# Incidence and survival of dementia in a general population of Japanese elderly: the Hisayama study

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## ABSTRACT

**Objective:** To estimate the incidence and survival rates of total and cause specific dementia in a general Japanese population.

**Methods:** A total of 828 subjects without dementia, aged 65 years or over, were followed-up prospectively for 17 years. Dementia was subdivided into cause specific subtypes: namely, Alzheimer's disease (AD), vascular dementia (VD), dementia with Lewy bodies (DLB), combined dementia and other types of dementia. During the follow-up, 275 subjects developed dementia; of these, 251 (91.2%) were evaluated morphologically, with 164 subjected to brain autopsy examination and the remaining 87 to neuroimaging.

**Results:** The incidences of total dementia, AD, VD, DLB, combined dementia and other types of dementia were 32.3 (n = 275), 14.6 (124), 9.5 (81), 1.4 (12), 3.8 (33), and 3.1 (16) per 1000 person years, respectively. The incidences of AD, combined dementia and other types of dementia rose with increasing age, particularly after the age of 85 years, but this tendency was not observed for VD or DLB. The survival curve of dementia cases aged 65–89 years was significantly lower than that of age and sex matched controls (10 year survival rate, 13.6% vs 29.3%; hazard ratio 1.67; 95% confidence interval 1.31 to 2.13). The 10 year survival rates were not significantly different among dementia subtypes.

**Conclusions:** Our findings suggest that the Japanese elderly population has a high risk for the development of dementia, specifically AD and VD, and once dementia is established, the risk of death is considerable.

Approximately 24.3 million people suffer from dementia globally, and this number is expected to double every 20 years to 81.1 million by 2040 because of the rapid increase in the number of elderly worldwide.[1] Effective prevention requires a strategy based on information about morbidity and mortality from dementia in general populations. Several population based studies have investigated the incidence[2–9] and fatality rates[10–13] of total and cause specific dementia but the current knowledge about the incidence and prognosis of dementia has derived mainly from studies done in Western populations, and it is unclear to what extent these findings apply to Japanese elderly populations. Here we present the incidence and survival of cause specific dementia in a 17 year follow-up study conducted in a Japanese community.

## METHODS

### Study population

Since 1985, a follow-up survey of dementia among individuals aged 65 years or older has been ongoing in the town of Hisayama, Japan.[9] The screening and assessment processes of the present analysis are shown in fig 1. In 1985, a total of 887 subjects aged 65 years or older (participation rate 94.6%) underwent a screening examination that included Hasegawa's dementia scale (HDS),[14] which is a neuropsychological test widely used in Japan comprised of 11 questions regarding orientation, memory function, common knowledge and calculation capacities, and questionnaires regarding psychological and medical symptoms, medical conditions and activities of daily living. Subjects with possible cognitive impairment underwent comprehensive investigations. After excluding 59 subjects with dementia at baseline, the remaining 828 subjects were enrolled in this study.

### Follow-up survey

Subjects were followed prospectively from November 1985 to October 2002 (fig 1). Detailed information about the follow-up survey of dementia has been described elsewhere.[9] Briefly, we established a daily monitoring system among the study team and local physicians or members of the town's Health and Welfare Office. Regular health checks were given annually to obtain information on any stroke or dementia missed by the monitoring network. Health status was also checked yearly by mail or telephone for any subject who did not undergo a regular examination or who had moved out of town.

Follow-up screening surveys of cognitive function were conducted in 1992,[15] 1998 and 2005. The screening surveys included neuropsychological tests (HDS,[14] HDS revised version (HDS-R)[16] or Mini-Mental State Examination (MMSE)[17]) and questionnaires similar to those used at the first screening. For subjects whose test scores were below the cut-off points (22/32.5 for HDS, 21/30 for the HDS-R and MMSE), comprehensive investigations, including interviews of the families or attending physicians, physical and neurological examinations, and a review of the clinical records were conducted.

When a subject died, an autopsy was performed at the Department of Pathology of Kyushu University. During the follow-up period, 553 subjects died, 439 of whom (79.4%) were subjected to autopsy. For dementia subjects with autopsy, detailed neuropathological evaluation was performed. No subject was lost to follow-up.

### Diagnosis of dementia

The diagnosis of dementia was made clinically based on the guidelines of the Diagnostic and

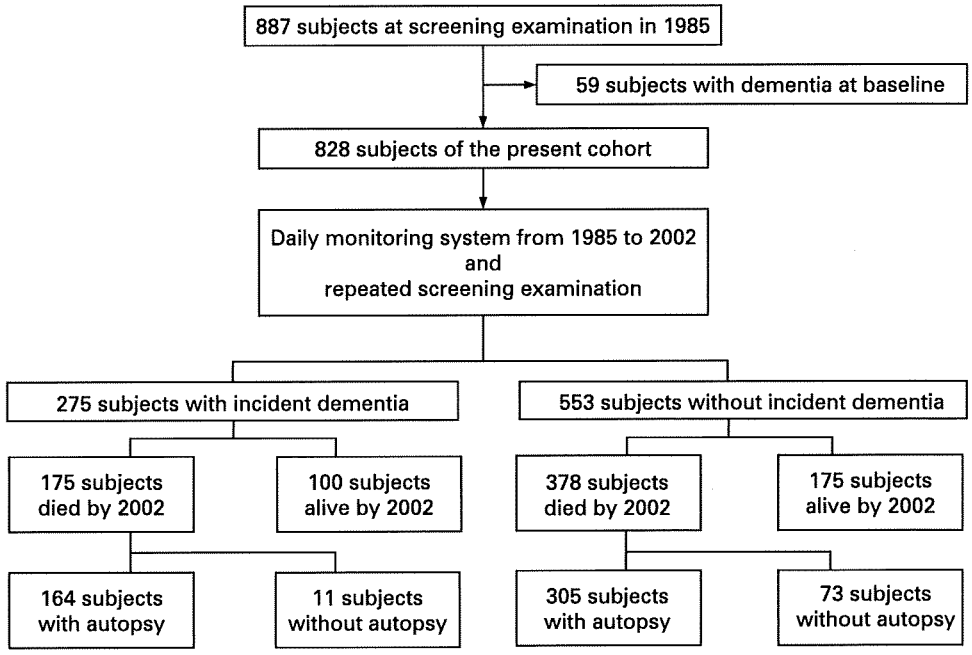


**Figure 1** Flow chart for screening and diagnostic procedures.

Statistical Manual of Mental Disorders, revised third edition (DSM-III-R).[18]

Alzheimer's disease (AD), vascular dementia (VD) and dementia with Lewy bodies (DLB) were diagnosed based on the criteria established by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA),[19] the Neuroepidemiology Branch of the National Institute of Neurological Disorders and Stroke with support from the Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN)[20] and the revised consensus guidelines described in the third report of the DLB consortium,[21] respectively.

For neuropathological evaluation of AD, the frequency of senile plaques and neurofibrillary tangles (NFT) was evaluated using the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria[22] and Braak stage.[23] The CERAD score and Braak stage were combined using the National Institute on Aging-Reagan Institute (NIA-RI) criteria,[24] and dementia cases with a "high likelihood" of AD pathology were defined as definite AD. Definite VD was defined as dementia with causative stroke or cerebrovascular change in neuroimaging and no neuropathological evidence of other forms of dementia. According to the DLB guidelines,[21] dementia cases with "high likelihood" criterion of DLB pathology were defined as definite DLB. Senile dementia of the neurofibrillary tangle type (tangle only dementia: SD-NFT) was diagnosed neuropathologically using Yamada's guideline.[25 26]

During the 17 year follow-up period, 275 subjects developed dementia. Of these, 175 cases died and 134 (76.6%) of these

**Table 1** Comparison of the clinical diagnosis of dementia subtype and the final diagnosis using neuropathological findings among 164 incident dementia cases with autopsy: the Hisayama Study, 1985–2002

| | Clinical diagnosis | | |
|---|---|---|---|
| **Final diagnosis using neuropathological findings** | **AD (n = 71)** | **VD (n = 47)** | **Other (n = 46)** |
| Pure AD | 35 | 16 | 11 |
| Pure VD | 17 | 21 | 12 |
| DLB | 2 | 1 | 6 |
| Combined dementia | 12 | 7 | 10 |
| AD+VD | 6 | 3 | 4 |
| AD+DLB | 3 | 2 | 1 |
| VD+DLB | 2 | 1 | 1 |
| AD+VD+DLB | 0 | 0 | 2 |
| AD+chronic subdural haematoma | 0 | 1 | 0 |
| DLB+SD-NFT | 0 | 0 | 1 |
| AD+VD+hypothyroid | 1 | 0 | 0 |
| SD-NFT+carbon monoxide poisoning | 0 | 0 | 1 |
| Other | 5 | 2 | 7 |

AD, Alzheimer's disease; DLB, dementia with Lewy bodies; SD-NFT, senile dementia of the neurofibrillary tangle type; VD, vascular dementia.

**Table 2** Frequency of each type of dementia among 275 incident dementia cases: the Hisayama Study, 1985–2002

| **Type of dementia** | **n (%)** |
|---|---|
| AD | 124 (45.1) |
| VD | 81 (29.5) |
| DLB | 12 (4.4) |
| Combined dementia | 33 (11.6) |
| AD+VD | 13 (4.7) |
| AD+DLB | 9 (3.3) |
| VD+DLB | 5 (1.8) |
| AD+VD+DLB | 2 (0.7) |
| AD+chronic subdural haematoma | 1 (0.4) |
| DLB+SD-NFT | 1 (0.4) |
| AD+VD+hypothyroid | 1 (0.4) |
| SD-NFT+carbon monoxide poisoning | 1 (0.4) |
| Other | 16 (6.2) |
| SD-NFT | 8 (2.9) |
| Chronic subdural haematoma | 2 (0.7) |
| Brain tumour | 2 (0.7) |
| Head trauma | 2 (0.7) |
| Pick's disease | 1 (0.4) |
| Hypoxic ischemic encephalopathy | 1 (0.4) |
| Unknown | 9 (3.3) |

AD, Alzheimer's disease; DLB, dementia with Lewy bodies; SD-NFT, senile dementia of the neurofibrillary tangle type; VD, vascular dementia.

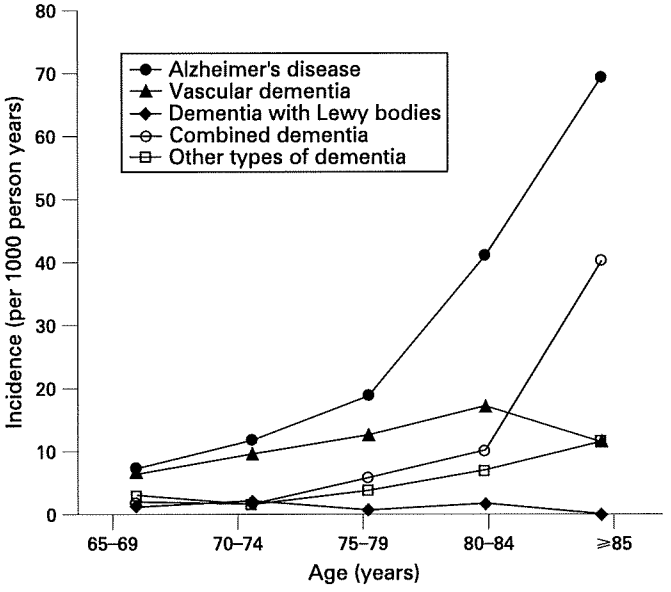


**Figure 2** Incidence rates of cause specific dementia by age group.

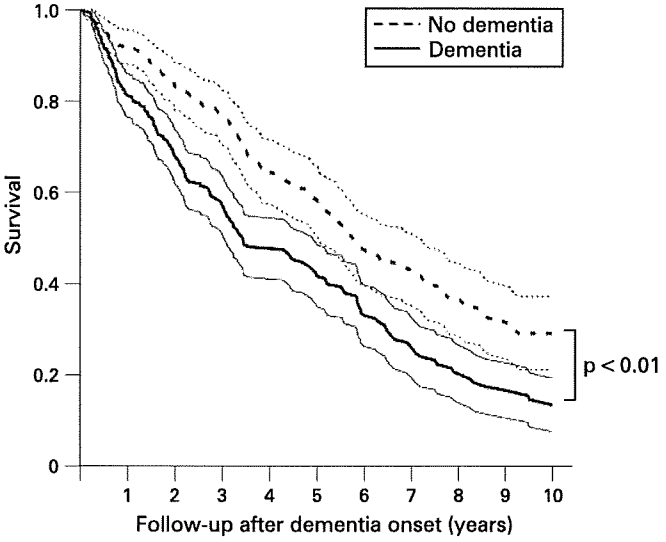


**Figure 3** Survival rates and 95% confidence intervals for new onset dementia cases and for age and sex matched control participants without dementia onset.

cases underwent brain autopsy examination (fig 1). The brains were evaluated neuropathologically in an additional 30 subjects with dementia who died after the end of the follow-up period, from November 2002 to October 2005. A total of 164 of the 275 subjects with dementia (59.6%) were examined neuropathologically. We performed evaluation with neuroimaging on 248 subjects with dementia (90.2%); among the 111 subjects with dementia who did not have an autopsy examination, 87 underwent a neuroimaging examination. Therefore, 251 subjects with dementia (91.2%) were evaluated morphologically.

In the present analysis, we used the final diagnosis of dementia subtypes, which was made based on the clinical and neuropathological information for dementia subjects with autopsy and clinical information, including neuroimaging only for those without autopsy. Table 1 shows a comparison of the clinical diagnosis of dementia subtype, which was made without information on neuropathological findings, and the final diagnosis, which was made using neuropathological findings, among 164 incident dementia cases with autopsy. Although the clinical diagnosis was not necessarily the same as the final diagnosis, moderate agreement was observed between the clinical and final diagnoses (agreement rate = 60%, kappa coefficient = 0.48 for AD; agreement rate = 59%, kappa coefficient = 0.53 for VD). Table 2 shows the frequency of each type of dementia among 275 incident dementia cases. We found 124 pure AD cases (definite 62; probable 52; possible 10), 81 pure VD cases (definite 50; probable 31) and 12 pure DLB cases (definite nine; probable two; possible one). When causes of cognitive impairment were attributed to two or more types of dementia, we classified the dementia as "combined dementia". This category accounted for 33 cases. There were 16 cases of other types of dementia.

The date of onset of VD was determined as the date when the responsible stroke occurred but the final diagnosis of VD was made more than 3 months after the stroke. The tentative time of onset, when the family or attending physician first noticed abnormal behaviour by the subject, was used for other types of dementia.

### Statistical analysis

The incidence of dementia was estimated using a person year approach. We estimated survival curves for the first 10 years after the onset of dementia for 221 new onset dementia cases with ages at dementia onset ranging from 65 to 89 years, and for 221 age and sex matched control subjects randomly selected from 553 subjects without incident dementia by the Kaplan–Meier product limit technique. We excluded subjects aged 90 years or over from this analysis because the number of control subjects for this age group was too small. Comparison of survival rates was done by log rank test. We also compared age and sex adjusted cumulative survival rates among cases with different types of dementia using Cox's proportional hazards model.

## RESULTS

The incidence of total dementia was 32.3 per 1000 person years. With regard to type, AD was the most frequent type of dementia (14.6 per 1000 person years), followed by VD (9.5) and then DLB (1.4). The incidences of AD, combined dementia and other types of dementia rose with increasing age, particularly after the age of 85 years, but this tendency was not observed for VD or DLB (fig 2).

Figure 3 shows the 10 year survival curves for new onset dementia cases and control subjects without dementia onset. The survival curve of dementia cases was significantly lower compared with that of the control subjects (10 year survival rate, 13.6% vs 29.2%; hazard ratio 1.67; 95% confidence interval 1.31 to 2.13; $p<0.0001$). Median survival time was 3.5 years in subjects with dementia and 5.8 years in those without dementia.

The age and sex adjusted survival curves for cases with different types of dementia are shown in fig 4. The survival rate of subjects with DLB tended to be lower than that of subjects with other types of dementia but the differences were not significant, probably because of the small number of subjects with DLB (10 year survival rates, 18.9% for AD, 13.2% for VD, 2.2% for DLB, 10.4% for combined dementia, 14.4% for other types of dementia).

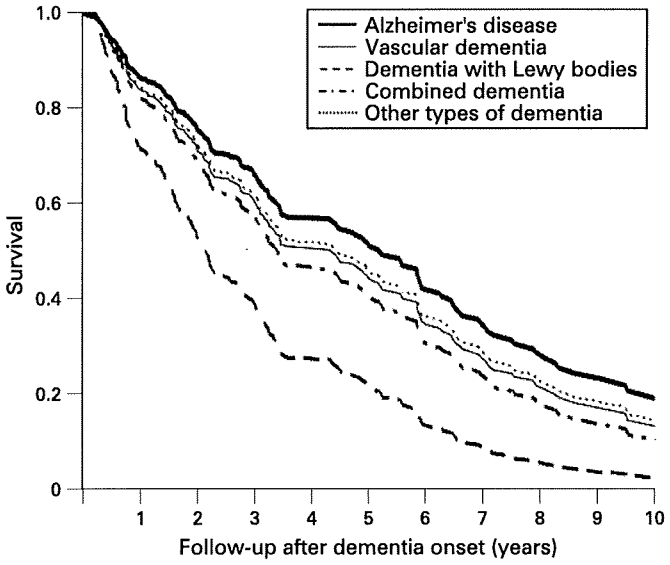


**Figure 4** Age and sex adjusted survival rates of cause specific dementia.

## DISCUSSION

The present analysis from a prospective cohort study has clearly demonstrated that the incidence of dementia was as high as 32.3 per 1000 person years in a general population of Japanese elderly, aged 65 years or older. We diagnosed dementia subtypes based on clinical and neuropathological examinations and found that AD, VD and DLB were the three major subtypes of dementia in this population. Another important finding was that the median survival time of subjects with new onset dementia was shorter than that of those without dementia onset.

Several population based cohort studies have reported the incidence of dementia for elderly populations.[2–9] The incidence of dementia of our study (32.3 per 1000 person years) was relatively higher than that obtained from the majority of other follow-up studies (13.5–25.5)[2–6] and similar to that of an Italian study (37.8)[7] and an African American study (32.4).[8] Possible reasons for the relatively higher incidence of dementia in our study were the frequently repeated screening surveys for dementia and the high follow-up rate.

In our subjects, DLB was the third most frequent type of dementia after AD and VD, with an incidence of 1.4 per 1000 person years. Although there have been several prevalence studies of DLB in general populations, little is known about the exact incidence of DLB.[27] Meich *et al* estimated the incidence of DLB as 0.57 per 1000 person years in a US population.[2] In contrast, no case of DLB was observed in the 4 year follow-up study of an Italian population.[7] It is possible that the higher incidence of DLB in our study resulted from a higher rate of neuropathological evaluation among subjects with dementia. Further cohort studies are needed to investigate the precise incidences of DLB.

In the present analysis, all types of dementia were associated with higher mortality, and the estimate of median survival time for subjects with total dementia was 3.5 years. This is shorter than that obtained from other population based cohort studies (5.2–7.6 years).[10–13] Most previous cohort studies estimated median survival time in follow-up surveys of subjects having dementia at the baseline examination. Therefore, it is possible that severe dementia cases with poor prognosis may not have been included, and that the survival time of patients with dementia may have been overestimated ("length bias"). In the Canadian Study of Health Aging, the crude median survival time was 6.6 years but the estimated survival time from the onset of dementia after controlling for "length bias" was 3.3 years.[13] This finding is comparable with the median survival time from the onset of dementia observed in the present analysis.

The strengths of our study include its longitudinal population based study design, long duration of follow-up, sufficient number of dementia events, 100% follow-up of subjects and examination of the brains of most dementia cases with autopsy and neuroimaging. A limitation of our study is that relatively low cut-off points of neuropsychological tests for comprehensive investigations of dementia in the follow-up examinations may have caused us to miss subjects in the early course of dementia. This limitation may have led to an underestimation of the incidence of dementia and survival time. Another limitation is that we compared the survival rates among subjects matched by age at dementia onset ranging only within 65–89 years because the number of control subjects aged 90 years or older without dementia was too small. However, subjects aged 90 years or older are not likely to live long, irrespective of the existence of dementia, and inclusion of subjects of this age group is not likely to have changed the findings of this study.

In conclusion, relatively more Japanese elderly suffer from dementia than the proportion expected based on the results of other follow-up studies. Once dementia is established, the risk of death is 1.7-fold higher compared with subjects without dementia. It is important to elucidate risk factors for each type of dementia and establish dementia prevention strategies, especially in countries such as Japan where the elderly population is increasing rapidly, as dementia places a burden on families and communities.

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**Competing interests:** None.

**Ethics approval:** The ethics committee of Kyushu University approved this study.

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# LDL Cholesterol and the Development of Stroke Subtypes and Coronary Heart Disease in a General Japanese Population

## The Hisayama Study

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***Background and Purpose***—Although the relation between serum LDL cholesterol level and coronary heart disease (CHD) is well established, its relation with stroke subtypes is less clear.

***Methods***—A total of 2351 inhabitants age ≥40 years in a Japanese community were followed up for 19 years.

***Results***—During follow-up, 271 subjects developed stroke and 144 developed CHD. Whereas the age- and sex-adjusted incidences of CHD significantly increased with increasing LDL cholesterol levels ($P$ for trend <0.001), the associations between LDL cholesterol level and the incidences of ischemic or hemorrhagic stroke were not significant. The age- and sex-adjusted incidences of atherothrombotic infarctions (ATIs) and lacunar infarctions (LIs) significantly increased with increasing LDL cholesterol level ($P$ for trend=0.03 for ATIs and=0.02 for LIs), but no such association was observed for cardioembolic infarction. After multivariate adjustment, the positive associations of LDL cholesterol level with the risks of ATI and CHD remained significant ($P$ for trend=0.02 for ATIs and=0.03 for CHD), whereas the association with LIs was not significant. The risk of ATI significantly increased in the fourth quartile of LDL cholesterol compared with the first quartile (multivariate-adjusted hazard ratio=2.84; 95% CI, 1.17 to 6.93). The multivariate-adjusted risks for developing nonembolic infarction (ATIs and LIs) and CHD were significantly elevated in the groups with elevated LDL cholesterol values with and without the metabolic syndrome.

***Conclusions***—Our findings suggest that an elevated LDL cholesterol level is a significant risk factor for developing ATI as well as CHD, and these associations are independent of the metabolic syndrome. (***Stroke.* 2009;40:382-388.)**

**Key Words:** epidemiology ■ cholesterol ■ lipoproteins ■ risk factors

Increased blood cholesterol levels are causally related to an increased risk of coronary heart disease (CHD).[1] In contrast, the relation between total cholesterol levels and the risk of stroke remains unclear because of conflicting results reported in the literature.[2,3] The inconsistent results may be due to several reasons. First, because stroke is a heterogeneous syndrome of different etiologic origins, lipid abnormalities may be important for some subtypes of stroke but not for others. An inverse association has been observed between total cholesterol and hemorrhagic stroke,[2,4] and there is a positive association between total cholesterol and ischemic stroke.[2,5] Furthermore, the association may be different for ischemic stroke subtypes.[6] Second, lipoprotein subfractions are considered to exert varying influence on stroke risk.[7] It is possible that the protective effect of HDL cholesterol against stroke weakens the positive association between total cholesterol and stroke in instances where lipoprotein subfractions are counted together. The association between cholesterol and stroke, therefore, needs to be discussed on the basis of stroke subtypes and lipoprotein subfractions.

Together with the results from prospective studies, the positive association between LDL cholesterol level and the risk of CHD has been confirmed by lipid-lowering randomized trials.[8] On the other hand, whereas statins significantly reduced the risk of stroke,[8] the risk reduction for stroke in trials in which subjects were treated with nonstatins was not significant,[9] suggesting that statins involve mechanisms other than cholesterol lowering for the prevention of stroke. Therefore, the true association between LDL cholesterol and the risk of stroke remains unknown. The purpose of this study was to evaluate the association between LDL cholesterol

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level and the development of stroke by its subtypes as well as of CHD in a prospective study of a general Japanese population.

## Subjects and Methods

### Study Population

Since 1961, we have been conducting a long-term, prospective cohort study of cardiovascular disease (CVD) in the town of Hisayama, a suburb of Fukuoka city in southern Japan. In 1983, a screening survey for the present study was performed in the town. A total of 2548 residents age ≥40 years (80.7% of the total population of this age group) consented to participate in the examination. Of these, 197 subjects were excluded for the following reasons: past history of stroke or myocardial infarction (MI; n=89), blood samples not being collected or collected after a meal (n=86), and excessively high value of triglycerides (≥4.48 mmol/L) for which the Friedewald formula loses its validity[10] (n=22). The remaining 2351 subjects (991 men, 1360 women) were included in this study.

### Follow-Up Survey

This population was followed up prospectively for 19 years, from November 1983 through October 2002, by annual health examinations. For subjects who did not undergo regular examinations or who moved out of Hisayama, health status was checked yearly by mail or telephone. We also established a daily monitoring system, which connected us with local physicians and the members of the Health and Welfare Office for the town, and through the system we gathered information on new events of CVD, including suspected cases. When stroke or CHD occurred or was suspected, physicians in the study team examined the subject and evaluated his/her detailed clinical information. The clinical diagnosis of stroke or CHD was based on the patient's history, physical and neurologic examinations, and ancillary laboratory examinations. When a subject died, an autopsy was performed at the Department of Pathology of Kyushu University. During the follow-up period, 1 subject was lost to follow-up, 707 subjects died, and 555 subjects (78.5%) underwent autopsy examination.

### Definition of Cardiovascular Events

The diagnosis and classification of stroke were determined on the basis of clinical information, including brain computed tomography and magnetic resonance imaging, cerebral angiography, echocardiography, carotid duplex imaging, or autopsy findings. In principle, stroke was defined as a sudden onset of nonconvulsive and focal neurologic deficits persisting for >24 hours, and the stroke was then classified as either hemorrhagic or ischemic. Hemorrhagic stroke included cerebral hemorrhage and subarachnoid hemorrhage. Ischemic stroke was further divided into 4 clinical categories: atherothrombotic infarction (ATI), lacunar infarction (LI), cardioembolic infarction (CEI), and undetermined subtype of ischemic stroke (UND), based on the Classification of Cerebrovascular Disease III proposed by the National Institute of Neurological Disorders and Stroke,[11] as well as on the basis of the diagnostic criteria of the Trial of Org10172 in Acute Stroke Treatment (TOAST) Study[12] and the Cerebral Embolism Task Force.[13]

Details of the diagnostic criteria for ischemic stroke subtypes have been described previously.[14] In brief, ATI was diagnosed when the subjects had significant stenosis (>50%) or occlusion of a major cerebral artery with infarct size ≥1.5 cm on brain imaging or autopsy. LI was diagnosed as the presence of a relevant brainstem, basal ganglia, or subcortical hemispheric lesion with a diameter <1.5 cm demonstrated on brain imaging or autopsy and no evidence of cerebral cortical or cerebellar impairment. The diagnosis of CEI was made on the basis of primary and secondary clinical features suggestive of CEI as reported by the Cerebral Embolism Task Force.[13] The category of UND included all ischemic stroke cases for which the subtype could not be determined because of insufficient clinical or morphologic information. We considered morphologic findings to be significant and used clinical features as reference information. Cases with cerebrovascular diseases with distinct pathology, such as collagen disease, hematologic disorder, trauma, chronic subdural hematoma, or moyamoya disease, were excluded from the evaluation.

During the follow-up period, we identified 271 first-ever stroke events. All of the stroke cases underwent morphologic evaluation that included brain imaging and autopsy; 269 subjects (99.3%) underwent brain imaging studies, and autopsies were performed on 128 subjects of 157 deceased stroke cases (81.5%), including 2 subjects who were not examined by brain imaging. When sufficient clinical and morphologic information was obtained, a diagnosis of cerebral infarction subtype was defined as "definite." When the amount of either type of information was insufficient, the diagnosis level was defined as "probable." On the basis of the aforementioned criteria, stroke cases were divided into 80 hemorrhagic strokes and 191 ischemic strokes (51 ATIs, 93 LIs, 46 CEIs, and 1 UND). Among 191 ischemic strokes, 182 were defined as definite and 9 as probable. In this study, we present the data regarding definite and probable stroke cases together, because these combined data were almost identical to those for definite cases only.

The criteria for the diagnosis of CHD included first-ever acute MI, silent MI, sudden cardiac death within 1 hour after the onset of acute illness, coronary artery angioplasty, and bypass grafting. The diagnosis of MI was based on detailed clinical information and at least 2 of the following findings: typical clinical symptoms, ECG evidence of MI, elevated cardiac enzymes, or morphologic findings including echocardiographic, scintigraphic, or angiographic abnormalities compatible with myocardial injury. Silent MI was defined as myocardial scarring without any historical indication of clinical symptoms and/or abnormal cardiac enzyme changes.[15] During the follow-up period, we identified 144 first-ever events of CHD.

### Risk Factors

Blood samples were drawn after an overnight fast of at least 12 hours. All measurements were done within 24 hours after venipuncture in the central study laboratory (Japan Medical Laboratory Inc, Fukuoka, Japan), which participated in the Centers for Disease Control and Prevention Lipid Standardization Program. Total cholesterol and triglyceride levels were measured enzymatically. Measurement of HDL cholesterol was performed after precipitation of VLDL and LDL with dextran sulfate and magnesium. LDL cholesterol concentration was calculated with the Friedewald formula.[10] Plasma glucose levels were determined by the glucose oxidase method. Sitting blood pressure (BP) was measured with a sphygmomanometer 3 times at the right upper arm after at least 5 minutes of rest, and the mean of the 3 measurements was used in the analysis. Hypertension was defined as a BP ≥140/90 mm Hg and/or current treatment with antihypertensive agents. ECG abnormalities were defined as left ventricular hypertrophy (Minnesota code 3-1), ST-segment depression (Minnesota codes 4-1,2,3), or atrial fibrillation (Minnesota code 8-3). Body height and weight were measured in light clothing without shoes, and body mass index (BMI; kg/m$^2$) was calculated. Information on alcohol consumption, smoking habits, and physical activity during leisure time was obtained by the use of a questionnaire. Alcohol consumption and smoking habits were classified as either current use or not. Those subjects who engaged in sports or other forms of exertion ≥3 times per week during their leisure time were designated the regular-exercise group. We defined the presence of the metabolic syndrome according to the National Cholesterol Education Program Expert Panel criteria[16] with a minor modification. The presence of the metabolic syndrome was based on the existence of 3 or more of the following components: (1) BMI ≥25 kg/m$^2$ as a substitute for waist circumference[17]; (2) fasting triglyceride concentration ≥1.68 mmol/L; (3) HDL cholesterol concentration <1.03 mmol/L in men and <1.29 mmol/L in women; (4) BP ≥130/85 mm Hg or use of antihypertensive drugs; and (5) fasting plasma glucose value ≥6.1 mmol/L or current use of antidiabetic drugs.

### Statistical Analysis

To analyze LDL cholesterol level as a categorical variable, we classified the subjects into 4 groups according to quartiles of LDL

**Table 1. Age- and Sex-Adjusted Mean Values or Frequencies of Risk Factors for CVD According to LDL Cholesterol Quartiles at Baseline**

| | Quartile of LDL Cholesterol Levels, mmol/L | | | | |
|---|---|---|---|---|---|
| Risk Factor | ≤2.65 (n=586) | 2.66 to 3.24 (n=591) | 3.25 to 3.88 (n=585) | ≥3.89 (n=589) | *P* Value for Trend |
| Men, % | 57.4 | 44.1 | 39.2 | 31.5 | <0.001 |
| Age, y | 56±11 | 57±11 | 57±11 | 59±11 | <0.001 |
| Total cholesterol, mmol/L | 4.03±0.57 | 4.81±0.41 | 5.40±0.43 | 6.45±0.68 | <0.001 |
| HDL cholesterol, mmol/L | 1.36±0.42 | 1.35±0.36 | 1.34±0.37 | 1.31±0.33 | <0.001 |
| Triglycerides, mmol/L | 1.15±0.75 | 1.07±0.51 | 1.12±0.53 | 1.32±0.58 | <0.001 |
| Fasting blood glucose, mmol/L | 4.66±0.92 | 4.75±0.96 | 4.76±0.93 | 4.96±1.14 | <0.001 |
| Systolic BP, mm Hg | 132±22 | 132±21 | 135±22 | 138±21 | <0.001 |
| Diastolic BP, mm Hg | 81±12 | 81±12 | 82±11 | 83±10 | <0.001 |
| Hypertension, % | 39.7 | 41.4 | 43.8 | 48.5 | 0.01 |
| ECG abnormalities,* % | 20.6 | 19.4 | 21.0 | 18.4 | 0.12 |
| BMI, kg/m$^2$ | 21.9±3.0 | 22.2±3.1 | 23.0±3.1 | 23.5±3.1 | <0.001 |
| Current drinking, % | 42.2 | 33.3 | 31.8 | 27.9 | <0.001 |
| Current smoking, % | 30.7 | 28.5 | 28.3 | 26.5 | <0.001 |
| Regular exercise,† % | 9.0 | 7.9 | 9.5 | 5.7 | 0.03 |

Data are mean±SD or percent. Percentage of men was age adjusted. Mean age was sex adjusted.

*Minnesota codes 3–1; 4–1, –2, –3; or 8–3.

†Engaging in sports or other forms of exertion regularly ≥3 times per week during leisure time.

cholesterol level: ≤2.65, 2.66 to 3.24, 3.25 to 3.88, and ≥3.89 mmol/L. Serum triglyceride levels were logarithmically transformed to improve the skewed distribution. Age- and sex-adjusted mean values of the possible risk factors were calculated by the ANCOVA method, and their trends across LDL cholesterol levels were tested by multiple-regression analysis. Frequencies of risk factors were adjusted for age and sex by the direct method and were examined for trends by the Cochran-Mantel-Haenszel test. The incidences of CVD were calculated by the person-year method and were adjusted for age and sex by the direct method according to 10-year age groups. Differences in age- and sex-adjusted incidences between LDL cholesterol quartiles were tested by Cox proportional-hazards regression analysis. The age- and sex-adjusted or multivariate-adjusted hazard ratios (HRs) and 95% CIs were also calculated by the Cox proportional-hazards model. All statistical analyses were performed with the SAS program package. $P<0.05$ was considered statistically significant in all analyses.

## Results

The age- and sex-adjusted mean values or frequencies of risk factors for CVD are listed by quartiles of LDL cholesterol levels at baseline in Table 1. The frequencies of male sex, current drinking, current smoking, and regular exercise and the mean values of HDL cholesterol declined with increasing LDL cholesterol level, whereas mean values of age, total cholesterol, triglycerides, fasting blood glucose, systolic and diastolic BPs, BMI, and frequency of hypertension significantly increased with rising LDL cholesterol level. The frequency of ECG abnormalities was not different among serum LDL cholesterol levels.

Table 2 shows the age- and sex-adjusted incidences of CVD according to quartiles of LDL cholesterol levels. No significant associations were observed between LDL cholesterol levels and the incidences of stroke, whether ischemic or hemorrhagic. In regard to subtypes of ischemic stroke, the incidences of ATI and LI significantly increased with increasing LDL cholesterol level ($P$ for trend=0.03 for ATI and=0.02 for LI), and there were significant differences between the first and fourth quartiles of LDL cholesterol for both subtypes (age- and sex-adjusted HR=2.31; 95% CI, 1.03 to 5.16; $P$=0.04 for ATI; age- and sex-adjusted HR=2.00; 95% CI, 1.05 to 3.80; $P$=0.03 for LI; Table 3). No such association was observed for CEI. The incidence of CHD also significantly increased with increasing LDL cholesterol level ($P$ for trend <0.001), and compared with the first quartile, the incidence was significantly higher in the third (age- and sex-adjusted HR=1.77; 95% CI, 1.07 to 2.91; $P$=0.03; Table 3) and fourth (age- and sex-adjusted HR=2.00; 95% CI, 1.22 to 3.28; $P$=0.006) quartiles.

As shown in Table 3, the positive associations between LDL cholesterol level and risk of ATI and CHD remained significant even after adjustment for age, sex, HDL cholesterol, triglycerides, systolic BP, ECG abnormalities, fasting blood glucose, BMI, current drinking, current smoking, and regular exercise ($P$ for trend=0.02 for ATI and=0.03 for CHD). Compared with the first quartile, the risk of ATI was significantly high in the fourth quartile after adjustment for the aforementioned confounding factors (multivariate-adjusted HR=2.84; 95% CI, 1.17 to 6.93; $P$=0.02). On the other hand, the negative association between LDL cholesterol and the risk of CEI appeared to be significant after multivariate adjustment ($P$ for trend=0.03), and the risk of CEI was significantly lower in the fourth quartile than in the first quartile (multivariate-adjusted HR=0.34; 95% CI, 0.12 to 0.96; $P$=0.04). A similar association was observed when LDL cholesterol was examined on a continuous scale.

Because not only LDL cholesterol but also other metabolic factors may be strong risk factors for CVD, we examined the combined as well as the separate effects of elevated LDL cholesterol level and the metabolic syndrome on the development of selected CVDs. As shown in the Figure, we

**Table 2. Age- and Sex-Adjusted Incidences (per 1000 Person-Years) of CVD According to LDL Cholesterol Quartiles**

| | Quartile of LDL Cholesterol Levels, mmol/L | | | | |
|---|---|---|---|---|---|
| | ≤2.65 (n=586) | 2.66 to 3.24 (n=591) | 3.25 to 3.88 (n=585) | ≥3.89 (n=589) | *P* Value for Trend |
| Stroke | | | | | |
| No. of events | 56 | 62 | 74 | 79 | |
| Age- and sex-adjusted incidence | 7.4 | 8.1 | 10.1 | 10.2 | 0.13 |
| Ischemic stroke | | | | | |
| No. of events | 37 | 47 | 47 | 60 | |
| Age- and sex-adjusted incidence | 4.9 | 6.3 | 6.8 | 7.9 | 0.07 |
| Atherothrombotic | | | | | |
| No. of events | 9 | 12 | 9 | 21 | |
| Age- and sex-adjusted incidence | 1.2 | 1.6 | 1.2 | 3.3* | 0.03 |
| Lacunar | | | | | |
| No. of events | 14 | 21 | 25 | 33 | |
| Age- and sex-adjusted incidence | 2.0 | 2.6 | 2.9 | 3.8* | 0.02 |
| Cardioembolic | | | | | |
| No. of events | 14 | 14 | 12 | 6 | |
| Age- and sex-adjusted incidence | 1.7 | 2.1 | 2.4 | 0.8 | 0.07 |
| Hemorrhagic stroke | | | | | |
| No. of events | 19 | 15 | 27 | 19 | |
| Age- and sex-adjusted incidence | 2.6 | 1.8 | 3.3 | 2.3 | 0.95 |
| CHD | | | | | |
| No. of events | 25 | 28 | 43 | 48 | |
| Age- and sex-adjusted incidence | 3.4 | 3.4 | 5.5* | 6.6† | <0.001 |

*$P<0.05$, †$P<0.01$ vs lowest quartile.

estimated the HRs for the occurrence of nonembolic infarction, including ATI and LI, as well as of CHD, by dividing the subjects into 4 groups according to the presence or absence of high LDL cholesterol levels (the fourth quartile, ≥3.89 mmol/L for nonembolic infarction; the third and fourth quartiles, ≥3.25 mmol/L for CHD) and the metabolic syndrome after adjustments for age, sex, ECG abnormalities, current drinking, current smoking, and regular exercise. Compared with a reference group with neither high LDL cholesterol levels nor the metabolic syndrome, the risk of developing nonembolic infarction was significantly high in the group with high LDL cholesterol levels alone and in the group with both high LDL cholesterol levels and the metabolic syndrome, whereas it was marginally significant for the group with the metabolic syndrome alone. Similarly, the risk for the development of CHD was elevated in both the group with high LDL cholesterol without the metabolic syndrome and the group with high LDL cholesterol and the metabolic syndrome. The risk of CHD was also significant for the group with the metabolic syndrome alone.

## Discussion

In a long-term, prospective study of a general Japanese population, we demonstrated positive and significant associations between serum LDL cholesterol level and risk for the development of ATI and CHD. These associations remained unchanged even after adjustment for other lipid fractions as well as other confounding factors, namely, age, sex, systolic BP, ECG abnormalities, fasting blood glucose, BMI, current drinking, current smoking, and regular exercise. In addition, the impact of high LDL cholesterol on CVD appeared to be similar to that of the metabolic syndrome. On the other hand, the association between LDL cholesterol level and the risk of CEI was negative and significant after adjusting for the aforementioned risk factors. To our knowledge, this is the first prospective cohort study to investigate the association between LDL cholesterol and the development of subtypes of ischemic stroke.

Several prospective studies have investigated the association between LDL cholesterol and ischemic stroke, but the results were not unanimous. The Cardiovascular Health Study[18] reported a positive association between LDL cholesterol and the risk of ischemic stroke, whereas the Atherosclerosis Risk in Communities Study[19] and the Framingham Study[20] found no clear associations. In the present analysis, LDL cholesterol level was not clearly associated with the risks of stroke and ischemic stroke, but these associations were heterogeneous across ischemic stroke subtypes. Because LI and CEI seem to have a less potent relation with elevated LDL cholesterol,[21,22] inclusion of those subtypes may mask the positive association between LDL cholesterol and ATI. This heterogeneity in the associations of LDL cholesterol level and ischemic stroke subtypes may be a reason for the controversial results obtained from previous studies that investigated the outcome of "total" ischemic stroke.

**Table 3. Age-, Sex-, and Multivariate-Adjusted HRs and 95% CIs for the Development of CVD According to LDL Cholesterol Quartiles**

| | Quartile of LDL Cholesterol Levels, mmol/L | | | | | |
|---|---|---|---|---|---|---|
| | ≤2.65 (n=586) | 2.66 to 3.24 (n=591) | 3.25 to 3.88 (n=585) | ≥3.89 (n=589) | *P* Value for Trend | Continuous Scale |
| Stroke | | | | | | |
| No. of events | 56 | 62 | 74 | 79 | | |
| Age- and sex-adjusted HR (95% CI) | 1.0 | 0.96 (0.66–1.37) | 1.12 (0.84–1.69) | 1.23 (0.86–1.75) | 0.13 | 1.08 (0.95–1.23) |
| Multivariate-adjusted HR (95% CI) | 1.0 | 0.94 (0.64–1.38) | 1.15 (0.79–1.67) | 1.23 (0.84–1.81) | 0.16 | 1.07 (0.93–1.24) |
| Ischemic stroke | | | | | | |
| No. of events | 37 | 47 | 47 | 60 | | |
| Age- and sex-adjusted HR (95% CI) | 1.0 | 1.08 (0.70–1.67) | 1.17 (0.75–1.80) | 1.45 (0.95–2.21) | 0.07 | 1.15 (0.99–1.35) |
| Multivariate-adjusted HR (95% CI) | 1.0 | 1.05 (0.66–1.66) | 1.05 (0.66–1.68) | 1.35 (0.85–2.14) | 1.19 | 1.11 (0.94–1.31) |
| Atherothrombotic | | | | | | |
| No. of events | 9 | 12 | 9 | 21 | | |
| Age- and sex-adjusted HR (95% CI) | 1.0 | 1.14 (0.48–2.71) | 0.98 (0.39–2.50) | 2.31 (1.03–5.16)* | 0.03 | 1.51 (1.14–1.99)§ |
| Multivariate-adjusted HR (95% CI) | 1.0 | 1.35 (0.54–3.35) | 1.19 (0.45–3.17) | 2.84 (1.17–6.93)* | 0.02 | 1.60 (1.19–2.16)§ |
| Lacunar | | | | | | |
| No. of events | 14 | 21 | 25 | 33 | | |
| Age- and sex-adjusted HR (95% CI) | 1.0 | 1.29 (0.65–2.54) | 1.58 (0.81–3.05) | 2.00 (1.05–3.80)* | 0.02 | 1.23 (1.00–1.53) |
| Multivariate-adjusted HR (95% CI) | 1.0 | 1.19 (0.57–2.50) | 1.41 (0.69–2.89) | 1.69 (0.83–3.43) | 0.11 | 1.13 (0.90–1.43) |
| Cardioembolic | | | | | | |
| No. of events | 14 | 14 | 12 | 6 | | |
| Age- and sex-adjusted HR (95% CI) | 1.0 | 0.83 (0.39–1.75) | 0.80 (0.37–1.75) | 0.39 (0.15–1.04) | 0.07 | 0.71 (0.51–1.00) |
| Multivariate-adjusted HR (95% CI) | 1.0 | 0.75 (0.34–1.63) | 0.59 (0.25–1.38) | 0.44 (0.12–0.96)* | 0.03 | 0.64 (0.44–0.94)‡ |
| Hemorrhagic stroke | | | | | | |
| No. of events | 19 | 15 | 27 | 19 | | |
| Age- and sex-adjusted HR (95% CI) | 1.0 | 0.69 (0.35–1.36) | 1.24 (0.68–2.24) | 0.83 (0.43–1.59) | 0.95 | 0.94 (0.74–1.20) |
| Multivariate-adjusted HR (95% CI) | 1.0 | 0.71 (0.35–1.47) | 1.41 (0.75–2.65) | 1.01 (0.50–2.05) | 0.53 | 1.02 (0.79–1.33) |
| CHD | | | | | | |
| No. of events | 25 | 28 | 43 | 48 | | |
| Age- and sex-adjusted HR (95% CI) | 1.0 | 1.02 (0.60–1.76) | 1.77 (1.07–2.91)* | 2.00 (1.22–3.28)† | <0.001 | 1.29 (1.08–1.53)§ |
| Multivariate-adjusted HR (95% CI) | 1.0 | 1.01 (0.56–1.80) | 1.68 (0.99–2.84) | 1.57 (0.91–2.73) | 0.03 | 1.15 (0.95–1.39) |

Multivariate adjustment was made for age, sex, HDL cholesterol, triglycerides, systolic BP, ECG abnormalities, fasting blood glucose, BMI, current drinking, current smoking, and regular exercise. For the continuous scale, HR is given for each 1-mmol/L increase in LDL cholesterol.

*$P<0.05$, †$P<0.01$ vs lowest quartile; ‡$P<0.05$, §$P<0.01$.

The atherogenesis of LDL cholesterol to large vessels, including coronary arteries and other peripheral arteries, is well known, and clinical studies have shown that an elevated LDL cholesterol level is also significantly related to the development of atherosclerotic lesions in extracranial or intracranial large vessels.[23,24] Because ATI is caused by atherosclerotic lesions of those large vessels, the significant association between elevated LDL cholesterol level and the risk of ATI observed in the present analysis is compatible with the evidence of the atherogenic role of LDL cholesterol.

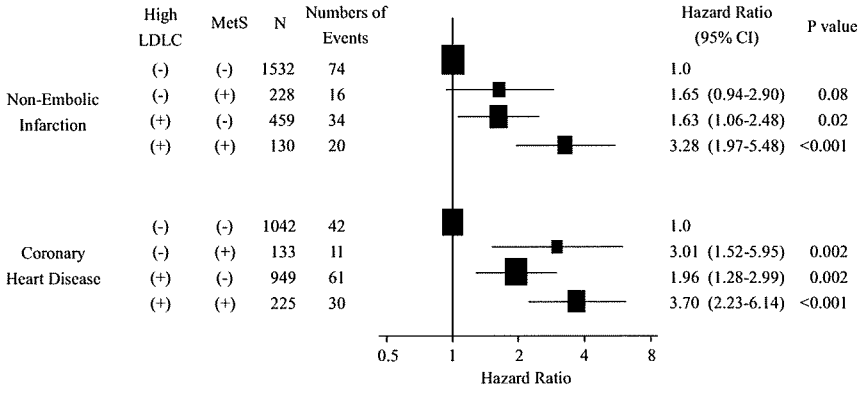


**Figure.** Multivariate-adjusted HRs for the development of nonembolic infarction and CHD according to the presence or absence of high LDL cholesterol and the metabolic syndrome. Multivariate adjustment was made for age, sex, ECG abnormalities, current drinking, current smoking, and regular exercise. Centers of the boxes are placed at the estimates of HRs. Horizontal lines indicate 95% CIs, and sizes of boxes are proportional to the numbers of events. LDLC indicates LDL cholesterol; MetS, metabolic syndrome.

In our cohort, the association between LDL cholesterol and the risk of LI was no longer significant after multivariate adjustment, suggesting that elevated LDL cholesterol was not an independent risk factor for the development of LI. Our previous report showed that multiple risk factors were related to the occurrence of LI,[14] and case-control studies on the relation between LDL cholesterol level and LI have reported varied associations.[21,22,25] One study reported a significant association between elevated LDL cholesterol and the risk of LI,[25] another study observed lower LDL cholesterol levels in LI cases,[22] and another study found no significant association.[21] Lacunar infarcts occur as a result of multiple mechanisms, such as (1) lipohyalinosis and/or fibrinoid necrosis, (2) microatheroma, (3) atherosclerosis of the basilar and middle cerebral artery stem or proximal division of large vessels, or (4) cardioembolic occlusion.[26] Lipohyalinosis is a vasculopathy caused by hypertension,[26] whereas large-vessel atherosclerosis is affected by risk factors including LDL cholesterol,[23,24] but cardioembolism seems less related to elevated LDL cholesterol.[22,25] These heterogeneous roles for LDL cholesterol in the multiple pathogenesis of LI occurrence might account for the weak association between LDL cholesterol and the risk of LI.

An inverse relation between LDL cholesterol level and the risk of CEI was observed in our earlier population in the 1960s,[14] and the same association was found in the present investigation after adjustment for confounding factors. Although the reason for this association is unknown, a plausible explanation is that a lowered cholesterol level might increase the risk of CEI through the increased occurrence of atrial fibrillation,[27] a predominant risk factor for CEI. Additional clinical and experimental evidence is needed to elucidate the mechanism underlying this association.

The results of previous prospective studies of the association between LDL cholesterol and hemorrhagic stroke have been inconsistent; a significant inverse association was reported in women in the Framingham Study,[20] whereas a nonsignificant association was observed in the Cardiovascular Health Study.[18] Lipid-lowering trials recently conducted in Japan[28,29] and a meta-analysis of >90 000 subjects enrolled in statin trials[8] found no apparent increase in the risk of hemorrhagic stroke. A nonsignificant association between LDL cholesterol and the risk of hemorrhagic stroke observed in our data was in accord with the findings of a previous prospective study[18] and intervention trials.[8,28,29]

Several prospective studies conducted in Western countries have reported positive associations between LDL cholesterol and the risk of CHD.[30] Among Japanese, no study has investigated the association between LDL cholesterol level and the risk of CHD, but several prospective studies have shown that total cholesterol is a strong risk factor for CHD.[31,32] The findings obtained from the present analysis support the results from those prospective studies and, for the first time, have demonstrated a positive association between calculated LDL cholesterol and the risk of CHD in a general Japanese population.

The metabolic syndrome has been shown to be a clear risk factor for CVD,[33] but LDL cholesterol level is not involved in the definition of the metabolic syndrome. In the present analysis, comparable and independent effects were observed for elevated LDL cholesterol and the metabolic syndrome on the risks of nonembolic infarction and CHD. The highest risk was observed for the subgroup with both an elevated LDL cholesterol value and the metabolic syndrome. Similar results were found in a prospective study of a Danish cohort.[34] All of these results imply that management of LDL cholesterol as well as the metabolic syndrome is important for the prevention of ischemic stroke and CHD.

The strengths of our study include its longitudinal population-based study design, long duration of follow-up, almost perfect follow-up of subjects, sufficient number of cardiovascular events, and accuracy for diagnosis of CVD, including ischemic stroke subtypes. One limitation of our study is that our findings are based on a 1-time measurement of serum lipids. Subsequent use of cholesterol-lowering agents could have altered lipid levels in some participants; however, this source of variability could not account for the relation observed in the present study, because a random misclassification of such nature would tend to cause an underestimation of study findings and bias the results toward the null hypothesis. Therefore, the true association could be stronger than that observed in our study. Another limitation is that the value of LDL cholesterol was not directly assayed but was calculated by the Friedewald equation. This equation has been adopted in substantial epidemiologic and clinical studies of LDL cholesterol and CVD. It is unlikely that the bias of LDL cholesterol values that occurred through calculation, if any, would have strengthened the association between LDL cholesterol and ATI or CHD observed in the present analysis.

In conclusion, we have shown that elevated LDL cholesterol is a significant risk factor for developing ATI as well as CHD in a general Japanese population. Because LDL cholesterol level is independent of the metabolic syndrome for the development of CVD, lowering a patient's LDL cholesterol level should be considered together with treatment of other metabolic disorders for the prevention of CVD.

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## Disclosures

None.

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# Anti-Inflammatory Effects of Antidepressants: Possibilities for Preventives Against Alzheimer's Disease

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**Abstract:** Increasing evidence of pro-inflammatory mediator expression in major depressions indicate that inflammatory changes may play a role. If this is true, the efficacy of antidepressants may be partially attributable to suppression of inflammation. Various types of antidepressants can suppress serum and plasma levels of pro-inflammatory mediators in patients with major depression. Therefore they can inhibit the production of pro-inflammatory mediators by immune cells. These include glial cells, which are the main sources and targets of cytokines in the brain. This review summarizes the evidence showing that antidepressants have an anti-inflammatory potential. The putative mechanisms are also discussed. Because of the anti-inflammatory effects of antidepressants, they might also act as preventives for neurodegenerative dementias including Alzheimer's disease, where the pathogenesis involves chronic inflammation associated with activated microglia.

**Keywords:** Antidepressants, major depression, Alzheimer's disease, inflammation, cytokines, microglia.

## INTRODUCTION

The history of antidepressant drug development has been unique and fortuitous. The monoamine oxidase inhibitor iproniazid and the tricyclic antidepressant (TCA) imipramine were originally developed as a tuberculosis remedy and as an antihistamine, respectively [1]. These drugs were serendipitously found to have an antidepressant effect in the 1950s, and soon thereafter were shown to increase synaptic levels of noradrenaline (NA) and 5-hydroxytryptamine (5-HT) [1]. Currently, it has been shown that antidepressants modulate not only the monoamine neurotransmitter system but also the inflammatory system.

The association between inflammation and major depression has been supported by the well-known observation that pro-inflammatory cytokines such as interferon (IFN)-α, which is used to treat patients with hepatitis C, and interleukin (IL)-2, which is used to treat patients with certain cancers, frequently induce depressive symptoms as side effects. In addition, depression is often found in inflammatory diseases such as multiple sclerosis, allergies of different types, and rheumatoid arthritis, in which pro-inflammatory cytokines are over-expressed [2]. Animal studies also support this idea. Chronic administration of the endotoxin lipopolysaccharide (LPS) or pro-inflammatory cytokines into rats has been shown to induce symptoms similar to depression. These symptoms are referred to as sickness behavior, which includes appetite loss, suppressed sexual behavior and apathy [3, 4].

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It can be hypothesized that if inflammation plays a causative role in the pathogenesis of major depression, antidepressants may partially act by suppressing such inflammation. The first evidence indicating that antidepressants have anti-inflammatory effects appeared four decades ago. Martelli *et al.* (1967) showed that administration of TCAs inhibited chemically induced edema in the standard rat paw assay [5]. Ten years later, Horrobin and colleagues reported that the TCA clomipramine was a powerful antagonist of prostaglandin (PG) $E_2$ [6] and then proposed that diverse antidepressants are inhibitors of PG synthesis [7]. In fact, a recent *in vitro* study revealed that the selective serotonin reuptake inhibitor (SSRI) paroxetine attenuated cyclooxygenase (COX)-2 expression in human T cells stimulated with phytohemagglutinin (PHA) [8]. Furthermore, experimental evidence is accumulating that various types of antidepressants exert anti-inflammatory effects by decreasing pro-inflammatory cytokine levels or increasing anti-inflammatory cytokine levels.

This review focuses on the influence of antidepressants on inflammatory mediator levels, particularly serum and plasma cytokine levels, in depressed patients. It also focuses on glial production of those mediators *in vitro* since glial cells are the major immune cells responsible for inflammation in the brain. We also discuss possible mechanisms of the anti-inflammatory action of antidepressants and the potential of antidepressants to act as preventives against Alzheimer's disease (AD).

## EVIDENCE FOR INFLAMMATION ASSOCIATED WITH MAJOR DEPRESSION

It has been reported that the levels of acute phase proteins such as C-reactive proteins (CRP), α2-macroglobulin, α1-acid glycoprotein, complement C4 and haptoglobin are

upregulated in major depression [9-14]. The levels of $PGE_2$ and thromboxane $B_2$ are also reported to be elevated in depressed patients [15-17]. Moreover, major depression is accompanied by increased levels of pro-inflammatory cytokines such as IL-1β, IL-6, IFN-γ and tumor necrosis factor (TNF)-α [18-23] whereas the anti-inflammatory cytokine transforming growth factor (TGF)-β1 has been shown to be decreased [24].

In contrast to many studies on serum and plasma levels, there have been few on cerebrospinal fluid (CSF) levels. Only IL-1β has been shown to be increased in major depression [25] while IL-6 was decreased [25] or not changed [26] and TNF-α was not changed [25].

It is uncertain whether inflammation is a cause or a result of major depression. In addition, it must be noted that not all studies have found such an association [27, 28]. Nevertheless, inflammation certainly appears to be a factor in at least some cases of major depression. Indeed, Müller *et al.* (2006) have recently shown interesting data that depressed patients treated for 6 weeks with the serotonin-noradrenaline reuptake inhibitor (SNRI) reboxetine plus the COX 2 inhibitor celecoxib showed significantly greater improvement in scores on the Hamilton Depression Scale compared to the reboxetine-alone group [29].

## EFFECT OF ANTIDEPRESSANTS ON INFLAMMATORY MEDIATOR LEVELS IN PATIENTS WITH MAJOR DEPRESSION

Several groups have studied serum or plasma levels of various cytokines and their receptors in patients with major depression before and after antidepressant pharmacotherapy (Table **1**, for a summery of studies before 2000 see [30]). Tuglu *et al.* (2003) showed that administration of SSRIs for 6 weeks decreased serum levels of TNF-α and CRP [23]. Basterzi *et al.* (2005) showed that similar SSRI treatment diminished serum IL-6 levels [31]. In keeping with such an anti-inflammatory effect, Myint *et al.* (2005) reported that 8-weeks of antidepressant treatment increased plasma TGF-β1 levels [24]. Interestingly, it has been shown that plasma levels of TNF-α and IL-6 in patients with SSRI-resistant depression are significantly higher than those in healthy controls [32]. However, Kubera *et al.* (2000) demonstrated that a 6-week antidepressant treatment which elicited successful clinical remissions did not change significantly the serum levels of IL-6, IL-10 and IL-1 receptor antagonist [33]. Two studies even described increases in the plasma TNF-α levels following antidepressant treatment. Kraus *et al.* (2002) reported that a 4-week treatment with a tetracyclic antidepressant (i.e., mirtazapine) increased the plasma levels of TNF-α and soluble TNF-α receptors significantly while a similar treatment with the SNRI venlafaxine did not influence those levels [34]. Kagaya *et al.* (2001) showed that plasma TNF-α level was increased after 1-month pharmacotherapy consisting mainly of clomipramine. They also examined the plasma levels of IL-1β and IL-6. Those levels after treatment were lower than before treatment, but not significantly [35].

Taken together, the effect of antidepressants on serum and plasma levels of inflammatory cytokines in depressed patients is still controversial. Such an inconsistency may stem from the difference in methodology employed and the limitation due to the small numbers tested in these clinical studies (e.g., $n<30$ in each study). In addition, Kennis and Maes (2002) pointed out the technical difficulty in detecting serum and plasma levels of cytokines since circulating cytokine levels are very low in human subjects [30]. Therefore, early studies on the cytokine concentrations before and after antidepressant treatment often employed *ex vivo* methods. Specifically, cytokine levels in the supernatants of cultured whole blood or cultured peripheral blood mononuclear cells (PBMCs) from depressed patients were measured by enzyme-linked immunosorbent assay (ELISA). In both cases, cytokine production was induced by stimulation with LPS and/or mitogens such as PHA and concanavalin A. Such *ex vivo* studies have shown inconsistent results on protein levels (for review see [30]).

Recently, Tsao *et al.* (2006) examined mRNA expression of inflammatory cytokines in non-stimulated PBMCs from depressed patients before and after 3-month SSRI (i.e., fluoxetine) treatment by using reverse transcriptase-polymerase chain reaction (RT-PCR) assy. They found that such pharmacotherapy significantly diminished the mRNA expression of IFN-γ. The mRNA expressions of IL-1β and TNF-α were also inhibited, but not significantly [36].

## EFFECT OF ANTIDEPRESSANTS ON GLIAL PRODUCTION OF INFLAMMATORY MEDIATORS *IN VITRO*

With regard to *in vitro* studies, various types of antidepressants have anti-inflammatory effects in terms of cytokine production by immune cells. Early studies focused on the effects of antidepressants on cytokine production by cultured PBMCs or cultured whole blood from healthy subjects or depressed patients. They demonstrated that *in vitro* treatment with various types of antidepressants decreased the production of pro-inflammatory cytokines including IFN-γ while increasing the production of such anti-inflammatory cytokines as IL-10 (for reviews see [30, 37]). Moreover, a TCA (amitriptyline) and a SSRI (fluoxetine) were shown to attenuate the production of pro-inflammatory cytokine-induced $PGE_2$ and nitric oxide (NO) by cultured human synovial cells [38].

Increasing evidence strongly suggests that changes in cytokine levels outside the brain cause changes in cytokine expression and activity in the brain, and *vice versa* [39]. In other words, the central and peripheral cytokine compartments are integrated but differently regulated [40]. In the brain, microglia and astrocytes are the major cell types that participate in the inflammatory system both as sources and targets of cytokines. This fact suggests that these glial cells may represent overlooked targets in the etiology of major depression. Several studies have recently investigated the effects of antidepressants on the glial production of inflammatory mediators *in vitro* (Table **2**).

Obuchowicz *et al.* (2006) examined the effects of amitriptyline and its metabolite nortriptyline on the production of IL-1β and TNF-α by rat microglial and mixed glial (i.e., microglia plus astrocytes) cultures stimulated with LPS, using both ELISA and quantitative RT-PCR. They found

**Table 1. Summary of Studies on Serum/Plasma Levels of Inflammatory Mediators in Depressed Patients Before and After Antidepressant Therapy**

| Study | n | Antidepressants | Target Studied | Result |
|---|---|---|---|---|
| Tuglu *et al.* (2003) | 26 | SSRIs (mostly Sertraline/Citalopram) | TNF-α | Decrease |
| | | | CRP | Decrease |
| Basterzi *et al.* (2005) | 23 | SSRIs (not specified) | IL-6 | Decrease |
| Myint *et al.* (2005) | 10 | Various types (mostly Paroxetine/Fluoxetine) | TGF-β1 | Increase |
| Kubera *et al.* (2000) | 9 | Not specified | IL-6 | No change |
| | | | IL-10 | No change |
| | | | IL-1RA | No change |
| Kraus *et al.* (2002) | 9 | SNRI (Venlafaxine) | TNF-α | No change |
| | | | sTNF-Rs | No change |
| | 11 | Tetracyclic (Mirtazapine) | TNF-α | Increase |
| | | | sTNF-Rs | Increase |
| Kagaya *et al.* (2001) | 12 | Mostly TCA (Clomipramine) | TNF-α | Increase |
| | | | IL-1β | No change |
| | | | IL-6 | No change |

IL-1RA, IL-1 receptor antagonist
sTNF-Rs, soluble TNF receptors

**Table 2. Summary of Studies that Examined the Effect of Antidepressants on Glial Production of Inflammatory Mediators *In Vitro***

| Study | Cell Used | Antidepressants | Target Studied | Result |
|---|---|---|---|---|
| Obuchowicz *et al.* (2006) | Rat microglia<br>Rat mixed glia | TCAs (Amitriptyline/Nortriptyline) | IL-1β | Decrease |
| | | | TNF-α | Decrease |
| | | | IL-1β mRNA | No change |
| | | | TNF-α mRNA | No change |
| Hashioka *et al.* (2007) | Mouse microglia (6-3) | TCA (Imipramine) | IL-6 | Decrease |
| | | | NO | Decrease |
| | | SSRI (Fluvoxamine) | IL-6 | Decrease |
| | | | NO | Decrease |
| | | SNRI (Reboxetine) | IL-6 | Decrease |
| | | | NO | Decrease |
| | | LiCl | IL-6 | Increase |
| | | | NO | Decrease |
| Vollmar *et al.* (2008) | Rat mixed glia | SNRI (Venlafaxine) | IL-6 | Decrease |
| | | | IFN-γ | Decrease |
| | | | TGF-β | Increase |
| | | | IL-10 | No change |

**(Table 2) contd....**

| Study | Cell Used | Antidepressants | Target Studied | Result |
|---|---|---|---|---|
| Ha *et al.* (2006) | Mouse microglia (BV2) | SSRI (Fluoxetine) | NO | Increase |
| | | | iNOS mRNA | Increase |
| | | | IL-6 mRNA | Increase |
| | | | TNF-α mRNA | Increase |
| | | | NF-κB activity | Increase |

that treatment with those antidepressants for 24 h significantly inhibited the secretion of both cytokines, but did not change the expression of the mRNAs [41].

We previously studied the effects of various types of antidepressants, as well as the mood stabilizer lithium chloride, on the release of the pro-inflammatory mediators IL-6 and NO from IFN-γ-activated murine 6-3 microglial cells by using ELISA and the Griess reaction, respectively [42]. We showed that 24-h pretreatment with the TCA imipramine, the SSRI fluvoxamine or the SNRI reboxetine significantly inhibited IL-6 and NO production in a dose-dependent manner. On the other hand, lithium chloride had a different spectrum of action, namely by enhancing IFN-γ-induced IL-6 production and inhibiting NO production.

Vollmar *et al.* (2008) measured IL-6, IL-10, IFN-γ and TGF-β concentrations in an astroglia-microglia co-culture treated with venlafaxine for 16 h by ELISA assay [43]. The culture system they employed allows mimicking of an inflammatory milieu by increasing the cultured microglial fraction without any inflammatory stimuli. They demonstrated an augmentation of TGF-β release with a concomitant reduction in the secretion of IL-6 and IFN-γ. Furthermore, they found a significant change of microglial phenotype from activated to resting morphology.

In contrast to those studies, Ha *et al.* (2006) demonstrated that treatment of murine microglial $BV_2$ cells with fluoxetine resulted in significant increases in NO and in the mRNAs of inducible NO synthase (iNOS), IL-6 and TNF-α [44]. They furthermore showed that fluoxetine increased the DNA binding activity of transcription factor nuclear factor-κB (NF-κB), whose activation mediates inflammatory responses. However, the study did not measure the concentrations of IL-6 and TNF-α released from microglial cells. Based on this study and the study by Obuchowicz *et al.*, it can be presumed that antidepressants inhibit the glial secretion of pro-inflammatory cytokines but do not decrease their mRNA levels. Thus, antidepressants may induce post-transcriptional changes in pro-inflammatory cytokines or increase their degradation as Obuchowicz *et al.* suggested.

Although the majority of studies have shown that antidepressants of various classes decrease the glial production of pro-inflammatory cytokines and increase the anti-inflammatory cytokine production, the limitation of such *in vitro* studies should be addressed. Considering the fact that antidepressant treatment needs at least 10-14 days for any clinical effectiveness to appear, the treatment of glial cells with antidepressants for 16-24 h appear to reflect only acute effects of the drugs. In addition, we should note the antidepressant concentrations those studies employed. Maes *et al.* (1999) indicated that 1 μM corresponds to the plasma concentrations attained during clinical treatment [45]. Pharmacokinetic studies in animals have shown that the concentrations of antidepressants detected in certain organs such as the brain and spleen are 10-20 times higher than plasma concentrations due to the lipophilic property of antidepressants [46, 47]. Nevertheless, the concentrations 50-100 μM used in some *in vitro* studies seem to be rather higher than clinically relevant concentrations.

## POSSIBLE MECHANISMS OF ANTI-INFLAMMATORY ACTIONS OF ANTIDEPRESSANTS

The exact mechanism by which antidepressants exert anti-inflammatory effects remains to be elucidated. Although one should remember the possible differences between the mechanism underlying anti-inflammatory effects of drugs *in vitro* and *in vivo*, several mechanisms are possible.

One of the most plausible involves an increase in intracellular cyclic adenosine monophosphate (cAMP) levels. A number of *in vivo* studies have shown that many antidepressants increase intracellular concentrations of cAMP through activation of monoamine receptors such as the receptors for 5-HT and NA [48, 49]. Also, *in vitro*, data indicate that antidepressants of several classes increase intracellular cAMP levels [50, 51]. We demonstrated that TCA, SSRI and SNRI inhibited IFN-γ-induced microglial production of IL-6 and NO *in vitro*. These inhibitions were reversed by the cAMP inhibitor SQ 22536 and by the protein kinase A (PKA) inhibitor Rp-adenosine3', 5'-cyclic monophosphorothioate triethylammonium salt (Rp-3', 5'-cAMPS), suggesting that the anti-inflammatory effects of various antidepressants on microglia are at least partially mediated by the cAMP-dependent PKA pathway [42]. These results are consistent with findings in a study on human whole blood [52]. We also demonstrated that lithium chloride reduced IFN-γ-induced microglial production of NO. Interestingly, the inhibition by lithium chloride was not reversed by either SQ 22536 or Rp-3', 5'-cAMPS, indicating such an inhibitory effect of lithium chloride is not mediated by the cAMP/PKA pathway.

In a number of cell types, the activation of the cAMP/PKA pathway has been shown to inhibit NF-κB activity [53], whose activation is known to induce the gene expression of iNOS and various pro-inflammatory cytokines. Specifically, in rat primary astrocytes [54] and human monocytes [55], the activation of the cAMP/PKA pathway

inhibits LPS-mediated induction of NF-κB binding activity. Activation of the cAMP/PKA pathway not only down-regulates NF-κB activity, it also down-regulates the Janus family kinase (JAK)/signal transducer and activator of transcription (STAT) 1 pathway. Upregulation of the pathway is known to transactivate IFN-γ-responsive genes including iNOS [56] and IL-12 [57]. Recently, Delgado *et al.* (2003) demonstrated in mouse microglia that vasoactive intestinal peptide inhibited IFN-γ-induced JAK/STAT1 activation through upregulation of the cAMP/PKA pathway [58]. Therefore, antidepressant induced upregulation of the cAMP/PKA pathway may mediate inhibitory effects of antidepressants on LPS or IFN-γ-evoked inflammatory transactivations in immune cells (Fig. **1**).

The manner in which *in vivo* anti-depressant treatment increases intracellular cAMP levels appears to be strait forward. Explicitly, it is believed that antidepressants increase synaptic levels of 5-HT and NA through inhibiting reuptake by their transporters on presynaptic neurons. Thus causes activation of their receptors which are coupled to G proteins that can regulate the cAMP system. Through G-protein activation of adenylate cyclase (i.e., through the activation of 5-HT or NA receptor subtypes positively coupled to adenylate cyclase), cAMP production is increased.

It remains unclear as to how antidepressants increase intracellular cAMP levels *in vitro*. Antidepressants may act on cells *in vitro* independently of monoamine receptors coupled to G proteins. A recent genetic study has shown that genes of phosphodiesterases (PDEs), which degrade cAMP, are associated with a susceptibility to major depression and to antidepressant treatment response [59]. Accordingly, antidepressants may directly affect PDE functions in cells and thus increase the intracellular cAMP *in vitro*. Alternatively, we can presume that antidepressants could have direct effects on G proteins.

Maes and colleagues hypothesized that the mechanism is related to the effect of antidepressants on the serotonergic system by 5HT influencing cytokine production [30, 60]. Obuchowicz *et al.* suggested that the mechanism might be nonspecific because antidepressants are potent inhibitors of sodium and calcium influx [61]. Further studies on this subject are clearly warranted.

## POTENTIAL OF ANTIDEPRESSANTS AS PREVENTIVES AGAINST ALZHEIMER'S DISEASE

Dementia and major depression are frequently comorbid among elder people. There is enough evidence from epidemiologic and neuropsychologic studies that major depression is associated with AD, even though it is uncertain whether major depression represents an early sign of dementia or a risk factor for dementia (for review see [62]).

It is well established that inflammatory processes are closely associated with the pathogenesis of a broad spectrum of neurodegenerative diseases [63, 64]. In AD, senile plaque is one of the neuropathological hallmarks of AD and a site of inflammatory processes, as evidenced by the presence of degenerating neurons and numerous reactive microglia and astrocytes [65, 66]. A number of *in vitro* studies have shown that amyloid-β-activated microglia damage or kill neurons by the release of inflammatory mediators such as pro-inflammatory cytokines, nitric oxide and superoxide radicals [67-70]. Therefore, chronic inflammation may be involved in the pathogenesis of both major depression and dementia.

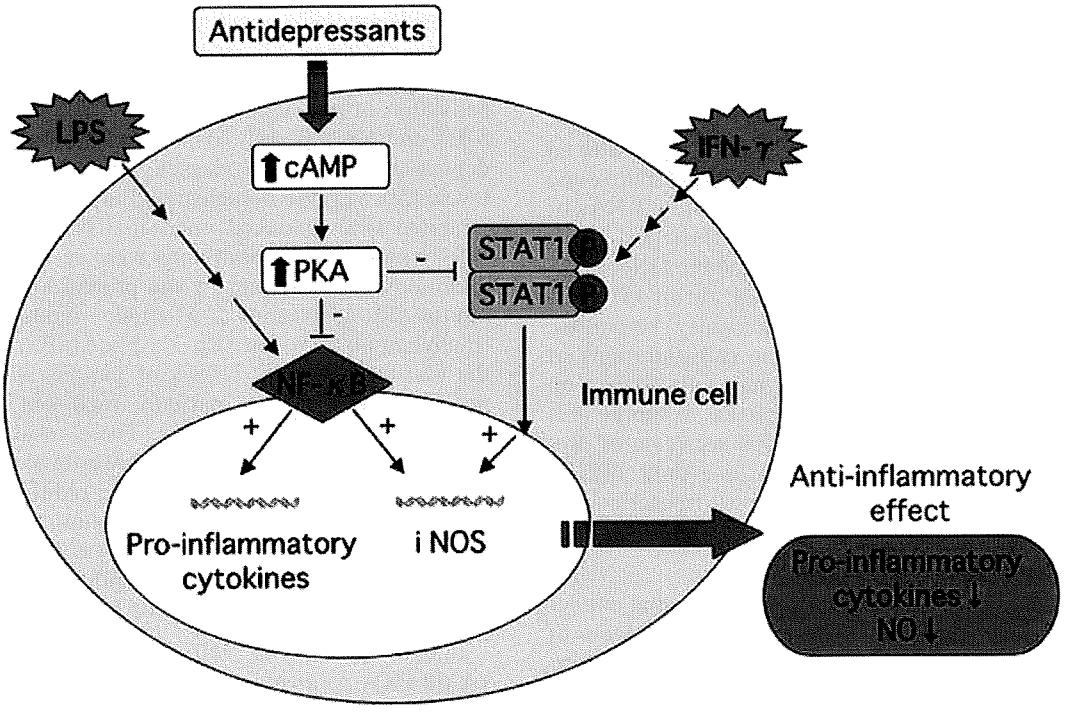


**Fig. (1). Scheme for possible mechanism by which antidepressants exert anti-inflammatory effect in immune cells.** Antidepressants may inhibit LPS or IFN-γ-evoked inflammatory transactivations through the up-regulation of cAMP/PKA pathway in immune cells. See text for details.

More than twenty epidemiological studies have shown that individuals are relatively spared from AD if they have been taking nonsteroidal anti-inflammatory drugs (NSAIDs) or have suffered from conditions where such drugs are routinely used (for review see [71]). In this regard, it is tempting to speculate that antidepressants with anti-inflammatory effects could be useful treatments for neurodegenerative diseases including AD. Interestingly, pre-symptomatic and chronic treatment with paroxetine has been shown to decrease AD-like pathology and reverse memory impairments in 3x transgenic AD mice [72]. Furthermore, in a small, 8-week double-blind placebo-controlled clinical study, fluoxetine was effective in reducing cognitive decline and behavioral abnormalities in patients with mild cognitive impairment [73]. This suggests that antidepressants could ameliorate AD or inhibit the progression of major depression to dementia.

It should be noted that such positive effects of antidepressants on memory and cognitive impairment might not be due to anti-inflammatory effects. Experimental evidence shows that chronic treatment with various antidepressants enhances neurogenesis in adult hippocampus [74, 75]. Clinical evidence indicates that long-term paroxetine treatment increases memory and hippocampal volume in patients with post traumatic stress disorder [76]. Accordingly, improvement of memory and cognition in the aforementioned two studies might be due to the hippocampal neurogenesis induced by antidepressants. Nevertheless, the anti-inflammatory properties of antidepressants may still be involved since the inflammation associated with LPS-activated microglia has been demonstrated to suppress hippocampal neurogenesis in adult rats [77].

## CONCLUSIONS

Accumulating evidence indicates that major depression is associated with inflammation and that various types of antidepressants possess anti-inflammatory properties even though the exact mechanisms remain to be elucidated. Association between major depression and neurodegenerative diseases including AD may be based on the importance of chronic inflammation in the pathogenesis. Some preliminary studies support the hypothesis that antidepressants could prevent AD or inhibit the progression of major depression to dementia. Further studies along these lines are clearly warranted, even though careful consideration of the side effects of antidepressants is required in studies on aged people.

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