

Effects of chronic alcohol consumption on expression levels of APP and A β -producing enzymes

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Chronic alcohol consumption contributes to numerous diseases, including cancers, cardiovascular diseases, and liver cirrhosis. Epidemiological studies have shown that excessive alcohol consumption is a risk factor for dementia. Along this line, Alzheimer's disease (AD) is the most common form of dementia and is caused by the accumulation of amyloid- β (A β) plaques in neurons. In this study, we hypothesized that chronic ethanol consumption is associated with pathological processing of APP in AD. To investigate the relationship between chronic alcohol consumption and A β production, brain samples from rats fed an alcohol liquid diet for 5 weeks were analyzed. We show that the expression levels of APP, BACE1, and immature nicastrin were increased in the cerebellum, hippocampus, and striatum of the alcohol-fed group compared to the control group. Total nicastrin and PS1 levels were induced in the hippocampus of alcohol-fed rats. These data suggest that the altered expression of APP and A β -producing enzymes possibly contributes to the chronic alcohol consumption-mediated pathogenesis of AD. [BMB reports 2011; 44(2): 135-139]

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia and is characterized by a progressive loss of memory and other detrimental cognitive changes (1). Amyloid- β (A β) plaques found in AD (2, 3) are produced by the sequential proteolytic cleavage of amyloid- β precursor protein (APP), a large, type I membrane protein, by β -secretase (BACE1) and γ -secretase. Oligomeric forms of overproduced A β lead to neuronal and synaptic loss, resulting in cognitive decline and AD.

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AD has multiple etiological factors, including genetic, environmental, and general lifestyle factors (1). Interestingly, there has been increasing support for diet playing a role in AD (4-6). A number of dietary factors such as saturated fatty acids, higher calorie intake, and excessive alcohol (7) have been reported to increase the risk of dementia and AD (8). In particular, a number of studies have shown the close association of alcoholic consumption and dementia, although the exact mechanisms are still not known (9-13). Studies have been shown that frequent alcohol uptake by rats can cause mitochondrial dysfunction in neurons, leading to neurodegeneration (14). Excessive alcohol consumption may contribute to structural changes in the brain as well as impairment of cognitive function (15). Chronic alcoholism results in global brain atrophy with typical points of predilection for neurotoxic effects (16). Alcohol can also induce cortical and subcortical cerebral atrophy (15, 16), atrophic changes in the cerebellum (17), enlargement of the cerebrospinal fluid space (15, 18), and hippocampal volume loss (16, 19-21). Moreover, genetic factors (22), gender (23), age (24), amount of consumed ethanol, and the duration of alcohol consumption (25) may all affect the degree of alcohol-related brain damage.

Recent research has focused on the pathophysiological mechanisms underlying alcohol-induced brain damage (26). However, the exact mechanisms by which alcohol causes brain tissue damage are still not known. The current study was designed to investigate the effects of chronic alcohol consumption on the expression of APP and APP processing enzymes, including β -secretase (BACE1) and γ -secretase.

RESULTS AND DISCUSSION

APP protein, a type I membrane protein, is cleaved by α -, β -, and γ -secretases (27, 28). Sequential cleavage of APP by β - and γ -secretases generates A β peptide, the main component of the cerebrovascular amyloid plaques that accompany AD (27, 28). Since excess alcohol consumption can be a risk factor for AD, we investigated the effects of chronic alcohol consumption on the expression of genes involved in A β production. We first compared the APP protein levels between the alcohol-fed

group and control group in different brain regions. Equal amounts of total protein were resolved by SDS-PAGE, and APP protein was detected by Western blotting. APP protein levels were significantly increased in the cerebellum, hippocampus, and striatum regions of the alcohol-fed group compared to the control group (Fig. 1). In the frontal cortex and cerebral cortex, a tendency for increased levels of APP protein was observed in the chronic alcohol-fed group, although it did not reach statistical significance (Fig. 1).

BACE1 fulfills most of the requirements expected for a candidate β -secretase (27, 28). We therefore examined BACE1 expression in the brains of rats (Fig. 2 and Fig. 3). Similar to the altered APP expression, we found that the levels of BACE1 protein were significantly higher in the cerebellum, hippocampus, and striatum brain regions of the alcohol-fed group compared to the control group. No changes were observed in the expression levels of BACE1 in the frontal cortex and cerebral cortex brain areas. Together, these data suggest that chronic ethanol consumption can affect the expression levels of APP and APP processing enzymes.

Oxidative stress-induced BACE1 expression is regulated by γ -secretase activity, providing evidence of a feed-forward pathogenic mechanism of AD in which oxidative stress increases A β production, which then enhances oxidative stress (29). Molecular identification of the γ -secretase complex has shown that four different members are essential for this enzymatic activity: presenilin 1 (PS1), anterior pharynx-defective (APH-1), presenilin enhancer 2 (PEN-2), and nicastrin (NCT) (27, 28). Molecular genetic analyses identified mutations in PS1 as the cause of many instances of early-onset familial AD and have established PS1 as an integral membrane protein as

well as a catalytic component of γ -secretase (3). To further investigate the relationship between alcohol consumption and APP processing, we measured the expression levels of PS1, the catalytic component of γ -secretase. We found that chronic alcohol consumption significantly increased PS1 immunoreactivity levels in the hippocampus with no detectable changes in the cerebral cortex when compared to the control group (Fig. 3). Of note, the levels of PS1 immunoreactivity were somewhat decreased in the frontal cortex, although it did

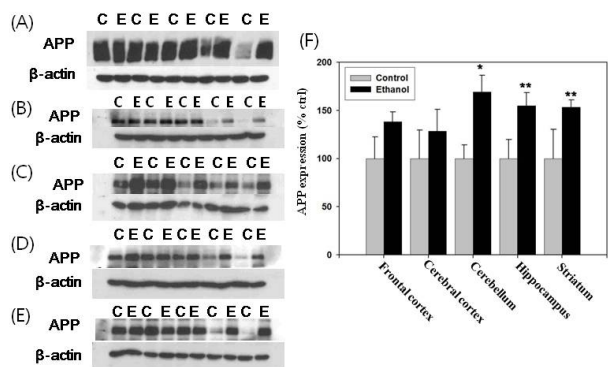


Fig. 1. Effects of chronic alcohol consumption on APP expression in various rat brain regions. Western blot analysis of APP expression was performed in different brain regions of the alcohol liquid diet-fed group and control isocaloric diet-fed group. Samples from the frontal cortex (A), cerebral cortex (B), cerebellum (C), hippocampus (D), and striatum (E) were analyzed. (F) APP expression was quantified by densitometry using NIH image J software, and data are represented as the means \pm SD, $n = 9$ (* $P < 0.05$, ** $P < 0.01$) compared to control group. C, control diet; E, ethanol diet.

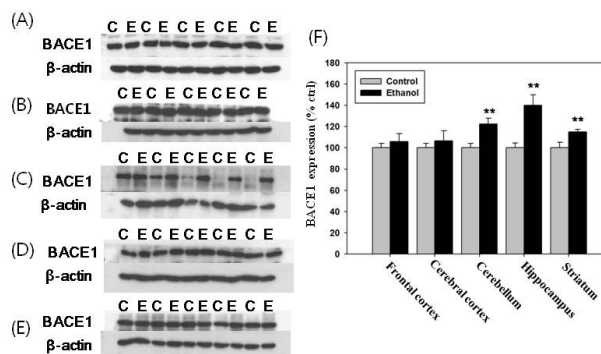


Fig. 2. Effects of chronic alcohol consumption on BACE expression in various rat brain regions. Western blot analysis of BACE1 expression was performed in different brain regions of the alcohol liquid diet-fed group and control isocaloric diet-fed group. Samples from the frontal cortex (A), cerebral cortex (B), cerebellum (C), hippocampus (D), and striatum (E) were analyzed. (F) BACE1 expression was quantified by densitometry using NIH image J software, and data are represented as means \pm SD, $n = 9$ (** $P < 0.01$) compared to control group. C, control diet; E, ethanol diet.

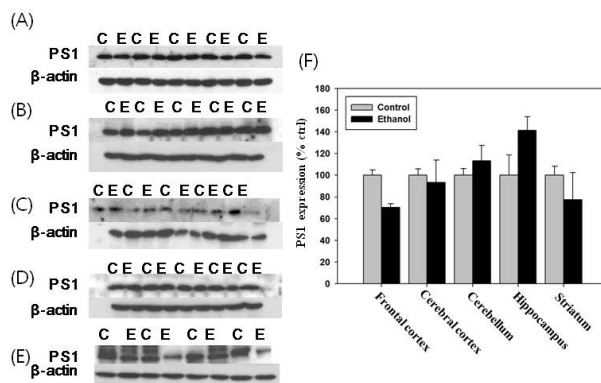


Fig. 3. Effects of chronic alcohol consumption on PS1 expression in various rat brain regions. Western blot analysis of PS1 expression was performed in different brain regions of the alcohol liquid diet-fed group and control isocaloric diet-fed group. Samples from the frontal cortex (A), cerebral cortex (B), cerebellum (C), hippocampus (D), and striatum (E) were analyzed. (F) PS1 expression was quantified by densitometry using NIH image J software, and data are represented as means \pm SD, $n = 9$ compared to control group. C, control diet; E, ethanol diet.

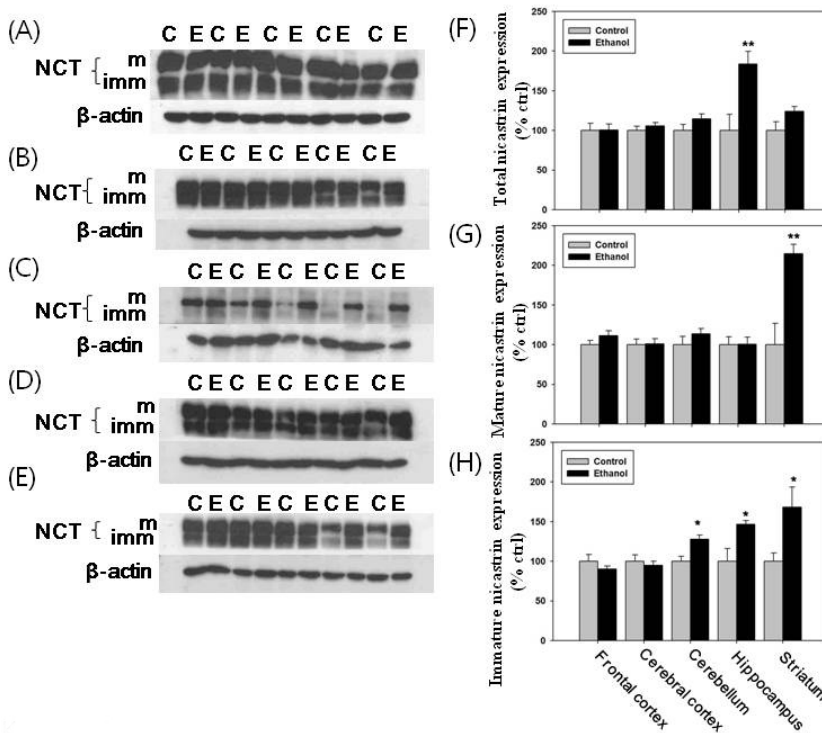


Fig. 4. Effects of chronic alcohol consumption on nicastrin expression in various rat brain regions. Western blot analysis of nicastrin expression was performed in different brain regions of the alcohol liquid diet-fed group and control isocaloric diet-fed group. Samples from the frontal cortex (A), cerebral cortex (B), cerebellum (C), hippocampus (D), and striatum (E) were analyzed. The immature form (I) migrated at ~105 kDa, and the mature form (M) migrated at ~125 kDa. Total (F), mature (G), and immature (H) nicastrin expression was quantified by densitometry using NIH image J software, and data are represented as means \pm SD, $n = 9$ (* $P < 0.05$, ** $P < 0.01$) compared to control group. C, control diet; E, ethanol diet.

not reach statistical significance (Fig. 3).

Nicastrin binds γ -secretase substrates directly, indicating that nicastrin is a receptor for γ -secretase substrates (30). Therefore, we decided to compare the nicastrin levels between the alcohol-fed group and control group in different brain regions. As expected, the mature form (~125 KDa) and immature form (~105 KDa) of nicastrin were detected in various brain regions (Fig. 4). Total nicastrin levels were increased in the hippocampus of the alcohol-fed group compared to the control group. The levels of immature nicastrin were higher in the hippocampus, striatum, and cerebellum brain regions of the alcohol-fed group, whereas the mature form of nicastrin was increased in the striatum.

Heavier alcohol intake at middle age is associated with increased risk of late-life dementia, especially among individuals that carry the apolipoprotein E gene (ApoE) 4 allele (31). However, the specific pathological mechanism involved remains unknown. This is the first study showing the possible link between altered APP processing and the development of neurodegeneration and AD upon chronic alcohol consumption. The current study shows that repeated administration of alcohol was able to modulate the immunoreactivity levels of APP, BACE1, and γ -secretase subunits, including PS1 and nicastrin. Our results also show that there was differential regulation of these subunits in several brain regions. Although chronic feeding with alcohol shifted the expression levels of

APP and APP processing enzyme subunits, future studies should clarify the functional significance of such changes either in cortical or subcortical brain structures to the development of AD.

In summary, we showed that the expression of APP and BACE1 was increased in the cerebellum, hippocampus, and striatum brain regions of the alcohol-fed group compared to the control diet group. PS1 expression and total nicastrin levels were slightly higher in the hippocampus. Immature nicastrin expression was higher in the cerebellum, hippocampus, and striatum brain regions of the alcohol-fed group compared to the control diet group. Mature nicastrin was detected more in the striatum of alcohol-fed rats. Brain region-specific changes in the expression of APP processing-related genes may be critical to developing alcohol-induced dementia and AD. The hippocampus, a region of the brain considered critical for learning and memory, appears to be extremely vulnerable in AD (32). Chronic stress and AD cause similar cognitive impairments and pathological hallmarks, specifically in the hippocampus (32). Along the same line, increased expression of all four examined genes in the hippocampus may be critical to the chronic alcohol-mediated pathogenesis of AD. These data suggest the possible link between altered APP processing and brain damage upon chronic alcohol consumption.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 150-170 g were purchased from Hyundai-Bio Animal Breeding Company (Korea). Rats were maintained in a room with controlled temperature and humidity ($25 \pm 1^\circ\text{C}$ and $\pm 5\%$, respectively) with a 12 h light-dark cycle. Animal studies were conducted in accordance with the Animal Research Committee of Sungkyunkwan University.

Liquid diets

After a 1-week adaptation period, experimental animals were given a liquid diet containing alcohol. Chronic ethanol feeding was carried out by incorporation of ethanol into a nutritionally adequate liquid diet obtained from Dyets Inc. (Pennsylvania, USA). The diet provided 1 kcal/ml, with 35% of total calories from fat, 47% from carbohydrates, and 18% from protein. Ethanol-treated animals were fed a liquid diet in which maltose dextrin was isocalorically replaced by ethanol, which provided 36% of the total calories (33). Animals at a body weight of 150-170 g were fed a liquid diet containing 40 g of ethanol per liter 2 days, followed by the final formula containing 50 g of ethanol per liter (34).

Western blotting analysis

Brain tissue was homogenized in PBS containing protease inhibitor cocktail (Roche Diagnostics) and phosphatase inhibitor cocktail (Roche Diagnostics) according to the manufacturer's instructions. Briefly, the homogenates were centrifuged for 10 min at 4°C , the pellet was discarded, and the supernatants were kept at -70°C . Then, 20 μg of total protein were separated by 10% SDS-PAGE and transferred to PVDF membrane (Millipore). Anti-APP (zymed), anti-BACE1 (ABR), anti-Nicastrin (epitomics), and anti-PS1-CTF (Chemicon) antibodies were used to measure the expression levels of each protein. Anti- β -actin antibody (Thermo) was used as a loading control.

Statistical analysis

All values were reported as the means \pm SD. The overall significance of the data was examined by one-way analysis of variance (ANOVA). Differences between the groups were considered significant at $P < 0.05$.

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REFERENCES

1. Kalaria, R. N., Maestre, G. E., Arizaga, R., Friedland, R. P., Galasko, D., Hall, K., Luchsinger, J. A., Ogunniyi, A., Perry, E. K., Potocnik, F., Prince, M., Stewart, R., Wimo, A., Zhang, Z. X. and Antuono, P. (2008) Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors. *Lancet Neurol.* **7**, 812-826.
2. Tanzi, R. E. and Bertram, L. (2005) Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. *Cell* **120**, 545-555.
3. Mattson, M. P. (2004) Pathways towards and away from Alzheimer's disease. *Nature* **430**, 631-639.
4. Gasior, M., Rogawski, M. A. and Hartman, A. L. (2006) Neuroprotective and disease-modifying effects of the ketogenic diet. *Behav. Pharmacol.* **17**, 431-439.
5. Lau, F. C., Shukitt-Hale, B. and Joseph, J. A. (2007) Nutritional intervention in brain aging: reducing the effects of inflammation and oxidative stress. *Subcell. Biochem.* **42**, 299-318.
6. Dosunmu, R., Wu, J., Basha, M. R. and Zawia, N. H. (2007) Environmental and dietary risk factors in Alzheimer's disease. *Expert Rev. Neurother.* **7**, 887-900.
7. Panza, F., Capurso, C. and Solfrizzi, V. (2008) Alcohol use, thiamine deficiency, and cognitive impairment. *JAMA* **299**, 2853-2854; author reply 2854-2855.
8. Ramesh, B. N., Rao, T. S., Prakasam, A., Sambamurti, K. and Rao, K. S. Neuronutrition and Alzheimer's disease. *J. Alzheimers. Dis.* **19**, 1123-1139.
9. Ruitenberg, A., van Swieten, J. C., Witteman, J. C., Mehta, K. M., van Duijn, C. M., Hofman, A. and Breteler, M. M. (2002) Alcohol consumption and risk of dementia: the Rotterdam Study. *Lancet* **359**, 281-286.
10. Mukamal, K. J., Kuller, L. H., Fitzpatrick, A. L., Longstreth, W. T., Jr., Mittleman, M. A. and Siscovick, D. S. (2003) Prospective study of alcohol consumption and risk of dementia in older adults. *JAMA* **289**, 1405-1413.
11. Galanis, D. J., Joseph, C., Masaki, K. H., Petrovitch, H., Ross, G. W. and White, L. (2000) A longitudinal study of drinking and cognitive performance in elderly Japanese American men: the Honolulu-Asia Aging Study. *Am. J. Public Health* **90**, 1254-1259.
12. Luchsinger, J. A. and Mayeux, R. (2004) Cardiovascular risk factors and Alzheimer's disease. *Curr. Atheroscler. Rep.* **6**, 261-266.
13. Truelsen, T., Thudium, D. and Gronbaek, M. (2002) Amount and type of alcohol and risk of dementia: the Copenhagen City Heart Study. *Neurology* **59**, 1313-1319.
14. Jaatinen, P., Riikonen, J., Riihioja, P., Kajander, O. and Hervonen, A. (2003) Interaction of aging and intermittent ethanol exposure on brain cytochrome c oxidase activity levels. *Alcohol.* **29**, 91-100.
15. de la Monte, S. M., Vonsattel, J. P. and Richardson, E. P., Jr. (1988) Morphometric demonstration of atrophic changes in the cerebral cortex, white matter, and neostriatum in Huntington's disease. *J. Neuropathol. Exp. Neurol.* **47**, 516-525.
16. Mann, K., Agartz, I., Harper, C., Shoaf, S., Rawlings, R. R., Momenan, R., Hommer, D. W., Pfefferbaum, A., Sullivan, E. V., Anton, R. F., Drobos, D. J., George, M. S., Bares, R., Machulla, H. J., Mundle, G., Reimold, M. and Heinz, A. (2001) Neuroimaging in alcoholism: ethanol and brain damage. *Alcohol. Clin. Exp. Res.* **25**, 104S-109S.
17. Ferrer, I., Fabregues, I., Pineda, M., Gracia, I. and Ribalta,

- T. (1984) A Golgi study of cerebellar atrophy in human chronic alcoholism. *Neuropathol. Appl. Neurobiol.* **10**, 245-253.
18. Fox, J. H., Ramsey, R. G., Huckman, M. S. and Proske, A. E. (1976) Cerebral ventricular enlargement. Chronic alcoholics examined by computerized tomography. *JAMA* **236**, 365-368.
19. Sullivan, E. V., Marsh, L., Mathalon, D. H., Lim, K. O. and Pfefferbaum, A. (1995) Anterior hippocampal volume deficits in nonamnestic, aging chronic alcoholics. *Alcohol. Clin. Exp. Res.* **19**, 110-122.
20. Bleich, S., Sperling, W., Degner, D., Graesel, E., Bleich, K., Wilhelm, J., Havemann-Reinecke, U., Javaheripour, K. and Kornhuber, J. (2003) Lack of association between hippocampal volume reduction and first-onset alcohol withdrawal seizure. A volumetric MRI study. *Alcohol Alcohol.* **38**, 40-44.
21. Kurth, C., Wegerer, V., Reulbach, U., Lewczuk, P., Kornhuber, J., Steinhoff, B. J. and Bleich, S. (2004) Analysis of hippocampal atrophy in alcoholic patients by a Kohonen feature map. *Neuroreport* **15**, 367-371.
22. Bleich, S., Wilhelm, J., Graesel, E., Degner, D., Sperling, W., Rossner, V., Javaheripour, K. and Kornhuber, J. (2003) Apolipoprotein E epsilon 4 is associated with hippocampal volume reduction in females with alcoholism. *J. Neural. Transm.* **110**, 401-411.
23. Hommer, D., Momenan, R., Kaiser, E. and Rawlings, R. (2001) Evidence for a gender-related effect of alcoholism on brain volumes. *Am. J. Psychiatry.* **158**, 198-204.
24. Pfefferbaum, A., Lim, K. O., Zipursky, R. B., Mathalon, D. H., Rosenbloom, M. J., Lane, B., Ha, C. N. and Sullivan, E. V. (1992) Brain gray and white matter volume loss accelerates with aging in chronic alcoholics: a quantitative MRI study. *Alcohol. Clin. Exp. Res.* **16**, 1078-1089.
25. Teichman, M., Richman, S. and Fine, E. W. (1987) "Dose/duration effect" relationship between alcohol consumption and cerebral atrophy: a psychological and neuro-radiological evaluation. *Am. J. Drug. Alcohol. Abuse.* **13**, 357-363.
26. Kril, J. J. and Homewood, J. (1993) Neuronal changes in the cerebral cortex of the rat following alcohol treatment and thiamin deficiency. *J. Neuropathol. Exp. Neurol.* **52**, 586-593.
27. Woo, H. N., Park, J. S., Gwon, A. R., Arumugam, T. V. and Jo, D. G. (2009) Alzheimer's disease and Notch signaling. *Biochem. Biophys. Res. Commun.* **390**, 1093-1097.
28. Woo, H. N., Baik, S. H., Park, J. S., Gwon, A. R., Yang, S., Yun, Y. K. and Jo, D. G. (2011) Secretases as therapeutic targets for Alzheimer's disease. *Biochem. Biophys. Res. Commun.* doi: 10.1016/j.bbrc.2010.11.132
29. Jo, D. G., Arumugam, T. V., Woo, H. N., Park, J. S., Tang, S. C., Mughal, M., Hyun, D. H., Park, J. H., Choi, Y. H., Gwon, A. R., Camandola, S., Cheng, A., Cai, H., Song, W., Markesbery, W. R. and Mattson, M. P. (2010) Evidence that gamma-secretase mediates oxidative stress-induced beta-secretase expression in Alzheimer's disease. *Neurobiol. Aging.* **31**, 917-925.
30. Shah, S., Lee, S. F., Tabuchi, K., Hao, Y. H., Yu, C., LaPlant, Q., Ball, H., Dann, C. E., 3rd, Sudhof, T. and Yu, G. (2005) Nicastrin functions as a gamma-secretase-substrate receptor. *Cell* **122**, 435-447.
31. Anttila, T., Helkala, E. L., Viitanen, M., Kareholt, I., Fratiglioni, L., Winblad, B., Soininen, H., Tuomilehto, J., Nissinen, A. and Kivipelto, M. (2004) Alcohol drinking in middle age and subsequent risk of mild cognitive impairment and dementia in old age: a prospective population based study. *BMJ* **329**, 539.
32. Rothman, S. M. and Mattson, M. P. (2010) Adverse stress, hippocampal networks, and Alzheimer's disease. *Neuromolecular Med.* **12**, 56-70.
33. Lieber, C. S. and DeCarli, L. M. (1989) Liquid diet technique of ethanol administration: 1989 update. *Alcohol Alcohol.* **24**, 197-211.
34. Kerai, M. D., Waterfield, C. J., Kenyon, S. H., Asker, D. S. and Timbrell, J. A. (1999) Reversal of ethanol-induced hepatic steatosis and lipid peroxidation by taurine: a study in rats. *Alcohol Alcohol.* **34**, 529-541.