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RESEARCH ARTICLE

Cognitive dysfunction associates with white matter hyperintensities and subcortical atrophy on magnetic resonance imaging of the elderly diabetes mellitus Japanese elderly diabetes intervention trial (J-EDIT)

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Abstract

Background Type 2 diabetes is associated with cognitive dysfunction and increases the risk of dementia in the elderly. The aim of this study was to explore, by means of magnetic resonance (MR) imaging, possible relationships among clinical profiles of diabetes, cognitive function, white matter hyperintensities (WMHs) and subcortical brain atrophy.

Methods Data were obtained from 95 nondemented type 2 diabetic participants aged 65 years or over, enrolled in an intervention trial for Japanese elderly diabetic patients. Cognitive function was measured with neuropsychiatric tests, including mini-mental state examination (MMSE), verbal memory, digit symbol substitution and Stroop tests. Hyperintensity was classified into periventricular, deep white matter, thalamic and basal ganglia. Four ventricle-to-brain ratios were used to measure subcortical atrophy. To identify clinical features of diabetes, indices of glycemic control, lipid metabolism, blood pressure and complications were examined. Canonical correlation analysis and regression analysis were used to assess correlation.

Results Scores for digit symbol substitution and MMSE negatively correlated with WMHs in the parietal lobe and hyperintensities in the thalamus, respectively. Lower scores for memory and digit symbol substitution showed positive association with enlarged subcortical atrophy adjacent to lateral ventricles. There was no association between clinical pictures of diabetic patients with cognitive dysfunction and of those with morphological changes in the brain.

Conclusions Impaired cognitive domains of the speed of mental processes and memory were associated with WMHs and subcortical atrophy. Degenerative changes in the cerebral small vessels may constitute predictive factors for the rate of cognitive dysfunction in elderly diabetic patients.

Keywords type 2 diabetes mellitus; elderly; cognitive dysfunction; periventricular hyperintensity; white matter hyperintensities; subcortical atrophy

Introduction

Type 2 diabetes is an age-related disease with a prevalence in Japan estimated at more than 5% of the population [1]. For elderly diabetic patients, the

purpose of treatment is not only to control plasma glucose levels, but also to prevent diabetic complications. Prospective intervention studies have provided evidence that intensive glycemic control effectively slows the onset and progression of diabetic vascular complications associated with type 2 diabetes [2]. However, these epidemiological investigations did not consider the various aspects of the prevention of cognitive decline in elderly diabetic patients.

The influence of diabetes on brain function has been of interest for more than 80 years [3,4]. Subjects with type 2 diabetes initially manifest deficits in abstraction, problem solving, memory and the completion of tasks involving speed and complex perceptual-motor responses. Recently, several epidemiological studies have shown that diabetes increases the risk for the most common forms of dementia, Alzheimer's disease and vascular dementia [5–9]. Hence, the most critical issue is to identify the factors responsible for diabetic cognitive impairment that lead to severe cognitive decline in the elderly.

Diabetes-related brain disorders have been considered multifactorial and attributed to genetic predisposition, nutritional factors, cerebrovascular disorders and the neurotoxic effects of hypoglycemia and hyperglycemia [10]. The so-called Rotterdam study, which is one of the largest population-based cohort studies, demonstrated conclusively that diabetic subjects with cerebrovascular diseases and with insulin treatment are more prone to dementia [5]. Recent biological findings have supported the view that several risk factors could be linked to diabetes and cognitive dysfunction in the elderly [10,11]. However, clinical pictures of elderly diabetes are various and elderly diabetic patients may have coincident neuropsychiatric disorders, thus making it difficult to identify the factors specifically responsible for cognitive decline.

To address these controversies regarding cognitive decline in elderly diabetic patients, we conducted a large-scaled prospective study of the Japanese Elderly Diabetes Intervention Trial (J-EDIT). J-EDIT was a prospective intervention study designed to investigate and identify the clinical characteristics of nondemented diabetic elderly. In the report presented here, we have analyzed the baseline measures of cognitive dysfunction in nondemented elderly with type 2 diabetes. The aim of this study was to explore possible associations among diabetic cognitive dysfunction, brain morphological changes detected on magnetic resonance (MR) imaging, and diabetic clinical features. To analyze brain MR images, we focused on white matter hyperintensities (WMHs) and subcortical brain atrophy because subcortical structural changes have been associated with cognitive impairment in demented and nondemented elderly subjects [12,13]. We classified hyperintensities into periventricular, deep white matter, thalamic and basal ganglia. The research questions were: (1) What diabetic indices are associated with cognitive dysfunction? (2) Which WMHs influence specific cognitive domains of elderly diabetic patients? (3) Do brain structural changes on MR imaging correlate with clinical measurements of diabetes? To address these questions, we adopted the canonical correlation analysis and regression analysis.

Materials and methods

Participants

J-EDIT started in 2001 as a prospective intervention study of Japanese elderly with diabetes mellitus to prevent the several diabetic complications. The study involved 1173 diabetic subjects from 42 institutes and hospitals in Japan who were 65 years or older (mean age was 71.8 ± 4.6) and whose serum HbA_{1c} levels were $\geqq 7.0\%$. Written informed consent was obtained from all patients. From these subjects enrolled in the J-EDIT, we selected 95 subjects with type 2 diabetes [14], who were treated at Kobe University Hospital, Nagoya University Hospital, Chiaki Hospital, Aoki Memorial Hospital, Nagoya Kyoritsu Hospital and Tokyo Metropolitan Geriatric Hospital. The diabetic participants who had difficulties in communicating, or showed signs of speech disturbance, deafness, severe disturbance of visual acuity, dementia and serious deterioration of the activities of daily life were excluded from this study. Clinical diagnosis of dementia was established according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV [15]. Subjects with chronic renal failure (serum creatinine >1.5 mg/dL), serious heart failure or symptomatic cerebral infarctions were also excluded from this study.

Assessment of diabetes mellitus, complications and comorbidities

The diagnosis and information of diabetes mellitus, blood examinations and complications were obtained from clinical charts [14]. Blood samples were obtained by vein puncture after overnight fasting to assess serum levels of glucose, HbA_{1c}, total cholesterol, triglyceride and HDL cholesterol. Serum insulin concentrations were measured in patients who were not receiving insulin therapy. Diabetic vascular complications were assessed for the coexistence of nephropathy, retinopathy, neuropathy and coronary diseases. Retinopathy was assessed fundoscopically through the dilated pupils by experienced ophthalmologists. The degree of retinopathy was classified into five categories; 0 (no retinopathy), 1 (intraretinal hemorrhages and hard exudates), 2 (soft exudates), 3 (intraretinal microvascular abnormalities, venous caliber abnormalities and venous beading), 4 (neovascularization of the disc or elsewhere in the retina, preretinal fibrous tissue proliferation, preretinal or vitreous hemorrhage and retinal detachment). Nephropathy was assessed in terms of the mean urinary albumin-to-creatinine ratio (ACR) and rated as 1 (no nephropathy: ACR <30 μg/mg), 2 (microalbuminuria: $30 \leqq$ ACR < 300 μg/mg), or 3

(persistent proteinuria: ACR ≧ 300 μg/mg or urinary protein ≧30 mg/dL). Diabetic neuropathy was classified as 1 (no neuropathy), 2 (loss of Achilles tendon reflex without neuropathic symptoms including paresthesia), or 3 (neuropathic symptoms). Coronary artery diseases were considered to be present when diabetic patients had at least one of the following: a history of myocardial infarction characterized by a typical clinical picture (chest pain, chest oppression, dyspnea, typical changes on ECG accompanied by pathological Q waves and/or localized ST variations), and typical enzymatic changes. Cardiovascular complications were classified into two categories, that is, presence or absence of coronary artery diseases.

Clinical diagnosis of hypoglycemia was based on the modified Whipple triad: symptoms and/or signs consistent with a low glucose concentration, low plasma glucose concentrations (<60 mg/dL), and relief of symptoms associated with the restoration of plasma glucose level [16]. Subjects with at least one hypoglycemic episode during the recent 12 months were considered to have hypoglycemia.

Procedures for analysis of the brain MR imaging: For every diabetic subject, a series of axial standard T1-weighted (repetition time [TR], 400 ms; echo time [TE], 12 ms), T2-weighted (TR, 3000 ms; TE, 90 ms; a 256 × 512 matrix) and fluid-attenuated inversion-recovery (FLAIR) (TR, 7500 ms; TE 110 ms; inversion time, 2200 ms; a 256 × 512 matrix) MR sequences of the brain were performed using 1.5 T MR units (Gyroscan NT-Intera and Gyroscan ASC-NT, both Philips, Eindhoven, The Netherlands; SIGUMA MR/I, General Electric, Milwaukee, WI). Scans in parallel with the anterior commissura–posterior commissura line were performed from the vertex to the foramen magnum with 7-mm thick slices and an interslice gap of 1.4 mm.

We analyzed WMHs and subcortical brain atrophy on MR images. WMHs appeared as hyperintense on T2-weighted images, but did not leave a clear hypointense hole on T1-weighted images (Figure 1(a)). FLAIR was used to obtain a clearer picture of the various WMHs [17], which were classified into subcortical WMHs and periventricular hyperintensity (PVH) (Figure 1(b)). WMHs were considered periventricular if the largest diameter was adjacent to the ventricular lining; otherwise, they were considered subcortical. PVH was rated semiquantitatively as 0 (none), 1 (pencil thin lining: <3 mm from the edge of ventricles), 2 (smooth halo: 3–10 mm), 3 (extending cap or thick lining: 10–25 mm), 4 (large confluent: >25 mm) for three separate regions; adjacent to frontal horns (frontal caps), adjacent to the wall of the lateral ventricles (bands), and adjacent to the occipital horns (occipital caps). The overall degree of PVH was calculated by adding up the scores for the three separate compartments (range 0–24) [18,19]. The number and size of subcortical WMHs were counted in the frontal, parietal, occipital and temporal lobes, as were the number and size of hyperintensities in the basal ganglia and thalamus. The size of subcortical WMHs was classified, according to the largest diameter, that is, small (1–3 mm), medium (3–10 mm), or large (>10 mm) [17]. To calculate the volume of subcortical hyperintensities, they were assumed to be spherical with a fixed diameter of 2, 6 and 12 mm for each of the three respective categories.

Linear analysis of subcortical brain atrophy, Evans ratio (ER), inverse cella media index (iCMI), caudate head index (CHI), and basal cistern index (BCI) were all calculated [20–23]. The following were measured with slide calipers: the maximum distance between the tips of the anterior horns (A), the width between the bilateral heads of the caudate nuclei (B), the maximum transverse inner diameter of the intracranial space (C), the maximum width of the cella mediae (D) and the maximum transverse inner diameter (E). Finally, the internal width between the bilateral temporal lobe (F) and the maximum transverse inner diameter (G) were calculated. The CHI, iCMI, ER and BCI were calculated with the following respective formulae: CHI = B/C, iCMI = D/E, ER = A/C and BCI = F/G, respectively (Figure 2).

Two raters who had no knowledge of the clinical data analyzed the brain MR imaging. To test the interrater reliability, the result of the two raters was subjected to correlation analysis for comparison in a random sample of 15 subjects. The analysis showed a strong correlation ($r = 0.85–0.89$, $P < 0.0001$), which suggested that the method of measurement used for this study was reliable.

Measurement of cognitive function

We used neuropsychiatric test batteries to examine the cognitive function of each of the subjects by assessing the speed of cognitive processes, as well as the extent of verbal memory, and global cognitive function. Two tests were used to assess the speed of mental processes: Stroop B (naming the color of the character that was printed in a color other than the one signified by the characters) and the digit symbol substitution test of the Wechsler Adult Intelligence Scale-Revised [24,25]. Verbal memory was assessed with the immediate and delayed word-list recall from the logical memory subtest of the Alzheimer's Disease Assessment Scale and of the paragraph from

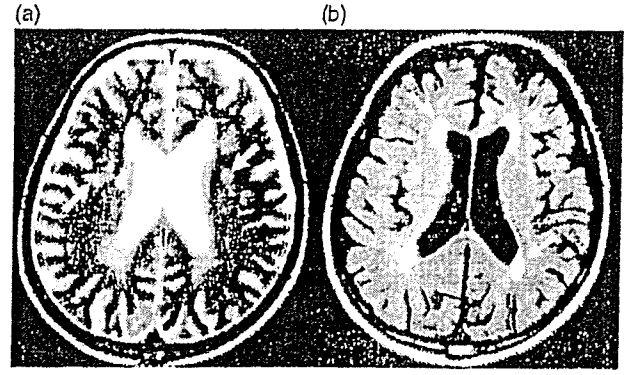

Figure 1. An example of subcortical (a) and periventricular (b) white matter hyperintensities on brain MR imaging

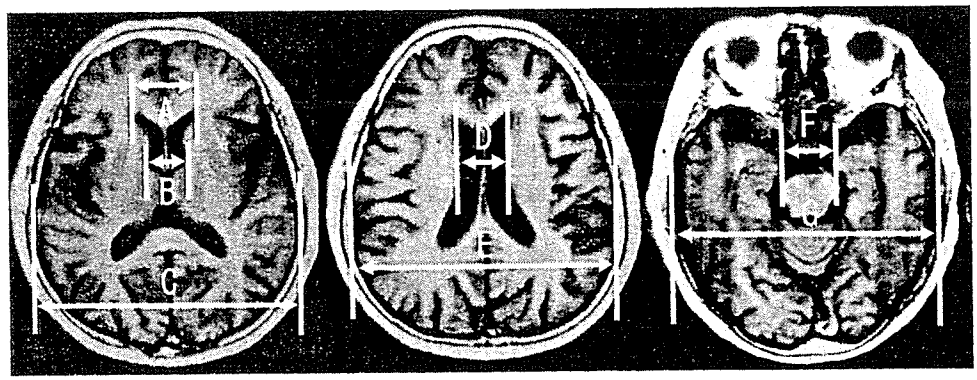

Figure 2. Measured portions on MR imaging. A, the maximum distance between the tips of the anterior horns; B, the width between the bilateral heads of the caudate nuclei; C, the maximum transverse inner diameter of the cranial space at the same MR section (left); D, the maximum width of the cella mediae; E, the maximum transverse inner diameter at the same section (center); F, the internal width between the bilateral temporal lobe at the level of basal cistern; G, the maximum transverse inner diameter at the same level (right). Evans ratio = A/C, caudate head index = B/C, inverse cella media index = D/E, basal cistern index = F/G

the neuropsychological tests of the National Center of Neurology and Psychiatry, Japan [26,27]. Finally, MMSE was used to measure global cognitive function [28].

Statistical analysis

To explore the association among cognitive profiles, clinical variables of diabetes, and morphological changes in MR imaging, we used canonical correlation analysis and regression analysis [29]. There were four sets of variables: seven cognitive tests, sixteen clinical variables of diabetes, eight brain WMH regions and four indices of brain atrophy. Since multiple test corrections would wipe out virtually any test significance, and variables within the same class often show a high degree of correlation, thus rendering the correlations redundant, we adopted the canonical correlation analysis to identify the direct relationship between two sets of variables. The fundamental principle behind canonical correlation analysis is the creation of a number of canonical solutions, each consisting of a linear combination of one set of variables, which has the form:

$$U_i = a_1(predictor_1) + a_2(predictor_2) + \cdots + a_m(predictor_m)$$

and a linear combination of the other set of variables, which has the form:

$$V_i = b_1(criterion_1) + b_2(criterion_2) + \cdots + b_n(criterion_n)$$

The purpose is to determine the coefficients (a's and b's) that maximize the correlation between canonical variates U_i and V_i. The first canonical correlation is the highest possible correlation between any linear combination of the variables in the predictor set and any linear combination of the variables in the criterion set.

A way of interpreting the canonical solutions is to look at the correlations between the canonical variates and the variables in each set. These correlations are called structure coefficients. The logic here is that variables that are highly correlated with a canonical variate have more in common with it and they should be considered more important when deriving a meaningful interpretation of the related canonical variates. As a substantial value of structure coefficients, an absolute value equal to or greater than 0.3 is often used [30,31].

For adjustment of confounders such as age and education, the correlation between cognitive function and MRI findings detected by the canonical correlations analysis was reanalyzed with multiple linear regression analysis. Statistical significance was defined as $p < 0.05$.

Results

Clinical characteristics of the 95 elderly diabetic patients are shown in Table 1. The mean age of the patients and duration of diabetes mellitus were 72.8 ± 0.5 years and 18.4 ± 1.2 years, respectively. Average HbA_{1c} was $7.9 \pm 0.1\%$, while other indices, such as blood pressure, serum cholesterol level and body mass index, were within reasonable limits. Eighteen diabetic subjects were receiving insulin therapy and 21 patients had hypoglycemic episodes. Because the number of patients in this study with insulin treatment or with hypoglycemic episodes was small, we could not analyze the effect of insulin therapy or hypoglycemia on cognitive dysfunction.

Canonical correlation analysis indicated a strong association of cognitive function with WMHs ($p = 0.004$) (Table 2). The canonical valuable of WMHs was contributed with the digit symbol substitution test, MMSE, immediate/delayed word-list recall, and Stroop tests (correlation of canonical variables: 0.76, 0.65, 0.33, 0.47, −0.41, respectively), and the canonical valuable of cognitive function was contributed with PVH and WMHs in the parietal, temporal and occipital lobes, total WMHs, and hyperintensities in the thalamus (correlation of canonical variables: −0.45, −0.33, −0.31, 0.33, −0.34, −0.39, respectively), but not with WMHs in frontal lobe. In other words, digit symbol substitution test, MMSE and immediate/delayed word-list recall had a negative

Table 1. Clinical characteristics of elderly patients with diabetes mellitus

	N	Mean ± SEM
Clinical character	-	-
Age (years)	95	72.8 ± 0.5
Male: Female	38:57	-
Education (years)	77	10.7 ± 0.3
Duration of diabetes mellitus (years)	92	18.4 ± 1.2
Body mass index (kg/m^2)	92	23.2 ± 0.4
Waist/hip ratio	92	0.92 ± 0.0
Systolic blood pressure (mmHg)	95	135.4 ± 1.2
Diastolic blood pressure (mmHg)	95	75.5 ± 0.9
Cardiovascular complication	95	0.16 ± 0.0
Retinopathy	88	0.70 ± 0.1
Nephropathy	87	0.59 ± 0.1
Neuropathy	87	0.66 ± 0.1
Fasting blood glucose (mg/dL)	82	164.2 ± 5.1
HbA_{1c} (%)	95	7.9 ± 0.1
Insulin	77	9.3 ± 1.3
Total cholesterol (mg/dL)	95	203.4 ± 3.2
Triglyceride (mg/dL)	95	150.1 ± 12.8
HDL cholesterol (mg/dL)	94	65.0 ± 2.7
Cognitive function	-	-
Mini-mental state examination	80	26.2 ± 0.3
Immediate word-list recall	81	8.2 ± 0.2
Delayed word-list recall	80	6.8 ± 0.3
Immediate paragraph recall	80	7.5 ± 0.3
Delayed paragraph recall	80	5.8 ± 0.3
Stroop test (B)	80	40.2 ± 1.6
Digit symbol substitution test	77	35.8 ± 1.2
MRI	-	-
White matter hyperintensities (WMHs)	-	-
Frontal lobe (mL)	95	1.8 ± 0.2
Parietal lobe (mL)	95	1.0 ± 0.1
Temporal lobe (mL)	95	0.8 ± 0.1
Occipital lobe (mL)	95	0.3 ± 0.1
Thalamus (mL)	95	0.1 ± 0.0
Basal ganglia (mL)	95	0.3 ± 0.0
Total (mL)	95	4.3 ± 0.4
Periventricular hyperintensity	86	9.7 ± 0.31
Evans ratio	95	26.0 ± 0.34
Caudate head index	95	13.5 ± 0.25
Inverse cella media index	95	26.3 ± 0.40
Basal cistern index	95	19.6 ± 0.27

Table 2. Canonical correlation between cognitive function and WMHs

Canonical correlation coefficient; 0.64			$P = 0.004$
Immediate word-list recall	0.33	WMHs of frontal lobe	−0.07
Delayed word-list recall	0.47	WMHs of parietal lobe	−0.33
Immediate paragraph recall	0.14	WMHs of temporal lobe	−0.31
Delayed paragraph recall	0.29	WMHs of occipital lobe	0.33
MMSE	0.65	Thalamus	−0.39
Stroop test (B)	−0.41	Basal ganglia	−0.23
Digit symbol substitution test	0.76	Total WMHs	−0.34
-	-	Periventricular hyperintensity	−0.45

Each value represents correlation of canonical valuables.

correlation with PVH and with WMHs in the parietal and temporal lobes, total WMHs and hyperintensities in the thalamus and positive correlation with WMHs in the occipital lobe. These results suggest that diabetic patients

Table 3. Canonical correlation between cognitive function and subcortical brain atrophy

Canonical correlation coefficient; 0.61			$p = 0.004$
Immediate word-list recall	−0.79	Evans ratio	0.83
Delayed word-list recall	−0.61	Caudate head index	0.79
Immediate paragraph recall	−0.28	Inverse cella media index	0.92
Delayed paragraph recall	−0.55	Basal cistern index	0.35
MMSE	−0.32	-	-
Stroop test (B)	0.30	-	-
Digit symbol substitution test	−0.71	-	-

Each value represents correlation of canonical valuables.

with more predominant WMHs except in the frontal lobe were more deficient in terms of speed of mental processes and in verbal memory.

The relationship between cognitive function and clinical indices was analyzed. No correlation could be established between cognitive function and clinical indices of diabetes, including glycemic control, lipid metabolism, blood pressure and complications of diabetes mellitus (data not shown). There was no correlation between WMHs and diabetic clinical pictures including diabetic control and complications (data not shown).

Table 3 shows a clear correlation of diabetic cognitive dysfunction with subcortical brain atrophy indices ($p = 0.004$). Lower scores for word-list recall and digit symbol substitution test, as well as delayed paragraph recall were positively associated with enlarged ER, CHI and iCMI. Canonical correlation did not detect a significant association between brain atrophy and diabetic clinical indices (data not shown).

The results were reanalyzed with regression analysis to adjust for age, education and systolic blood pressure, because these factors were generally considered to have effects on the cognitive function and/or brain structural changes on MR images [32,33]. The variables that strongly contributed to the canonical variates between cognitive functions and MRI findings were selected as the criterion variables of the regression analysis. In these analyses, digit symbol substitution test was associated with WMHs of parietal lobe, and MMSE was correlated with hyperintensities in the thalamus after adjustment for age, education and systolic blood pressure (Table 4). Table 5 shows the significant relationship between immediate word-list recall and CHI and the association between digit symbol substitution test and inverse cella media index.

Discussion

The study presented here represents the first investigation analyzing the possible associations between cognitive dysfunction and clinical features, with simultaneous evaluation of brain morphological changes detected on MR images in nondemented elderly with type 2 diabetes. It was found that WMHs and subcortical brain atrophy

Table 4. Multivariate regression analysis for subcortical WMHs with adjustment for age, education and blood pressure

Digit symbol substitution test	*P*-value	95% CI
WMHs of frontal lobe	0.13	−0.0004–0.0033
WMHs of parietal lobe	<0.05	−0.0057–0.0002
WMHs of temporal lobe	0.99	−0.0039–0.0038
WMHs of occipital lobe	0.56	−0.0048–0.0087
Thalamus	0.34	−0.0187–0.0065
Basal ganglia	0.31	−0.0092–0.0030
Periventricular hyperintensity	0.54	−1.34–0.70
MMSE	*P*-value	95% CI
WMHs of frontal lobe	0.85	−0.0004–0.0005
WMHs of parietal lobe	0.10	−0.0012–0.0001
WMHs of temporal lobe	0.47	−0.0006–0.0012
WMHs of occipital lobe	0.18	−0.0005–0.0027
Thalamus	<0.05	−0.0062–0.0002
Basal ganglia	0.57	−0.0018–0.0010
Periventricular hyperintensity	0.93	−0.23–0.25

Each variable is adjusted for age, education, and systolic blood pressure.
CI: confidence intervals

Table 5. Multivariate regression analysis for subcortical atrophy with adjustment for age, education, and blood pressure

Immediate word-list recall	*P-value*	95% CI
Evans ratio	0.50	−0.24–0.12
Caudate head index	<0.05	−0.33–0.02
Inverse cella media index	0.28	−0.22–0.07
Basal cistern index	0.77	−0.14–0.11
Digit symbol substitution test	*P*-value	95% CI
Evans ratio	0.87	−1.15–1.35
Caudate head index	1.00	−1.07–1.08
Inverse cella media index	<0.05	−2.07–0.08
Basal cistern index	0.13	−1.54–0.20

Each variable is adjusted for age, education, and systolic blood pressure
CI: confidence intervals.

strongly correlate with several domains of diabetic cognitive impairment, such as impaired speed of cognitive processes and memory. Our study also indicates that the various and separate subcortical hyperintensities in the parietal lobes and in the thalamus, but not in the frontal lobe, are associated with diabetic cognitive impairment. However, we could not detect the diabetic factors responsible for cognitive dysfunction, nor for the morphological changes on MR images, in spite of our thorough investigation of the various diabetic indices, including diabetic control, complications and comorbidities. These findings were established with the canonical correlation analysis used for our study.

Previous studies have investigated the relationship between WMHs and cognitive decline in nondemented and demented elderly [18,34–38]. Cognitive test scores in older adults were found to be worse in the presence of severe WMHs, even after adjustments for age, gender and education [18]. PVH is more likely than subcortical WMHs to be associated with speed of cognitive processes and memory [18,34,35]. The white matter of the subcortical structure can be divided into the area just beneath the neocortex and the area surrounding the ventricles. The periventricular region contains many long association fibers that connect the cerebral cortex with subcortical nuclei such as those found in the striatum and in more distant cortical areas. On the other hand, the subcortical region close to the neocortex features a high density of short looped U-fibers connecting adjacent cortical areas [39]. Periventricular WMHs damage the long-tract white matter pathways connecting many cortical areas, which might explain their effects on multiple domains of cognition.

Impaired cognitive function in elderly diabetic patients was correlated with subcortical WMHs in the parietal lobe as well as hyperintensities in the thalamus in this study. The thalamus is recognized to be associated with cognitive functions such as learning, memory and executive function [32,40]. The parietal lobe constitutes association areas that are the sites of cortical integration for all behavior such as vision, body awareness and spatial orientation and for abstract and complex cognitive functions. Recent neuroradiological studies have demonstrated reduced regional cerebral blood flow and metabolism in Alzheimer's disease especially in the parieto-temporal cortical areas and correlated with the distribution of Alzheimer's pathological features, while the primary sensorimotor and visual cortical areas were relatively preserved [41,42]. Symptoms of early stage Alzheimer's disease originate from the impaired temporal and parietal lobe functions. Incidental onset of Alzheimer's disease in elderly diabetic patients can be expected to exacerbate the functional loss and clinical symptoms related to temporal and parietal lobes, as also observed in the case of diabetic cognitive dysfunction.

The pathophysiological origins of WMHs are still unclear, with vascular and nonvascular contributions likely to be causative factors. Subcortical WMHs on T2-weighted MR imaging correlate with several pathological changes such as myelin pallor, dilatation of the perivascular space, myelin or axonal loss, scattered cystic infarcts and angionecrosis. Periventricular hyperintensities on MR scans are associated with partial breakdown of the ependymal cell lining and subependymal gliosis in addition to the pathological changes of subcortical WMHs [43,44]. Although characteristic pathological features of the diabetic brain have yet not been identified, vascular compromise is common in the elderly and is accompanied by damage to white matter pathways [12,45]. Age and hypertension have been the most consistent predictors of WMHs [32,33,46,47], while some other studies have indicated that diabetes increases the risk of WMHs [13,36,48]. The increased WMHs in elderly diabetic patients, presumably accompanied by as yet unidentified clinical variables, may account, at least in part, for diabetic cognitive dysfunction.

To reduce the rate of dementia in elderly diabetic patients, it is crucial to identify the factors responsible for the progression toward severe cognitive decline. Degenerative changes in cerebral small vessels may affect diabetic cognitive dysfunction, while it seems likely that it is also influenced by diabetic metabolic abnormalities and complications with or without unidentified genetic susceptibility. However, the results of our study do not

support the notion of any relationship between cognitive dysfunction and diabetic clinical characteristics. Whether variations in glucose homeostasis influence cognitive function remains controversial [49–51], although it has been found that the cognitive function of diabetic and nondiabetic subjects fluctuates in accordance with the serum glucose levels [50,51]. At the same time, increased insulin resistance is associated with atrophy of medial temporal lobe structures in elderly diabetic patients [52]. For these reasons, new surrogate markers that reflect chronic hyperglycemia in the diabetic brain are needed. Hyperglycemia causes oxidative stress via the polyol pathway, enhances advanced glycation end products (AGE), and increases lipid peroxidation and imbalances in the generation of reactive oxygen species and their scavengers [53,54]. N^{ε}-Carboxymethyllysine (CML), the most prominent AGE product, is crucially involved in the development of diabetic microangiopathy [55], and the level of CML expression is high in the blood vessels and brain of diabetic patients, but low in aging controls [56]. Oxidative stress has also been implicated in the pathophysiology of Alzheimer's disease and hypoxic brain insults [57–59]. Progression of diabetic retinopathy strongly correlates with the total sum of blood glucose control, and may constitute a predictor for cerebral small vessel disease [60,61].

Certain limitations of our study need to be considered. The first limitation is that this analysis was a cross-sectional study. The second is the possibility that participants with some other cognitive dysfunction could be involved in this study. The mean MMSE score of our diabetic patients was 26.2 ± 0.3, which means that most of the subjects did not attain the full MMSE score. In particular, we could not completely exclude the patients with mild cognitive impairment. The third limitation is the method for measuring brain atrophy. We used linear measurements to evaluate subcortical atrophy adjacent to the lateral ventricles, and this procedure is outdated and less accurate than the recently developed volumetric analysis of MR images [62]. However, volumetric MR analyses of a number of diabetic patients from different institutes and hospitals could not be performed. Finally, we did not estimate the effects of treatment *per se* for diabetes, hypertension and lipid abnormalities on diabetic cognitive domains. The Rotterdam study has suggested that the use of oral medication and insulin treatment for diabetes increases the association with dementia, while MR imaging has demonstrated that hypertension is associated with cognitive dysfunction and WMHs [63,64]. Recent prospective intervention studies have indicated that appropriate blood pressure control delays the progression of cognitive decline [65]. Furthermore, there are indications that impaired cholesterol transport may play a pathophysiological role in Alzheimer's disease and that HMG-CoA reductase inhibitors (statin) may have a protective effect on cognitive dysfunction in the elderly [66–68]. Most of the diabetic subjects enrolled in this study were successfully treated for their blood pressure and lipid abnormalities, which could diminish the impact of hypertension and lipid abnormalities on diabetic cognitive impairment and brain structural changes. The effects of these limitations of our study will be analyzed in the prospective intervention J-EDIT study.

In summary, WMHs and subcortical brain atrophy observed on MRI scans of elderly diabetic patients without symptomatic brain infarctions were found to be associated with impaired speed of mental processes and memory, while WMHs are thought to be responsible for degenerative changes of cerebral small vessels and to be implicated in the pathogenesis of cognitive impairment. These findings suggest that hyperintensities in the parietal lobe and thalamus and subcortical atrophy, in particular, constitute predictors of the rate of cognitive dysfunction in elderly diabetic patients and may underlie procession toward severe cognitive impairment. Our prospective J-EDIT study should help to determine the factors that can prevent cognitive dysfunction in elderly diabetic patients.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Outcome of One-year of Specialist Care of Patients with Type 2 Diabetes: A Multi-Center Prospective Survey (JDDM 2)

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Abstract

OBJECTIVE Specialist care is reportedly associated with favorable therapeutic results, although detailed outcomes of recent large-scale prospective surveys of specialist care have yet to be published. The goal of this study was to elucidate the effects of one year's specialist care on the management of type 2 diabetes.

PATIENTS AND METHODS A multi-centered, prospective observational study was undertaken. 754 type 2 diabetes patients, who made their first visit to one of eleven participating outpatient clinics specializing in diabetes care, were enrolled. Routine structured diabetes care according to established guideline, including diabetes self-management education, was provided to all patients at each clinic visit. Parameters relating to glycemic control, serum lipids, blood pressure, patient follow-up status and others were followed for twelve months.

RESULTS The HbA_{1c} level had improved significantly from 8.4±2.2% at baseline to 6.8±1.2% after six months and was 7.0±1.3% after twelve months (mean±SD). The higher the baseline HbA_{1c} level, the greater the subsequent improvement. Moreover, the most dramatic improvements in HbA_{1c} levels were seen within the first three months. The proportion of patients satisfying all of the therapeutic goals was extremely low at baseline and remained at less than 10% after twelve months of specialist care.

CONCLUSIONS Diabetic patients under specialist care experienced substantial improvement, especially in glycemic control, as early as a few months after the first visit. However, 35 percent of patients dropped out during the 12-month study period and this is one area that needs to be improved.

Key words: diabetes specialist, diabetes clinic, quality of care, diabetes self-management education (DSME), pharmacological therapy

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Introduction

Continuing medical care, including diabetes self-management education (DSME) provided by medical professionals with expertise in diabetes, is essential to minimize the risk of long-term complications in patients with diabetes (1-3). Specialist diabetes care has been shown to deliver a better glycemic control outcome than care provided by general practitioners (4-11). However, the outcome assessment of specialist routine care needs to be regularly updated to take into account the continual changes in modern diabetes care and pharmacotherapy (12). Other than a postal survey of secondary care services (13), very few large prospective surveys regarding the outcome of recent specialist care are available.

The Japan Diabetes Clinical Data Management Study Group (JDDM) is a large network of diabetes specialists in Japan. It consists of approximately seventy clinical diabetic specialists, most of whom are board certified and have their

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own clinics. The ultimate goal of the JDDM is to acquire clinical evidence that can be used to optimize diabetes care. To achieve this goal, the JDDM is developing a cohort of diabetic patients who are receiving care at the participating clinics, and by January 2005 approximately 60,000 patients were registered. Clinical and treatment information is stored on a standardized database system and from the cohort of the registered patients, we evaluated the clinical outcomes of new patients consecutively registered on the JDDM database during a particular period. The goal of this study was to elucidate the effects of one year of specialist care on the management of type 2 diabetes. Analyses based on prior treatment history and baseline glycemic status were also made.

Patients and Methods

Recruitment of patients

Eleven JDDM clinics throughout Japan (as listed in the appendix) that specialize in diabetes care voluntarily participated in this study. All type 2 diabetic patients who made their first visit to any of the participating clinics during the study period (January to June 2001) were consecutively recruited. A total of 754 patients entered the study, all of whom provided informed consent to participate. The protocol was consistent with the Japanese Government's "Ethical Guidelines Regarding Epidemiological Studies" and received ethical approval from the JDDM ethics committee. Patients were classified as having type 2 diabetes mellitus according to the Japan Diabetes Society (JDS) criteria (14) which are similar to the WHO criteria (15) in terms of glucose threshold levels. Patients with impaired glucose tolerance were not included in this study. The study follow-up period was 12 months from the patient's first visit.

Diabetes management and care

The patients took part in a comprehensive, structured program in accordance with JDS guidelines (16) and the care package included a comprehensive diabetes self-management education (DSME) program with an emphasis on the importance of lifestyle modifications which was conducted by Certified Diabetes Educators (CDE). Topics covered included good dietary habits, physical activities, treatment adherence, and standard medication including oral hypoglycemic agents and/or insulin. The therapeutic goals, mostly based on JDS guidelines at the time of the study, for the study participants were: a stable HbA_{1c} level <6.5%; a body mass index (BMI) $\leq$24 kg/m^2; blood pressure <130/85 mmHg; serum total cholesterol level <5.17 mmol/L (200 mg/dL); serum HDL cholesterol level $\geq$1.03 mmol/L (40 mg/dL); serum triglyceride level <1.68 mmol/L (150 mg/dL); smoking cessation; and, decreased alcohol consumption (16). Patients were requested to return to the clinic for follow-up care once a month (preferably) or at least once every two months. Changes in patient medication were made in an effort to reach the therapeutic goals outlined above on a treat to target basis. Standard JDS meal plans using diabetic food exchange lists (17) were used. Dieticians also provided individual nutritional guidance. All patients, except those with medical complications for whom a strenuous exercise regime was contraindicated, were encouraged to engage in physical exercise, for a minimum of 30 minutes at least three times a week, that was vigorous enough for them to work up a sweat. A diary to record the progress of laboratory and other data was distributed to the patients to provide feedback on the results of their therapy program.

Clinical and laboratory parameters

Body weight, blood pressure, HbA_{1c}, fasting plasma glucose, serum lipids/creatinine/urea nitrogen, and urine analysis results were obtained at scheduled clinic visits during the study period. Ophthalmological and neurological examinations were done at baseline. JDS guidelines were used to assess the development of microvascular complications. Neuropathy was defined as having three or more of the following: (i) absence of ankle tendon reflex; (ii) absence of knee tendon reflex; (iii) decreased vibration sensation; (iv) abnormal results for monofilament touch test (18); or (v) abnormal subjective symptoms. Nephropathy was defined as having an albumin excretion of more than 30 mg/g creatinine in two or more consecutive urine testings. Retinopathy was defined to involve simple, non-proliferative retinopathy. HbA_{1c} levels were determined by high-pressure liquid chromatography (HPLC) with 5.8% as the upper normal limit. Plasma glucose levels were determined by the glucose oxidase technique. All other laboratory tests were determined by standard methods.

Data processing and statistical analysis

Clinical data was input to a bespoke, standardized software system "CoDiC™" (19) which was distributed to each participating clinic. Data were collected from each institute on an anonymous basis and stored centrally for statistical analysis using SPSS, version 10.05 (SPSS Inc., Chicago, IL, USA). The F-test was used to determine whether the variance of each group was equivalent. Student's paired and unpaired t-tests, one-way ANOVA and a post hoc multiple comparison test (Dunnett) were used to compare continuous variables between groups. A P-value of less than 0.05 was considered significant. All values are presented as means ± standard deviations unless otherwise stated.

Results

Background characteristics and baseline analysis

Baseline measurements broken down according to prior or first time treatment are shown in Table 1. Of the previously treated patients, 93% were direct referrals from primary care physicians with the remainder discontinuing their previous medical care. Among the previously untreated patients, 62%

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Table 1. Patient characteristics at baseline and 12 months later. Baseline data of the patients that completed the 12-month study period are shown in [ ]

(Mean±S.D., n.a.; not applicable, n.d.; not done)

	Total		Newly treated patients		Previously treated patients	
	Baseline[a]	12th month[b]	Baseline[c]	12th month[d]	Baseline[e]	12th month[f]
Number of patients	754	491	341	194	413	297
Men/Women	496/258	311/180	241/100	134/60	255/158**	177/120
Age (yr.)	58.0±11.9 [58.8±11.6]	n.a.	56.2±11.1 [57.1±10.9]	n.a.	59.6±12.3*** [59.8±11.9]	n.a.
Diabetes duration (yr.)	9.1±8.8 [9.4±8.6]	n.a.	6.3±7.1 [6.7±7.5]	n.a.	10.7±9.3*** [10.6±8.8]	n.a.
BMI (kg/m2)	24.1±4.1 [24.2±4.4]	24.2±3.8*	24.7±4.2 [24.7±4.7]	24.4(3.9	23.7±4.1** [23.9±4.1]	24.1±3.7**
HbA1C (%)	8.4±2.2 [8.6±2.2]	7.0±1.3***	8.5±2.3 [9.0±2.4]	6.8(1.3***	8.4±2.1 [8.4±2.0]	7.2±1.3***+++
Systolic blood pressure (mmHg)	136.9±21.8 [136.9±21.3]	131.2±17.8***	136.8±21.2 [136.4±20.9]	130.4±17.1***	138.1±22.6 [137.2±21.7]	131.9±18.3***
Diastolic blood pressure (mmHg)	79.6±12.9 [79.5±12.5]	75.7±11.9***	81.4±12.8 [81.0±12.8]	76.3±12.7***	78.7±12.5** [78.4±12.2]	75.4±11.2***
Total cholesterol (mmol/l)	5.48±1.04 [5.37±1.00]	5.21±0.88***	5.61±0.98 [5.48±0.93]	5.23±0.80***	5.39±1.06 [5.31±1.05]	5.21±0.88**
HDL cholesterol (mmol/l)	1.41±0.39 [1.42±0.40]	1.40±0.36	1.42±0.37 [1.44±0.38]	1.40±0.31	1.41±0.39 [1.42±0.41]	1.40±0.36
Triglycerides (mmol/l)	1.72±1.24 [1.63±1.20]	1.73±1.63	1.80±1.39 [1.66±1.30]	1.41±0.82***	1.66±1.13 [1.60±1.12]	1.73±1.13++
Patients with retinopathy (%)	31.1 [34.0]	n.d.	20.0 [23.6]	n.d.	39.4 [40.7]	n.d.
Patients with nephropathy (%)	30.6 [31.2]	n.d.	21.1 [21.0]	n.d.	38.1 [37.9]	n.d.
Patients with neuropathy (%)	25.2 [25.8]	n.d.	17.6 [20.0]	n.d.	31.2 [29.7]	n.d.
Medication for hypertension (%)	17.4 [19.0]	33.8***	11.9 [13.8]	28.1***	21.7 [22.3]*	37.5***+
Medication for hyperlipidemia (%)	6.7 [6.9]	18.2***	3.5 [3.8]	13.8***	9.2 [8.8]*	21.0***+
Medications for both of the above (%)	3.4 [3.2]	8.7***	2.9 [2.9]	6.7***	3.8 [3.4]	10.1***
		*p<0.05, ***p<0.001 (a vs.b)		***p<0.001 (c vs.d)	*p<0.05, **p<0.01, ***p<0.001 (e vs.c)	**p<0.01, ***p<0.001 (e vs.f) +p<0.05, ++p<0.01, +++P<0.001 (d vs. f)

visited the clinics because of elevated FPG and/or HbA_{1c} levels found at a medical check-up, while 8% attended because of the development of diabetic symptoms. The remainder were referred from other speciality clinics or hospitals. Baseline HbA_{1c} was similar in patients with and without a previous history of diabetes care. However, the previously untreated patients were significantly younger with a shorter duration of diabetes and a lower BMI (Table 1).

Patient follow-up status and dropout

The proportions of patients making repeat clinic visits, sub-grouped according to whether they had received prior treatment for diabetes and by baseline HbA_{1c} levels, are shown in Fig. 1A and Fig. 1B, respectively. Approximately 35% of all participants defaulted from follow-up during the first year (Fig. 1A). At twelve months, patients with HbA_{1c}

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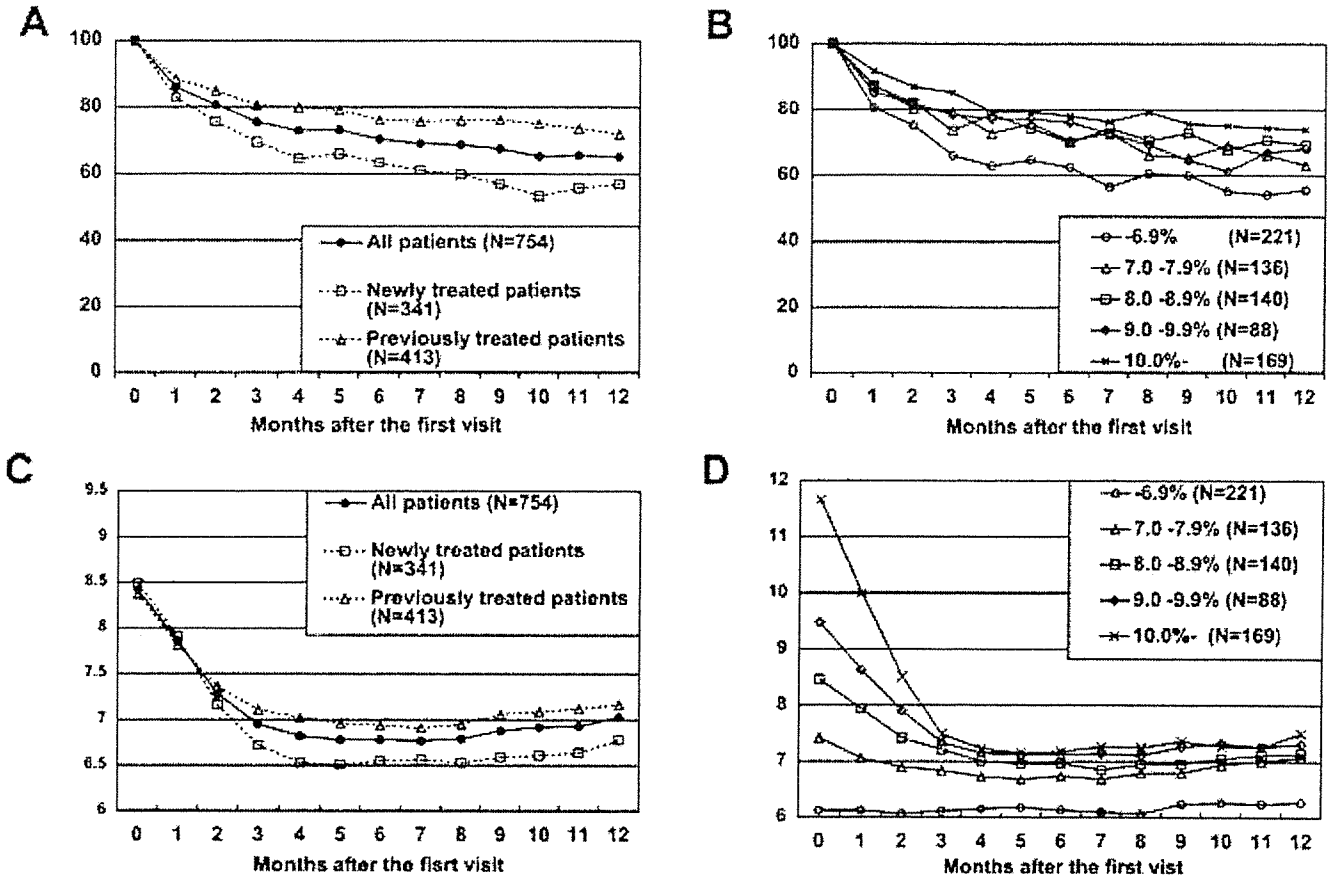

Figure 1. Sequential changes in the proportion of patients under follow-up (A, B), and HbA_{1C} levels (C, D) during the twelve months following the first visit to a specialist clinic. The results were stratified according to the patients' previous follow-up status (A, C) or HbA_{1C} levels at baseline (B, D).

levels of 6.9% or less had the greatest dropout rate (44.3%) while patients with levels of 10% or more had the lowest rate (26.0%) ($P<0.001$ between the two subgroups; Fig. 1B). A comparison of patient backgrounds at baseline between those who completed (N=491) and those who were lost to follow-up (N=263) showed that baseline HbA_{1C} levels were significantly lower ($P=0.039$) in those who dropped out (8.1±2.2%) than in those who completed treatment (8.6± 2.2%). However, there were no significant differences in age, gender, diabetes duration or baseline BMI between these two groups (data not shown). Patients being treated for diabetes for the first time had a significantly higher dropout rate (43%) than previously treated patients (28%) (Table 1 and Fig. 1A) ($P<0.001$). The reasons given for patient dropout included the pressure of official (28%) or private business (11%), misunderstanding regarding diabetes therapy (13%), moving out of town (11%), and economic reasons (6%).

Changes in glycemic and other control

The mean HbA_{1C} levels of all patients who completed 12-month follow-up improved significantly from 8.4±2.2% at baseline to 6.8±1.2% after six months, and 7.0±1.3% after twelve months (Fig. 1C). Newly treated patients showed significantly greater improvements in HbA_{1C} levels during the first year than the previously treated patients ($P<0.001$; Fig. 1C). As shown in Fig. 1D, the higher the initial HbA_{1C} level the greater the improvement that was seen. For patients with the highest baseline HbA_{1C} (10% or more), mean HbA_{1C} levels fell dramatically from 11.7±1.3% to 7.5±1.6% in the first three months and remained stable thereafter. Conversely, only very limited improvement was found in patients with HbA_{1C} levels of 7.9% or less. In general, decreases in HbA_{1C} levels were almost exclusively observed in the three months following the first visit. There was no significant correlation between the final HbA_{1C} levels and the frequency of DSME (data not shown). Total cholesterol and systolic/diastolic blood pressure significantly decreased regardless of treatment history, while HDL cholesterol did not show any significant changes during the 12-month study period. Significant improvement in triglycerides was seen only in newly treated patients (Table 1).

Pharmacological therapy and adherence to guidelines

The pharmacological therapy of patients is shown in

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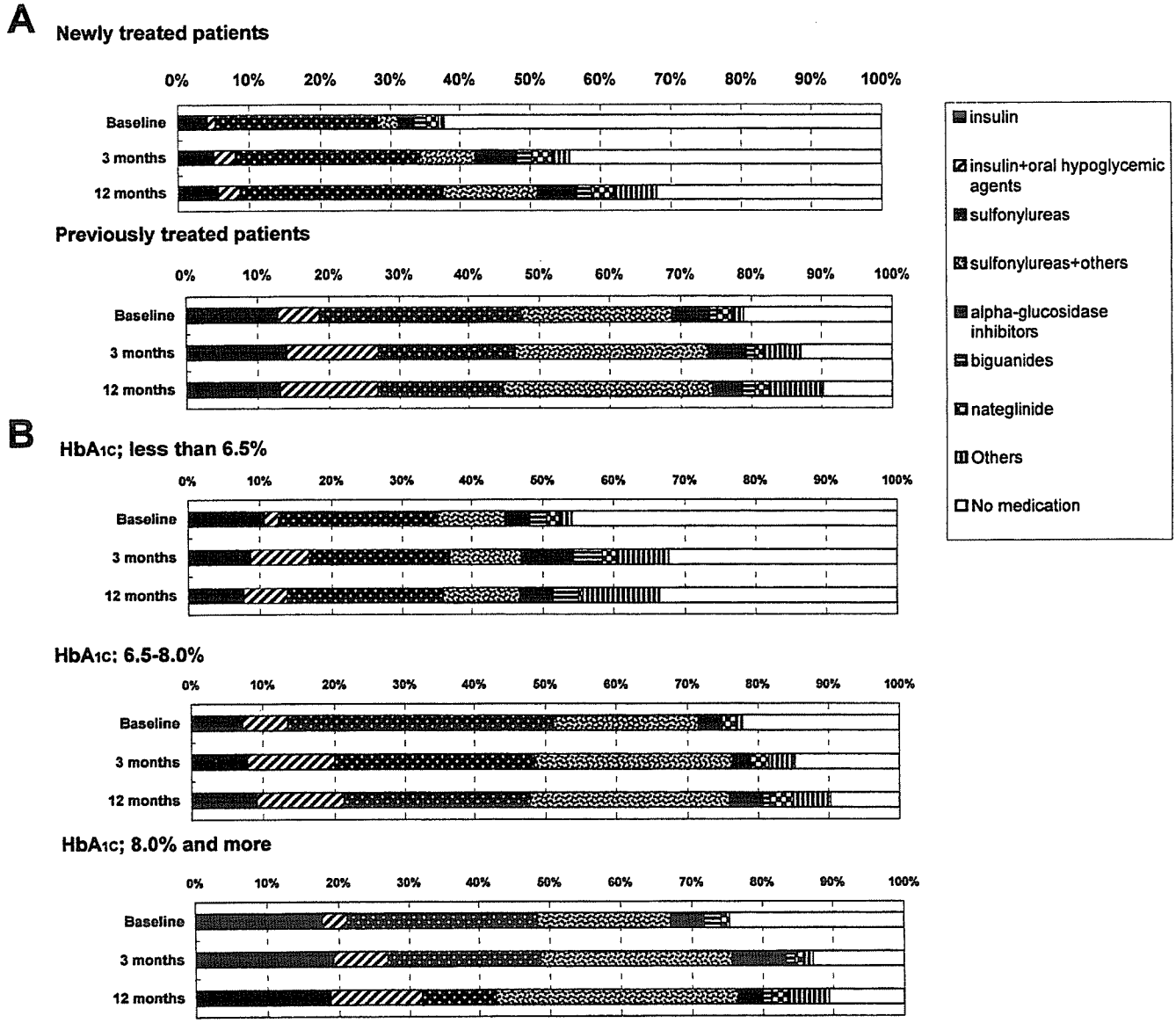

Figure 2. **Pharmacotherapeutic status of the patients at baseline, three months and twelve months after start of the study, stratified according to previous follow-up status (A) or HbA_{1C} levels at baseline (B).**

Fig. 2A and Fig. 2B, broken down according to previous treatment status or baseline HbA_{1C} level, respectively. The use of hypoglycemic agent, including insulin differ, according to a patient's prior treatment history. Patients who had been previously treated used more insulin than previously untreated patients and the proportion of patients using two or more agents was higher in previously treated patients (Fig. 2A). The proportion of patients using sulfonylureas was increased in patients with baseline HbA_{1C} levels of 6.5% or higher and insulin usage was higher in patients with initial HbA_{1C} levels of 8.0% and more than in the other subgroups (Fig. 2B). The proportion of patients taking medications for hypertension, hyperlipidemia, or both significantly increased two- or three-fold during the twelve months of study regardless of prior treatment history (Table 1). To investigate the adherence of each clinic to the guidelines, the HbA_{1C} levels used by clinics to trigger the start of medication were surveyed. The medication thresholds were as follows: 6.5% or more (2 clinics), 7.0% or more (2), 8.0% or more (3), 9.0% or more (1), and patient-by-patient assessment (3). The survey also revealed that clinic HbA_{1C} target levels were as follows: 5.8% or less (1 clinic), 6.5% or less (7) and 7.0% or less (3).

Therapeutic goal achievement rates

In assessing the proportions of patients who achieved either the individual or all of the therapeutic goals, comparisons were made at 12 months with two baseline groups; one containing all the patients who participated in the study, and the other containing only those patients who completed the 12-month study (Table 2). Of the patients who completed 12 months of follow-up, the proportion achieving the HbA_{1C}

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Table 2. **Proportion of patients satisfying the therapeutic goals (except smoking and drinking) at baseline and 12 months later. Baseline (1) includes all patients enrolled at registration, and baseline (2) includes only patients who remained until the end of the 12 month study. (P < 0.05*, 0.01**, 0.001*** Compared to the group of baseline (2) by McNeuar test)**

		Total (%)	Newly treated (%)	Previously treated (%)
HbA_{1C} < 6.5%	Baseline (1)	20.4	23.4	17.9
	Baseline (2)	16.5	17.9	15.7
	At 12th month	36.5 ***	46.9 ***	29.9 ***
HbA_{1C} < 7.0%	Baseline (1)	29.3	32.3	26.8
	Baseline (2)	24.6	25.1	24.2
	At 12th month	54.1 ***	63.1 ***	48.4 ***
BMI < 24 kg/m^2	Baseline (1)	55.7	49.5	60.7
	Baseline (2)	54.4	47.9	58.6
	At 12th month	51.3 *	49.1	52.7
Systolic blood pressure < 130 mmHg and diastolic blood pressure < 85 mmHg	Baseline (1)	32.2	33.9	30.9
	Baseline (2)	33.3	34.3	32.5
	At 12th month	43.5 ***	44.8 *	42.6 *
Total cholesterol < 200 mg/dl	Baseline (1)	45.0	44.8	45.0
	Baseline (2)	38.5	37.1	39.5
	At 12th month	50.0 ***	51.7 **	48.9 *
HDL cholesterol > 40 mg/dl	Baseline (1)	86.6	86.5	86.6
	Baseline (2)	87.4	85.7	88.5
	At 12th month	88.3	89.3	87.6
Triglycerides < 150 mg/dl	Baseline (1)	67.8	67.7	67.9
	Baseline (2)	65.3	63.6	66.4
	At 12th month	69.4	74.1 *	66.4
All of the above (Regarding HbA_{1C}, goal of < 6.5% was adapted)	Baseline (1)	2.1	2.4	1.9
	Baseline (2)	1.2	1.0	1.3
	At 12th month	3.5	6.2	1.9
All of the above (Regarding HbA_{1C}, goal of < 7.0% was adapted)	Baseline (1)	2.5	2.4	2.5
	Baseline (2)	1.2	1.0	1.3
	At 12th month	4.7*	9.3**	1.9

goal of 6.5% or less increased significantly from 16.5% at baseline to 36.5% at 12 months. The improvement was particularly evident in newly-treated patients (from 17.9% at baseline to 46.9% at 12 months). The proportion of patients achieving the HbA_{1C} goal of 7.0% or less was 24.6% at baseline and 54.1% at 12 months. On the other hand, the proportion of patients who achieved the BMI goal ($\leq$24 kg/m^2) decreased significantly from 54.4% at baseline to 51.3% after 12 months. The proportions of patients achieving the blood pressure or total cholesterol goals increased significantly by approximately 10% during the 12-month period. Only newly-treated patients showed a significant improvement in achieving the triglyceride goal.

When adopting the HbA_{1C} goal of 6.5% for analysis, the proportion of patients satisfying all of the therapeutic goals (except smoking and alcohol drinking) at baseline was 2.1%, when all patients who participated in the study were included, and 1.2% when only patients who completed the 12-month follow-up were included. The proportion increased to 3.5% after 12 months but this increase was not statistically significant. However, when the HbA_{1C} goal of 7.0% was adopted for analysis, the proportion of patients satisfying all of the therapeutic goals increased significantly from 1.2% at baseline to 4.7% after 12 months.

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Discussion

Glycemic control and weight control

This prospective study highlights the current Japanese standards of diabetes management and care provided in specialist clinics and demonstrated, a reasonable improvement in the glycemic control of patients, especially in those with severe diabetes (Fig. 1). Most of the improvements in HbA_{1c} levels seen in the first year occured within the first three months of commencing management (Fig. 1C). It should be emphasized that even patients who had previously been treated in primary care settings showed improvement in HbA_{1c} levels. Of the patients receiving treatment for the first time, less than 40% started medication after their first visit, while at twelve months nearly 70% of those patients had been prescribed one or more medications (Fig. 2A). This probably accounts for the rapid improvement in HbA_{1c} in these patients (Fig. 1).

Several issues still remain concerning our care of glycemic control. The first is that the proportions of patients achieving the HbA_{1c} goals were still very low even after 12 months of care (Table 2). The second issue is that the improvement in HbA_{1c} was limited to patients with baseline HbA_{1c} levels of 8% or higher (Fig. 1D). The third issue is that the HbA_{1c} levels in patients with a baseline of 7.0% or more converged above the 7.0% level at the midpoint of the study and tended to increase (deteriorate) after that point (Fig. 1D). Finally, a slight but significant increase in BMI, which was possibly related to the effects of pharmacological therapy (20), was observed during the 12 months of care (Tables 1, 2). It is true that the mean BMI of Japanese patients with type 2 diabetes is much lower than that of the United Kingdom Prospective Diabetes Study (UKPDS) patients (21). However, the BMI cut-off for being overweight is now 23 kg/m^2 in Asian subjects (22), which is lower than the mean BMI of the present patients.

Other therapeutic goals

At baseline, the proportion of patients satisfying all of the therapeutic goals (except smoking and alcohol drinking) was only 2.5%, even when a HbA_{1c} goal of less than 7.0% was adopted (Table 2). Only 32.2% and 45.0% of the patients at baseline fulfilled the target goals for blood pressure and total cholesterol levels, respectively, which were lower than the proportions reported in the U.S. (35.8% for blood pressure and 51.8% for total cholesterol) (23), suggesting that under-treatment of cardiovascular risk factors in diabetic patients was common also in Japan.

After 12 months of specialist care, total cholesterol, HDL cholesterol, triglycerides and blood pressure, all critical factors associated with diabetic vascular complications (12, 24-26), were controlled at levels close to the treatment goals set by the JDS (Table 1). However, our results also demonstrated that, in spite of the dramatic increase in the proportion of patients taking medications for hypertension and/or hyperlipidemia (Table 1) only a small proportion of more patients achieved the treatment goals than at baseline, which was notably lower than the achievement rate for glycemic control noted above (Table 2). Although the prevalence of cardiovascular complications in Japanese patients with type 2 diabetes is known to be lower than in patients from other countries (27, 28), the incidences of cerebral infarction and coronary heart disease in Japanese patients with diabetes are both approximately three times higher than in non-diabetic subjects (29), suggesting that we also need to improve our management of hypertension and serum cholesterol in order to prevent macrovascular complications at the same time as we seek to control glycemia.

Effects of DSME

A recent meta-analysis (30) and the results of the JDCS, the largest and longest trial focusing on the effects of lifestyle intervention (31, 32), have demonstrated a moderate, beneficial impact of DSME. However, a meta-analysis of educational intervention on the management of diabetes (33) failed to show a significant correlation between management effects and the number of visits or education type. We could not find a significant correlation between the frequency of DSME and glycemic control results (data not shown). However, this does not necessarily refute the significance of DSME since the quality of the DSME cannot be represented as a frequency measure. DSME is inevitably involved in a specialist's routine care and it is difficult to extract the genuine effects of DSME (34). It is conceivable that the potent effects of pharmacological therapy on glycemic control in the first few months of the study period masked the moderate effects of DSME. As a matter of fact, even previously treated patients (mostly direct referrals from primary care physicians) who underwent only limited changes in pharmacotherapy after starting specialist care (Fig. 2A) showed significant improvement in HbA_{1c} levels (Table 1), suggesting that the DSME element of specialist care had some positive effects.

Issues regarding lost to follow-up

A common barrier to improved patient care is that a considerable proportion of patients are lost to follow-up (35-38) and these defaulting patients have poorer outcomes than patients who continue to attend clinics (2, 35, 39, 40). Our study demonstrated a dropout rate of 35% which was close to that observed in many other studies (35). Unlike the situation in many other countries, visiting a clinic every month or two is a common characteristic of the Japanese healthcare system and reflects the facts that the government-based health insurance covers all citizens and extra patient expenditure for specialist care is unnecessary. However, the health insurance system does not seem to contribute to an improvement in the patient dropout rate. The significant differences in baseline HbA_{1c} levels between the patients who dropped-out and those who completed the care program suggests that

patients with milder diabetes need to be encouraged not to abandon medical care.

Limitation of the study and future strategy

There are several important limitations in our study. First, this is only a one-year prospective study and longer-term results, including chronic complications, need to be evaluated, especially as there was a slight deterioration in HbA_{1c} during the last 6 months of the study period. A further study of the outcome of long-term care including actual changes in lifestyle parameters is necessary since only a few substantial studies lasting longer than two years (2, 41) are currently available. Second, the high dropout rate could affect the study result. Although those with lower HbA_{1c} showed the highest dropout rate, a rapid deterioration in their glycemic control cannot be ruled out. Third, individual compliance to the DSME was not monitored but should be investigated in relation to the therapeutic outcome of each patient. At the same time, an analysis of adherence to practice guidelines in each clinic and their patient outcomes should be analyzed in more detail. Fourth, differences in ethnic (21, 42-47), socioeconomic or cultural background need to be considered as a possible source of bias when applying these results to other regions. Finally, a control group of patients treated by primary care physicians was not available and the eleven clinics that participated did so voluntarily. Consequently, we cannot tell from this particular study whether specialist care is superior to that of primary care physicians, although patient selection bias was minimized by registering all newly visited patients consecutively.

In conclusion, enhanced management and care of patients, especially those with relatively mild hyperglycemia, and ongoing therapy for those with HbA_{1c} levels approaching 6.5%, together with continuing efforts to eliminate obesity and patient dropout, and to manage hypertension and dyslipidemia more carefully will probably result in an improved diabetes care outcome.

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Appendix: The following members of the JDDM group participated in this study (in alphabetical order); Dr. Hiroshi Hayashi (Matsuzaka), Dr. Koichi Hirao (Yokohama), Dr. Koichi Kawai (Tsukuba), Dr. Mikihiko Kudo (Aomori), Dr. Yoshio Kurihara (Sapporo), Dr. Mariko Oishi (Kyoto), Dr. Fuminobu Okuguchi (Sendai), Dr. Takeshi Osonoi (Naka), Dr. Hideo Sasaki (Niigata), Dr. Hiromichi Sugiyama (Shizuoka), Dr. Katsuya Yamazaki (Toyama). The JDDM consists of many investigators at participating institutes all over Japan.

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Dysfunction of Endothelial Nitric Oxide Synthase and Atherosclerosis

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Abstract—Atherosclerosis is associated with an impairment of endothelium-dependent relaxations, which represents the reduced bioavailability of nitric oxide (NO) produced from endothelial NO synthase (eNOS). Among various mechanisms implicated in the impaired EDR in atherosclerosis, superoxide generated from dysfunctional eNOS has attracted attention. Under conditions in which vascular tissue levels of tetrahydrobiopterin (BH4), a cofactor for NOS, are deficient or lacking, eNOS becomes dysfunctional and produces superoxide rather than NO. Experimental studies in vitro have revealed that NO from eNOS constitutes an anti-atherogenic molecule. A deficiency of eNOS was demonstrated to accelerate atherosclerotic lesion formation in eNOS knockout mice. In contrast, eNOS overexpression with hypercholesterolemia may promote atherogenesis via increased superoxide generation from dysfunctional eNOS. Thus, eNOS may have 2 faces in the pathophysiology of atherosclerosis depending on tissue BH4 metabolisms. An improved understanding of tissue BH4 metabolisms in atherosclerotic vessels is needed, which would help in developing new strategies for the inhibition and treatment of atherosclerosis. (*Arterioscler Thromb Vasc Biol.* **2004;24:998-1005.**)

Key Words: endothelial nitric oxide synthase ■ atherosclerosis ■ tetrahydrobiopterin ■ superoxide ■ nitric oxide

Nitric oxide (NO) is generated from the conversion of L-arginine to L-citrulline by the enzymatic action of an NADPH-dependent NO synthase (NOS), which requires Ca^{2+}/calmodulin, FAD, FMN, and tetrahydrobiopterin (BH4) as the cofactors.[1-4] In the vessels, NO is produced from the endothelium by constitutive expression of the endothelial isoform of NOS (eNOS), which is activated by mechanical stress such as blood shear-stress and stimulation with agonists such as bradykinin and acetylcholine. NO has a variety of functions, but its action as the endothelium-derived relaxing factor (EDRF) is the most important for the maintenance of vascular homeostasis.[5] An impairment of endothelium-dependent relaxations (EDR) is present in atherosclerotic vessels even before vascular structural changes occur and represents the reduced eNOS-derived NO bioavailability. Endothelial dysfunction as characterized by an impairment of EDR, and thereby reduced eNOS-derived NO bioactivity, is the critical step for atherogenesis. Among various mechanisms responsible for the impaired EDR, the increased NO breakdown by superoxide is important, and there is augmented production of superoxide in atherosclerotic vessels. Recently, it was revealed that under certain circumstances, eNOS becomes dysfunctional and produces superoxide rather than NO. The pathophysiological role of dysfunctional eNOS has attracted attentions in vascular disorders, including atherosclerosis. This review focuses on the role of dysfunctional eNOS on atherosclerotic vessels and refers to the possible role of dysfunctional eNOS on atherogenesis.

Impaired EDR in Atherosclerosis

All major risk factors for atherosclerosis such as hyperlipidemia, diabetes, hypertension, and smoking are associated with impaired EDR.[6-8] Although the underlining mechanisms of the reduced EDR are multifactorial, its most important cause is a derangements of the eNOS/NO pathway, which include the reduced activity and expression of eNOS, decreased sensitivity to NO, and increased degradation of NO by reaction with superoxide.[8] Regarding the expression of eNOS at the vessel wall, it may be reduced in advanced atherosclerosis, possibly because of reduced transcription and/or increased instability of eNOS mRNA caused by cytokines.[9] However, most animal models with atherosclerosis demonstrate the unchanged or rather augmented expression of eNOS, at least in early atherosclerosis, despite the presence of impaired EDR.[10,11]

The enzymatic activity of eNOS is inhibited by various mechanisms associated with atherosclerosis and hyperlipidemia. Pro-atherogenic lipids, such as oxidized low-density lipoprotein (oxLDL) and lysophosphatidylcholine, inhibit signal transduction from receptor activation to eNOS activation.[12-14] Hypercholesterolemic serum and LDL upregulate caveolin abundance, augments caveolin–eNOS heterocomplex, and thereby attenuates NO production from the endothelial cells.[15,16] Endogenous NOS inhibitors such as asymmetric dimethylarginine (ADMA) and N-monometylarginine (NMA) are also revealed to be involved in the mechanisms of reduced EDR in atherosclerosis.[17,18]

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The accelerated degradation of NO by increased superoxide from vessel wall is demonstrated as another important mechanism of the reduced EDR in hyperlipidemia and atherosclerosis.[8] Superoxide production from atherosclerotic vessels is augmented in human and animal models with atherosclerosis.[19-22] The endothelium is important as a source of superoxide production, and its denudation decreases superoxide production from vessels with atherosclerosis but has no effects in normal vessels without atherosclerosis.[19] Animal models of hyperlipidemia and atherosclerosis demonstrate an excess vascular superoxide flux that is linked to reduced NO bioactivity. As an evidence for the involvement of superoxide in the impaired EDR in atherosclerotic vessels, the restoration of EDR by antioxidants and superoxide dismutase has been shown.[20,23,24] In rabbit aortas with high-cholesterol diet-induced atherosclerosis, the impaired vasodilatory responses to acetylcholine and A23187 were restored by chronic treatment with polyethylene-glycolated SOD.[20] Antioxidants improve EDR in human and animal models with atherosclerosis.[25-27] In particular, vitamin C is effective in the restoration of EDR associated with most risk factors for atherosclerosis, including hypercholesterolemia, hypertension, diabetes mellitus, and smoking.[28-30]

Superoxide Production From Vessels

Superoxide is produced by a variety of enzymes, including xanthine oxidase, cyclooxygenase, and NADPH oxidase. Among them, NADPH oxidase plays a major role in vascular cells.[31,32] In normal vessels, NADPH oxidase is present in adventitial fibroblasts. In atherosclerotic vessels, increased expression of subcomponents of NADPH oxidase has been found.[33-36] In the early stage of atherosclerosis, superoxide seems to be produced from NADPH oxidase localized in the endothelium; in advanced atherosclerosis, vascular smooth muscle cells serve as the major source of NADPH oxidase-derived superoxide.[37]

However, in vitro biochemical studies demonstrated that NOS can independently produce superoxide under certain conditions.[38-41] The catalytic mechanisms of NOS involve flavin-mediated electron transport from C-terminal–bound NADPH to the N-terminal heme center, where oxygen is reduced and incorporated into the guanidine group of L-arginine, giving rise to NO and L-citrulline. The eNOS-mediated superoxide generation is primarily regulated by BH4 availability. In the presence of suboptimal concentrations of BH4, activation of NOS leads to "uncoupling of NOS" and subsequent production of superoxide.[42-45] In "uncoupled NOS," electrons flowing from the reductase domain to the heme are diverted to molecular oxygen rather than to L-arginine; thereby, production of superoxide occurs. The ability of NOS to produce superoxide was first demonstrated in neuronal NOS (nNOS) and then extended to eNOS.[46,47] In the recombinant bovine eNOS, the heme moiety was identified as the main source for superoxide production.[45] In endothelial cells, a close link between cellular BH4 levels and NO synthesis was demonstrated, suggesting that an optimal concentration of BH4 is essential for NO production. The precise role of BH4 in the formation of NO is not completely understood, but it is postulated that BH4 donates electrons from the reductase domain to the ferrous–dioxygen complex in the oxygenase domain.[48,49] It is also demonstrated that addition of exogenous BH4 increases NO production and decreases superoxide production from endothelial cells.[40] As mentioned later in this article, there is an interaction between NADPH oxidase and eNOS, and it is thought that superoxide produced by NADPH is involved in the uncoupling of eNOS.

Exogenous BH4 and eNOS Function

It has been demonstrated in clinical and animal studies that acute administration of BH4 improves endothelial dysfunction associated with hypercholesterolemia, atherosclerosis, hypertension, and cigarette smoking.[50-53] These data have been presented as evidence for the presence of "uncoupled eNOS," which produces superoxide rather than NO, leading to impaired EDR. Laursen et al clearly demonstrated the production of superoxide from eNOS.[54] In apolipoprotein E-knockout (apoE-KO) mice, they showed the increased vascular superoxide production from the endothelium, which was associated with impaired EDR. Incubation of vessels with sepiapterin, a precursor to BH4, improved EDR and decreased superoxide production.

As in the study of Laursen et al, sepiapterin has been shown to restore endothelial function in acute studies, however, sepiapterin may not always be effective when vessels are exposed to it for a long time.[55-57] Sepiapterin is an oxidized BH4 analogue that generates BH4 by enzymatic reduction of sepiapterin reductase and dihydrofolate reductase. It is reported that relatively long-term (6 hours) incubation of hyperlipidemic rabbit vessels with sepiapterin resulted in a further derangement of vasodilatory response to endothelium-dependent agonists.[58] In addition, incubation of canine cerebral arteries with high levels of sepiapterin was shown to reduce EDR significantly, despite an increase in vascular BH4 levels. It is revealed that a high concentration of sepiapterin can serve as a pro-oxidant and thereby oxidizes BH4 to dihydrobiopterin (BH2).[49] Sepiapterin may increase BH2 rather than BH4 in the tissues, and the increased BH4 levels potentially compete with BH4 for eNOS binding and worsen eNOS uncoupling.

Vascular Pteridine Metabolism in Atherosclerosis

The presence of eNOS dysfunction as a mechanism of impaired endothelial function seems to be well-recognized now. However, only limited information is available on pteridine metabolism in the vessel wall in diseased states. In normal vascular tissue, >60% of total BH4 is present in the endothelium.[38,56] Endothelial cells from diabetic BioBreeding (BB) rats have a marked reduction in BH4 contents.[59] In the insulin resistance rat model induced by high-fructose diet, a modest reduction of BH4 levels in the aortas was associated with impaired EDR.[60] Furthermore, as compared with control rats, the levels of 7,8-dihydrobiopterin and biopterin, the oxidized form of BH4, were increased in the aortas of diabetic BB rats. Plasma BH4 levels were decreased in SHR with established hypertension.[61] Recently, it was reported that BH4 content was reduced and the content of oxidized forms of BH4 was increased in vessels from mice with deoxycorticosterone (DOCA)-salt hypertension.[62]