

## APP and PS-1 mutations induce brain oxidative stress independent of dietary cholesterol: implications for Alzheimer's disease

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### Abstract

Epidemiological and biochemical studies strongly implicate a role for cholesterol in the pathogenesis of Alzheimer's disease (AD). Mutation in the PS-1 and APP genes, which increases production of the highly amyloidogenic amyloid  $\beta$ -peptide (A $\beta$ 42), is the major cause of familial AD. The AD brain is under significant oxidative stress, including protein oxidation and lipid peroxidation. In the present study, protein oxidation and lipid peroxidation were compared in the brain homogenates from knock-in mice expressing mutant human PS-1 and APP in relation to the intake of dietary cholesterol. The APP and PS-1 mice displayed increased oxidative stress as measured by protein oxidation and lipid peroxidation, independent of dietary cholesterol. These results are discussed with reference to proposed therapeutic strategies of AD. © 2004 Elsevier Ireland Ltd. All rights reserved.

**Keywords:** A $\beta$ (1–42); Cholesterol; Presenilin-1; Amyloid precursor protein; Protein carbonyl; Lipid peroxidation; Oxidative stress; Alzheimer's disease

Cholesterol has been implicated in the pathogenesis of amyloid plaques in Alzheimer's disease (AD). Recent epidemiological studies suggest that cholesterol plays a significant role in the development of Alzheimer's disease (AD), because patients taking cholesterol synthesis inhibitors (statins) have reduced incidence of the disease [9,19]. Longitudinal studies have suggested a relationship between elevated midlife cholesterol levels and late-life cognitive impairment or AD [10,14]. In the case of increased cholesterol, a change in membrane fluidity/dynamics and other effects due to increased membrane cholesterol may lead to increased production of A $\beta$  [15]. Today, considerable evidence suggests that brain cholesterol homeostasis is strongly coupled with brain amyloid metabolism [20], although cholesterol's role within the pathogenetic cascade of excessive A $\beta$  deposition in the brain of AD patients is currently unclear.

In vivo, a cholesterol-rich diet is reported to influence central steps of brain amyloid pathogenesis in animals [17]. Amyloid  $\beta$ -peptide (A $\beta$ ) is the main component of senile plaques, which are a pathologic hallmark of AD. The 42-mer (A $\beta$ 42) aggregates trigger the amyloid cascade and considerable data that supports this hypothesis comes from genetic studies of autosomal dominant inherited forms of AD, as disease-linked mutations in the genes of APP and PS-1 result in increased production of A $\beta$ 42 [21]. In the present study, we investigated the interaction of the presence of the APP [8] and PS-1 [6] mutations and elevated dietary cholesterol upon oxidative stress.

Animals were fed regular mouse chow (chow) or a high cholesterol diet (H). High cholesterol diet was from Test diets (#7178) and contained 5% cholesterol by weight, which we then further diluted by 50% with standard Purina lab chow for mice. The final cholesterol concentration was therefore 2.5% so that the mice would actually consume it. Brain samples were obtained from Tuscon, where the tissues were flash frozen and shipped overnight on dry ice to Lexington. All

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chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless stated otherwise. The 7-week-old mice were sacrificed and the brain was dissected to isolate sections of the anterior sensorimotor cortex (as this region had more concentration of plaques and also because of its cholinergic innervations from the forebrain), followed by homogenizing and sonicating the brains in media-I (0.32 M sucrose, 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 0.1 mM MgCl<sub>2</sub> and 1 mM PMSF). The amount of protein in the samples was measured by the BCA method using bovine serum albumin as a standard [3]. Protein carbonyls are an index of protein oxidation and were determined as described previously [5]. Briefly, the brain homogenates (5 µg of protein) were derivatized with 10 mM 2,4-dinitrophenylhydrazine in the presence of 5 µl of 12% SDS for 20 min at room temperature. The samples were neutralized with 7.5 µL of the neutralization solution (2 M Tris in 30% glycerol). Derivatized protein samples were blotted onto nitrocellulose membrane with a slot-blot apparatus (250 ng/lane). The membrane was then washed with wash buffer (10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% Tween 20), blocked by incubation in the presence of 5% BSA, followed by incubation with rabbit polyclonal anti-DNP antibody (from Chemicon International) as primary antibody for one hour. The membranes were washed with wash buffer and further incubated with alkaline phosphatase (ALP)-conjugated goat anti-rabbit antibody as the secondary antibody for one hour. Blots were developed using Sigma fast tablets (BCIP/NBT) and were quantified using Scion Image (PC version of Macintosh compatible NIH Image) software. Levels of HNE were quantified by slot-blot analysis as described previously [11]. Anti-HNE antibody (from Alpha Diagnostics) raised in rabbit was used as the primary antibody. Statistical significance was determined using ANOVA. Chow-diet-fed wild-type mice were taken as a control in this study and the *P* values <0.05 were considered significant.

The results with chow diet suggests that, APP, APP/PS-1, and PS-1 mutations all lead to significant increased protein oxidation indexed by protein carbonyls (Fig. 1) and increased lipid peroxidation (Fig. 2) indexed by lipid peroxidation product, HNE, with the effects of PS-1 mutation and APP/PS-1 mutation larger than that for just APP mutation. In the high cholesterol diet group, cholesterol by itself, in the wild-type, APP, APP/PS-1, and PS-1 significantly increases protein oxidation (Fig. 1) and HNE levels (Fig. 2) compared to wild-type mouse receiving chow diet. If the comparison of high cholesterol diets only is made, then in both protein carbonyls and HNE measures, there is no change in either oxidative stress marker when wild-type is compared to APP, APP/PS-1, or PS-1 mutant mice brain. These results suggest that, once the oxidative stress due to mutations occurs in brain, no further effects of cholesterol were found.

Brain cholesterol homeostasis is thought to be independent of peripheral fluctuations in cholesterol content. This implies that dietary cholesterol should have no effect on brain cholesterol concentrations and therefore should not affect cerebral processing of APP. However, Sparks et al. [18]

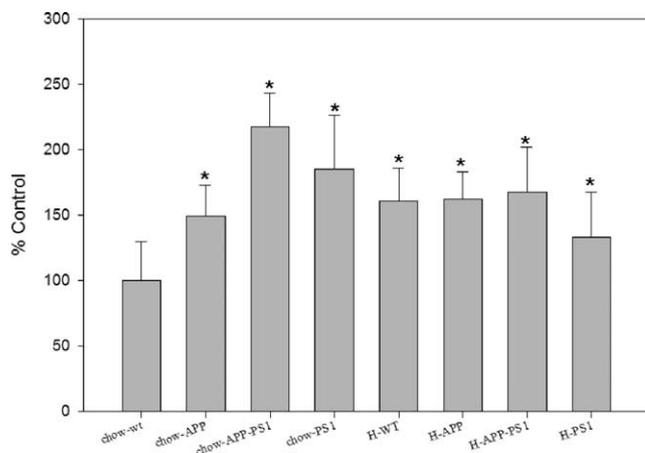


Fig. 1. Protein carbonyl content was determined as described in “Materials and Methods”. Chow: regular mouse chow diet; H: high cholesterol diet. The results presented are the mean  $\pm$  S.E.M. expressed as percentage of control values. Each data point is an average of five independent determinations. The mean absolute value of protein oxidation in arbitrary units in the chow-wild-type (control) was 266. Statistical comparison was made using ANOVA ( $n = 5$  independent measurements from different mice; \* $P < 0.005$ ).

provided the first evidence that increased dietary cholesterol affected amyloid production. Our present findings are consistent with the above findings, since we observed a significant increase in protein carbonyls and HNE, indices of oxidative stress, presumably due to A $\beta$  [4], in the high cholesterol diet compared to chow diet. Studies on PS/APP transgenic mice using 7-dehydrocholesterol- $\Delta$ 7-reductase inhibitor (a class of cholesterol-lowering drug inhibiting the final step of cholesterol biosynthesis) have shown that amyloidosis can be prevented by treatment with these types of drugs [17]. Epidemiological data suggest that statin treatment is effective at reducing the risk of AD, and statins have been shown to re-

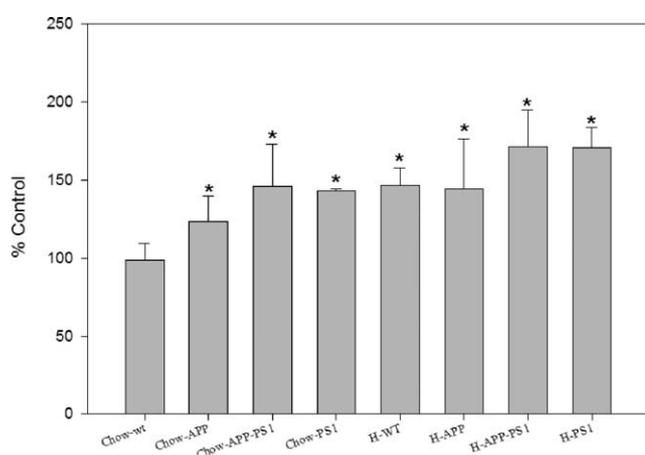


Fig. 2. HNE levels were determined as described in “Materials and Methods”. Chow: regular mouse chow diet; H: high cholesterol diet. The results presented are the mean  $\pm$  S.E.M. expressed as percentage of control values. Each data point is an average of five independent determinations from different mice. The mean absolute value of lipid peroxidation in arbitrary units in the chow-wild-type (control) was 235. Statistical comparison was made using ANOVA ( $n = 5$ ; \* $P < 0.005$ ).

duce both intracellular and extracellular levels of A $\beta$ 40 and A $\beta$ 42 peptides in primary cultures of hippocampal neurons transfected with human APP [7]. It is known that cholesterol accumulates in senile plaques and tangles of AD patients and in APPsw transgenic mice [13] and that cholesterol does not bind to free A $\beta$ , but binds avidly to aggregated A $\beta$  [1]. Thus, extracellular cholesterol may be a seed required for the initiation of the deposition of aggregated A $\beta$ . Binding of cholesterol to aggregated A $\beta$  may also prevent clearance of A $\beta$  to the periphery because of which we see an increased oxidative stress in the high-cholesterol dietary samples than the chow-dietary samples. Further elucidation of this hypothesis is underway.

An apparent complexity about cholesterol and AD exists. On one hand, elevated cholesterol leads to increased A $\beta$  production [18]. On the other hand, the brain makes its own cholesterol, which is protected, from exchange with plasma lipoproteins [2]. Statins may be effective in reducing the risk of AD by more than one mechanism. In addition to lowering the brain cholesterol levels by blocking HMG-CoA reductase, statins have other protective effects. For example, statins may increase the concentration of Tau protein and possibly A $\beta$  in the CSF, thereby making both proteins less available to brain structures [16]. Statins also are reported to upregulate endothelial nitric oxide synthase, which, through increased NO production, could scavenge superoxide radical anion, thereby reducing oxidative stress [12]. Additional studies will be necessary to determine if these notions have merit.

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