

by the systemic injection of scopolamine and changes in ventral hippocampal ACh release in rats. The present results indicate that memory impairment, once induced by acute cholinergic dysfunction, was reversed by the enhancement of dopaminergic transmission. Thus, the ameliorative effect of quinpirole on mnemonic impairment observed in the present study may have been achieved via ACh-DA interaction. The average time spent by rats on each arm in the radial arm maze task was also not affected by the intracerebral administration of dopaminergic agents (Table 1). Therefore, we concluded that the ameliorative effect of the D₂R agonist on mnemonic impairment did not improve locomotor activity, which would be expected when a dopaminergic agonist is administered systemically (Millan et al. 2004). Moreover, Gimenez-Llort et al. (2002) also showed that local raclopride administration to the ventral hippocampus induced no significant changes in locomotor activity.

A number of anatomical, physiological, and behavioral studies (Amaral and Winter 1989; Moser et al. 1993; Trivedi and Coover 2004) have indicated that the hippocampus is functionally heterogeneous along the dorsal-ventral axis; therefore, hippocampal formation is divided into two subregions of the ventral hippocampus (VH) and the dorsal hippocampus (DH). The VH contains the highest density of hippocampal dopamine innervation (Verney et al. 1985). Furthermore, D₂R was shown to be abundant in the VH (Bruinink and Bischoff 1993). Therefore, in the current study, dopaminergic agents were injected into the VH, but not into the DH, and we demonstrated that D₂R in the VH was associated with mnemonic function. On the other hand, it is generally known that the DH, but not the VH, plays an important role in learning and memory, and in particular in spatial memory (Bannerman et al. 2004). However, the relationship of the VH to mnemonic function shown in the present study could also account for recent experimental findings by other research groups. Lorenzini et al. (1996, 1997) showed that a functional inactivation of the rat VH by means of local tetrodotoxin administration induced memory impairment in a passive avoidance task. It was reported that nicotinic antagonist administration to the VH induced spatial memory impairment in rats performing tasks in a radial-arm maze (Felix and Levin 1997; Kim and Levine 1996; Levin et al. 2002). Also, Mishima et al. (2000) revealed that scopolamine administration to the VH induced spatial memory impairment in rats performing a radial-arm maze task. Such experimental evidence has indicated that the VH is associated with learning and memory, at least in the performance of passive avoidance tasks and radial-arm maze tasks. The VH is closely associated with memory-related brain sites, such as the prefrontal cortex (Bannerman et al. 2004). Moreover, the extensive connectivity between the dorsal and ventral hippocampus may also be reflected in some degree of functional interdependence between the two subregions (Amaral and Winter 1989). Further investigation of the involvement of the dopaminergic system in memory function in the VH is still necessary.

Both Alzheimer's disease and dementia with Lewy bodies, two major types of senile and degenerative dementia, are known to be associated with severe cholinergic deficits. Thus, cholinergic enhancers, represented by AChEI, remain a mainstay of pharmacological therapeutics for these diseases (McKeith et al. 1996, 2004; Cummings 2004). Meanwhile, both the pathological and clinical evidence reported to date are suggestive of the involvement of dopaminergic systems in the clinical course of dementia. For example, a loss of hippocampal D₂R in Alzheimer's disease and its correlation with memory have been reported (Joyce et al. 1993; Kemppainen et al. 2003). Empirically, anti-dopaminergic agents have been conveniently used to control various psychological and behavioral symptoms that occur with the progression of dementia (American Psychiatric Association 1997). However, the use of major tranquilizers, in particular D₂R antagonists, reportedly deteriorates cognitive function in patients with dementia (McShane et al. 1997). In agreement with such findings, chronic administration of haloperidol, a non-specific D₂R antagonist, reduces cholinergic fiber immunoreactivity in the rat hippocampus and impairs spatial learning performance of rats performing a water maze task (Terry et al. 2003; Parikh et al. 2004). The present findings appear to indicate that the excessive use of dopaminergic antagonists might potentially exacerbate cognitive decline in patients with dementia. On the other hand, the ameliorative effect observed in this study may suggest that dopaminergic agents could potentially be of therapeutic value in patients with cholinergic deficits, e.g., those with Alzheimer's disease, dementia with Lewy bodies.

Conclusions

The present study indicated the possible involvement of hippocampal ACh-DA interactions in mnemonic processing, as suggested by the finding that the retention of passive avoidance behavior was impaired by the focal injection of a D₂R antagonist into the ventral hippocampus; however, this finding was observed only when the cholinergic system was compromised by dietary manipulation. Furthermore, it was revealed that memory impairment induced by injection of scopolamine could be ameliorated by the focal injection of a D₂R agonist into the same site, i.e., the ventral hippocampus. Thus, the present findings may help us to better understand the therapeutic significance of dopaminergic agents in the treatment of patients with dementia associated with cholinergic deficits.

References

- Acquas E, Chiara GD (2002) Dopamine-Acetylcholine interactions in the basal ganglia. In: Chiara GD (ed) *Dopamine in the CNS II*. Springer, Berlin Heidelberg New York, pp 85-103
- Amaral DG, Winter MP (1989) The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience* 31:571-591

- American Psychiatric Association (1997) Practice guideline for the treatment for patients with Alzheimer's disease and other dementias of late life. *Am J Psychiatry* 154(5 Suppl):1-39
- Bannerman DM, Rawlins JNP, McHugh SB, Deacon RMJ, Yee BK, Bast T, Zhang W-N, Pothuizen HHJ, Feldon J (2004) Regional dissociations within the hippocampus—memory and anxiety. *Neurosci Biobehav Rev* 28:273-283
- Bruinink A, Bischoff S (1993) Dopamine D₂ receptors are unevenly distributed in the rat hippocampus and are modulated differently than in striatum. *Eur J Pharmacol* 245:157-164
- Cummings JL (2004) Alzheimer's disease. *N Engl J Med* 351:56-67
- Daniel JM, Winsauer PJ, Moerschbaecher JM (2003) Castration in rats impairs performance during acquisition of working memory task and exacerbates deficits in working memory produced by scopolamine and mecamlamine. *Psychopharmacology* 170:294-300
- Day J, Damsma G, Fibiger HC (1991) Cholinergic activity in the rat hippocampus, cortex and striatum correlates with locomotor activity: an in vivo microdialysis study. *Pharmacol Biochem Behav* 38:723-729
- Eidi M, Zarrindast M-R, Eidi A, Oryan S, Parivar K (2003) Effects of histamine and cholinergic systems on memory retention of passive avoidance learning in rats. *Eur J Pharmacol* 465:91-96
- Felix R, Levin ED (1997) Nicotinic antagonist administration into the ventral hippocampus and spatial working memory in rats. *Neuroscience* 81:1009-1017
- Frey U, Schroeder H, Matthies H (1990) Dopaminergic antagonists prevent long-term maintenance of posttetanic LTP in the CA1 region of rat hippocampal slices. *Brain Res* 522:69-75
- Gasbarri A, Sulli A, Innocenzi R, Pacitti C, Brioni JD (1996) Spatial memory impairment induced by lesions of the mesohippocampal dopaminergic system in the rat. *Neuroscience* 74:1037-1044
- Gimenez-Llort L, Wang F-H, Ögren OS, Ferré S (2002) Local dopaminergic modulation of the motor activity induced by *N*-methyl-D-aspartate receptor stimulation in the ventral hippocampus. *Neuropsychopharmacology* 26:737-743
- Joyce NJ, Kaeger C, Ryoo H, Goldsmith S (1993) Dopamine D₂ receptors in the hippocampus and amygdala in Alzheimer's disease. *Neurosci Lett* 154:171-174
- Kempainen N, Laine M, Laakso MP, Kaasinen V, Nagren K, Vahlberg T, Kurki T, Rinne JO (2003) Hippocampal dopamine D₂ receptors correlate with memory functions in Alzheimer's disease. *Eur J Neurosci* 18:149-154
- Kim JS, Levine ED (1996) Nicotinic, muscarinic and dopaminergic actions in ventral hippocampus and the nucleus accumbens: effects on spatial working memory in rats. *Brain Res* 725:231-240
- Kurotani S, Umegaki H, Ishiwata K, Suzuki Y, Iguchi A (2003) The age-associated changes of dopamine-acetylcholine interaction in the striatum. *Exp Gerontol* 38:1009-1013
- Levin ED, McGurk SR, Rose JE, Butcher LL (1989) Reversal of a mecamlamine-induced cognitive deficit with the D₂ agonist, LY171555. *Pharmacol Biochem Behav* 33:919-922
- Levin ED, Bradley A, Addy N, Sigurani N (2002) Hippocampal $\alpha 7$ and $\alpha 4\beta 2$ nicotinic receptors and working memory. *Neuroscience* 109:757-765
- Lorenzini CA, Baldi E, Bucherelli C, Sacchetti B, Tassoni G (1996) Role of dorsal hippocampus in acquisition, consolidation and retrieval of rat's passive avoidance response: a tetrodotoxin functional inactivation study. *Brain Res* 730:32-39
- Lorenzini CA, Baldi E, Bucherelli C, Sacchetti B, Tassoni G (1997) Role of ventral hippocampus in acquisition, consolidation and retrieval of rat's passive avoidance response memory trace. *Brain Res* 768:242-248
- McGurk SR, Levin ED, Butcher LL (1989) Radial-arm maze performance in rats is impaired by a combination of nicotinic-cholinergic and D₂ dopaminergic antagonist drugs. *Psychopharmacology* 99:371-373
- McKeith IG, Galasko D, Kosaka K, Perry EK, Dickson DW, Hansen LA, Salmon DP, Lowe J, Mirra SS, Byrne EJ, Lennox G, Quinn NP, Edwardson JA, Ince PG, Bergeron C, Burns A, Miller BL, Lovestone S, Collerton D, Jansen EN, Ballard C, de Vos RA, Wilcock GK, Jellinger KA, Perry RH (1996) Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. *Neurology* 47:1113-1124
- McKeith I, Mintzer J, Aarsland D, Burn D, Chiu H, Cohen-Mansfield J, Dickson D, Dubois B, Duda JE, Feldman H, Gauthier S, Halliday G, Lawlor B, Lippa C, Lopez OL, Carlos Machado J, O'Brien J, Playfer J, Reid W; International Psychogeriatric Association Expert Meeting on DLB (2004) Dementia with Lewy bodies. *Lancet Neurol* 3:19-28
- McShane R, Gedling K, Fairburn C, Jacoby R, Hope T (1997) Do neuroleptic drugs hasten cognitive decline in dementia? Prospective study with necropsy follow up. *Br Med J* 314:266-270
- Millan MJ, Seguin L, Gobert A, Cussac D, Brocco M (2004) The role of dopamine D₃ compared with D₂ receptors in the control of locomotor activity: a combined behavioural and neurochemical analysis with novel, selective antagonists in rats. *Psychopharmacology* 174:341-357
- Mishima K, Iwasaki K, Tsukikawa H, Matsumoto Y, Egashira N, Abe K, Egawa T, Fujiwara M (2000) The scopolamine-induced impairment of spatial cognition parallels the acetylcholine release in the ventral hippocampus in rats. *Jpn J Pharmacol* 84:163-173
- Mishima K, Tsukikawa H, Inada K, Fujii M, Iwasaki K, Matsumoto Y, Abe K, Egawa T, Fujiwara M (2001) Ameliorative effect of vasopressin-(4-9) through vasopressin V(1A) receptor on scopolamine-induced impairments of rat spatial memory in the eight-arm radial maze. *Eur J Pharmacol* 427:43-52
- Moser E, Moser M-B, Anderson P (1993) Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. *J Neurosci* 13:3916-3925
- Mufson EJ, Ginsberg SD, Ikonovic MD, DeKosky ST (2003) Human cholinergic basal forebrain: chemoanatomy and neurologic dysfunction. *J Chem Neuroanat* 26:233-242
- Nakamura A, Suzuki Y, Umegaki H, Ikari H, Tajima T, Endo H, Iguchi A (2001) Dietary restriction of choline reduces hippocampal acetylcholine release in rats: in vivo microdialysis study. *Brain Res Bull* 56:593-597
- Olton DS, Samuelson RJ (1976) Remembrance of places past: spatial memory in rats. *J Exp Psychol Anim Behav Process* 2:97-116
- Parikh V, Terry AV, Khan MM, Mahadik SP (2004) Modulation of nerve growth factor and choline acetyltransferase expression in rat hippocampus after chronic exposure to haloperidol, risperidone, and olanzapine. *Psychopharmacology* 172:365-374
- Paul EG (2003) Acetylcholine modulation of neural systems involved in learning and memory. *Neurobiol Learn Mem* 80:194-210
- Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2nd edn. Academic, San Diego
- Rogers LJ, Kesner PR (2003) Cholinergic modulation of the hippocampus during encoding and retrieval. *Neurobiol Learn Mem* 80:332-342
- Terry VA, Hill DW, Parikh V, Waller LJ, Evans RD, Mahadik PS (2003) Differential effects of haloperidol, risperidone, and clozapine exposure on cholinergic markers and spatial learning performance in rats. *Neuropsychopharmacology* 28:300-309
- Trivedi AM, Coover DG (2004) Lesions of the ventral hippocampus, but not the dorsal hippocampus, impair conditioned fear expression and inhibitory avoidance on the elevated T-maze. *Neurobiol Learn Mem* 81:172-184

- Umegaki H, Munoz J, Meyer RC, Spanger EL, Yoshimura J, Ikari H, Iguchi A, Ingram DK (2001) Involvement of dopamine D₂ receptors in complex maze learning and acetylcholine release in ventral hippocampus of rats. *Neuroscience* 103:27–33
- Verney C, Baulac M, Berger B, Alvarez C, Vigny A, Helle KB (1985) Morphological evidence for a dopaminergic terminal field in the hippocampal formation of young and adult rat. *Neuroscience* 14:1039–1052
- Walsh TJ, Herzog CD, Gandhi C, Stackman RW, Wiley RG (1996) Injection of IgG 192-saporin into the medial septum produces cholinergic hypofunction and dose-dependent working memory deficits. *Brain Res* 726:69–79
- Wilkerson A, Levine ED (1999) Ventral hippocampal dopamine D₁ and D₂ system and spatial working memory in rats. *Neuroscience* 89:743–749
- Wilson JW, Cook AJ (1994) Cholinergic manipulations and passive avoidance in the rat: effects on acquisition and recall. *Acta Neurobiol Exp* 54:377–391
- Yanagihashi R, Yamanouchi K, Ishikawa T (1991) The effects of apomorphine on the hippocampal field potential in freely moving rats: pharmacological evidence of the involvement of D₂ receptors. *Neuropharmacology* 30:177–182
- Zhang HT, O'Donnell JM (2000) Effects of rolipram on scopolamine-induced impairment of working and reference memory in the radial-arm maze tests in rats. *Psychopharmacology* 150:311–316

Association between insulin resistance and cognitive function in elderly diabetic patients

Mari Suzuki,¹ Hiroyuki Umegaki,¹ Tomoko Uno,² Ookhor Oyun,² Nanaka Mogi,¹ Hitoshi Maeno,³ Kunio Yamanouchi,⁴ Akihisa Iguchi¹ and Yuzo Sato²

Departments of ¹Geriatrics, Nagoya University Graduate School of Medicine, Nagoya, ²Department of Health Science, Faculty of Psychological and Physical Science, Aichi Gakuin University, Nissin, ³Meitou Hospital, Nagoya, and ⁴Yamanouchi Clinical Preventive Research Office of Diabetes Mellitus, Aichi-gun, Aichi, Japan

Background: Recently, cognitive impairment in elder diabetic subjects has sparked considerable interest. Insulin resistance (IR) is one of the central pathologies in diabetes mellitus, and several studies have shown that IR is associated with cognitive impairment in non-diabetic elderly subjects. However, the involvement of IR in cognitive dysfunction in the diabetic elderly has remained to be elucidated.

Methods: In the current study we measured IR with the euglycemic insulin clamp technique, and assessed cognitive function in 13 elderly diabetic patients (mean age, 69.1 ± 4.4). Several tests to assess cognitive function including Mini-Mental State Examination (MMSE) were performed, and clinical indices were evaluated. IR was evaluated by metabolic clearance rates (MCR).

Results: The Spearman's rank correlation coefficient between MCR and MMSE scores was 0.587 ($P = 0.035$). When subjects were divided into two groups at the median MCR (5.0 mL/kg/min), the lower MCR (high IR) group ($n = 5$) had significantly lower MMSE scores than the higher group ($n = 8$). The Spearman's rank correlation coefficient was -0.641 ($P = 0.018$) between hs-CRP and MMSE scores. When subjects were divided into two groups at the median of high-sensitivity C-reactive protein (hs-CRP) (594.0 $\mu\text{g/dL}$), the higher hs-CRP group ($n = 6$) had significantly lower MMSE scores than the lower group ($n = 7$).

Conclusion: The current study shows that higher IR measured with the euglycemic insulin clamp technique and higher hs-CRP is associated with lower MMSE scores in non-demented diabetic elderly patients.

Keywords: C-reactive protein, type 2 diabetes mellitus, euglycemic clamp technique, insulin resistance, neuropsychological test.

Introduction

The number of elderly diabetic patients has been increasing in most developed countries including Japan.

In diabetic pathology there are several kinds of abnormalities of the metabolism, mainly the glucose metabolism; these induce systemic complications, namely microangiopathy and macroangiopathy. At the center of the pathophysiology of type 2 diabetes is insulin resistance and compensatory hyperinsulinemia; these pathologies accelerate atherosclerosis through abnormal vascular relaxation, increase of oxidative stress, activation of the sympathetic nervous system, and increased sodium reabsorption. Recently, cognitive impairment in elder diabetic subjects has sparked

Accepted for publication 1 August 2006.

Correspondence: Dr Hiroyuki Umegaki, Department of Geriatrics, Nagoya University Graduate School of Medicine, 65 Tsuruma-Cho, Showa-ku, Nagoya, Aichi 466-8550, Japan.
Email: umegaki@med.nagoya-u.ac.jp

considerable interest. Several studies have shown that diabetes is a risk for cognitive decline and incidents of dementia, not only of vascular origin, but also of Alzheimer-type.^{1,2}

The pathogenesis of diabetes-related cognitive impairment has not been fully elucidated; however, several hypotheses have been raised. The involvement of insulin resistance (IR) is one of these. Increasing evidence suggests that IR and/or elevated insulin levels may be associated with adverse effects on cognition in non-diabetic elderly subjects.³⁻⁵

Although IR is evaluated by several methods such as the homeostatic model assessment (HOMA-R), the euglycemic insulin clamp technique is the gold standard for IR measurement.⁶ While some studies investigated the association between IR and cognition,³⁻⁵ no studies have used the euglycemic insulin clamp technique to assess the relationship between IR and cognitive function in diabetic patients.

In the current study we measured IR with the euglycemic insulin clamp technique, and assessed cognitive function in non-demented elderly diabetic patients.

Methods

Subjects

Thirteen elderly diabetic outpatients (six males and seven females) whose ages ranged between 65 and 77 (mean age, 69.1 ± 4.4) were enrolled in the study. Subjects with a diagnosis of dementia according to the Diagnostic and Statistical Manual of Mental Disorders Third Edition Revised (DSM-III-R) for dementia⁷ and/or whose score on the Mini Mental State Examination (MMSE) was 23 or lower were excluded, as were those who had a clinical history and/or neurological symptoms of stroke. Subjects with the diagnosis or treatment of depression were not involved. No subjects had difficulty in undergoing a cognitive functional assessment of audio-visual deficiency. The users of thiazolidine derivatives or metformin were excluded.

An ethical committee in the Department of Health Science, Faculty of Psychological and Physical Science, Aichi Gakuin University approved our study, and all patients gave their written informed consent prior to participation. Cognitive functional tests were administered individually to each subject. Assessment was performed in the morning, after the subjects had eaten their usual breakfast and had been examined by a doctor to ascertain their physical condition.

Cognitive tests

Cognitive function was assessed by structured performance tests that were selected to represent a broad range of cognitive domains, including those measured

in previous studies of type 2 diabetes, as reviewed by Strachan *et al.*⁸ The MMSE,⁹ which assesses orientation, registration, attention, calculation, language and recall with a score range from 0 to 30, was used to assess mental status. For verbal memory, we used the Word List, a subtest of the Alzheimer's Disease Assessment Scale (ADAS).¹⁰ Paragraph recall (immediate and delayed) from the neuropsychological tests of the National Center of Neurology and Psychiatry, Japan¹¹ was also assessed. Complex psychomotor skills were evaluated by the Digit Symbol Test, a subtest of the Wechsler Adult Intelligence Scale-Revised (WAIS-R).¹² We used the Stroop Color-Word Test¹³ (Japanese version) to assess attention. Seconds to completion are recorded and the difference between the time required to read the word card and that required to read the dots card is calculated.

A well-trained psychological tester administered all four tests in the same order for all subjects. The doctors checked the physical conditions of the subjects before the assessment and confirmed that they were not hypoglycemic.

Glucose infusion rates (GIR) and metabolic clearance rates (MCR) were determined by the euglycemic clamp procedure.

Insulin action was evaluated using the euglycemic clamp, a method that has already been described in detail.¹⁴ Briefly, after an overnight fast, an intravenous catheter was inserted into an antecubital vein for infusion of insulin and glucose. Regular human insulin (Novolin-R, Novo Nordisk A/S, Denmark) was continuously infused using a continuous-injection pump at a rate of 40 mU/m²/min. The blood glucose concentration was measured at 5-min intervals during the clamp procedure using a glucose monitor (G sensor, GLUCOCARD G meter, ARKLAY, Inc., Japan), and it was maintained at the fasting level by adjusting the infusion rate of a 20% glucose solution. Given that the hepatic glucose output has been shown to be suppressed more than 90% at 40 mU/m²/min insulin infusion, the GIR could be regarded as a measure of the peripheral glucose uptake.¹⁵ In order to compare *in vivo* insulin action of subjects with different basal plasma glucose levels, MCR were calculated by dividing GIR by the mean plasma glucose.¹⁶ Generally, a lower MCR means a higher IR.

The euglycemic clamp and the cognitive assessment were performed on the same day.

Clinical characteristics

Covariants examined were age, sex, years of education, duration of diabetes, HbA1c, fasting blood sugar (FBS), immunoreactive insulin (IRI), body mass index (BMI), total cholesterol (TC), high density lipoprotein (HDL) cholesterol, triglyceride (TG), systolic blood pressure (SBP), diastolic blood pressure (DBP), statin use,

antihypertensive medication and smoking. IRI was measured only in non-insulin users. High-sensitivity C-reactive protein (hs-CRP) was measured by latex-enhanced assay.¹⁷

Statistical analysis

All data are presented as mean ± standard deviation (SD). All variables were divided into two groups at the median value, and compared by Mann-Whitney's U analysis. Spearman's rank correlation coefficient was calculated to confirm the relationship between the variables. A P-value of less than 0.05 was considered to indicate statistical significance.

Results

The characteristics of the patients are shown in Table 1. The mean HbA1c and BMI were 7.6 ± 0.6% and 24.9 ± 8.1, respectively. The mean score of MMSE was 26.9 ± 1.5, and the mean MCR was 4.9 ± 1.2 mL/kg/min.

MMSE scores and MCR were significantly correlated (Spearman's rank correlation coefficient = 0.587, P = 0.035) (Fig. 1). The subjects were divided into two groups at the median MCR (5.0 mL/kg/min), and all other variables were compared. MMSE scores were significantly lower in the group with lower MCR values (higher IR) (25.5 ± 0.6, n = 8) than in those with higher MCR (27.8 ± 1.0, n = 5).

The MMSE scores and hs-CRP were significantly inversely correlated (Spearman's rank correlation

coefficient = -0.641, P = 0.018) (Fig. 2). The subjects were divided into two groups at the median hs-CRP (594.0 µg/dL), and all other variables were compared. MMSE scores were significantly lower in the group with higher hs-CRP (26.2 ± 1.2, n = 7) than in that with lower hs-CRP (28.2 ± 1.3, n = 6).

No significant association was found between MCR and hs-CRP (P = 0.256). Other cognitive test scores had no significant associations with MCR or hs-CRP. BMI did not have significant correlations with MCR or hs-CRP (P = 0.115, 0.291, respectively). Specific data are shown in Table 2.

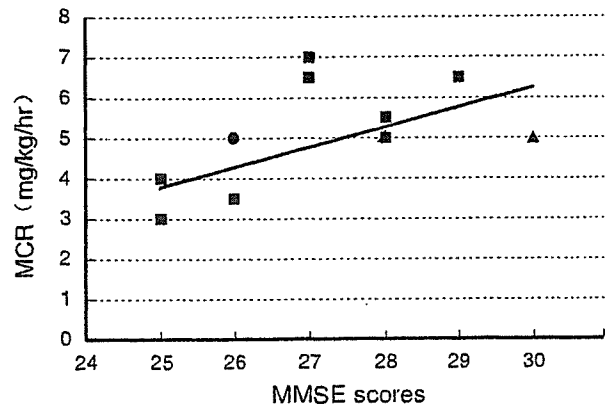


Figure 1 The association of Mini-Mental State Examination (MMSE) scores and metabolic clearance rates (MCR). The MMSE scores and MCR measurement were significantly correlated. Spearman's rank correlation coefficient was 0.587 (P = 0.035). ●, diet; ■, oral hypoglycemic agents (OHA); ▲, insulin.

Table 1 Clinical characteristics of the participants

Sex (male/female)	6/7
Age (years)	69.1 ± 4.4
Duration of diabetes (years)	16.9 ± 7.9
Therapy (diet/OHAs/insulin)	4/6/3
Body mass index (kg/m ²)	22.5 ± 8.2
Systolic blood pressure (mmHg)	128.0 ± 12.6
Diastolic blood pressure (mmHg)	78.1 ± 6.6
HbA1c (%)	7.6 ± 0.6
FBS (mg/dL)	132.6 ± 33.0
IRI (µU/mL)	11.7 ± 9.0
TC (mg/dL)	204.2 ± 25.8
HDL (mg/dL)	54.1 ± 14.6
TG (mg/dL)	126.4 ± 76.4
MCR (ml/kg/min)	4.9 ± 1.2
hs-CRP(µg/dL)	846.2 ± 219.2

Values are expressed as means ± SD.

BMI, body mass index; FBS, fasting blood sugar; HDL, high density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; IRI, immunoreactive insulin; MCR, metabolic clearance rates; OHA, oral hypoglycemic agents; TC, total cholesterol; TG, triglyceride.

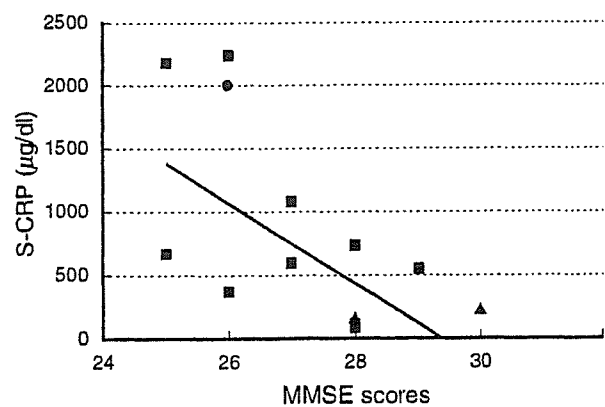


Figure 2 The association of Mini-Mental State Examination (MMSE) scores and high-sensitivity C-reactive protein (hs-CRP). The MMSE scores and metabolic clearance rates (MCR) measurement were significantly correlated. Spearman's rank correlation coefficient was -0.641 (P = 0.018). ●, diet; ■, oral hypoglycemic agents (OHA); ▲, insulin.

Table 2 Results of individual data

	MCR		hs-CRP	
	MCR < 5	MCR ≥ 5	S-CRP ≥ 594	S-CRP < 594
Sex (male/female)	3/1	3/6	2/4	4/3
Age (years)	69.1 ± 4.1	69.0 ± 5.7	66.9 ± 3.7	71.7 ± 3.8
Duration of diabetes (years)	16.3 ± 8.4	18.3 ± 7.7	16.4 ± 6.9	17.5 ± 9.6
HbA1c (%)	7.6 ± 0.7	7.5 ± 0.5	7.5 ± 0.8	7.7 ± 0.4
Body mass index (kg/m ²)	27.9 ± 9.0	23.2 ± 5.3	29.6 ± 9.0	22.8 ± 5.6
Systolic blood pressure (mmHg)	132.9 ± 10.59	117.0 ± 10.1	129.3 ± 14.7	126.5 ± 10.7
Diastolic blood pressure (mmHg)	80.0 ± 6.9	73.8 ± 3.8	78.4 ± 5.5	77.7 ± 8.3
TC (mg/dL)	208.2 ± 28.8	195.3 ± 17.5	212.3 ± 29.0	194.8 ± 19.8
HDL (mg/dL)	56.2 ± 14.6	49.3 ± 15.435	49.0 ± 13.5	60.0 ± 14.6
TG (mg/dL)	118.9 ± 58.2	143.3 ± 117.4	160.7 ± 62.4	86.3 ± 75.9
IRI (μU/mL)	13.6 ± 10.1	7.5 ± 4.3	11.4 ± 9.5	12.1 ± 9.4
FBS (mg/dL)	127.6 ± 33.1	144.0 ± 34.4	131.9 ± 30.2	133.5 ± 38.9
MCR (ml/kg/min)	4.3 ± 0.8	6.4 ± 0.6	4.9 ± 1.5	5.1 ± 1.0
hs-CRP(μg/dL)	966.2 ± 906.6	576.3 ± 407.9	1355.9 ± 752.6	251.7 ± 177.8
MMSE	26.9 ± 1.7	27.8 ± 1.0	26.3 ± 1.1	28.2 ± 1.3
ADAS (immediate)	7.1 ± 1.1	6.3 ± 1.0	7.0 ± 1.1	6.6 ± 1.2
ADAS (delayed)	6.8 ± 2.0	7.3 ± 1.9	7.1 ± 2.0	6.7 ± 1.9
WAIS-R Symbol	48.1 ± 9.8	40.5 ± 13.2	41.6 ± 12.3	50.7 ± 7.4
Paragraph recall (immediate)	8.3 ± 3.0	5.6 ± 1.8	7.4 ± 3.5	7.6 ± 2.3
Paragraph recall (delayed)	6.6 ± 3.7	4.4 ± 1.4	6.0 ± 3.8	5.8 ± 3.0
Stroop test (seconds)	12.9 ± 6.5	13.1 ± 7.0	14.1 ± 7.1	11.6 ± 5.7

Values are expressed as means ± SD.

ADAS, Alzheimer's Disease Assessment Scale; BMI, body mass index; FBS, fasting blood sugar; HDL, high density lipoprotein cholesterol; hs-CRP, high sensitivity c reactive protein; IRI, immunoreactive insulin; MMSE, Mini-Metal State Examination; TC, total cholesterol; TG, triglyceride.

Discussion

In the present study MMSE scores in non-demented elderly diabetic subjects were significantly correlated with MCR and hs-CRP. The group with lower MCR had significantly lower MMSE scores, and the higher hs-CRP group had significantly lower MMSE scores. These results suggested that in the non-demented elderly diabetic subjects who are at high risk for cognitive impairment, IR and/or inflammation were associated with cognitive function.

Previous studies have reported an association between IR and cognitive impairment in non-diabetic subjects based on plasma insulin measurement, HOMA-R, or the Quantitative Insulin Sensitivity Check Index.¹⁸ The current study assessed IR using the euglycemia clamp technique, which is a gold standard for IR assessment, and demonstrated significant correlation between IR and MMSE scores in non-demented elderly diabetic patients.

The mechanism of the involvement of IR in cognitive impairment remains to be elucidated; however, several hypotheses have been raised. First, IR accelerates atherosclerosis,¹⁹ and asymptomatic cerebrovascular lesions may lead to cognitive decline. Second, IR-

induced compensatory hyperinsulinemia may be involved. Amyloid beta is a neurotoxic substrate that plays a critical role in the pathogenesis of dementia of Alzheimer type. Insulin degrading enzyme (IDE) reportedly degrades in the central nervous system, and an excess amount of insulin may lead to a decrease of amyloid beta degeneration by IDE with a competitive mechanism.²⁰ The current study showed that IR was associated with cognitive decline in elderly diabetic patients; however, the mechanism still remains unknown, and further study is required to investigate how IR is associated with cognitive decline in non-diabetic and diabetic elderly persons.

Several studies have reported that inflammation is associated with cognitive impairment in non-diabetic subjects,^{21,22} and recently in elderly subjects with metabolic syndrome,²³ a higher inflammatory marker was shown to be a risk factor for cognitive decline. The links between systemic inflammation and cognitive decline also remain largely unknown. Inflammation is associated with atherosclerosis,²⁴ again, inflammation may be associated with cognitive impairment through a vascular mechanism. Indeed, one study has demonstrated a relationship between inflammatory proteins and silent brain infarctions.²⁵ Because overexpression of IL-6, which is

an inflammatory cytokine, showed progressive neuronal loss and decreased learning in an animal model,²⁶ it is possible that inflammation itself could affect cognitive ability. One study demonstrated that hyperinsulinemia provokes an increase of inflammatory cytokines and amyloid beta in the periphery and the brain.²⁷

In the current study we did not find a statistically significant relationship between IR (MCR) and inflammation (hs-CRP). Many previous reports have indicated a definite relation between IR and inflammation;²⁸ therefore, further investigation is required.

The reasons why only MMSE scores but not other types of cognitive functional assessment were associated with IR or inflammation were not clear; however, this may suggest that factors other than IR or inflammation contribute to diabetes-related cognitive dysfunction.

The current study had several limitations. First, because of the cross-sectional study design, it was unclear whether treatment on IR and/or inflammation leads to cognitive functional recovery. Many studies have demonstrated that exercise improves IR in the elderly.^{29,30} Interestingly, several observational studies reported that higher physical activities were associated with reduced risk for dementia.^{31,32} It is important to investigate whether improving IR by exercise can ameliorate cognitive impairment. Second, because the number of patients involved was relatively small, the influence of antidiabetic medication and/or other possible confounding factors could not be assessed. In the current study the users of thiazolidine derivatives or metformin were excluded, however, other drugs including statins or the inhibitors of the angiotensin system could affect IR or inflammation.³³ Therefore, the effects of these drugs and other possible confounding factors will be studied with more subjects in the future. The Rotterdam study reported that patients with insulin treatment had a higher risk of dementia.³⁴ The effects of exogenous insulin on cognitive function should also be further investigated. Third, brain imaging was not available in the current study although the subjects with a history of stroke were excluded. Asymptomatic brain infarctions may be involved in IR-mediated cognitive dysfunction, and future studies will be needed. Finally, because the subjects with very mild Alzheimer's disease and other degenerative diseases could not be excluded in the current study, further follow-up study may be needed.

In summary, higher IR measured with the euglycemic insulin clamp technique and higher hs-CRP were both associated with lower MMSE scores in the diabetic elderly.

Acknowledgments

This research was supported by a Longevity Science Research Grant from the Ministry of Health and Labor

of Japan (H13-009) and a Grant in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (11670066). We would like to thank all subjects who participated in this study. No conflicts of interest exist.

References

- 1 Cukierman T, Gerstein HC, Williamson JD. Cognitive decline and dementia in diabetes-systematic overview of prospective observational studies. *Diabetologia* 2005; **48**: 2460-2469.
- 2 Mogi N, Umegaki H, Hattori A *et al.* Cognitive function in Japanese elderly with type 2 diabetes mellitus. *J Diabetes Complicat* 2004; **18**: 42-46.
- 3 Geroldi C, Frisoni GB, Paolisso G *et al.* Insulin resistance in cognitive impairment: the InCHIANTI study. *Arch Neurol* 2005; **62**: 1067-1072.
- 4 Kalmijn S, Feskens EJ, Launer LJ, Stijnen T, Kromhout D. Glucose intolerance, hyperinsulinaemia and cognitive function in a general population of elderly men. *Diabetologia* 1995; **38**: 1096-1102.
- 5 Vanhanen M, Koivisto K, Kuusisto J *et al.* Cognitive function in an elderly population with persistent impaired glucose tolerance. *Diabetes Care* 1998; **21**: 398-402.
- 6 Wallace TM, Matthews DR. The assessment of insulin resistance in man. *Diabet Med* 2002; **19**: 527-534.
- 7 American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 3rd edn. revised. Washington, DC: American Psychiatric Association, 1987.
- 8 Strachen MW, Ewing FM, Deary IJ, Frier BM. Is type 2 diabetes associated with an increased risk of cognitive dysfunction? A critical review of published studies. *Diabetes Care* 1997; **20**: 438-445.
- 9 Folstein MF, Folstein SE, McHugh PR. 'Mini-Mental State': a practical method of grading the cognitive function of patients for the clinician. *J Psychiatr Res* 1978; **12**: 189-198.
- 10 Mohs RC, Rosen WG, Davis KL. The Alzheimer's disease assessment scale: an instrument for assessing treatment efficacy. *Psychopharmacol Bull* 1983; **19**: 448-450.
- 11 Ueda M, Takayama Y, Sasanuma S. Memory disorders in the elderly stage of dementia of the Alzheimer type: preliminary findings. *Jap J Neuropsychol* 1996; **12**: 178-186.
- 12 Shinagawa F, Kobayashi S, Fujita K, Mackawa H. Japanese manual for the Wechsler Adult Intelligence Scale-Revised. *Nihon-Bunka-kagaku-sya* 1990; 115-118.
- 13 Stroop JR. Studies of interference in serial verbal reactions. *J Exp Psychol* 1935; **18**: 643-662.
- 14 Uno T, Ohsawa I, Tokudome M, Sato Y. Effects of Goshajinkigan on insulin resistance in patients with type 2 diabetes. *Diabetes Res Clin Pract* 2005; **69**: 129-135.
- 15 DeFronzo RA, Ferrannini E, Sato Y, Felig P, Wahren J. Synergistic interaction between exercise and insulin on peripheral glucose uptake. *J Clin Invest* 1981; **68**: 1468-1474.
- 16 Doberne L, Greenfield MS, Rosenthal M, Widstrom MA, Reaven G. Effect of variations in basal plasma glucose concentration on glucose utilizations (M) and metabolic clearance (MCR) rates during insulin clamp studies in patients with non-insulin-dependent diabetes mellitus. *Diabetes* 1982; **31**: 396-400.
- 17 Rifai N, Russel PT, Ridker PM. Clinical efficacy of an automated high-sensitivity C-reactive protein assay. *Clin Chem* 1999; **45**: 2136-2141.

- 18 Messier C. Impact of impaired glucose tolerance and type 2 diabetes on cognitive aging. *Neurobiol Aging* 2005; 26: 26–30.
- 19 Mankovsky BN, Ziegler D. Stroke in patients with diabetes mellitus. *Diabetes Metab Res Rev* 2004; 20: 268–287.
- 20 Qiu WQ, Folstein MF. Insulin, insulin-degrading enzyme and amyloid-beta peptide in Alzheimer's disease: review and hypothesis. *Neurobiol Aging* 2006; 27: 190–198.
- 21 Schmidt R, Schmidt H, Curb JD, Masaki K, White LR, Lawner LJ. Early inflammation and dementia: a 25-year follow-up of the Honolulu-Asia Aging Study. *Ann Neurol* 2002; 52: 168–174.
- 22 Weaver JD, Huang MH, Albert M, Harris T, Rowe JW, Seeman TE. Interleukin-6 and risk of cognitive decline: MacArthur studies of successful aging. *Neurology* 2002; 59: 371–378.
- 23 Yaffe K, Kanaya A, Lindquist K *et al*. The metabolic syndrome, inflammation, and risk of cognitive decline. *JAMA* 2004; 292: 2237–2242.
- 24 Jain MK, Ridker PM. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. *Nat Rev Drug Discov* 2005; 4: 977–987.
- 25 Hoshi T, Kitagawa K, Yamagami H, Furukado S, Hougaku H, Hori M. Relations of serum high-sensitivity C-reactive protein and interleukin-6 levels with silent brain infarction. *Stroke* 2005; 36: 768–772.
- 26 Campbell IL, Abraham CR, Masliah E *et al*. Neurological disease induced in transgenic mice by cerebral overexpression of interleukin 6. *Proc Natl Acad Sci USA* 1993; 90: 10 061–10 065.
- 27 Fishel MA, Watson GS, Montine TJ *et al*. Hyperinsulinemia provokes synchronous increases in central inflammation and beta-amyloid in normal adults. *Arch Neurol* 2005; 62: 1539–1544.
- 28 Sjöholm A, Nystrom T. Endothelial inflammation in insulin resistance. *Lancet* 2005; 365: 610–612.
- 29 Kubota M, Nagasaki M, Tokudome M, Shinomiya Y, Ozawa T, Sato Y. Mechanical horseback riding improves insulin sensitivity in elder diabetic patients. *Diabetes Res Clin Pract* 2006; 71: 124–130.
- 30 Rimbart V, Boirie Y, Bedu M, Hocquette JF, Ritz P, Morio B. Muscle fat oxidative capacity is not impaired by age but by physical inactivity: association with insulin sensitivity. *FASEB* 2004; 18: 737–739.
- 31 Laurin D, Verreault R, Lindsay J, MacPherson K, Rockwood K. Physical activity and risk of cognitive impairment and dementia in elderly persons. *Arch Neurol* 2001; 58: 498–504.
- 32 Larson EB, Wang L, Bowen JD *et al*. Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. *Ann Intern Med* 2006; 144: 73–81.
- 33 Hsueh WA, Lyon CJ, Quinones MJ. Insulin resistance and the endothelium. *Am J Med* 2004; 117: 109–117.
- 34 Ott A, Stolk RP, Hofman A, van Harskamp F, Grobbee DE, Breteler MM. Association of diabetes mellitus and dementia: the Rotterdam Study. *Diabetologia* 1996; 39: 1392–1397.

Cognitive Function in the Elderly with Diabetes Mellitus

Hiroynuki Umegaki* and Akihisa Iguchi

Department of Geriatrics, Nagoya University Graduate School of Medicine, 65 Tsuruma-Cho, Showa-Ku, Nagoya, Aichi 466-8550, Japan

Received 28 September, 2005; Accepted 31 October, 2005

Summary Impaired cognitive function in the elderly with diabetes mellitus has been reported, and many, but not all, studies showed that diabetes mellitus is a risk factor for dementia. Although the mechanism of cognitive dysfunction in elderly diabetes mellitus subjects remains to be elucidated, several lines of evidence indicate that insulin and/or inflammation may be involved.

Key Words: diabetes mellitus, dementia, Alzheimer's disease, insulin, TNF- α

Introduction

The number of patients with diabetes mellitus (DM), especially type 2 DM, is increasing all over the world, including Japan. DM is an age-related condition, and one-third of the DM patients in Japan are over 60. Although the influence of DM on brain functions has been studied for more than 80 years [1], the increase of elderly DM patients has recently caused more interest to be paid to the cognitive functions of these subjects.

Cognitive Decline in DM Elderly

DM-related cognitive decline has been reported in many cross-sectional and in several longitudinal studies [2–4]. We studied the cognitive functions of Japanese non-demented elderly DM patients and compared them to age-matched non-DM subjects (Table 1) [5]. Subjects with a diagnosis of dementia and/or whose score on the Mini Mental State Examination (MMSE) [6] was 23 or lower were excluded, as were those who had a clinical history and/or neurological symptoms of stroke. The cognitive assessment included the MMSE, a Word List recall (immediate and delayed) activity

from a subtest of the Alzheimer's Disease Assessment Scale (ADAS) [7], a Digit Symbol Test, which is a subtest of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) [8], and the Stroop Color-Word Test [9]. In this study, slight but significant cognitive impairment in elderly DM subjects was found in Japanese DM elderly (Table 2).

Dementia in DM Elderly

Several cross-sectional and longitudinal studies showed that DM is associated with dementia. The Rotterdam study reported that DM is a risk factor for Alzheimer's type dementia [10], and several longitudinal data are in agreement with this finding [11–13]; however, other studies showed that DM is associated with vascular dementia, not with Alzheimer's type dementia [14–16].

The association of AD pathology and DM is also controversial; the Honolulu-Asia Aging study reported that DM subjects had more neuritic plaques and neurofibrillary tangles in the hippocampus [17], but in contrast with this, autopsy samples from a Jewish nursing home showed less AD-related pathology in DM subjects [18].

Brain Atrophy in DM Elderly

In 1994, a Japanese group reported that elderly DM subjects (6th to 8th decade) had more frequent cerebral atrophy than non-DM subjects [19]. We also reported the

*To whom correspondence should be addressed.

Tel: +81-52-744-2365 Fax: +81-52-744-2371

E-mail: umegaki@med.nagoya-u.ac.jp

Table 1. Characteristics of participants by diabetes status

Variable	DM subjects	Non-DM subjects	P value
N	69	27	—
Age	71.6 ± 5.6	73.4 ± 6.6	0.164
Gender (% female)	70.4	52.2	0.107
Education (years)	10.4 ± 2.7	11.4 ± 3.0	0.167
Hypertension (%)	52.5	50.0	0.845
Hyperlipidemia (%)	36.5	60.0	0.074
HbA1c (%)	8.0 ± 1.0	5.7 ± 0.4	P<0.01

Data are the mean ± SD unless otherwise indicated.

Student's unpaired *t*-test (age, education, HbA1c) and Kruskal-Wallis analysis (other variables).

Table 2. Performance on measures of cognitive function by diabetes status

Measure	DM subjects	Non-DM subjects	P value
MMSE	27.1 ± 2.2	28.3 ± 1.7	P<0.05
Word List (immediate)	5.7 ± 1.7	6.2 ± 1.7	0.254
(delayed)	7.1 ± 2.2	6.7 ± 2.0	0.364
WAIS-R Digit Symbol	36.3 ± 10.9	43.0 ± 12.1	P<0.05
Stroop Color Word Test	19.2 ± 12.8	15.0 ± 6.7	0.113

Data are the means ± SD, unless otherwise indicated.

A higher score indicates better performance, except in the case of the Stroop Color Word Test.

Student's unpaired *t*-test.

WAIS-R: Wechsler Adult Intelligence Scale-Revised.

Table 3. Values of brain CT measurements in DM and CR subjects

Indices	DM	CR	P value
Evan's ratio	0.264 ± 0.024	0.248 ± 0.029	0.032
i-CM	0.239 ± 0.033	0.230 ± 0.028	0.344
temporal horn (right) (mm)	10.3 ± 4.6	7.9 ± 3.0	0.048*
temporal horn (left) (mm)	10.7 ± 5.8	7.7 ± 3.0	0.046*
maximal width of Sylvian fissure (right) (mm)	24.3 ± 5.1	21.7 ± 5.5	0.091
maximal width of Sylvian fissure (left) (mm)	23.8 ± 4.0	21.7 ± 4.7	0.218

**p*<0.05 by ANOVA followed by Scheffe's post hoc test

Indices used to assess brain CT were: a) Evan's Ratio, the maximal width of the frontal horns divided by the maximal internal width of the skull on the same slice level, b) inverse Cella Media Index, the minimum width of the lateral ventricles at the cella media region divided by the maximal internal width of the skull on the same level, c) width of the third ventricle (mm), d) temporal horns, the maximal width of [the] temporal horn tips (mm) on both sides, e) the maximal width of the Sylvian fissure (mm) at the insula on both sides.

widening of the temporal horns on both sides in DM subjects (mean age 71.2), suggesting the atrophy of the hippocampus (Table 3) [20], results which were in agreement with those from the Rotterdam study. In this study, MRI volumetric techniques were used to demonstrate the atrophy of the hippocampus and amygdala in elderly DM subjects [21].

A large multicenter collaborative study in Europe demonstrated that DM in the elderly (65–75) was associated with cortical brain atrophy, and that a strong interaction between diabetes and hypertension existed [22]. A large study in the USA also demonstrated that DM was associated with brain atrophy in a relatively young cohort (mean 62.3) [23].

Pathological Mechanism of DM-related Cognitive Decline

Involvement of insulin

The Rotterdam study reported that DM subjects with insulin treatment had a stronger risk for dementia [10]. Several studies demonstrated that hyperinsulinemia or insulin resistance is associated with cognitive decline or AD risk [24, 25]. Although the mechanism of this association is not clear, several hypotheses have been proposed. High insulin levels may be a reflection of a sedentary life-style, which is associated with a higher risk of dementia [26]. Peripheral insulin is transported to the brain across the blood-brain barrier, and insulin receptors exist in the hippocampus and other brain regions [27]. Insulin may have some effects on the neurons in the CNS. Recently, insulin degrading enzyme (IDE) has been found to play a role in regulating amyloid beta, a peptide which is closely involved in AD pathology, and insulin may inhibit the activity of the degradation of amyloid beta by IDE to increase the levels of amyloid beta in the brain [28]. Craft *et al.* reported that insulin stimulates inflammation in the CNS [29].

Involvement of cytokines

We performed a study to elucidate the mechanism of DM-related cognitive decline [30]. Inflammatory mechanisms have been hypothesized to play a role in the pathogenesis of several age-associated diseases, including Alzheimer's disease [31]. Additionally, high plasma levels of inflammatory proteins reportedly increase the risk of cognitive decline in both non-DM [32, 33] and DM subjects [34], although the involvement of inflammatory cytokines in DM-related cognitive impairment has not yet been investigated.

We recruited 45 outpatients (25 males and 20 females) ranging in age from 65 to 85 years old (mean age, 72.6 ± 5.7). The average HbA1c and BMI were 7.0% and 24.6, respectively. The same exclusion criteria and cognitive assessment as those in Mogi *et al.* [5] were applied.

The clinical variables assessed were age, sex, years of education, DM duration, HbA1c, fasting serum glucose, immunoreactive insulin, body mass index, total cholesterol (TC), high-density lipoprotein cholesterol, triglyceride, systolic blood pressure, diastolic blood pressure, statin use, antihypertensive medication and smoking, and the existence of diabetic microangiopathic complications (neuropathy, nephropathy, retinopathy). The levels of serum tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) were determined by commercially available enzyme-linked immunosorbent assays (Quantikine HS TNF α and Quantikine HS IL-6, R & D Systems, Minneapolis, MN, USA). High-sensitivity C-reactive protein (hs-CRP) was measured by latex-enhanced assay. Comparisons between two groups were made using the Student's t-test and χ -square analysis.

Logistic regression analysis was performed to determine if the significant variables identified by the Student's t-test and the χ -square analysis were significant factors which would predict that the scores of the cognitive tests would be in the lowest quartiles.

The lowest quartile of WAIS-R scores was compared with the other three quartiles for every index (Table 4), and the group of subjects in the lowest quartile was found to have significantly higher serum levels of TC and TNF- α . Multivariate analysis using a model including significantly different factors from previous analyses showed that TNF- α was a significant and independent predictor of lower scores on the WASI-R symbol (Table 5).

The highest quartile of serum TNF- α levels showed significantly lower scores on the WAIS-R symbol test and MMSE (31.9 ± 9.1 vs. 38.9 ± 9.0 and 26.5 ± 1.1 vs. 27.9 ± 1.4 , respectively).

Several studies on non-DM subjects have demonstrated that inflammatory markers are associated with cognitive impairment [35, 36], and a recent study on elderly people with metabolic syndrome revealed that high inflammation was a significant factor associated with cognitive impairment [37]. Because the overexpression of IL-6 showed progressive neuronal loss and decreased learning in an animal model [38], is possible that inflammation itself could affect cognitive ability.

Another potential mechanism could be through atherosclerosis. Inflammatory markers, including TNF- α , have been suggested to be involved in the process of atherogenesis [39, 40], and a recent trial showed that treatment with TNF- α blockers decreased cardiovascular events [41]. Serum inflammatory markers, including TNF- α , were found to be high in patients with brain infarction [42], and one study has demonstrated a relationship between inflammatory proteins and silent brain infarctions [43]. In the present study, all potential subjects with a clinical history of strokes and/or focal neurological signs were excluded, but subjects with high serum TNF- α might have silent brain infarctions, which could affect cognitive functions.

A recent population-based study reported that moderate-to-high physical activity was associated with low inflammatory markers [44]. Interestingly, physical activity is reportedly a protective factor in cognitive decline and dementia [45, 46].

Conclusion

The accumulating evidence indicates that DM is associated with cognitive decline, and may be a risk factor for dementia of the Alzheimer's type and vascular origin. We do not yet know, however, how to intervene to prevent DM-related cognitive decline or dementia, or how to reverse it once it occurs. The mechanism of brain dysfunction asso-

Table 4.

	Higher	Lowest	P value
n	34	11	
Age (yrs)	72.1 ± 5.3	74.7 ± 7.3	0.19
Education (yrs)	10.2 ± 2.2	8.7 ± 1.8	0.53
DM duration	16.9 ± 7.3	17.9 ± 6.2	0.68
HbA1c (%)	7.2 ± 1.4	6.6 ± 1.2	0.20
FBS (mg/dl)	144 ± 38.0	138.2 ± 50.6	0.68
TRI	10.9 ± 6.3	8.3 ± 5.5	0.22
BMI (kg/m ²)	24.4 ± 3.4	25.3 ± 3.9	0.45
TC (mg/dl)	193.1 ± 41.7	221.9 ± 29.9	0.04
HDL (mg/dl)	48.7 ± 12.0	53.3 ± 16.4	0.31
TG (mg/dl)	148.9 ± 84.2	215 ± 299.5	0.24
SBP (mmHg)	132.2 ± 14.8	134.0 ± 13.0	0.72
DBP (mmHg)	74.4 ± 8.4	70.3 ± 10.2	0.18
TNF-α (Log)	-0.09 ± 0.31	0.18 ± 0.31	0.01
IL-6 (Log)	0.35 ± 0.21	0.46 ± 0.15	0.11
H CRP (Log)	-1.03 ± 0.51	-1.03 ± 0.37	0.99
Sex (male %)	19 (55.8)	7 (63.6)	0.84
Neuropathy (%)	20 (58.8)	7 (63.4)	0.98
Retinopathy (%)	20 (58.8)	8 (72.7)	0.57
Nephropathy (%)	9 (26.5)	5 (45.5)	0.39
Antihypertensive medication (%)	18 (52.9)	9 (81.8)	0.15
Statin use (%)	13 (38.2)	3 (27.3)	0.81
Smoker (%)	12 (35.3)	6 (54.5)	0.40

Data are the means ± SD

Table 5. Odds ratio

	Exp (95% CI)	P value
TNF-α	44.49 (1.38–1426.30)	0.03
TC	1.02 (1.00–1.05)	0.06

ciated with DM needs to be investigated fully.

References

- [1] Miles, W.R. and Root, H.F.: Psychologic tests applied to diabetic patients. *Arch. Intern. Med.*, **30**, 767–777, 1922.
- [2] Strachan, M.W., Ewing, F.M., Deary, I.J., and Frier, B.M.: Is type 2 diabetes associated with an increased risk of cognitive dysfunction? A critical review of published studies. *Diabetes Care*, **20**, 438–445, 1997.
- [3] Gregg, E.W., Yaffe, K., Cauley, J.A., Rolka, D.B., Blackwell, T.L., Narayan, K.M., and Cummings, S.R.: Is diabetes associated with cognitive impairment and cognitive decline among older women? Study of Osteoporotic Fractures Research Group. *Arch. Intern. Med.*, **160**, 174–180, 2000.
- [4] Kanaya, A.M., Barrett-Connor, E., Gildengorin, G., and Yaffe, K.: Change in cognitive function by glucose tolerance status in older adults: a 4-year prospective study of the Rancho Bernardo study cohort. *Arch. Intern. Med.*, **164**, 1327–1333, 2004.
- [5] Mogi, N., Umegaki, H., Hattori, A., Maeda, N., Miura, H., Kuzuya, M., Shimokata, H., Ando, F., Ito, H., and Iguchi, A.: Cognitive Function in Japanese Elderly with Type 2 Diabetes Mellitus. *J. Diabetes Complicat.*, **18**, 42–46, 2004.
- [6] Folstein, M.F., Folstein, S.E., and McHigh, P.R.: 'Mini-Mental State': a practical method of grading the cognitive function of patients for the clinician. *J. Psychiatr. Res.*, **12**, 189–198, 1978.
- [7] Mohs, R.C., Rosen, W.G., and Davis, K.L.: The Alzheimer's disease assessment scale: an instrument for assessing treatment efficacy. *Psychopharmacol.*, **19**, 448–450, 1983.
- [8] Shinagawa, F., Kobayashi, S., Fujita, K., and Maekawa, H.: Japanese manual for the Wechsler Adult Intelligence Scale-Revised. *Nihon-bunka-kagaku-sya*, Tokyo, pp. 115–118, 1990.
- [9] Stroop, J.R.: Studies of interference in serial verbal reactions. *J. Exp. Psychol.*, **18**, 643–662, 1935.
- [10] Ott, A., Stolk, R.P., van, Harskamp, F., Pols, H.A.P., Hofman, A., and Breteler, M.M.B.: Diabetes mellitus and the risk of dementia: the Rotterdam Study. *Neurology*, **53**, 1937–1942, 1999.
- [11] Leibson, C.L., Rocca, W.A., Hanson, V.A., Cha, R., Kokmen,

- E., O'Brien, P.C., and Palumbo, P.J.: Risk of dementia among persons with diabetes mellitus: a population-based cohort study. *Am. J. Epidemiol.*, **145**, 301–308, 1997.
- [12] Peila, R., Rodriguez, B.L., and Launer, L.J.: Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: the Honolulu-Asia aging study. *Diabetes*, **51**, 1256–1262, 2002.
- [13] Brayne, C., Gill, C., Huppert, F.A., Barkley, C., Gehlhaar, E., Girling, D.M., O'Connor, D.W., and Paykel, E.S.: Vascular risk and incident dementia: results from a cohort study of the very old. *Dement. Geriatr. Cogn. Disord.*, **9**, 175–180, 1998.
- [14] Hassing, L.B., Johansson, B., Nilsson, S.E., Berg, S., Pedersen, N.L., Gatz, M., and McClearn, G.: Diabetes mellitus is a risk factor for vascular dementia, but not for Alzheimer's disease: a population-based study of the oldest old. *Int. Psychogeriatr.*, **14**, 239–248, 2002.
- [15] Macknight, C., Rockwood, K., Awalt, E., and McDowell, L.: Diabetes mellitus and the risk of dementia, Alzheimer's disease and vascular cognitive impairment in the Canadian Study of Health and Aging. *Dement. Geriatr. Cogn. Disord.*, **14**, 77–83, 2002.
- [16] Xu, W.L., Qiu, C.X., Wahlin, A., Winblad, B., and Fratiglioni, L.: Diabetes mellitus and risk of dementia in the Kungsholmen project: a 6-year follow-up study. *Neurology*, **63**, 1181–1186, 2004.
- [17] Peila, R., Rodriguez, B.L., and Launer, L.J.: Honolulu-Asia Aging Study. Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: The Honolulu-Asia Aging Study. *Diabetes*, **51**, 1256–1262, 2002.
- [18] Beeri, M.S., Silverman, J.M., Davis, K.L., Marin, D., Grossman, H.Z., Schmeidler, J., Purohit, D.P., Perl, D.P., Davidson, M., Mohs, R.C., and Haroutunian, V.: Type 2 diabetes is negatively associated with Alzheimer's disease neuropathology. *J. Gerontol. A Biol. Sci. Med. Sci.*, **60**, 471–475, 2005.
- [19] Araki, Y., Nomura, M., Yamamoto, H., Yamamoto, T., Tsukaguchi, I., and Nakamura, H.: MRI of the brain in diabetes mellitus. *Neuroradiology*, **36**, 101–103, 1994.
- [20] Ushida, C., Umegaki, H., Hattori, A., Mogi, N., Aoki, S., and Iguchi, A.: Assessment of brain atrophy in elderly subjects with diabetes mellitus by computed tomography. *Geriatr. Gerontol. Int.*, **1**, 33–37, 2001.
- [21] den, Heijer, T., Vermeer, S.E., van Dijk, E.J., Prins, N.D., Koudstaal, P.J., Hofman, A., and Breteler, M.M.: Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI. *Diabetologia*, **46**, 1604–1610, 2003.
- [22] Schmidt, R., Launer, L.J., Nilsson, L.G., Pajak, A., Sans, S., Berger, K., Breteler, M.M., de, Ridder, M., Dufouil, C., Fuhrer, R., Giampaoli, S., and Hofman, A.: CASCADE Consortium. Magnetic resonance imaging of the brain in diabetes: the Cardiovascular Determinants of Dementia (CASCADE) Study. *Diabetes*, **53**, 687–692, 2004.
- [23] Knopman, D.S., Mosley, T.H., Catellier, D.J., and Sharrett, A.R.: Atherosclerosis Risk in Communities (ARIC) Study. Cardiovascular risk factors and cerebral atrophy in a middle-aged cohort. *Neurology*, **65**, 876–881, 2005.
- [24] Geroldi, C., Frisoni, G.B., Paolisso, G., Bandinelli, S., Lamponi, M., Abbatecola, A.M., Zanetti, O., Guralnik, J.M., and Ferrucci, L.: Insulin resistance in cognitive impairment: the InCHIANTI study. *Arch. Neurol.*, **62**, 1067–1072, 2005.
- [25] Luchsinger, J.A., Tang, M.X., Shea, S., and Mayeux, R.: Hyperinsulinemia and risk of Alzheimer disease. *Neurology*, **63**, 1187–1192, 2004.
- [26] Fratiglioni, L., Paillard-Borg, S., and Winblad, B.: An active and socially integrated lifestyle in late life might protect against dementia. *Lancet Neurol.*, **3**, 343–353, 2004.
- [27] Schwartz, M.W., Figlewicz, D.P., Baskin, D.G., Woods, S.C., and Porte, D., Jr.: Insulin in the brain: a hormonal regulator of energy balance. *Endocr. Rev.*, **3**, 387–414, 1992.
- [28] Qiu, W.Q., Walsh, D.M., Ye, Z., Vekrellis, K., Zhang, J., Podlisny, M.B., Rosner, M.R., Safavi, A., Hersh, L.B., and Selkoe, D.J.: Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. *J. Biol. Chem.*, **273**, 32730–32738, 1998.
- [29] Fishel, M.A., Watson, G.S., Montine, T.J., Wang, Q., Green, P.S., Kulstad, J.J., Cook, D.G., Peskind, E.R., Baker, L.D., Goldgaber, D., Nie, W., Asthana, S., Plymate, S.R., Schwartz, M.W., and Craft, S.: Hyperinsulinemia Provokes Synchronous Increases in Central Inflammation and beta-Amyloid in Normal Adults. *Arch. Neurol.*, **62**, 1539–1544, 2005.
- [30] Suzuki, M., Umegaki, H., Ieda, S., Mogi, N., and Iguchi, A.: Factors associated with cognitive impairment in elderly diabetes mellitus patients. *J. Am. Geriatr. Soc.*, in press, 2005.
- [31] Akiyama, H., Barger, S., Barnum, S., Bradt, B., Bauer, J., Cole, G.M., Cooper, N.R., Eikelenboom, P., Emmerling, M., Fiebich, B.L., Finch, C.E., Frautschy, S., Griffin, W.S., Hampel, H., Hull, M., Landreth, G., Lue, L., Mucke, R., Mackenzie, I.R., McGeer, P.L., O'Banion, M.K., Pachter, J., Pasinetti, G., Plata-Salaman, C., Rogers, J., Rydel, R., Shen, Y., Streit, W., Strohmeyer, R., Tooyoma, I., Van Muiswinkel, F.L., Veerhuis, R., Walker, D., Webster, S., Wegrzyniak, B., Wenk, G., and Wyss-Coray, T.: Inflammation and Alzheimer's disease. *Neurobiol. Aging*, **21**, 383–421, 2000.
- [32] Yaffe, K., Lindquist, K., Penninx, B.W., Simonsick, E.M., Pahor, M., Kritchevsky, S., Launer, L., Kuller, L., Rubin, S., and Harris, T.: Inflammatory markers and cognition in well-functioning African-American and white elders. *Neurology*, **61**, 76–80, 2003.
- [33] Engelhart, M.J., Geerlings, M.I., Meijer, J., Kilian, A., Ruitenberg, A., van Swieten, J.C., Stijnen, T., Hofman, A., Witteman, J.C., and Breteler, M.M.: Inflammatory proteins in plasma and the risk of dementia: the Rotterdam study. *Arch. Neurol.*, **61**, 668–672, 2004.
- [34] King, D.E., Mainous, A.G., 3rd, Buchanan, T.A., and Pearson, W.S.: C-reactive protein and glycemic control in adults with diabetes. *Diabetes Care*, **26**, 1535–1539, 2003.
- [35] Schmidt, R., Schmidt, H., Curb, J.D., Masaki, K., White, L.R., and Launer, L.J.: Early inflammation and dementia: a 25-year follow-up of the Honolulu-Asia Aging Study. *Ann. Neurol.*, **52**, 168–174, 2002.
- [36] Weaver, J.D., Huang, M.H., Albert, M., Harris, T., Rowe, J.W., and Seeman, T.E.: Interleukin-6 and risk of cognitive

- decline: MacArthur studies of successful aging. *Neurology*, **59**, 371–378, 2002.
- [37] Yaffe, K., Kanaya, A., Lindquist, K., Simonsick, E.M., Harris, T., Shorr, R.I., Tylavsky, F.A., and Newman, A.B.: The metabolic syndrome, inflammation, and risk of cognitive decline. *JAMA*, **292**, 2237–2242, 2004.
- [38] Campbell, I.L., Abraham, C.R., Masliah, E., Kemper, P., Inglis, J.D., Oldstone, M.B., and Mucke, L.: Neurological disease induced in transgenic mice by cerebral overexpression of interleukin 6. *Proc. Natl. Acad. Sci. USA*, **90**, 10061–10065, 1993.
- [39] Young, J.L., Libby, P., and Schonbeck, U.: Cytokines in the pathogenesis of atherosclerosis. *Thrombosis and Haemostasis*, **88**, 554–567, 2002.
- [40] Ridker, P.M., Hennekens, C.H., Buring, J.E., and Rifai, N.: C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N. Engl. J. Med.*, **342**, 836–843, 2000.
- [41] Jacobsson, L.T., Turesson, C., Gulfe, A., Kapetanovic, M.C., Petersson, I.F., Saxne, T., and Geborek, P.: Treatment with tumor necrosis factor blockers is associated with a lower incidence of first cardiovascular events in patients with rheumatoid arthritis. *J. Rheumatol.*, **32**, 1213–1218, 2005.
- [42] Hoshi, T., Kitagawa, K., Yamagami, H., Furukado, S., Hougaku, H., and Hori, M.: Relations of serum high-sensitivity C-reactive protein and interleukin-6 levels with silent brain infarction. *Stroke*, **36**, 768–772, 2005.
- [43] Castellanos, M., Castillo, J., Garcia, M.M., Leira, R., Serena, J., Chamorro, A., and Davalos, A.: Inflammation-mediated damage in progressing lacunar infarctions: a potential therapeutic target. *Stroke*, **33**, 982–987, 2002.
- [44] Elosua, R., Bariali, B., Ordovas, J.M., Corsi, A.M., Lauretani, F., and Ferrucci, L.: Association between physical activity, physical performance, and inflammatory biomarkers in an elderly population: The InCHIANTI Study. *J. Gerontol. A Biol. Sci. Med. Sci.*, **60**, 760–767, 2005.
- [45] Abbott, R.D., White, L.R., Ross, G.W., Masaki, K.H., Curb, J.D., and Petrovitch, H.: Walking and dementia in physically capable elderly men. *JAMA*, **22**, 1447–1453, 2004.
- [46] Weuve, J., Kang, J.H., Manson, J.E., Breteler, M.M., Ware, J.H., and Grodstein, F.: Physical activity, including walking, and cognitive function in older women. *JAMA*, **22**, 1454–1461, 2004.