare Figure 2A and 2B). That also applied to its APOE4 isoform, but not Tau protein, whose levels were altered upon vitamin C treatment. Yet, in HNDF cultured under inflammatory conditions vitamin C reduced the level of APOE, while not affecting the APOE4 isoform. The inverse correlation between expression of *APOE* gene and the APOE protein level was also very noticeable in the presence of other test compounds such as genistein and phosphatidylserine in cells from young and old AD donors under non-inflammatory conditions. Interestingly, however this difference again was not observed at the levels of APOE4 isoform. Less surprisingly, in normal HNDF, correlation between APOE protein level and expression of *APOE* gene was noticed upon treatment with test compounds, except for vitamin C as well as inositol and choline. These two last compounds reduced APOE molecule level. Interestingly however, SERM compound daidzein as well as phosphatidylserine and inositol markedly decreased Tau protein levels in both AD cell lines. The same compounds, though, increased Tau protein content in HNDF.

An even more interesting outcome was achieved when these studied cells were cultured in inflammatory conditions. For example, phosphatidylserine decreased APOE protein content in HNDF, although no such effect was revealed at its mRNA level, and the levels of APOE4. Also, inverse correlation between mRNA and APOE protein expression was detected upon treatment with genistein, and upon inositol treatment, which shows that it inhibited *APOE* gene expression while APOE protein had a substantial increase compared to IL1β induced control. Strong correlation was observed in fibroblasts derived from a young AD donor with daidzein that decreased APOE gene expression and APOE protein level, and mildly diminished APOE4 isoform expression (compared with IL1β induced control). In fibroblasts from an old AD donor only choline showed increasing effect on *APOE* gene expression with modest inhibition of the APOE molecule and no effect on its APOE4 form.

The inverse correlation between the number of *APOE* transcripts expression and APOE protein levels in the central nervous system (CNS) has been described. For example, Gottschalk *et al.* reported augmented levels of *APOE* mRNA in postmortem brain tissues of AD patients compared to control tissue samples. However, studies analyzing APOE protein levels in the CNS proved to be inconstant by showing either higher, lower, or unchanged levels of APOE protein in cerebrospinal fluid of AD patients compared to healthy individuals. [56, 57, 58, 59, 60] This demonstrates the difficulty in correlating *APOE* mRNA with APOE protein levels. The differences can be attributed, but not limited to protein accumulation in the cytoplasm, post-translational modifications such as lipidation, the lag time difference in measuring transcriptional and translational response and its relation to circadian rhythm, and/or by APOE post-transcriptional control of gene expression. [61] Moreover, APOE molecules are found in different subcellular compartments such as the nucleus, mitochondria, and mitochondrial ER-associated membranes (MAMs), where the synthesis of phospholipids occurs. [62] However, nuclear localization signals (NLS) or mitochondria localization signals (MLS) in APOE protein have not yet been found, [5] thus we could speculate that it is possible that the mRNA of the *APOE* gene could be transported to the cell target

compartments for its subsequent rapid, on-site translation according to the cell biological needs. [63]

Since loss of female sex hormones (estrogens) together with APOE4 homozygosity are important risk factors for AD development in post-menopausal women, our findings that compounds known as SERMs (i.e., genistein and daidzein), affect *APOE* gene expression, is encouraging.

Interestingly, in this work we found out that in the absence of IL1β, genistein and daidzein have cell specific effect on *APOE* gene transcription in cells derived from a young and old AD patient. In cells from a young AD patient, both genistein and daidzein inhibited *APOE* gene expression, but in old AD donor cells genistein down-regulated *APOE* gene expression and daidzein up-regulated it. It is worth noticing that in normal HNDF the *APOE* gene expression pattern was similar under genistein and daidzein treatments. Other natural substances like chaste tree extract, contain phytoestrogens mimicking endogenous estrogen function, thus reports about its alleviating menstrual pain can be found. [64] Our study did not focus on specific cellular mechanisms but since SERMs are ligands, like estrogen (E2), of ERα or ERβ, they induce specific conformational changes of the liganded ER-coactivator complex, thus, activating or repressing transcriptional and non-genomic estrogenic responses. Importantly, the outcome of these responses is specific depending on the cell type or tissue's relative content and expression of nuclear receptors and their coactivators (SRC1, SRC2 and/or SRC3) within the cell. [40,65]

Regarding the effects of phosphatidylserine, inositol, and choline on normal HNDF cells cultured in non-inflammatory conditions, we only noticed significant inhibitory effect of phosphatidylserine on *APOE* at mRNA level and the protein level (Figure 2A, and Figure 2B), without any effect on APOE4 isoform. Although inositol and choline did not reveal any effect on *APOE* gene expression, it was noticed that they significantly inhibited APOE protein, with APOE4 isoform largely unaffected. Interestingly, in cells from a young donor we observed that both phosphatidylserine and inositol inhibited *APOE* at mRNA and the protein level while phosphatidylserine had a stimulatory effect on APOE protein and inositol had an inhibitory effect. Moreover, no effect on the levels of APOE4 isoform was observed. In fibroblasts from an old AD donor, phosphatidylserine down-regulated *APOE* gene expression, whereas choline increased its expression and inositol did not change it. At the protein level, all these compounds elevated APOE but not APOE4. In pro-inflammatory conditions, none of these compounds revealed a profound effect. Interestingly, a modest increase in APOE4 molecule was observed in a young AD cell upon treatment with IL1β, but a co-treatment with all test compounds, respectively, did not affect its level. It is worth noticing that in normal HNDF, inositol hexa-phosphate inhibited the IL1β induced *APOE* gene expression, and increased APOE protein levels in the cell extracts. However, these effects of inositol at the protein level were not observed in AD patients' cells suggesting a possible regulation of phospholipid metabolism under stress conditions. [66]

Since microtubule associated protein Tau (MAPT) is an important biomarker of AD and its association with APOE is known, we included testing our selected groups of natural compounds on the Tau protein expression. The western blots probed with anti-Tau (2N4R) antibody, produced a band of 50 kDa (i.e., the projected molecular weight) in young AD, old AD, and HNDF cultured in non-inflammatory conditions. In cells from a young AD donor, a Tau level decreased, upon treatment with all tested compounds, except for vitamin C, genistein, and choline, but its almost complete abolishment was obtained with daidzein and phosphatidylserine, respectively. In cells from an old AD donor a Tau 50 kDa band was observed in non-treated cells upon vitamin C and genistein, which augmented Tau level in the cell extracts, but inositol and choline had markedly inhibitory effect. In HNDF, vitamin C decreased the level of Tau, in contrast to the rest of test compounds, which increased its cellular level. Interestingly, in pro-inflammatory conditions, increased level of Tau protein upon treatment with Ilβ was observed only in cells from AD donors. In addition, in a young AD cell line, two bands of around 50 kDa or less were detected, as opposed to the old AD cell line and HNDF, where only one expected 50 kDa band was spotted. We did not explore this issue in more detail; however, it could be due to degradation or phosphorylation status Tau. In the same young AD cells, vitamin C, genistein, daidzein, inositol, and choline inhibited Tau protein level, but in old AD cells, genistein, daidzein, and phosphatidylserine revealed similar inhibitory effects compared to IL1β induced control. In contrast, in HNDF Tau has been elevated by daidzein as well as phosphatidylserine, inositol, and choline, but not by genistein. Thus, this data provides evidence for a promising effect of genistein and daidzein as an agent inhibiting Tau protein expression in AD cells lines under pro-inflammatory conditions.

Summary: We have characterized fibroblasts from a young AD donor, an old AD donor and normal HNDF, in their response to selected natural compounds including vitamins, isoflavones (known as selective estrogen receptor modulators or SERMs) and precursors of the phospholipid synthesis, such as inositol hexa-phosphate, and choline, including the main membrane structural phospholipid, phosphatidylserine. The effects of test compounds were evaluated on the *APOE* gene transcriptional level and the protein expression of the AD biomarkers such as APOE, APOE4 and the microtubule associated protein Tau. The studies were performed under non-inflammatory and pro-inflammatory conditions (in the absence of or the presence of cytokine IL1β). The effects of the natural compounds were cell specific in their response to modulate the *APOE* gene transcription or APOE, APOE4 and Tau protein content in the total cellular extracts, and dependent on the state of the cells (non-inflammatory versus pro-inflammatory conditions). Our work demonstrated that APOE and Tau differentially responded to various nutritional stimuli, whereas APOE4 isoform was insensitive to these compounds' treatment (except for daidzein) and support the hypothesis that APOE4 carriers may have diminished response due to lower content of APOE and/or APOE4 loss of function. [67]. Our study indicates that daidzein and inositol warrant further investigations due to their promising activities relevant in AD.

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Conflict of Interest

No conflict of interest declared.

Abbreviations

Alzheimer's disease (AD); apolipoprotein E (APOE); cerebrospinal fluid (CSF); high-density lipids (HDL); cysteine (C) or arginine (R); Late Onset Alzheimer's Disease (LOAD); amyloid-β (Aβ); neurofibrillary tangles (NTF); paired helical filaments (PHF); stress activated protein kinases (SAPK); granulocyte-macrophage colony stimulating factor-2 (GM-CSF, CSF2); cyclooxygenase-2 (COX2); selective estrogen receptor modulators (SERM); estrogen receptor (ER); human normal dermal fibroblasts (HNDF); polyvinylidene difluoride 0.2 μm membrane (PVDF membrane); nuclear localization signals (NLS); mitochondria localization signals (MLS); microtubule associated protein Tau (MAPT)

References

[1] https://www.alz.org/media/images/2017-facts-and-figures.pdf (Accessed March 2024)

[2] Barron A M, Pike CJ, "Sex hormones, aging, and Alzheimer's disease." *Frontiers in bioscience (Elite edition),* vol. 4,(3). 976-997. Jan 2012.

[3] Mahley, RW, Rall SC Jr. "Apolipoprotein E: far more than a lipid transport protein." *Annual review of genomics and human genetics,* vol. 1. 507-537. 2000.

[4] Tudorache IF, Trusca VG, Gafencu AV. "Apolipoprotein E - A Multifunctional Protein with Implications in Various Pathologies as a Result of Its Structural Features." *Computational and structural biotechnology journal,* vol. 15. 359-365. Jun. 2017.

[5] Rueter J, Rimbach G, Huebbe P. "Functional diversity of apolipoprotein E: from subcellular localization to mitochondrial function." *Cellular and molecular life science:CMLS,* vol.79(9). 499. Aug. 2022.

[6] Weisgraber KH, Mahley RW. "Apoprotein (E –A-II) complex of human plasma lipoproteins. I. Characterization of this mixed disulfide and its identification in a high-density lipoprotein subfraction." *The Journal of biological chemistry* vol. 253(17). 6281-6288. 1978.

[7] Kockx M, Traini M, Kritharides L. "Cell-specific production, secretion, and function of apolipoprotein E." *Journal of molecular medicine (Berlin, Germany)* vol. 96(5). 361-371. 2018.

[8] Mahley RW. "Apolipoprotein E: from cardiovascular disease to neurodegenerative disorders." *Journal of molecular medicine (Berlin, Germany),* vol. 94(7). 739-746. 2016.

[9] Chernick D, Ortiz-Valle S, Jeong A, Swaminathan SK, Kandimalla KK, Rebeck GW, et al., "High-density lipoprotein mimetic peptide 4F mitigates amyloid-β-induced inhibition of apolipoprotein E secretion and lipidation in primary astrocytes and microglia." *Journal of neurochemistry* vol. 147(5). 647-662. 2018.

[10] Lane-Donovan C, Herz J. "High-Fat Diet Changes Hippocampal Apolipoprotein E (ApoE) in a Genotype- and Carbohydrate-Dependent Manner in Mice." *PloS one,* vol.11(2), e0148099. 1 Feb. 2016.

[11] Fernández-Calle R, Konings SC, Frontiñán-Rubio J, García-Revilla J, Camprubí-Ferrer L, Svensson M, et al., "APOE in the bullseye of neurodegenerative diseases: impact of the APOE genotype in Alzheimer's disease pathology and brain diseases." *Molecular neurodegeneration,* vol. 17(1). 62. 24 Sep. 2022.

[12] Huebbe P, Nebel A, Siegert S, Moehring J, Boesch-Saadatmandi C, Most E, et al., "APOE ε4 is associated with higher vitamin D levels in targeted replacement mice and humans." *FASEB journal: official publication of the Federation of American Societies for Experimental Biology* vol. 25(9). 3262-3270. 2011.

[13] Chin D, Hagl S, Hoehn A, Huebbe P, Pallauf K, Grune T, et al., "Adenosine triphosphate concentrations are higher in the brain of APOE3- compared to APOE4-targeted replacement mice and can be modulated by curcumin." *Genes & nutrition* vol. 9(3). 397. 2014.

[14] Huebbe P, Lange J, Lietz G, Rimbach G. "Dietary beta-carotene and lutein metabolism is modulated by the APOE genotype." *BioFactors (Oxford, England)* vol. 42(4). 388-396. 2016.

[15] Grimm MOW, Michaelson DM, Hartmann T. "Omega-3 fatty acids, lipids, and apoE lipidation in Alzheimer's disease: a rationale for multi-nutrient dementia prevention." *Journal of lipid research,* vol. 58(11). 2083-2101. 2017.

[16] Chen Y, Strickland MR, Soranno A, Holtzman DM. "Apolipoprotein E: Structural Insights and Links to Alzheimer Disease Pathogenesis." *Neuron,* vol. 109(2). 205-221. 2021.

[17] Nagy Z, Esiri MM, Jobst KA, Morris JH, King EM, McDonald B, et al., "Relative roles of plaques and tangles in the dementia of Alzheimer's disease: correlations using three sets of neuropathological criteria." *Dementia (Basel, Switzerland)* vol. 6(1). 21-31. 1995.

[18] Ferrer I, Gomez-Isla T, Puig B, Freixes M, Ribé E, Dalfó E, et al., "Current advances on different kinases involved in tau phosphorylation, and implications in Alzheimer's disease and tauopathies." *Current Alzheimer research* vol. 2(1). 3-18. 2005.

[19] Fleming LM, Weisgraber KH, Strittmatter WJ, Troncoso JC, Johnson GV. "Differential binding of apolipoprotein E isoforms to tau and other cytoskeletal proteins" *Experimental neurology,* vol. 138(2). 252-260. 1996.

[20] Strittmatter WJ, Saunders AM, Goedert M, Weisgraber KH, Dong LM, Jakes R, et al., "Isoform-specific interactions of apolipoprotein E with microtubule-associated protein tau: implications for Alzheimer disease." *Proceedings of the National Academy of Sciences of the United States of America,* vol. 91(23). 11183-11196. 1994.

[21] Theendakara V, Peters-Libeu CA, Spilman P, Poksay KS, Bredesen DE, Rao RV. "Direct Transcriptional Effects of Apolipoprotein E." *The Journal of neuroscience: the official journal of the Society for Neuroscience,* vol. 36(3). 685-700. 2016.

[22] Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al., "Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families." *Science (New York, N.Y.)* vol. 261(5123). 921-923. 1993.

[23] Troutwine BR, Hamid L, Lysaker CR, Strope TA, Wilkins HM. "Apolipoprotein E and Alzheimer's disease." *Acta pharmaceutica Sinica B,* vol. 12(2). 496-510. 2022.

[24] Li Y, Macyczko JR, Liu CC, Bu G. "ApoE4 reduction: An emerging and promising therapeutic strategy for Alzheimer's disease." *Neurobiology of aging* vol. 115. 20-28. 2022.

[25] Mahley RW, Huang Y. "Small-molecule structure correctors target abnormal protein structure and function: structure corrector rescue of apolipoprotein E4-associated neuropathology." *Journal of medicinal chemistry* vol. 55(21). 8997-9008. 2012.

[26] Costa-Mattioli M, Walter P. "The integrated stress response: From mechanism to disease." *Science (New York, N.Y.)* vol. 368(6489). 2020. eaat5314.

[27] Kim EK, Choi EJ. "Compromised MAPK signaling in human diseases: an update." *Archives of toxicology,* vol. 89(6). 867-882. 2015.

[28] Moser VA, Workman MJ, Hurwitz SJ, Lipman RM, Pike CJ, Svendsen CN. "Microglial transcription profiles in mouse and human are driven by APOE4 and sex." *iScience* , vol. 24(11). 103238. 5 Oct. 2021.

[29] de Leeuw SM, Kirschner AWT, Lindner K, Rust R, Budny V, Wolski WE, et al., "APOE2, E3, and E4 differentially modulate cellular homeostasis, cholesterol metabolism, and inflammatory response in isogenic iPSC-derived astrocytes." *Stem cell reports* vol. 17(1). 110-126. 2022.

[30] Tan ZS, Beiser AS, Vasan RS, Roubenoff R, Dinarello CA, Harris TB, Benjamin EJ, Au R, Kiel DP, Wolf PA, Seshadri S. "Inflammatory

markers and the risk of Alzheimer disease: the Framingham Study." *Neurology* vol. 68(22). 1902-1908. 2007.

[31] Andreasson KI, Bachstetter AD, Colonna M, Ginhoux F, Holmes C, Lamb B, et al. "Targeting innate immunity for neurodegenerative disorders of the central nervous system." *Journal of neurochemistry* vol. 138(5). 653-693. 2016.

[32] Novoa C, Salazar P, Cisternas P, Gherardelli C, Vera-Salazar R, Zolezzi JM, et al., "Inflammation context in Alzheimer's disease, a relationship intricate to define." *Biological research* vol. 55(1) 39. 23 Dec. 2022.

[33] Iannucci J, Sen A, Grammas P. "Isoform-Specific Effects of Apolipoprotein E on Markers of Inflammation and Toxicity in Brain Glia and Neuronal Cells In Vitro." *Current issues in molecular biology* vol. 43(1). 215-225. 27 May. 2021.

[34] Pamies D, Sartori C, Schvartz D, González-Ruiz V, Pellerin L, Nunes C, et al., "Neuroinflammatory Response to TNFα and IL1β Cytokines Is Accompanied by an Increase in Glycolysis in Human Astrocytes In Vitro." *International journal of molecular sciences* vol. 22(8) 4065. 14 Apr. 2021.

[35] Newton R, Hart LA, Stevens DA, Bergmann M, Donnelly LE, Adcock IM, et al., "Effect of dexamethasone on interleukin-1beta-(IL-1beta)-induced nuclear factor-kappaB (NF-kappaB) and kappaB-dependent transcription in epithelial cells." *European journal of biochemistry* vol. 254(1). 81-89. 1998.

[36] Dutertre M, Smith CL. "Molecular mechanisms of selective estrogen receptor modulator (SERM) action." *The Journal of pharmacology and experimental therapeutics* vol. 295(2). 431-437. 2000.

[37] Fernández-Suárez ME, Daimiel L, Villa-Turégano G, Pavón MV, Busto R, Escolà-Gil JC, et al., "Selective estrogen receptor modulators (SERMs) affect cholesterol homeostasis through the master regulators SREBP and LXR." *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* vol. 141. Sep. 2021. 111871.

[38] Katzenellenbogen BS, Choi I, Delage-Mourroux R, Ediger TR, Martini PG, Montano M, Sun J, et al., "Molecular mechanisms of estrogen action: selective ligands and receptor pharmacology." *The Journal of steroid biochemistry and molecular biology* vol. 74,5 (2000): 279-85.

[39] Dasgupta S, Lonard DM, O'Malley BW. "Nuclear receptor coactivators: master regulators of human health and disease." *Annual review of medicine* vol. 65. 279-292. 2014.

[40] Johnson AB, O'Malley BW. "Steroid receptor coactivators 1, 2, and 3: critical regulators of nuclear receptor activity and steroid receptor modulator (SRM)-based cancer therapy." *Molecular and cellular endocrinology* vol. 34(2). 430-439. 2012.

[41] Walsh CT, Tang Y, *The Chemical Biology of Human Vitamins*. Published by The Royal Society of Chemistry Publisher, London, UK. 2019.

[42] Fenech M, Baghurst P, Luderer W, Turner J, Record S, Ceppi M, et al., "Low intake of calcium, folate, nicotinic acid, vitamin E, retinol, beta-carotene and high intake of pantothenic acid, biotin and riboflavin are significantly associated with increased genome instability--results from a dietary intake and micronucleus index survey in South Australia." *Carcinogenesis* vol. 26(5). 991-999. 2005.

[43] Koppula S, Akther M, Haque ME, Kopalli SR. "Potential Nutrients from Natural and Synthetic Sources Targeting Inflammaging-A Review of Literature, Clinical Data and Patents." *Nutrients* vol. 13(11). 4058. 13 Nov. 2021.

[44] Klotz LO, Carlberg C. "Nutrigenomics and redox regulation: Concepts relating to the Special Issue on nutrigenomics." *Redox biology* vol. 68. 102920. 2023.

[45] Carlberg C, Velleuer E. "Nutrition and epigenetic programming." *Current opinion in clinical nutrition and metabolic care* vol. 26(3). 259-265. 2023.

[46] Kumar RR, Singh L, Thakur A, Singh S, Kumar B. "Role of Vitamins in Neurodegenerative Diseases: A Review." *CNS & neurological disorders drug targets* vol. 21(9). 766-773. 2022.

[47] Jabeen K, Rehman K, Akash MSH, Nadeem A, Mir TM. "Neuroprotective and Cardiometabolic Role of Vitamin E: Alleviating Neuroinflammation and Metabolic Disturbance Induced by $AlCl_3$ in Rat Models." *Biomedicines* vol. 11(9). 2453. 4 Sep. 2023.

[48] Sattarov R, Toresson H, Orbjörn C, Mattsson-Carlgren N. "Direct Conversion of Fibroblast into Neurons for Alzheimer's Disease Research: A Systematic Review." *Journal of Alzheimer's disease: JAD* vol. 95(3). 805-828. 2023.

[49] Foncin JF, Salmon D, Supino-Viterbo V, Feldman RG, Macchi G, Mariotti P, et al., "Démence présénile d'Alzheimer transmise dans une famille étendue" [Alzheimer's presenile dementia transmitted in an extended kindred]. *Revue neurologique* vol. 141(3). 194-202. 1985.

[50] St George-Hyslop PH, Tanzi RE, Polinsky RJ, Haines JL, Nee L, Watkins PC, et al., (Myers RH, Feldman RG, Pollen D, Drachman D, et al.) "The genetic defect causing familial Alzheimer's disease maps on chromosome 21." *Science (New York, N.Y.)* vol. 235(4791). 885-90. 1987.

[51] Rossi A, Rigotto G, Valente G, Giorgio V, Basso E, Filadi R, et al. "Defective Mitochondrial Pyruvate Flux Affects Cell Bioenergetics in Alzheimer's Disease-Related Models." *Cell reports* vol. 30(7). 2332-2348. 2020. e10.

[52] Mendonsa G, Dobrowolska J, Lin A, Vijairania P, Jong YJ, Baenziger NL. "Molecular profiling reveals diversity of stress signal transduction cascades in highly penetrant Alzheimer's disease human skin fibroblasts." *PloS one* vol. 4(2). e4655. 2009.

[53] Livak KJ, Schmittgen TD. "Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method." *Methods (San Diego, Calif.)* vol. 25(4). 402-408. 2001.

[54] Wang WY, Tan MS, Yu JT, Tan L. "Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease." *Annals of translational medicine* vol. 3(10). 136. 2015.

[55] Ballaz SJ, Rebec GV. "Neurobiology of vitamin C: Expanding the focus from antioxidant to endogenous neuromodulator." *Pharmacological research* vol. 146. 104321. 2019.

[56] Gottschalk WK, Mihovilovic M, Roses AD, Chiba-Falek O. "The Role of Upregulated APOE in Alzheimer's Disease Etiology." Journal of Alzheimer's disease & Parkinsonism vol. 6(1). 209. 2016.

[57] Baker-Nigh AT, Mawuenyega KG, Bollinger JG, Ovod V, Kasten T, Franklin EE, et al., "Human Central Nervous System (CNS) ApoE Isoforms Are Increased by Age, Differentially Altered by Amyloidosis, and Relative Amounts Reversed in the CNS Compared with Plasma." *The Journal of biological chemistry* vol. 291(53). 27204-27218. 2016.

[58] Cruchaga C, Kauwe JS, Nowotny P, Bales K, Pickering EH, Mayo K, et al., "Cerebrospinal fluid APOE levels: an endophenotype for genetic studies for Alzheimer's disease." *Human molecular genetics* vol. 21(20). 4558-4571. 2012.

[59] Talwar P, Sinha J, Grover S, Agarwal R, Kushwaha S, Srivastava MV, et al. "Meta-analysis of apolipoprotein E levels in the cerebrospinal fluid of patients with Alzheimer's disease." *Journal of the neurological sciences* vol. 360. 179-187. 2016.

[60] Martínez-Morillo E, Hansson O, Atagi Y, Bu G, Minthon L, Diamandis EP, et al. "Total apolipoprotein E levels and specific isoform composition in cerebrospinal fluid and plasma from Alzheimer's disease patients and controls." *Acta neuropathologica* vol. 127(5). 633-643. 2014.

[61] Srivastava RA. "Regulation of the apolipoprotein E by dietary lipids occurs by transcriptional and post-transcriptional mechanisms." *Molecular and cellular biochemistry* vol. 155(2). 153-162. 1996.

[62] Vance JE. "Phospholipid synthesis in a membrane fraction associated with mitochondria." *The Journal of biological chemistry* vol. 265(13). 7248-7256. 1990.

[63] Jung H, Gkogkas CG, Sonenberg N, Holt CE. "Remote control of gene function by local translation." *Cell* vol. 157(1). 26-40. 2014.

[64] Berger D, Schaffner W, Schrader E, Meier B, Brattström A. "Efficacy of Vitex agnus castus L. extract Ze 440 in patients with pre-menstrual syndrome (PMS)." *Archives of gynecology and obstetrics* vol. 264(3). 150-153. 2000.

[65] Jiang Y, Gong P, Madak-Erdogan Z, Martin T, Jeyakumar M, Carlson K, et al., (Khan I, Smillie TJ, Chittiboyina AG, Rotte SC, Helferich WG, Katzenellenbogen JA, Katzenellenbogen BS.) "Mechanisms enforcing the estrogen receptor β selectivity of botanical estrogens." *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* vol. 27(11). 4406-4418. 2013.

[66] Suliman M, Case KC, Schmidtke MW, Lazcano P, Onu CJ, Greenberg ML. "Inositol depletion regulates phospholipid metabolism and activates stress signaling in HEK293T cells." *Biochimica et biophysica acta. Molecular and cell biology of lipids* vol. 1867(6). 159137. 2022.

[67] Yang LG, March ZM, Stephenson RA, Narayan PS. "Apolipoprotein E in lipid metabolism and neurodegenerative disease." *Trends in endocrinology and metabolism: TEM* vol. 34(8). 430-445. 2023.

[68] Muñoz SS, Li H, Ruberu K, Chu Q, Saghatelian A, Ooi L, et al. "The serine protease HtrA1 contributes to the formation of an extracellular 25-kDa apolipoprotein E fragment that stimulates neuritogenesis." *The Journal of biological chemistry* vol. 293(11). 4071-4084. 2018.

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