without abdominal obesity if accompanied by clustering of metabolic disorders. The AHA/NHLBI definition of MetS proved to be the most useful to predict cardiovascular disease in the diabetic elderly.

Introduction

Metabolic syndrome (MetS) consists of multiple, interrelated risk factors of metabolic origin that appear to directly promote the development of cardiovascular disease. Recently, MetS has been reported to be associated with impaired activities of daily living (ADLs) and cognitive decline of the elderly [1, 2]. Thus, management of risk factors and prevention of MetS seem to constitute an efficient strategy to attain successful ageing. Although insulin resistance and visceral adiposity could play a pivotal role in promoting atherosclerosis, the real cause may be a more complex interaction between genetic and environmental factors [3, 4].

The clinical usefulness of MetS for risk prediction for individuals with type 2 diabetes remains a matter of debate. It has been reported that insulin resistance and MetS are predictive of accelerated atherosclerosis in type 2 diabetic patients [5–14]. On the other hand, a recent reappraisal of MetS endorsed by the American Diabetes Association and the European Association for the Study of Diabetes argues that MetS is an entity of little or no prognostic use for diabetic patients [15]. They emphasized that it remains unclear whether identification of MetS confers a clinical advantage over identification and treatment of its individual components. Although the term MetS may not be applicable to diabetic subjects, even detractors agree that there are diabetic elderly who have increased insulin resistance associated with MetS-type cardiometabolic risk factor clustering [5–14].

Moreover, ageing is associated with increased insulin resistance in addition to type 2 diabetes [16]. Thus, the impact of abdominal obesity on insulin resistance and clustering of metabolic risk factors in diabetic elderly remains unknown. To date, for Asian elderly with type 2 diabetes, there is limited information on age-associated changes in abdominal obesity, insulin resistance and clustering of metabolic risk factors, and their association with vascular complications.

To address the need for elucidation concerning metabolic risk factor clustering in diabetic elderly, we conducted a large-scale prospective study of the Japanese Elderly Diabetes Intervention Trial (J-EDIT) [17, 18]. The questions we addressed were: (1) prevalence of abdominal obesity, insulin resistance, and MetS-type risk factor clustering as defined by different sets of criteria; (2) possible connections between abdominal obesity and insulin resistance, and (3) the predictive power of MetS-type clustering of metabolic risk factors for cardiovascular diseases in diabetic elderly. To answer these questions, we analyzed the baseline measures of the J-EDIT.

Methods

Participants

J-EDIT started in 2001 with an enrolment of 1,173 diabetic subjects aged 65 years or over and with serum HbA1c levels of ≥7.0% from 42 institutes in Japan. The J-EDIT protocol, which is in accordance with the provisions of the Declaration of Helsinki, received ethical approval from the institutional review boards of all of the participating institutes. Written informed consent was obtained from all patients. All examinations relevant for this study were completed by 812 subjects, 371 of whom were men. The remaining 361 subjects were excluded because some of their data were missing.

Diagnostic Criteria for MetS

In this study, we applied the three different sets of criteria proposed for the diagnosis of MetS by the International Diabetes Federation (IDF), the Japanese Society of Internal Medicine (JSIM) and the American Heart Association and the National Heart, Lung, and Blood Institute (AHA/NHLBI) [19–21]. According to IDF and JSIM criteria, there is a strong correlation between abdominal obesity and insulin resistance, which makes the presence of abdominal obesity a condition for diagnosis of MetS [19, 20]. A Japanese study concluded that a visceral fat area in excess of 100 cm^2 measured by means of CT scanning corresponds to a waist circumference of 85 cm for men and ≥90 cm for women [22]. In 2005, moreover, the IDF recognized specific cutoffs by sex and ethnicity [23]. However, new data support the use of alternative waist circumference cutoffs for the prediction of cardiovascular complications [24–26]. In 2007, the IDF recommended new waist circumference cutoffs for Japanese, 90 cm for men and 80 cm for women [19]. However, these cutoffs for waist circumference in the definition of MetS for Japanese have remained a matter of debate. For our study, we therefore adopted two criteria, one from the IDF (2007) and one from the JSIM. On the other hand, AHA/NHLBI has introduced alternative criteria, which have the advantage of avoiding emphasis on a single cause [21]. The resulting three definitions for MetS are as follows.

(1) The IDF definition of MetS (IDF-MetS) specifies abdominal obesity with waist circumference cutoffs of ≥90 cm for men or ≥80 cm for women plus any one of the following factors [19]: (a) elevated triglyceride (≥150 mg/dl) or specific treatment for this lipid abnormality; (b) reduced HDL-cholesterol of <40 mg/dl for men or <50 mg/dl for women, and (c) elevated systolic blood pressure (≥130 mm Hg) or diastolic blood pressure (≥85 mm Hg) or treatment for previously diagnosed hypertension.

Table 1. Demographic and clinical parameters of the patients

	Male	Female	All subjects
n	371	441	812
Age, years	71.4 ± 4.5	72.0 ± 4.6	71.8 ± 4.6
Duration of diabetes, years	16.7 ± 10.2	15.7 ± 8.8	16.2 ± 9.5
Systolic blood pressure, mm Hg	137.1 ± 15.8	138.6 ± 17.3	137.9 ± 16.7
Diastolic blood pressure, mm Hg	76.2 ± 9.6	75.5 ± 10.0	75.8 ± 9.9
HbA1c, %	8.0 ± 0.9	8.0 ± 0.9	8.0 ± 0.9
Fasting plasma glucose, mg/dl	169.7 ± 50.5	165.1 ± 50.7	167.2 ± 50.6
Fasting plasma insulin, μU/ml	9.3 ± 10.0	10.2 ± 8.5	9.8 ± 9.3
Urine Alb, mg/g Cr	226.4 ± 597.9	184.5 ± 499.7	203.7 ± 546.8
Serum cholesterol, mg/dl	192.7 ± 31.2	209.5 ± 34.5	201.8 ± 34.1
Serum HDL-C, mg/dl	52.9 ± 15.5	60.3 ± 19.4	56.9 ± 18.1
Serum LDL-C, mg/dl	114.9 ± 28.0	123.7 ± 32.0	119.7 ± 30.5
Serum triglyceride, mg/dl	129.2 ± 84.1	129.4 ± 66.4	129.3 ± 74.9
Current smokers, %	28.4	5.8	16.2
OHA use, %	62.5	61.7	62.1
Insulin use, %	25.3	31.1	28.5
Medication for hypertension, %	47.3	61.9	55.1
Medication with fibrates, %	3.2	4.3	3.8
Medication with statin, %	18.9	43.1	32.0
History of CHD, %	15.5	15.6	15.6
History of stroke, %	14.7	11.3	12.9

Data are presented as means ± SD or as percentages. OHA = Oral antihyperglycemic agents; CHD = coronary heart disease.

(2) The JSIM definition of MetS (JSIM-MetS) specifies abdominal obesity with waist circumference cutoffs of ≥85 cm for men or ≥90 cm for women, plus any one of the following factors [20]: (a) elevated triglyceride (≥150 mg/dl) or reduced HDL-cholesterol (<40 mg/dl) or specific treatment for these lipid abnormalities, and (b) elevated systolic blood pressure (≥130 mm Hg) or diastolic blood pressure (≥85 mm Hg) or treatment for previously diagnosed hypertension.

(3) The AHA/NHLBI definition of MetS (AHA/NHLBI-MetS) specifies two or more of the following conditions [21]: (a) waist circumference of ≥90 cm for men or ≥80 cm for women; (b) elevated triglyceride (≥150 mg/dl) or specific treatment for lipid abnormality; (c) reduced HDL-cholesterol of <40 mmol/l for men or 50 mmol/l for women, and (d) elevated systolic blood pressure (≥130 mm Hg) or diastolic blood pressure (≥85 mm Hg) or treatment for previously diagnosed hypertension.

Assessment of Diabetes Mellitus and Complications

Information about diabetes mellitus, blood examinations and complications were obtained from clinical charts. Waist circumference was measured at the umbilicus level. Information regarding cigarette smoking was collected using a standardized questionnaire.

After overnight fasting, blood samples were taken by vein puncture to assess serum levels of glucose, HbA1c, total cholesterol, triglyceride, and HDL-cholesterol. Insulin resistance was assessed from levels of fasting glucose and insulin concentration by means of the homeostasis model assessment (HOMA) formula: fasting insulin (μU/ml) × fasting glucose (mg/dl)/405 [27]. This method was not applicable to subjects treated with insulin. Serum LDL-cholesterol levels were calculated using Friedewald's equation, except for triglyceride levels of >400 mg/dl, in which case the LDL cholesterol data were recorded as 'missing'.

Information about a previous history of coronary heart disease (CHD) and stroke and findings from a 12-lead electrocardiogram (ECG) were obtained for all patients to assess cardiovascular disease at baseline. CHD was considered to be present when diabetic patients had at least one of the following: a history of myocardial infarction and angina characterized by a typical clinical picture (chest pain, chest oppression, dyspnea, typical ECG alteration). Stroke events were defined as a constellation of neurological deficits of sudden or rapid onset for which there was no apparent cause other than a vascular accident. Cases with asymptomatic lesions detected by brain imaging were not included.

Statistical Analysis

Data are presented as means ± SD or as percentages unless otherwise specified. Association of waist circumference with HOMA insulin resistance (HOMA-IR) was tested using simple and multiple logistic regression. Variables among the MetS subgroups were compared using ANOVA and statistical differences were tested with Dunnett's statistical test. Backward logistic regression analysis was used to calculate the adjusted odds ratio (OR) and 95% confidence interval (CI) for risk factors with cardiovascular diseases. The SAS software package (Version 8.0; SAS, Cary, N.C., USA) was used for all analyses. $p < 0.05$ was considered significant.

Table 2. Age-associated changes in BMI, waist circumference, HOMA-IR, and prevalence of MetS risk factors

	Men, age group				Women, age group			
	65–69	70–74	75–79	80–85	65–69	70–74	75–79	80–85
BMI	23.8 ± 3.2	23.9 ± 3.1	23.9 ± 3.1	23.6 ± 2.7	23.6 ± 3.5	24.1 ± 3.8	24.5 ± 3.7	22.8 ± 3.3
Waist circumference, cm	85.8 ± 8.9	85.0 ± 8.1	87.6 ± 8.1	88.3 ± 10.0	80.6 ± 10.4	82.7 ± 10.7	84.5 ± 11.2	79.6 ± 10.2
HOMA-IR	3.81 ± 4.0	3.37 ± 3.1	4.37 ± 5.8	2.83 ± 2.0	3.17 ± 2.7	3.49 ± 3.0	4.80 ± 3.6	3.54 ± 4.1
IDF-MetS, %	28.6	30.4	33.3	42.1	47.9	59.6	59.6	46.9
JSIM-MetS, %	51.7	47.4	59.7	68.4	19.4	23.0	31.7	25.0
AHA/NHLBI-MetS, %	56.5	52.6	59.7	57.9	72.2	83.2	80.8	65.6
Hypertension, %	79.6	74.6	84.5	84.2	77.8	87.6	91.3	96.9
IDF and AHA/NHLBI								
Low HDL-C, %	38.1	30.6	35.2	31.6	60.4	65.8	65.4	46.9
High triglyceride, %	26.5	30.6	25.4	36.8	29.2	36.6	30.8	25.0
JSIM dyslipidemia, %	36.1	37.3	33.8	47.4	30.6	39.1	32.7	28.1

Data are presented as means ± SD or as percentages. BMI = Body mass index; HOMA-IR = homeostasis model assessment, insulin resistance; MetS = metabolic syndrome; IDF = International Diabetes Federation; JSIM = Japanese Society of Internal Medicine; AHA/NHLBI = American Heart Association and the National Heart, Lung, and Blood Institute.

Results

Age-Associated Increase in Abdominal Obesity, Insulin Resistance and MetS-Type Risk Factor Clustering

Demographic and clinical parameters of the 812 study participants are listed in table 1. Age-associated changes in BMI, waist circumference, and HOMA-IR are listed in table 2. BMI did not show any apparent changes for men, although waist circumference increased with age. Waist circumference also increased for women, followed by a decrease at the age of 80 or over. HOMA-IR similarly increased with age, but decreased from the age of 80 for both men and women. The increase in insulin resistance seemed to correlate with the age-associated increase in abdominal obesity of diabetic elderly.

The overall prevalence of MetS-type risk factor clustering based on IDF, JSIM and AHA/NHLBI criteria was 44.0, 37.1 and 67.7%, respectively. The incidence of IDF-MetS and JSIM-MetS, which specify abdominal obesity, increased with age, but decreased at age 80 or over for women (table 2). In contrast, the prevalence of AHA/NHLBI-MetS did not show any apparent change with ageing.

As for the individual components of MetS, the prevalence of hypertension was highest and increased with age. Among diabetic elderly aged 80–85 years, hypertension was found in 84.2% of men and 96.9% of women. The prevalence of low HDL-cholesterol also increased with age, but started to decrease at the age of 80 and over.

We also investigated whether diabetic patients with a longer history of diabetes and/or more serious hyperglycemia might show an increased prevalence of MetS-type risk factor clustering, but no such trend could be found in any type of criteria-defined MetS (data not shown).

Possible Connections between Abdominal Obesity and Insulin Resistance

Overall, HOMA-IR was 4.2 ± 5.0 for diabetic elderly. The relationship between waist circumference and insulin resistance is shown in figure 1. Simple regression analysis showed that log-transformed HOMA-IR was associated with waist circumference in a crude linear manner (coefficient = 0.051, $p < 0.0001$, $R^2 = 0.105$ for men; coefficient = 0.034, $p < 0.0001$, $R^2 = 0.116$ for women). This was demonstrated by an increase in HOMA-IR of 1.1 for men and 0.6 for women for every 10-cm increment in waist circumference. After adjustment for sex, age, systolic blood pressure, HbA1c, triglyceride, and HDL-C, the association remained statistically significant (coefficient = 0.034, $p < 0.0001$).

For any of the three definitions, HOMA-IR was higher for subjects with MetS than for those without MetS (4.34 ± 3.65 for IDF-MetS and 3.21 ± 3.50 for non-IDF-MetS, $p = 0.0003$; 4.33 ± 3.95 for JSIM-MetS and 3.30 ± 3.33 for non-JSIM-MetS, $p = 0.0022$; 4.03 ± 3.58 for AHA/NHLBI-MetS and 3.04 ± 3.57 for non-AHA/NHLBI-MetS, $p = 0.0022$).

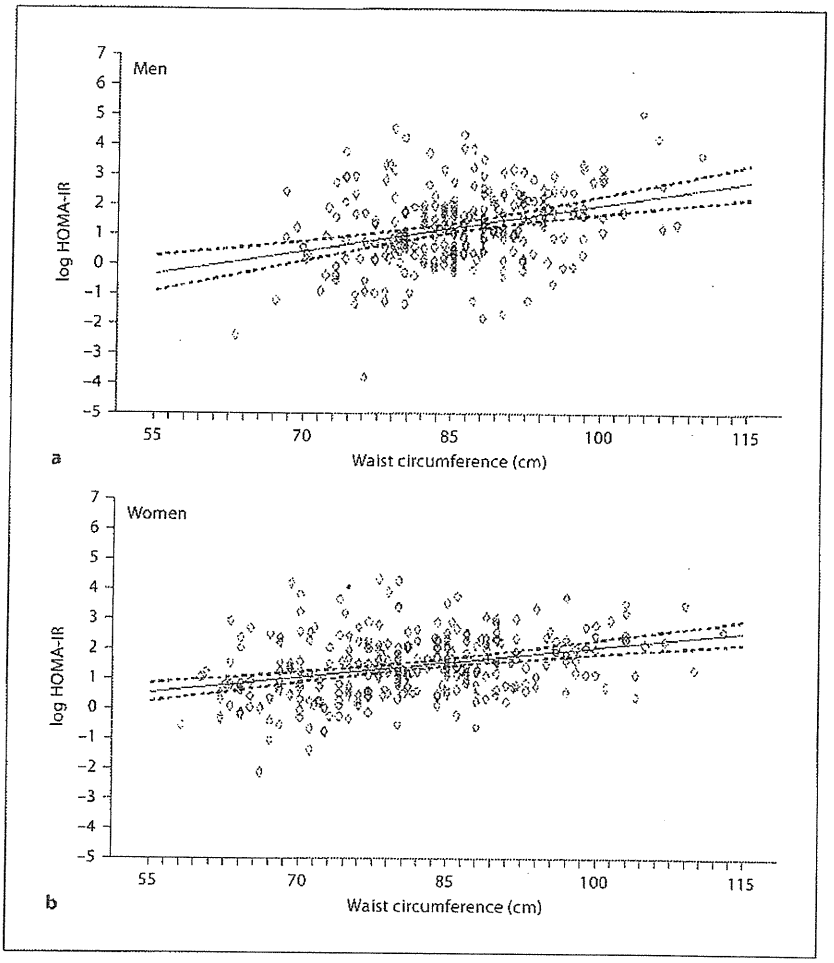

Fig. 1. Relationship between waist circumference and insulin resistance. Association of HOMA-IR with waist circumference of men (**a**) and women (**b**) in the J-EDIT. Log-transformed HOMA-IR (log HOMA-IR) was associated with waist circumference in a crude linear manner (coefficient = 0.051, $p < 0.0001$, $R^2 = 0.105$ for men; coefficient = 0.034, $p < 0.0001$, $R^2 = 0.116$ for women).

It has been proposed that HOMA-IR is useful for estimating insulin resistance of type 2 diabetic patients [28], but the degree of association between HOMA-IR and clamp insulin resistance for diabetic patients treated with oral antihyperglycemic agents of the class insulin secretagogues has remained unclear. In this connection, Emoto et al. [29] have reported that HOMA-IR strongly correlates with clamp insulin resistance in type 2 diabetic patients treated with sulfonylureas (SUs) as well as in those treated with diet alone. Furthermore, Spearman's correlation coefficients for HOMA-IR and waist circumference were similar for subjects taking SU drugs and those who had used neither SU drugs nor glinides (data not shown). Such evidence indicates that it seems likely that waist circumference is associated with insulin resistance in diabetic elderly, regardless of treatment with oral antihyperglycemic agents of the class insulin secretagogues.

Insulin Resistance of Metabolic Factor Clustering with and without Abdominal Obesity

We compared the clinical characteristics of IDF-MetS and AHA/NHLBI-MetS by dividing the study population into 3 subgroups, non-MetS, AHA/NHLBI-only, and IDF&AHA/NHLBI (table 3). There was no difference in age among the subgroups. HOMA-IR was especially elevated in the IDF&AHA/NHLBI group, as was waist circumference. Interestingly, in the AHA/NHLBI-only group, HOMA-IR was moderately elevated without an accompanying increase in waist circumference. Furthermore, the mean duration of diabetes for MetS with overlapping patterns was significantly shorter than that for

Table 3. Clinical characteristics of subgroups of IDF-MetS and AHA/NHLBI-MetS risk factor clustering

	Non-MetS	AHA/NHLBI only	IDF&AHA/NHLBI
Number	263	192	357
Age, years	71.9 ± 4.7	71.0 ± 4.5	72.1 ± 4.5
Waist circumference, cm	78.2 ± 7.9	77.7 ± 6.8	91.4 ± 7.3*
HOMA-IR	3.04 ± 3.6	3.46 ± 3.4	4.34 ± 3.7*
Duration of diabetes, years	16.1 ± 10.3	14.0 ± 8.2	13.6 ± 8.7‡

For abbreviations see table 2. Data are means ± SD or actual numbers.

* $p < 0.001$, † $p = 0.004$, ‡ $p = 0.012$, in comparison with non-MetS.

non-MetS. These results suggest that there are two distinct ways for insulin resistance to increase in diabetic elderly, one is in association with abdominal obesity and the other is not relevant to abdominal obesity.

Association of MetS-Type Risk Factor Clustering with Cardiovascular Disease

We examined the independent association of MetS-type risk factor clustering with cardiovascular diseases (table 4). Because sex is reportedly an independent factor associated with MetS [30–32], MetS and sex were included in the independent variables, while the other risk factors for atherosclerotic disease, such as age, HbA1c, duration of diabetes, smoking, total cholesterol, LDL-cholesterol, triglyceride, systolic blood pressure and diastolic blood pressure, were analyzed with backward stepwise regression. Age was found to be consistently associated with CHD, while JSIM-MetS and AHA/NHLBI-MetS, but not IDF-MetS, were also associated with CHD. When MetS was eliminated from the independent variables, age and diastolic blood pressure proved to be significantly associated with CHD, suggesting these factors independently correlate with CHD in diabetic elderly. For stroke, AHA/NHLBI-MetS was identified as a predictive factor. When MetS was eliminated from the independent variables, sex (men) and triglyceride showed a significant correlation with stroke. On the other hand, IDF-MetS and JSIM-MetS, which both specify the presence of abdominal obesity for MetS, were not associated with stroke. These results indicate that MetS of AHA/NHLBI definition is the most consistent predictor for CHD and stroke for diabetic elderly, even after adjustment for the risk factors of age, sex, blood pressure, dyslipidemia, and indices of diabetes.

Table 4. Association of MetS and other risk factors with cardiovascular disease

Previous history of CHD			Previous history of stroke		
	OR	95% CI		OR	95% CI
IDF					
MetS	1.44	0.91–2.28	MetS	1.16	0.70–1.92
Sex	1.39	0.88–2.19	Sex	1.69	1.03–2.75
Age	1.06	1.01–1.12	Age	1.05	0.99–1.10
DBP	0.98	0.95–0.99	TG	1.01	1.00–1.01
			DM duration	1.25	0.89–1.75
JSIM					
MetS	1.80	1.14–2.85	MetS	1.32	0.79–2.19
Sex	1.10	0.70–1.73	Sex	1.51	0.92–2.47
Age	1.06	1.01–1.11	Age	1.05	0.99–1.10
DBP	0.97	0.95–0.99	TG	1.01	1.00–1.01
			DM duration	1.27	0.90–1.78
AHA/NHLBI					
MetS	1.90	1.13–3.19	MetS	1.86	1.07–3.24
Sex	1.48	0.94–2.33	Sex	1.84	1.13–3.00
Age	1.06	1.01–1.12	Age	1.05	0.99–1.11
DBP	0.97	0.95–0.99			
Factors other than MetS					
Sex	1.26	0.82–1.95	Sex	1.63	1.01–2.61
Age	1.01	1.01–1.12	Age	1.05	0.99–1.11
DBP	0.98	0.96–0.99	TG	1.01	1.00–1.01
			DM duration	1.25	0.89–1.75

MetS = Metabolic syndrome; CHD = coronary heart disease; SBP = systolic blood pressure; DBP = diastolic blood pressure; TG = triglyceride; DM = diabetes mellitus. For other abbreviations, see table 2.

The association of IDF-MetS, JSIM-MetS and AHA/NHLBI-MetS with cardiovascular disease was examined. MetS and sex were included in the independent variables, and age, HbA1c, duration of diabetes, smoking, total cholesterol, LDL-cholesterol, TG, SBP and DBP were analyzed with backward stepwise regression.

Discussion

This J-EDIT study first provided evidence of MetS-type risk factor clustering in Asian (Japanese) elderly with type 2 diabetes. Several new findings are reported: (1) abdominal obesity, insulin resistance and prevalence of MetS-type risk factor clustering evidently increased with age, but somewhat decreased at the age of 80 and over; (2) overall insulin resistance was substantially elevated in diabetic elderly [28, 30], and there was a significant crude linear association between waist circumference and insulin resistance; (3) insulin resistance was elevated not only in cases with but also without abdominal

obesity if accompanied by clustering of metabolic disorders, and (4) AHA/NHLBI-MetS, comprising both abdominal obese and non-abdominal obese metabolic factor clustering cases, was the most useful for prediction of cardiovascular disease in diabetic elderly.

The incidence of MetS in the general population reportedly differs widely among ethnic groups and according to the definition of MetS [13, 33–38]. It has also been reported that the prevalence of MetS increases with age [39, 40]. However, the prevalence of MetS-type risk factor clustering among patients with known diabetes is consistently high regardless of ethnicity or definition [6–13, 30, 33, 41–48]. In the Japan Diabetes Complications Studies (JDCS), which was concerned with relatively younger diabetic patients aged 40–70 years, the prevalence of IDF-MetS risk factor clustering was 32% for men and 9.2% for women [47]. Although the inclusion criteria of JDCS and JEDIT were not the same, it seems likely that prevalence of MetS-type risk factor clustering in Japanese patients with type 2 diabetes increases with age, at least until the age of 80. To our knowledge, there are no epidemiological data of MetS-type risk factor clustering of diabetic elderly in the other ethnic groups.

Although we did not measure insulin resistance directly in this study, HOMA-IR has been shown to correlate well with direct methods in subjects with various degrees of glucose tolerance, including patients who have already developed diabetes [27]. The averages of HOMA-IR of younger diabetic subjects have been reported as 2.9–3.3 [28, 30]. In our diabetic elderly, insulin resistance was evidently high (4.2 ± 5.0), which may be due to the diabetic state itself and/or age-associated changes in body composition such as increases in fat mass and decreases in fat-free mass [16]. We therefore expected that the correlation of insulin resistance with abdominal obesity might become weaker in diabetic elderly, but there was in fact a significant linear association of insulin resistance with waist circumference, and the former was found to be higher in JSIM-MetS and IDF-MetS. In this respect, it should be pointed out that insulin resistance also increased moderately in MetS-type risk factor clustering without abdominal obesity, so that the mechanism for the increase in insulin resistance associated with non-obese type metabolic factor clustering remains to be clarified [40].

Evidence is accumulating that MetS is clinically relevant for the prediction of cardiovascular disease in non-diabetic elderly [49–51]. Our study is the first to demonstrate that AHA/NHLBI-MetS correlates independently with cardiovascular disease in diabetic elderly after adjustment for the other risk factors for atherosclerotic disease. It seems plausible that non-obese metabolic factor clustering together with increased insulin resistance has a major impact on the risk of cardiovascular diseases of diabetic elderly, because MetS with abdominal obesity does not always appear to be associated with cardiovascular diseases [10, 52–54]. Definitions of MetS-type risk factor clustering that specify abdominal obesity have not yet been developed for Asian (Japanese) diabetic elderly. Other studies have also identified the usefulness of the National Cholesterol Education Program (NCEP)-MetS and AHA/NHLBI-MetS for the prediction of cardiovascular disease in younger subjects with type 2 diabetes [9–10, 13]. On the other hand, Sone et al. [30] have demonstrated that NCEP-MetS has limited clinical usefulness as a predictor for Asian diabetic patients. Further prospective analyses are thus needed to investigate the clinical significance of MetS-type risk factor clustering without abdominal obesity for diabetic elderly.

There are certain limitations to our study. First, we performed a cross-sectional evaluation and our results are therefore subject to survival bias. Second, our study subjects were hospital-based patients with diabetes of relatively long duration, so that any inferences are of necessity limited to similar patient groups. On the other hand, this population sample represents the real-world scenario of type 2 diabetes in Japan.

In conclusion, abdominal obesity and insulin resistance were found to increase with age, at least until the age of 80, in Asian diabetic elderly, and a relationship between waist circumference and HOMA-IR was demonstrated. An important finding was that MetS-type metabolic factor clustering without abdominal obesity also showed elevated insulin resistance. AHA/NHLBI-MetS, comprising both obese and non-obese metabolic disease clustering, was found to be the most effective for the prediction of cardiovascular disease, whilst the significance of MetS with abdominal obesity in this respect remains unclear. An on-going prospective study of J-EDIT may help to clarify the pathophysiology of metabolic disease clustering and its association with cardiovascular disease and geriatric syndromes of diabetic elderly.

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Short Report

Amyloid-β neurotoxicity restricts glucose window for neuronal survival in rat hippocampal slice cultures

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ABSTRACT

Diabetes may increase the risk of Alzheimer's disease (AD). However, a preventive strategy to combat cognitive decline in diabetic elderly with preexisting AD has remained unknown. The aim of this study was to determine the effects of metabolic perturbation on amyloid-β (Aβ) neurotoxicity and the optimal glucose range for improved neuronal survival, which is referred to as the "glucose window". Organotypic hippocampal slice cultures were incubated in either normoglycemic or hyperglycemic medium for 48 h, and subsequently treated in experimental media containing 0–30 mM glucose, with and without $A\beta_{25-35}$. Neuronal survival was evaluated by the propidium iodide method. Aβ neurotoxicity was exacerbated during hypoglycemia/hyperglycemia (≦2 mM/≧30 mM) without Aβ and ≦3 mM/≧20 mM with Aβ. ROS elevated in the respective glucose ranges and supplementation of ROS scavengers effectively improved neuronal survival. Interestingly, a sharp and sudden drop in glucose levels from preceding hyperglycemia further increased Aβ neurotoxicity. Supplementation of pyruvate protected exacerbated Aβ neurotoxicity. These results indicate that increased oxidative stress during severe hypoglycemia, hyperglycemia and fluctuation of blood glucose enhances neuronal cell death, resulting in the extremely limited glucose window, and therefore suggest that careful management of glucose avoiding hypoglycemia is needed to prevent brain degeneration in diabetic patients with AD.

1. Introduction

Diabetes and dementia are two of the most common and devastating health problems of the elderly. A systematic review of population-based studies has provided accumulated evidence that diabetes is associated with cognitive decline and increases the risk of developing Alzheimer's disease (AD) in people who do not have dementia [1].

It has been postulated that diabetes and AD share a number of features. Although the exact pathogenesis remains somewhat unclear, several mechanisms through which diabetes may affect the brain have been identified [1]. First, the relationship between diabetes and AD can be explained by diabetic vasculopathy and its sequelae. Second, toxic effects of hyperglycemia are thought to be involved in the development of diabetic end-organ damage to the brain. An increase in advanced glycation end products, disturbances of intracellular second messenger pathways, and an imbalance in the generation and scavenging of reactive oxygen species (ROS) would be crucial in the pathogenesis of AD [2,3]. Third, iatrogenic hypoglycemia during aggressive treatment of diabetes is considered to have a specific impact on cognitive function [4]. Finally, deficits in brain insulin signaling have been recently postulated in AD pathogenesis [5].

To date, an effect of diabetes on the rate of cognitive decline in patients with preexisting AD is unclear. A previous study found no difference in the rate of cognitive decline of the Mini-mental state examination (MMSE) score in patients with and without diabetes [6]. Another studies found an unexpected slower rate of cognitive decline of the MMSE in patients with a history of diabetes with AD [7,8], while a history of diabetes was associated with faster annual cognitive decline in patients with incident AD [9]. This controversial result could be result from the age of patients and/or the difference in treatment and metabolic abnormalities of diabetes [7].

A preventive strategy to combat cognitive decline in diabetic elderly with preexisting AD has remained a matter of debate. In healthy subjects, fasting glucose and 2 h postglucose levels are less than 5.5 mM and 7.8 mM, respectively, while fasting glucose ≧7.0 mM or 2 h postloaded glucose ≧11.1 mM in diabetic patients [10]. Several studies have suggested that blood glucose levels should be normalized for better cognitive function, but special attention is needed to avoid hypoglycemia [4,11]. The lower and upper limits of blood glucose levels required to avoid irreversible neuronal degeneration remain unclear. Moreover, there is little information on the molecular basis of metabolic perturbation in diabetes that could influence AD pathology.

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To address these matters, we performed in-vitro experiments to identify the optimal glucose range, which is referred to as the "glucose window", for improved neuronal survival. We hypothesized that amyloid-β (Aβ) neurotoxicity is exacerbated during both hypoglycemia and hyperglycemia. The goals of our study were thus 1) identification of the glucose window in the presence and absence of Aβ, 2) to clarify the underlying mechanism of exacerbated Aβ-neurotoxicity during glucose metabolism abnormalities, and 3) to identify substrates for enlargement of the glucose window to improve neuronal survival in the presence of Aβ.

2. Materials and methods

2.1. Preparation of organotypic hippocampal slice cultures (OHCs)

All animals were treated according to the guidelines for animal experimentation of the Kobe University School of Medicine. OHCs were prepared from 9-to-11-day-old Wistar rats (SLC Japan, Hamamatsu, Japan) using a standard method [12] and incubated in the medium containing 36 mM glucose before the 12th day of culture (Fig. 2A). Then, OHCs were incubated in either normoglycemic (10 mM) or hyperglycemic (20 mM) medium for 48 h, and subsequently treated for another 48 h in various experimental media containing 0–30 mM glucose, with and without Aβ, trolox (1 mM) or sodium pyruvate (10 mM).

2.2. Preparation of $A\beta_{25-35}$

$A\beta_{25-35}$ is a short synthetic peptide and suitable for studying Aβ neurotoxicity. $A\beta_{25-35}$ and $A\beta_{1-42}$ oligomers have been shown to induce neuronal damage through similar mechanisms [13]. Since the effect of $A\beta_{25-35}$ at 50 μM on neuronal survival was found to be equivalent to that of $A\beta_{1-42}$ oligomer at 1 μM (data not shown), $A\beta_{25-35}$ was used at a concentration of 50 μM in all subsequent experiments.

2.3. Measurement of neuronal death and ROS

The propidium iodide (PI) method, was used for the assessment of neuronal death in the CA1 region of OHCs [12]. 4.6 μg/ml PI (Sigma, St. Louis, USA) was added to the wells of the culture microplates. After obtaining PI images after 48 h treatment in the conditioning mediums, all living neurons were killed by adding 10 μM N-methyl-D-aspartic acid. Final PI fluorescence intensity was adjusted to 100%, which is equivalent to total neuronal cell death, and cell death observed at 48 h was expressed as a percentage of the maximum fluorescence.

ROS levels were measured by using a non-fluorescent compound, 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA, Invitrogen, USA), following procedures previously utilized to estimate β-amyloid-induced ROS production in neurons [14]. Because ROS levels significantly increased after 1–3 h treatment with $A\beta_{25-35}$, but not after 6 h (data not shown), we measured ROS contents 3 h after each experimental treatment.

2.4. Statistical analysis

Data analysis was conducted using the ANOVA and Tukey statistics (SPSS 15.0 J). A value of $P<0.05$ was considered statistically significant.

3. Results

3.1. Exacerbation of Aβ neurotoxicity during hypoglycemia/hyperglycemia

First, the effects of a variety of extracellular glucose concentrations on neuronal survival were tested in the absence of $A\beta_{25-35}$. In comparison with control (10 mM glucose), neuronal survival was not affected when the medium glucose concentration was 3–25 mM, while severe hypoglycemia (≦2 mM) and hyperglycemia (≧30 mM) caused neuronal death (Fig. 1A and B). Impairment of neuronal survival during hyperglycemia was not due to elevated osmolarity, because adjustment of osmolarity with sucrose to make it equivalent to glucose concentration did not affect neuronal survival at any glucose concentration (data not shown). Compared to control, neuronal death in the presence of $A\beta_{25-35}$ increased when medium glucose concentration was ≦3 mM and ≧20 mM.

Because our slice culture was a static system without dynamic flow of medium, we measured glucose concentrations at the termination of experiments ($n=4$) and found that glucose concentrations were reduced to approximate 60–70% of the original concentrations. Mean glucose concentrations during 48 h observation were 0.2, 1.7, 2.3, 4.1, 8.6, 12.7, 17.4, and 28.9 (mM) in media containing 0, 2, 3, 5, 10, 15, 20, and 30 (mM) glucose at start of experiments, respectively. These findings demonstrate that hyperglycemia as well as hypoglycemia had adverse effects on neuronal survival and exacerbated Aβ neurotoxicity during hypoglycemia/hypoglycemia, resulting in a restricted glucose window (5–15 mM).

3.2. Effect of oxidative stress on Aβ neurotoxicity elicited during hypoglycemia and hyperglycemia

The contribution of Aβ-induced oxidative stress on neuronal death during hypoglycemia/hyperglycemia was examined. When the medium glucose concentration was ≦2 mM and ≧30 mM, ROS concentration in the OHCs without Aβ increased compared with control (Fig. 1C). In the presence of $A\beta_{25-35}$, ROS levels were further elevated when glucose concentration was ≦3 mM and ≧20 mM. Supplementation of trolox, an ROS scavenger, partially reversed the increment in ROS as a result of glucose deprivation, as well as improved neuronal survival (Fig. 1D). Trolox completely eliminated hyperglycemia-induced ROS accumulation accompanied by a recovery of neuronal survival to control levels. In the presence of $A\beta_{25-35}$, trolox also reduced ROS accumulation and reversed neuronal death during hypoglycemia/hyperglycemia, suggesting that oxidative stress plays a pivotal role in the increase in Aβ neurotoxicity elicited during hypoglycemia/hyperglycemia.

3.3. Sharp and sudden drop in glucose levels from preceding hyperglycemia further increases Aβ neurotoxicity

Finally, the effects of fluctuations in glucose levels on neuronal survival were investigated (Fig. 2A). In the absence of $A\beta_{25-35}$, reduction of glucose to 3–10 mM from preceding hyperglycemia (20 mM) did not cause any apparent changes in neuronal survival, but severe hypoglycemia (2 mM) after hyperglycemia exacerbated neuronal death (Fig. 2B). In the presence of $A\beta_{25-35}$, a sharp and sudden drop in glucose caused deterioration in neuronal damage (Fig. 2C). Reduction of glucose to 5 mM, which is equivalent to the physiological concentration of glucose, caused significant neuronal death in association with elevated levels of ROS (Fig. 2C and D). Severe hypoglycemia (2–3 mM) induced an increase in neuronal death after the drop in glucose from previous hyperglycemia.

Supplementation with trolox and pyruvate diminished Aβ neurotoxicity (Fig. 2D and E), to enlarge the restricted glucose window. Several beneficial effects of pyruvate on neuronal survival have been reported, including its function as an antioxidants and an energy substrate [12,15]. Protective effect of pyruvate was more pronounced than that of trolox ($P=0.003$ at 2 mM glucose). Our findings indicated that Aβ neurotoxicity was exacerbated after the drop in glucose level from the preceding hyperglycemia accompanied by an increase in ROS accumulation. It is of note that the glucose window was 10–15 mM for this preparation.

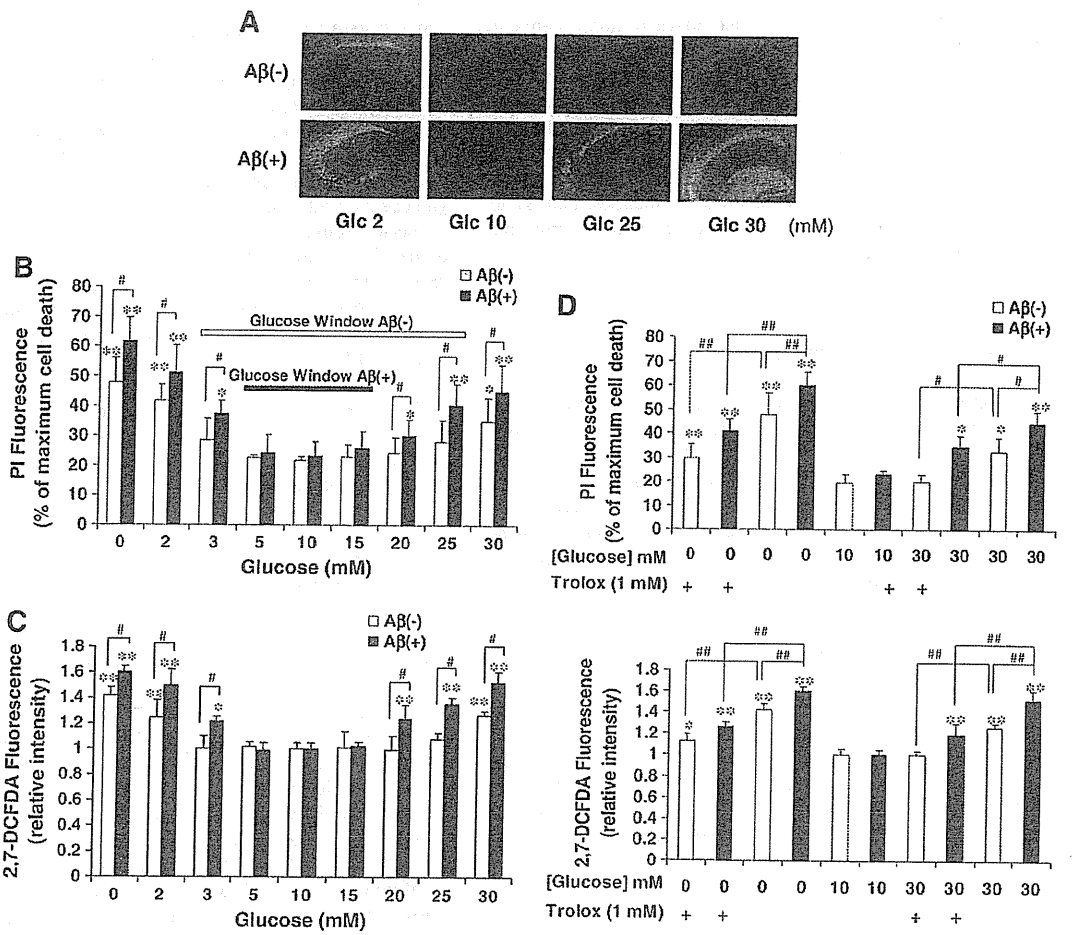

Fig. 1. Effects of glucose concentrations on neuronal death in the presence and absence of amyloid-β (Aβ) in organotypic hippocampal slice cultures (OHCs) (A) Representative images of propidium iodide (PI) fluorescence in OHCs at various glucose concentrations, with and without $Aβ_{25–35}$. (B) Neuronal death with and without Aβ at a variety of glucose concentrations. After 12 days in culture, OHCs were incubated for 48 h in a medium containing 10 mM glucose, and subsequently treated in various experimental media containing 0–30 mM glucose with and without 50 μM $Aβ_{25–35}$ (black and white bars, respectively). Neuronal survival was affected in severe hypoglycemia/hyperglycemia (≦2 mM/≧30 mM) without Aβ, while neuronal death increased when glucose concentration was ≦3 mM/≧20 mM in the presence of $Aβ_{25–35}$. Normal glucose window (3–25 mM) was seriously restricted in the presence of Aβ (5–15 mM). Data are presented as averages and SEMs (vertical bars) ($n=12$). Asterisks indicate significant differences from control (10 mM glucose) for each group (ANOVA followed by Tukey post-hot test; $^{*}p<0.05$, $^{**}p<0.01$). $^{\#}p<0.05$ denotes the difference between the findings in the presence and absence of Aβ for the various glucose concentrations. (C) Changes in reactive oxygen species (ROS) contents of OHCs during hypoglycemia/hyperglycemia with and without Aβ. OHCs were exposed to conditioned media for 3 h and ROS levels were measured with an H_2DCFDA assay. Data are expressed as fold increases over control (10 mM glucose) in the presence and absence of Aβ (black and white bars, respectively) ($n=12$). Asterisks indicate significant differences from control ($^{*}p<0.05$, $^{**}p<0.01$). $^{\#}p<0.05$ denotes the difference between the findings in the presence and absence of Aβ for the various glucose concentrations. (D) Protective effects of trolox (1 mM), a vitamin E analogue, on neuronal death (upper panel) and ROS levels (lower panel) during glucose deprivation and hyperglycemia (30 mM glucose) with (black bars) and without (white bars) $Aβ_{25–35}$. Asterisks indicate significant differences from control (10 mM glucose) in the presence and absence of Aβ ($^{*}p<0.05$, $^{**}p<0.01$) ($n=12$). $^{\#}p<0.05$ and $^{\#\#}p<0.01$ denote differences among the four subgroups (Aβ +/− and trolox +/−) in glucose-free and 30 mM glucose medium.

4. Discussion

The primary conclusions of this study are that: (1) optimal glucose concentration for neuronal survival in OHCs was found to be 3–25 mM, and a glucose window of 5–15 mM in the presence of Aβ; (2) Aβ and hypoglycemia/hyperglycemia additively increased oxidative stress, resulting in an increase in neuronal death; (3) a drop in glucose from preceding hyperglycemia further exacerbated neuronal death with Aβ; (4) pyruvate was capable of restoring Aβ neurotoxicity and to enlarge the glucose window. These results provide evidence that the glucose window resistant to Aβ neurotoxicity is extremely restricted and metabolic substrates such as pyruvate reverse the glucose window in OHCs model of AD.

This study clearly demonstrates that hyperglycemia can cause neuronal damage in hippocampal neurons through an ROS-mediated mechanism. Impaired metabolic glucose pathways have been implicated in the pathogenesis of diabetic complications, which results in an increase in cellular oxidative stress [2]. Recently, Suh et al. [16] identify glucose as the requisite electron donor for reperfusion-induced neuronal superoxide production in stroke. Furthermore, the association of ROS accumulation with hypoglycemia-induced neuronal death was also demonstrated in our study. Hypoglycemia-induced brain degeneration is not the result of fuel deprivation per se. It has been recently postulated that hypoglycemia triggers a cascade of events in vulnerable neurons, including free radical generation [15,17]. Because Aβ links to the production of oxidative stress in AD pathogenesis [13], it seems likely that Aβ-induced free radical production during hypoglycemia/hyperglycemia can easily overcome the antioxidant defense system in OHCs.

Surprisingly, hippocampal neuronal cells were seen to be extremely vulnerable to acute glucose reduction from preceding hyperglycemia with Aβ. It is plausible that even moderate hypoglycemia after

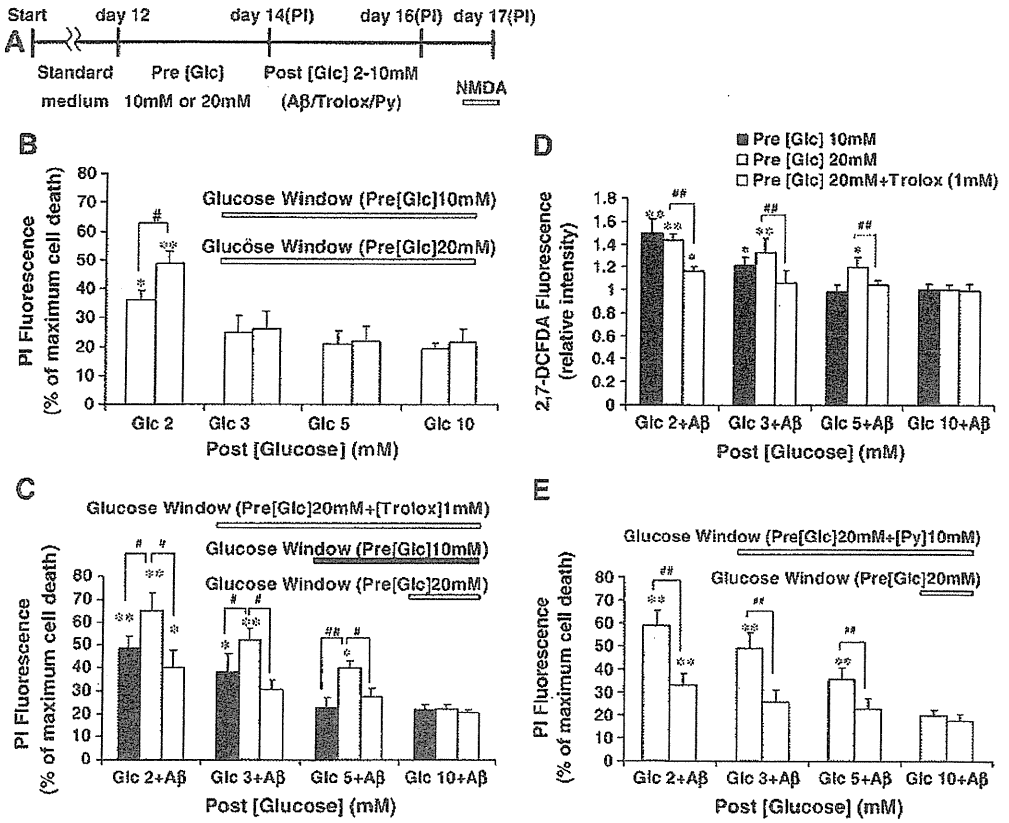

Fig. 2. Exacerbated Aβ neurotoxicity after a sharp and sudden drop in glucose level following hyperglycemia. (A) Schematic presentation of experimental protocols for acute glucose reduction from preceding hyperglycemia. On day 12, OHCs were incubated in normoglycemic or hyperglycemic medium for 48 h (Pre [Glc] 10 mM or 20 mM). On day 14, OHCs were treated with experimental media containing various concentrations of glucose (Post [Glc] 2–10 mM), with and without Aβ, trolox and sodium pyruvate (Py). PI fluorescence images were obtained on days 14, 16 and 17. (B) Neuronal death after acute changes in glucose following hyperglycemia without Aβ. Reduction of glucose to 3–10 mM did not cause any apparent changes in neuronal survival between Pre [Glc] 10 mM and Pre [Glc] 20 mM (white and gray bars, respectively) ($n = 12$). However, severe hypoglycemia (2 mM glucose) after previous hyperglycemia exacerbated neuronal death compared with control (10 mM glucose) ($^{**}p<0.01$), and a significant difference was observed between the groups ($^{\#\#}p<0.01$). (C) Exacerbation of neuronal damage after acute glucose reduction from hyperglycemia in the presence of 50 μM $A\beta_{25-35}$. A sharp and sudden reduction in glucose level from 20 mM to less than 5 mM caused significant neuronal death (gray bars) compared with identical hypoglycemia following Pre [Glc] 10 mM (black bars). The glucose window was 10–15 mM for this preparation. Supplementation with trolox (1 mM) reversed the exacerbation of Aβ neurotoxicity (white bars) ($^{*}p<0.05$, $^{**}p<0.01$ compared with 10 mM glucose; $n = 12$). $^{\#}p<0.05$ and $^{\#\#}p<0.01$ denote significant differences among the three subgroups for each of the glucose concentrations. (D) Oxidative stress after acute glucose reduction from hyperglycemia with Aβ. The acute drop in glucose to 2 mM and 5 mM from previous hyperglycemia apparently increased ROS accumulation in OHCs in the presence of $A\beta_{25-35}$ (gray bars) compared with the 10 mM glucose level ($^{*}p<0.05$, $^{**}p<0.01$, $n = 12$). Supplementation with trolox effectively reversed ROS accumulation (white bars) ($^{\#\#}p<0.01$). (E) Beneficial effects of pyruvate on exacerbated Aβ neurotoxicity after acute glucose reduction from hyperglycemia. Administration of pyruvate (10 mM) appeared to improve Aβ neurotoxicity after an acute drop in glucose from hyperglycemia (white bars), resulting in an enlarged glucose window ($^{**}p<0.01$ compared with control). $^{\#\#}p<0.01$ represents a difference between the presence and absence of pyruvate at each of the glucose concentrations ($n = 12$).

prolonged hyperglycemia may cause irreversible neurological changes in the presence of Aβ, thus endorsing the need for gradual and moderate normalization of hyperglycemia. In this connection, Suh et al. [18] provided evidence that hypoglycemic neuronal death is increased during glucose reperfusion as a result of elevated superoxide production through NADPH-oxidase activation. This implies that the adverse effects of fluctuations in glucose levels from hyperglycemia to hypoglycemia and vice versa should be stressed in the management of diabetes.

Finally, our results have raised the question concerning what is the optimal glucose ranges for neuronal survival in the pathological conditions? A substantial body of evidence supports the benefits of strict glucose management with insulin therapy in general critical practice [19]. However, the effects of intensive insulin therapy on the outcome of critically ill neurologic patients have not been fully investigated [20,21]. Recent report involving the human brain suggests that tight systemic glucose control (4.4–6.7 mM) is associated with reduced cerebral glucose availability and increased prevalence of brain energy crisis, which in turn correlates with increased mortality in patients with severe brain injury, when compared with intermediate glucose range (6.8–10.0 mM) [22]. Based on these investigations, optimal glucose utilization for better neuronal survival and neurological outcomes appears to vary in pathological conditions.

Our experiments clearly indicated that hippocampal neurons were particularly sensitive to metabolic perturbation in diabetes, which exacerbates Aβ neurotoxicity, leading to the restricted glucose window. This study examines disease propagation, rather than incidence of AD, and therefore suggests that hypoglycemia and hyperglycemia, as well as fluctuation of blood glucose should be avoided for better neuronal survival. It may be the case in the human brain with Aβ pathology. In this context, a protective effect of pyruvate against Aβ neurotoxicity seems fascinating as an available and economical tool to enlarge the restricted glucose window, because it is usually difficult to control diabetes in such restricted glucose ranges in the demented elderly.

It is noted that higher concentration of Aβ (50 μM) was used in this study. Most estimates for the concentration of the human brain have been in the low nanomolar range [23]. To span this large concentration gap, several potential mechanisms have been proposed. Recently, Hu et al. [24] reported that Aβ at low physiologically relevant concentrations

of extracellualr Aβ can be taken up by neurons, and then concentrated into endosomes/lysosomes (≥2.5 μM). Our experiments might accelerate this process using higher concentrations of Aβ, leading to increased Aβ neurotoxicity. However, detailed studies are needed to reveal mechanism of Aβ induced neuronal degeneration.

In summary, increased oxidative stress during hypoglycemia, hyperglycemia and/or fluctuation of blood glucose induce the greater rate of Aβ-induced neuronal damage of the cultured hippocampal slices. Multifactorial impacts of metabolic abnormalities in diabetes on neuronal survival have to be examined in further experiments.

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特　集

認知症と身体疾患

糖尿病と認知症

櫻井　孝

特　集　認知症と身体疾患

糖尿病と認知症

櫻井　孝

抄　録

認知症は，幾多の血管合併症を切り抜けてきた糖尿病患者が，高齢者になって向き合う最後の合併症である．高齢者糖尿病の脳機能を守るために，高血糖・低血糖の管理，高インスリン血症，脳血管病変を抑制することが重要である．糖尿病に合併する認知症では，代謝性脳症の改善により脳機能が改善する余地があり，積極的な治療介入が必要となる．中壮年期からの認知症のリスクを見据えた糖尿病の治療戦略が求められている．

Key words：アルツハイマー型認知症，血管性認知症，糖尿病，認知症予防

老年精神医学雑誌 21：308-315，2010

はじめに

高齢者糖尿病では認知症の合併がなくても脳機能は軽度低下する．しかしこの認知障害は糖尿病の療養を阻害するほどではないため，長らく多くの関心を集めることはなかった．1990年代後半になって，糖尿病と認知症の関連について多くの疫学調査の結果が報告され，糖尿病は血管性認知症（vascular dementia；VaD）のみならず，アルツハイマー型認知症（Alzheimer's disease；AD）のリスクでもあることが明らかになってきた[2]．今日，認知症は糖尿病の新たな合併症として大きな関心を集めている．

わが国でも高齢者糖尿病は急速に増加している．糖尿病"患者"で認知症をいかに早期発見するか，また糖尿病をどのようにコントロールすれば認知症の発症・進行を抑制できるかについてのエビデンスを積み上げていくことが今後の課題である．

そこで本稿では，糖尿病の認知障害から認知症が発症する機序，糖尿病を合併した認知症の特徴，認知症予防のための糖尿病治療について現在の知見をまとめた．

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1 高齢者糖尿病と認知症の疫学的関連

最近のメタアナリシスでは，糖尿病は認知症の危険因子であることが示されている（表1）[2]．糖尿病は脳血管障害の危険因子であることは確立しており，VaDとの関連は理解しやすい．しかし最近の研究では，糖尿病がADの発症により密接に関連していることが明らかになってきた．多くの前向き縦断研究（表1）で，ADの相対危険はおおむね1.5～2.0倍程度であり，有意な関連を示す報告，示さない報告が混在する．しかしこれらの研究のなかで最も信頼性が高いとされるロッテルダム研究では，ADとの有意な関連が示された[11]．さらに治療法別にADの相対危険を検討したところ，インスリン使用者では相対危険が4.2倍であったという．わが国の久山町研究の報告でも，耐糖能異常，高血圧はVaDのリスクであったが，ADに対しては耐糖能異常のみが有意な危険因子

表1　糖尿病と認知症の疫学的関連

	文　献	相対危険(95%CI)	血管性危険因子での補正後の相対危険
全認知症	Ott	1.9 (1.3-2.8)	
	Brayne	OR 2.6 (0.9-7.8)	
	Peila	1.5 (1.0-2.2)	1.5 (1.0-2.2)
	MacKnight	1.2 (0.9-1.7)	1.3 (0.9-1.8)
	Xu	HR 1.5 (1.1-2.1)	HR 1.5 (1.0-2.1)
	Leibson	SMR 1.6 (1.3-2.0)	
	Hassing		1.2 (0.8-1.7)
アルツハイマー型認知症	Ott	1.9 (1.2-3.1)	
	Brayne	OR 1.4 (1.1-17.0)	
	Yoshitake	2.2 (1.0-4.9)	
	Peila	1.7 (1.0-2.8)	1.8 (1.1-2.9)
	MacKnight	1.2 (0.8-1.8)	1.3 (0.8-2.0)
	Xu	HR 1.3 (0.8-1.9)	HR 1.3 (0.9-2.1)
	Leibson	SMR 1.6 (1.3-2.0)	
	Luchsinger	HR 2.4 (1.8-3.2)	HR 2.0 (1.4-2.9)
	Arvanitakis	HR 1.7 (1.1-2.5)	
	Katzman	OR 0.5 (0.1-2.3)	
	Hassing		0.8 (0.5-1.5)
血管性認知症	Ott	2.0 (0.7-5.6)	
	Yoshitake	2.8 (2.6-3.0)	
	Peila	2.2 (1.1-4.7)	2.3 (1.1-5.0)
	MacKnight	2.2 (1.3-3.6)	2.0 (1.2-3.6)
	Xu	HR 2.2 (1.1-5.0)	HR 2.6 (1.2-6.1)
	Luchsinger	HR 4.2 (2.2-8.3)	HR 3.4 (1.7-6.9)
	Hassing		2.5 (1.4-4.8)

OR：オッズ比，HR：ハザード比，SMR：標準化死亡比

(Biessels GJ, Staekenborg S, Brunner E, Brayne C, et al.: Risk of dementia in diabetes mellitus ; A systematic review. *Lancet Neurol*, 5 : 64-74, 2006 より引用改変)

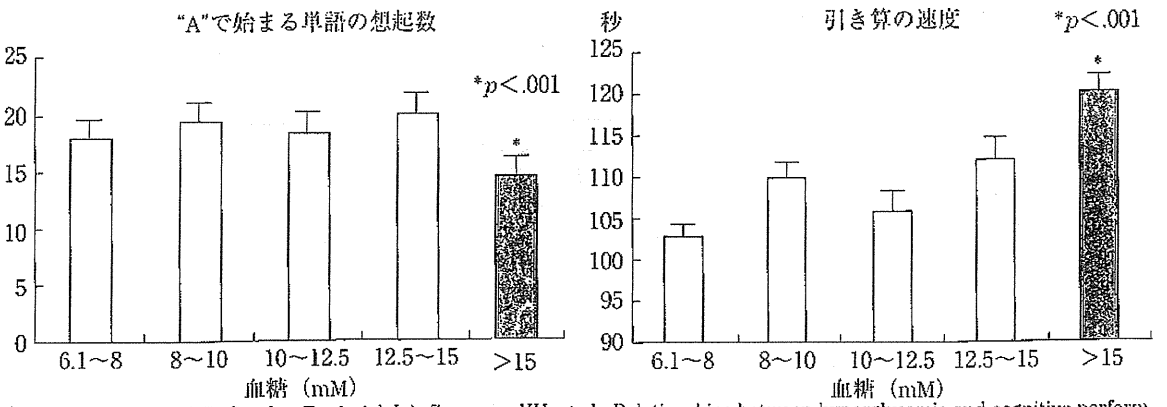
であるという[8]．これらの報告を総括すると，糖尿病はVaD，ADともに重要なリスクであることはほぼコンセンサスを得たと考えられる．

筆者らは神戸大学病院に通院する高齢者糖尿病480人を対象に認知症の有病率を調査した．その結果，認知症は13.1%（ADは10.4%）と推計された．わが国の認知症の有病率は8～10%（ADが約50%）とする報告が多いが，今回の調査で，糖尿病では約1.5～2.0倍認知症が多いと推計された．

2 糖尿病による認知機能障害

高齢者糖尿病では認知症がなくても，脳機能は軽度低下することが1922年にMiles[10]により初めて報告された．1997年のStrachanら[16]の総説によると，記憶，注意，前頭葉機能に低下が多いとされる．実際，糖尿病では思考速度が低下し，記憶障害を訴える患者をしばしば経験する．

Sommerfieldら[15]はグルコースクランプ法を用いて，同一の対象で16.5 mM（300 mg/dl）と4.5 mM（80 mg/dl）のブドウ糖濃度における認知機能の変化を報告している．高血糖では正血糖と比べて，情報処理速度，作動記憶，注意などの課題で低下がみられた．また2型糖尿病で高血糖の認知機能への影響を検討した研究では，血糖が15 mM（270 mg/dl）を超えると，語想起，引き算課題の成績が低下していた（図1）[4]．すなわち，“ヒト”では高血糖による代謝性脳症は可逆性であり，



(Cox DJ, Kovatchev BP, Gonder-Frederick LA, Summers KH, et al.: Relationships between hyperglycemia and cognitive performance among adults with type 1 and type 2 diabetes. *Diabetes Care*, 28: 71-77, 2005)

図1　2型糖尿病における血糖と認知機能

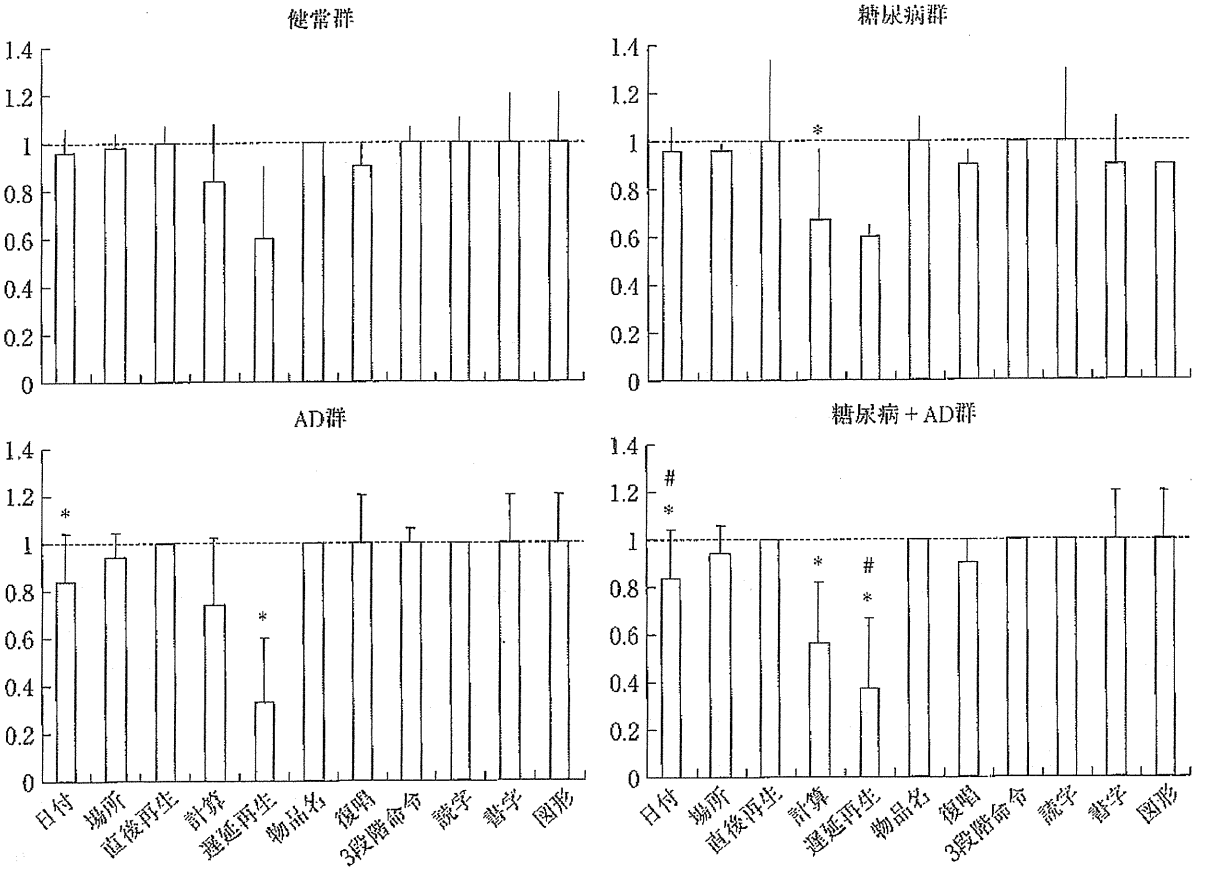
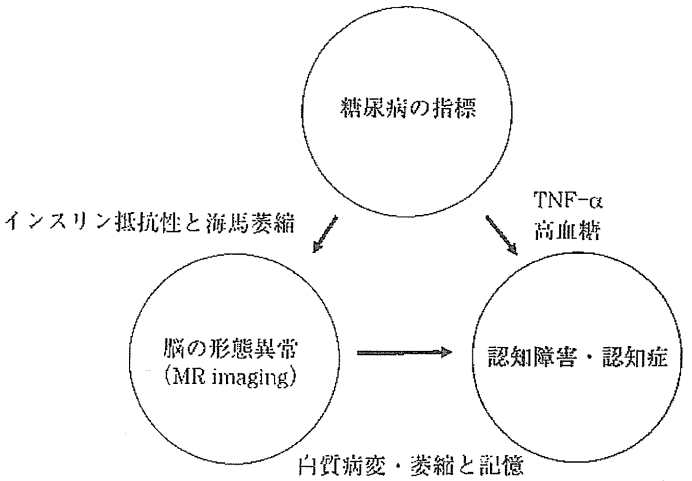


図2　MMSE下位項目の比較

おおむね270～300 mg/dl程度が，認知障害の閾値であると考えられる．

筆者らは健常者群，糖尿病群，AD群，糖尿病＋AD群においてミニメンタルテスト（Mini-Mental State Examination；MMSE）の成績を比較した（年齢，性別，教育年数は一致）[4]．AD群はNINCDS-ADRDAのprobable ADでMMSE 24点以上を対象とした．その結果，糖尿病群のMMSE総点の成績は健常群よりやや低下していた．またMMSEの下位項目を比較したところ（図2），



(Akisaki T, Sakurai T, Takata T, Umegaki H, et al.: 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). *Diabetes Metab Res Rev*, 22: 376-384, 2006より引用改変)

図3　糖尿病の認知障害の発症機序

糖尿病群では計算・注意の課題の失点が目立ち，AD群では時間見当識，遅延再生の成績が有意に低値であった．糖尿病＋AD群では両者の特徴を重ね合わせたプロフィールを示した．すなわち，糖尿病では主に前頭葉関連の認知能がより低下し，ADでみられる強い短期記憶障害とは異なる症候を示した．

糖尿病の認知障害の発症機序では，高血糖などの代謝性要因，脳の形態変化，認知機能の3つのカテゴリーを層別化して考える必要がある（図3）[1,13]．これまでの研究により，インスリン抵抗性と海馬萎縮，高血糖と認知障害などについてエビデンスがみられる．筆者らは3つのカテゴリーを階層的に解析したところ，脳の形態異常（白質病変，中心性萎縮）と記憶障害に有意な関連が認められた[1]．

3 糖尿病での認知症の発症機序

糖尿病に認知症の合併が多い機序として，図3に示した糖尿病による認知障害を基盤に，遺伝的な素因，脳血管障害，高血糖に伴う代謝異常，脂質異常，高インスリン血症，低血糖が関与すると考えられる．これらの血管，代謝ストレスによりVaD，AD，あるいはその他の病型の認知症のリスクが上昇すると考えられる（図4）．とくにADでは，脳で高血糖の持続，advanced glycation end products（AGEs）や酸化ストレスの増加が，ADの神経病変を加速させることが報告されている．また高インスリン血症は，ADの発症の根幹にかかわる可能性が指摘されている．

ここでインスリン抵抗性改善薬により認知機能の改善がみられた自験例を呈示する．図5は81歳女性で，肥満，高血圧，2型糖尿病を有する，いわゆるメタボリックシンドローム型の高齢者糖尿病である（HOMA-R 5.1）[9]．数年前からもの忘れがあり，NINCDS-ADRDAのprobable ADと診断された．糖尿病に対してグリメピリド，メトホルミンを投与したところ，血糖は一時改善したが再び悪化した．ADに対してドネペジル塩酸塩を投与したが，18か月後にはMMSE 19点，改訂長谷川式簡易知能評価スケール（HDS-R）17点まで低下した．そこで血糖改善を目的にインスリン抵抗性改善薬であるピオグリタゾンを投与したところ，糖尿病コントロールはHbA1c 6.3%

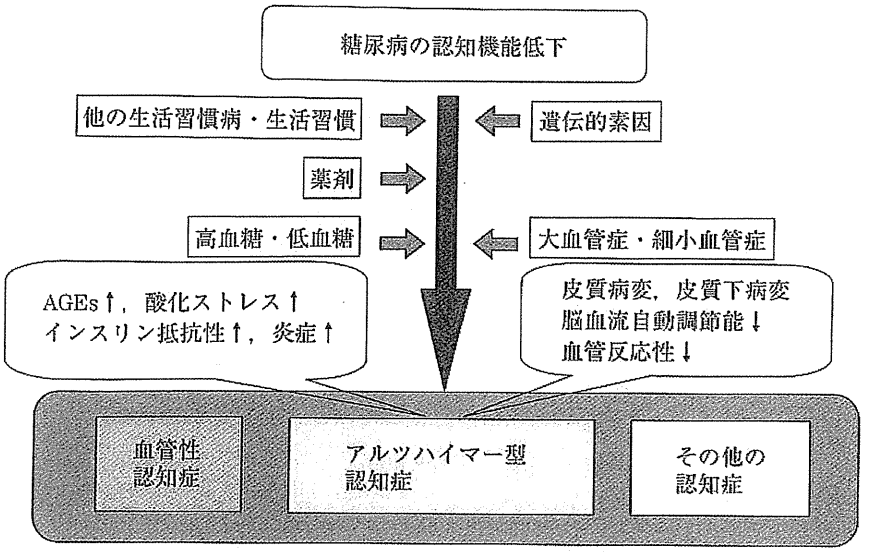
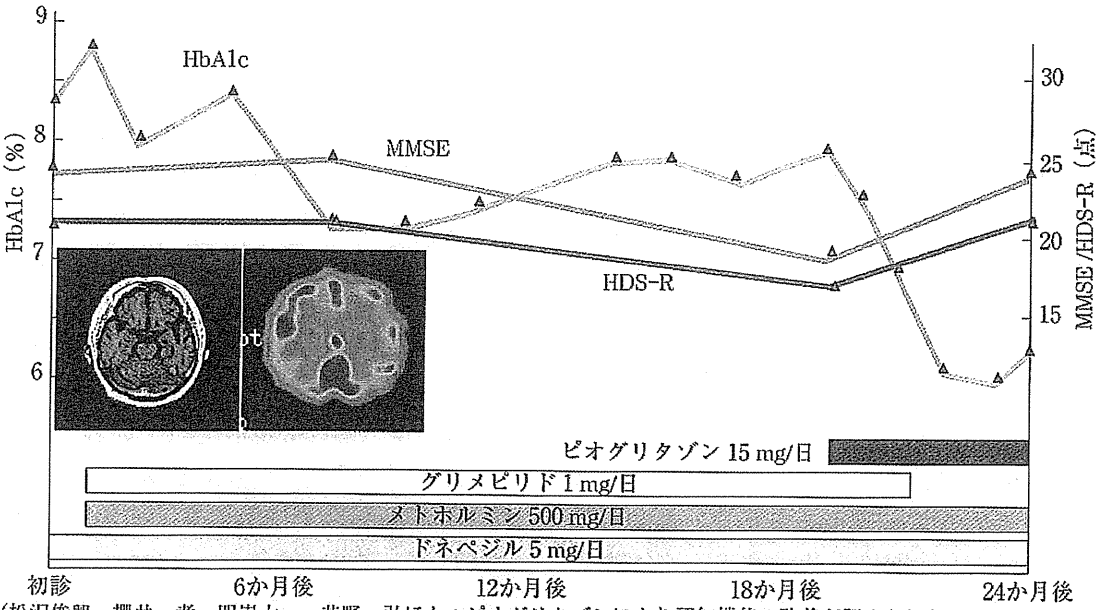


図4　糖尿病における認知症の発症機構



（松沢俊興，櫻井　孝，明嵜太一，芳野　弘ほか：ピオグリタゾンにより認知機能の改善が認められたアルツハイマー病を合併した高齢者糖尿病の1例．糖尿病，50：819-823，2007）

図5　インスリン抵抗性改善薬により認知機能の改善がみられた自験例（81歳，女性）

（HOMA-R 2.9）にまで急速に改善した．同時に脳機能の改善を示唆するエピソードが介護者から得られ，心理検査でも認知機能（見当識，遅延再生）の改善が確認された．しかし本例では，脳機能の改善がチアゾリジンの効果か，代謝性脳症の改善によるものかについては不明である．

2例目は，79歳女性でADが合併した2型糖尿病である（図6）．厳格なインスリン療法が行われており，HbA1cはほぼ6～7%に管理されていた．脳機能の変化をAlzheimer's Disease Assessment Scale（ADAS）の成績で示したが，ADの診断後，ドネペジル塩酸塩を約1年使用していたが，その間はほとんどADASの成績に変化はなかった．そこでピオグリタゾン15 mgを追加したところ，

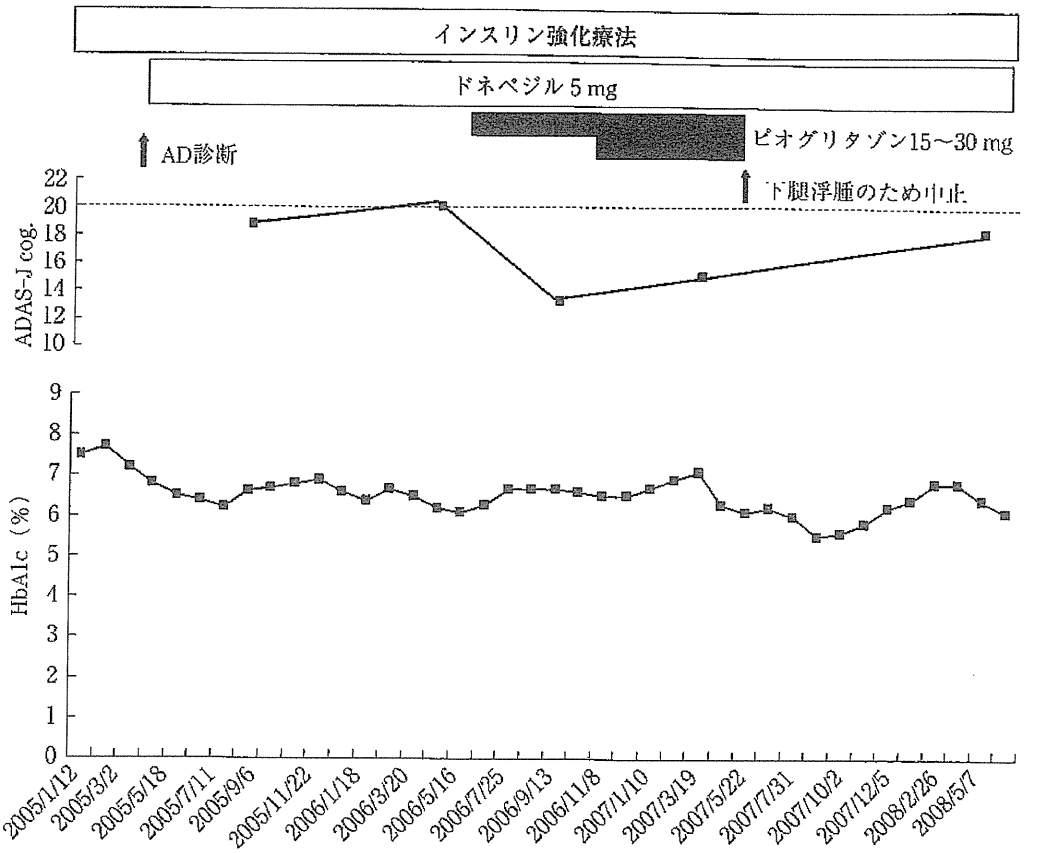


図6　インスリン抵抗性改善薬により認知機能の改善がみられた自験例（79歳，女性）

半年後には6点以上の改善が認められた．ピオグリタゾンを30 mgに増量したところ，下肢に強い浮腫が生じ中止した．その後ADASの成績は再び悪化した．本例では，脳機能改善にチアゾリジンが有用であったと考えられた．

高インスリン血症では，脳内でも炎症性サイトカインが増加し神経傷害を誘導する．インスリン分解酵素（insulin degrading enzyme；IDE）はアミロイドβタンパク（amyloid β-protein；Aβ）をも基質とするが，高インスリン血症ではAβの分解が拮抗阻害される．一方，インスリンは血液脳関門を通過し，海馬や大脳皮質に高濃度に分布しているインスリン受容体に結合する．慢性の高インスリン血症では脳内へのインスリンの移行が抑制され，脳内インスリンは低値となる．脳でのインスリンシグナルは，記憶や学習にも重要な役割を果たしており，インスリンの作用不足は，Aβやタウ病変などの病理過程を促進し，ADの発症に深く関与している[5,6,12]．チアゾリジン誘導体は，高インスリン血症の改善，炎症抑制などを介して神経保護的な作用を有すると考えられる．

脳内にインスリンをより多く移行させることができる点鼻インスリン製剤（わが国では未承認）による認知機能の改善も報告されており，中枢神経でのインスリン作用の改善は，AD治療の1つのポイントとなるかもしれない．一方，インスリン抵抗性改善作用を有するビグアナイドについては，細胞レベルの実験で，逆にAβの産生を増加させるとの報告があり，今後のヒトでの解析が待たれている[3]．

糖尿病と認知症との関連において，低血糖の関与は重要である．低血糖が遷延すると非可逆的な脳機能障害をきたすが，このような重症低血糖は通常まれである．2型糖尿病で，低血糖と認知症