回当たり60分間，動的バランス，筋力，持久力，柔軟性，歩行機能の向上および転倒回避のための集団指導にhome exerciseを加えながら36週行ったところ，運動指導期間中に発生した転倒数は運動群が対照群に比べて31%(incidence rate ratio：0.69，95%CI 0.50～0.96，p＝0.029)減ったことを指摘し，転倒経験者の転倒予防手段としては，運動が有効であると強調している．

Hauerら[3]は，医療処置を要する転倒負傷あるいは転倒が原因で入院した75歳以上の高齢女性57名を運動群31名，対照群26名に分け，運動群に週3回の筋力強化，バランス訓練を3カ月行った後，3カ月の追跡データを分析し，報告している．その結果によれば，介入群で筋力，歩行速度，バランスなどの身体機能は有意に改善されている．さらに，介入群の転倒率は対照群に比べて25%(relative risk：0.753，95%CI 0.455～1.245)減少したが，統計学的に有意ではない．転倒率の低下が有意ではない原因は，集団の数が少ないことに起因すると指摘している．

Linら[4]は，過去4週間で転倒した65歳以上の地域在住高齢者150名を運動群50名，環境改善群50名，教育群50名に分けて，2週1回，4カ月指導後，2カ月および4カ月の2回の追跡データを分析している．その結果，運動群でQOLおよびバランス，歩行，転倒恐怖感は教育群に比べて有意に改善されたが，6カ月間の1,000人当たりの転倒率は教育群2.4，環境改善1.1，運動群1.6と，3群間で統計学的な有意差はみられなかったと報告している．

以上の結果より，転倒経験者の転倒予防を目的とした介入プログラムを提供するときには，対象者数や追跡期間のみならず対象者個々人の特徴を十分把握した上で，可変因子の改善を目的としたプログラムを提供することによってSkeltonらが検討したように，転倒経験者の場合でも運動中心介入は転倒率の減少に寄与する手段になると考える．

地域在住転倒経験者の転倒予防を目的とした取組みの実際と課題について

1．概要

過去1年間で1回以上転んだ経験をもっている70歳以上の高齢女性125名を対象に，3カ月間の介入終了1年後に行った聞き取り調査と体力測定の追跡データを分析し，運動中心の取組みが転倒率抑制に及ぼす効果を検証する．

聞き取り調査は，既往歴，痛み，過去1年間の転倒・骨折歴，転倒恐怖感，健康度自己評価，服薬，運動習慣，基本的生活機能(移動，食事，入浴，着替え，トイレ)，老研式活動能力指標13項目などであり，体力測定は，身長，体重，血圧，身体組成，握力，開眼片足立ち，歩行速度，膝伸展力，足背屈力である．

運動指導は，週2回，1回当たり60分間，3カ月間の筋力向上，バランスおよび歩行機能の改善を目指す運動である．

2．得られた成果

介入1年後の追跡率は74.4%(運動群：73.0%，対照群75.8%)である．介入前，介入後，追跡後における体力の変化を検討した結果を**表2**に示す．**表2**に示したように，分散分析の結果，介入群と対照群の間で有意差がみられた項目は足背屈力(F＝10.821，p＝0.002)であり，最大歩行速度においても介入群の低下は対照群の低下に比べて有意ではなかったが，低下の抑制傾向が観察されている(F＝3.645，p＝0.060)．

図1は，介入群と対照群における追跡1年間の転倒率の比較を示したものである．**図1**に示したように，介入群の転倒率(19.6%)は対照群の転倒率(38.3%)に比べて有意に低い値を示している(Z＝1.979，p＝0.048)．

3．データの解釈および今後の課題

運動を中心とした3カ月間の介入終了1年後の追跡データを分析した結果，運動指導を受けた群の転倒率はそうではない群に比べて，転倒率は有意に抑制される結果を得ている．

多くの先行研究で，運動中心の介入は転倒の

表2　介入群と対照群における事前・事後・追跡間の身体機能変化

		事前	事後	追跡	分散分析	
変　数	群	M±SD	M±SD	M±SD	群×回数	p 値
握力(kg)	介入群(n=38)	19.55±6.00	18.50±6.08	21.92±5.63	F(1,72)=1.391	0.242
	対照群(n=36)	20.92±8.94	18.83±5.60	21.58±5.57		
足背屈力(kg)	介入群(n=38)	9.61±2.93	11.11±3.95	8.77±2.65	F(1,72)=10.821	0.002
	対照群(n=36)	10.57±2.62	10.86±2.38	7.59±2.21		
最大歩行速度(m/sec)	介入群(n=36)	1.82±0.37	1.86±0.37	1.84±0.37	F(1,68)=3.645	0.060
	対照群(n=34)	1.82±0.43	1.83±0.35	1.72±0.39		
開眼片足立ち(秒)	介入群(n=43)	28.77±23.74	32.26±24.54	26.47±22.54	F(1,82)=2.768	0.100
	対照群(n=41)	32.32±23.53	29.00±23.54	26.00±23.68		

危険因子として指摘されている筋力，歩行機能，バランス能力の改善に有効であり，介入期間中に改善された体力要素はその後の転倒率の抑制につながるかについて，Buchner ら[5]は，介入群に週3回，1回1時間の運動指導を24～26週間実施した後，25カ月間の追跡調査を行った結果，筋力と有酸素能力が有意に改善されるとともに，初回転倒までの時間が有意に延長され，転倒率の顕著な低下，入院期間や医療費の改善効果も得られたことを報告し，介入効果は身体機能の改善のみならず転倒関連要因の改善にも有効であることを指摘している．さらに，Suzuki ら[6]は，74～89歳の高齢女性52名を無作為割り付けにより介入群(28名)，対照群(24名)に分けて，介入群には2週1回の頻度，1回当たり60分，6カ月間で10回指導後，追跡調査を2回(8カ月後，20カ月後)行い，介入前・後・追跡期間中の身体機能の変化と転倒率について分析し，開眼片足立ち，最大歩行速度，膝伸展力，Functional reach は事前より高い機能水準を維持していることを確認するとともに，20カ月間の累積転倒率は，対照群54.5%，介入群13.6%であったことを報告している．これらの結果は，介入期間中に改善された身体機能の維持は，その後の転倒率の低下につながることを示唆する結果として，高齢者の転倒予防戦略を立てるときに，運動中心の取組みが有効であることを支持する核心要素である．

これらの研究成果は，地域在住の一般高齢者の報告であり，多くの研究で転倒経験が転倒の危険因子(RR=3.0)であると指摘されているが，転倒経験者に対する取組みの成果についての報告は極めて少ない状況である．さらに，高齢者の転倒原因について調べた結果によれば(図2)[7]，高齢者転倒の多くは「歩行中のつまずき」によって発生することが明らかになっている．したがって，運動プログラムにはすり足の改善に有効な運動要素を必ず入れるべきである．本研究では，すり足と深く関わっている「前脛骨筋」の力を推定する評価項目として「足背屈力」採用し，足背屈力が向上すれば，すり足の改善に寄与すると仮定している．これらの仮説を裏づけるために本研究で検討した結果，足背屈力は介入群で低下が抑制される傾向が観察され，介入群の転倒率が対照群の転倒率より有意

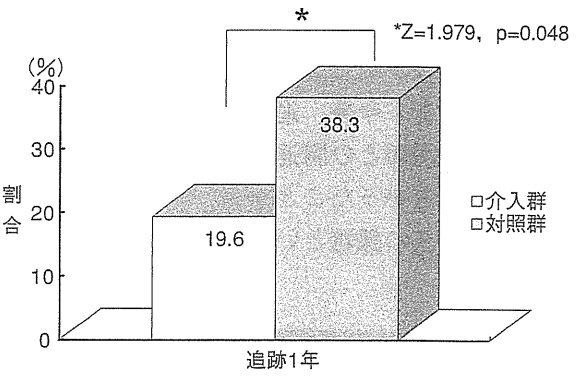

図1　地域在住転倒経験高齢者における運動指導後1年間の転倒率

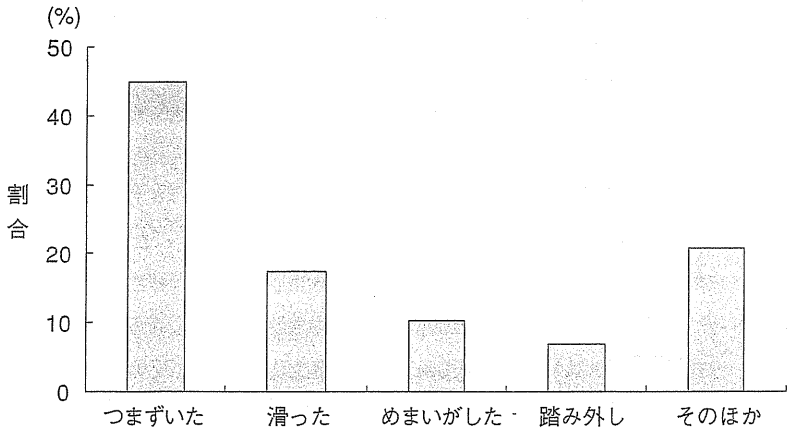

図2　転倒の主な原因

に低下している背景要因として，介入期間中に改善された足背屈力が追跡期間中に維持され，すり足が改善されたことに起因した結果であると推測できる．今後，追跡期間中のコンプライアンスと転倒率との関連性の検討および1年後の追跡率が低かったことから，不参加者に対する調査が今後の課題といえよう．

おわりに

転倒歴は再転倒の危険因子として指摘されているが，転倒経験者に対する転倒予防戦略の成果についての検討は極めて少ないのが現状である．しかし，転倒経験者の可変因子の改善を目指す運動指導を行うことによって，転倒危険因子と指摘されている筋力，バランス，歩行能力が改善され，改善された身体機能は転倒を抑制する方向へと働くことが検証されている．これらの結果から，地域在住転倒経験者の転倒予防を目的としたプログラムの提供は，地域在住虚弱高齢者における介護予防の観点からみたときの意義は大であると判断する．

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essary. This includes the "bench to bedside" tools but also must include epidemiologic, economic, and clinical efficiency instruments as outlined in phases 2 and 3.

No doubt, as scientific and medical knowledge grows, populations evolve, and the health care environment changes, stakeholders will need to be able to use present day tools while developing new methods that can evaluate each phase so as to feed forward to improve health care and feedback to inform innovation in the other phases. Dr Jaffe highlights excellent (and controversial) points regarding the future of medical education, fiscal debt of burgeoning physicians, and health care economics in a changing political landscape. This article was not intended to offer an opinion as to which type of payer system should be favored, nor was it intended to analyze the benefits and risks of different payer systems currently in place in this country and others; this has been done in prior articles.[1,2] *We hope that we have offered thoughts that must be applied in any payer system, such as the application of clinical trial data for best outcomes in wider populations, improving efficiency of health care delivery, and facilitating use of electronic medical records for patient care. As this evolution in evaluating options in health care continues, the way we teach ourselves, our colleagues, our students, our patients, and our policy makers will enable us to incorporate translational medicine into the way we take care of patients.*

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Targets of the Peroxisome Proliferator–Activated Receptor γ Agonist Trials for the Prevention of Alzheimer Disease

The recent article by Geldmacher et al[1] describes the peroxisome proliferator–activated receptor γ (PPARγ) agonist pioglitazone trial for patients with Alzheimer disease (AD). Pioglitazone was generally well tolerated during 18 months of exposure, but no efficacy was demonstrated on clinical outcome measures.

Thiazolidinediones have beneficial effects in animal models of AD, but the efficiency of PPARγ agonists for treatment in mild to moderate AD remains unclear. Beneficial effects of rosiglitazone were first described in *APOE-4*–negative patients with AD.[2] However, repeated trials with rosiglitazone have not demonstrated consistent results.[2] Pioglitazone treatment in diabetic patients with AD improved cognitive function as well as regional cerebral blood flow after a 6-month interval.[3] I have experienced several cases in which pioglitazone has been protective against cognitive decline in AD over 24 months.[4] In this connection, there might be unidentified factors that influence thiazolidinedione therapy for AD.

Thiazolidinediones exert neuroprotective effects through suppression of inflammatory responses and reduction of insulin resistance. In patients with greater insulin resistance, the more consistent the effects of this class of drug could be expected. As discussed in the present article, disease severity and *APOE-4* in the study participants can be critical components. In addition, I would suggest the importance of impaired function of cerebral vessels, which are frequently concomitant with Alzheimer-related neuropathology. Cerebral small-vessel disease is often associated with cognitive dysfunction and accelerates the progression of AD; this was not fully described in the previous articles. Pioglitazone has been reported to prevent ischemic stroke, although its precise effects on cerebral small-vessel disease have not been yet investigated.[3] Thus, the next clinical trials of thiazolidinediones should target the prevention of new onset of AD in patients with greater insulin resistance and be augmented by evaluation of cerebral small-vessel disease.

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In reply

We thank Dr Sakurai for his insightful comments. The observation that pioglitazone was associated with improvement in clinical and biomarker outcomes in patients with AD and diabetes mellitus reinforces the potential value of this line of therapeutics.[1] *There is increased risk for AD among patients with diabetes.*[2] *Taken together, these observations suggest additional means of enriching samples for future trials of PPARγ agonists and other insulin-sensitizing agents beyond the typical* APOE *genotyping. We also concur that these agents are likely to be most useful in prevention, rather than response to, symptomatically expressed AD. Therefore, trials of patients with mild cogni-*

tive impairment (or biomarker evidence of incipient AD) and relative insulin resistance might have the highest likelihood of detecting clinical efficacy for insulin-sensitizing drugs as antidementia agents.

The interactions between microvascular disease and Alzheimer disease are complex and provide multiple potential therapeutic targets for prevention.[3] Given the important regulatory role of PPARγ in many cell lines, it will likely be impossible to segregate the effects of pioglitazone in neuronal, glial, and vascular compartments on AD pathogenesis or progression in living humans. However, studies of insulin sensitizers without the broad effects of PPARγ agonists would help clarify the role of insulin resistance in the progression of AD.

Traditional clinical criteria for AD, which exclude patients with clinically significant cerebrovascular disease, make studies of the vascular contributions to AD more difficult. The emergence of molecular imaging for amyloid as an outcome measure in clinical trials will ease that difficulty and should allow a greater understanding of the role of vascular factors in AD progression. For instance, therapeutic approaches to cerebrovascular function that have no direct effects on amyloid pathways could be studied for their effects on cerebral blood flow or metabolism as well as amyloid burden. Thus, despite the absence of overt clinical effectiveness in our pilot study of pioglitazone,[4] or in the phase III study of rosiglitazone,[5] much remains to be learned about the potential of PPARγ agonists and other insulin sensitizing agents as interventions for AD.

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Methodological Remarks Concerning the Recent Meta-analysis of Carotid Artery Stenting vs Carotid Endarterectomy

We read with great interest the comprehensive meta-analysis by Bangalore et al[1] that reached important conclusions regarding outcomes after carotid artery stenting and carotid endarterectomy. Nevertheless, a variety of methodological issues seem worth addressing.

Concerning short-term outcomes, the numbers from the Stenting and Angioplasty With Protection in Patients at High Risk for Endarterectomy trial (Figure 2A) seem questionable, as the original total number of events was 8 of 167 (carotid artery stenting) vs 16 of 167 (carotid endarterectomy); evidently the numbers provided by Bangalore et al[1] in symptomatic and asymptomatic patients do not sum to those above. Rather surprisingly, the numbers for the Stent-Protected Angioplasty vs Carotid Endarterectomy (SPACE) trial in 2 distinct outcomes (Figure 2, A and B) were identical (46 of 607 vs 38 of 589); this would necessitate that no myocardial infarction has occurred periprocedurally in SPACE. Regarding Trial of Endarterectomy vs Stenting to Carotid Atherovascular Stenosis–China (TESCAS-C) the numbers provided by Bangalore et al[1] seem in discrepancy with the original ones; Figure 2A should ideally read 4 of 82 vs 7 of 84, whereas Figure 2B should read 3 of 82 vs 5 of 84.

The TESCAS-C trial seemed to represent a problematic entity concerning data extraction. Apart from the aforementioned discrepancy, TESCAS-C should have appeared in Figure 3A and 3B where the appropriate numbers are 2 of 82 vs 3 of 84 and 1 of 82 vs 2 of 84, respectively. Baseline characteristics (Table 1) regarding TESCAS-C should be 61.5% (hypertension) and 22.9% (diabetes mellitus). Similarly inaccurate data seem to have appeared in Table 1 regarding hypertension, diabetes mellitus, and coronary artery disease in the 2 studies by Brooks et al (89.4%-29.8%-67.3% for the first study and 89.4%-14.1%-64.7% for the second study) and CAVATAS (hypertension, 54.8%; diabetes mellitus, 13.3%).

Moreover, 3 discrepancies regarding the Endarterectomy vs Angioplasty in Patients With Symptomatic Severe Carotid Stenosis data seem worth noting; the proportions in Figure 3A, 6A, and 6B should be 24 of 265 vs 9 of 262, 71 of 265 vs 54 of 262, and 37 of 265 vs 20 of 262, respectively. Additionally, in Figure 6A, numbers pertaining to Carotid and Vertebral Artery Transluminal Angio-

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ORIGINAL ARTICLE

Causes of decreased activity of daily life in elderly patients who need daily living care

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Aim: The causes of decreased activity of daily life (ADL) in elderly patients include cerebrovascular diseases, bone fracture by falls, and dementia. The present study was conducted among elderly patients with decreased ADL who were hospitalized in nursing wards in order to investigate the causes of becoming early bedridden and to determine precautionary measures against decreased ADL.

Methods: The study subjects were 224 elderly patients with decreased ADL (mean age: 83.3 ± 8.0 years) and 49 outpatients without decreased ADL (mean age: 76.8 ± 5.3 years). Current age, age at the start of ADL decrease, medical history and history of smoking were investigated.

Results: In the groups with decreased ADL, current age and the age of becoming bedridden in non-diabetic versus diabetic groups were 84.7 ± 7.9 versus 80.3 ± 7.5 and 82.7 ± 8.3 versus 77.6 ± 8.0 years, respectively, both showing significantly lower values in the diabetic group ($P < 0.05$). Multiple regression analysis revealed that sex difference and diabetes were the factors determining the age of becoming early bedridden. Diabetic patients with smoking habit were significantly younger than diabetic and non-diabetic patients without smoking habit.

Conclusion: Sex difference, smoking habit and presence of diabetes mellitus are independent risk factors of becoming early bedridden. Therefore, the major targets of medical care among elderly should be diabetic men with a smoking habit to lower the risks of decreased ADL. **Geriatr Gerontol Int 2011; 11: ••–••.**

Keywords: activity of daily life, bedridden, diabetes mellitus, elderly, smoking habit.

Introduction

In our country, an aging population is already prominent and we will face further increase in the elderly population who need daily living care. The financial and psychological burden of families as well as the rise of medical expenditure in the national budget have become serious social problems demanding urgent countermeasures.

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The causes of decreased activities of daily living (ADL) of Japanese elderly include cerebrovascular diseases (27.7%), bone fracture by falls (11.8%) and dementia (10.7%), all of which result from complicated or overlapped lifestyle diseases.[1]

On the other hand, the incidence of metabolic syndrome, which is a combination of lifestyle diseases, has continued to increase with age in Japan, with high rates among men (29.7%) and women (19.3%) alike after 70 years of age.[2] Failure to intervene in metabolic syndrome is usually followed by the onset of type 2 diabetes mellitus in a short time. It has been reported that, once the diagnosis of diabetes mellitus is made, overall life expectancy is shortened by approximately 7 years.[3]

In older populations, failure of independent living or self-support increases with disturbed ADL or cognitive functions due to major and minor vascular diseases.

Because these conditions significantly compromise quality of life (QOL), early and vigorous control of lifestyle diseases is required to maintain QOL among the elderly.

According to the World Health Organization, the health age, which refers to the age without decreased ADL, of the Japanese is 74.1 years, while the average life expectancy is approximately 80 years (men 78.6, women 85.6 years).[4] In particular, falls among the elderly is one of the important causes of decreased ADL, which is experienced by 30% of the US population aged 75 years or older.[5,6] Investigation on the risk factors of falls, therefore, would be helpful in reducing mortality and morbidity in this age group. The National Service Framework for the elderly also emphasizes the prevention of falls, especially in the high-risk group.[7–10]

However, comprehensive studies have rarely been conducted on the causes of decreased ADL such as nutritional status and atherosclerotic conditions as well as the presence of lifestyle diseases including type 2 diabetes among the elderly. It is well-known that patients with diabetes mellitus develop complications such as retinopathy at late stage, neuropathy and nephropathy, which may lead to decreased ADL. Therefore, we hypothesized that age of becoming bedridden in diabetic patients is younger than non-diabetic patients.

Consequently, this study was conducted on elderly patients with decreased ADL who were hospitalized in nursing wards in order to investigate the causes of decreased ADL, to evaluate nutritional status and atherosclerotic conditions, and to determine precautionary measures against decreased ADL.

Methods

The study subjects consisted of 224 elderly patients (mean age: 83.3 ± 8.0 years) with decreased ADL who were hospitalized in Inamino Hospital, Hyogo, Japan (Table 1). A total of 155 patients were non-diabetic (113 female) and 69 patients had diabetes mellitus (47 female). Sixty non-diabetic and 29 diabetic patients with decreased ADL were excluded from the analysis of age of decreased ADL, because of the lack of exact information concerning the age at decreased ADL from their families.

On the other hand, 49 outpatients (mean age: 76.8 ± 5.3 years) at Kobe University Hospital with favorable ADL were enrolled as the control group, of which 22 patients were non-diabetic (15 female) and 27 patients had diabetes mellitus (10 female). Informed consent was signed by the families of all hospitalized

Table 1 Characteristics of 2 study groups

	Decreased ADL (n = 224)	Favorable ADL (n = 49)
Age (years)	83.3 ± 8.0	76.8 ± 5.3
Age of decreased ADL (years)	81.2 ± 8.5 (n = 135)	
BMI (kg/m^2)	18.4 ± 3.4	21.0 ± 2.9
Alb (g/dL)	3.4 ± 0.4	4.1 ± 0.3
TC (mg/dL)	171.3 ± 37.6	202.4 ± 34.6
TG (mg/dL)	90.5 ± 40.9	126.8 ± 70.1
HDL-C (mg/dL)	53.0 ± 16.9	64.4 ± 21.6
LDL-C (mg/dL)	99.9 ± 30.7	113.9 ± 27.8
SBP (mmHg)	122.2 ± 20.6	131.7 ± 17.4
DBP (mmHg)	67.2 ± 11.8	67.4 ± 11.0
IMT (mm)	1.2 ± 0.5 (n = 112)	
HDS-R	10.8 ± 8.1 (n = 117)	
CVD	39.2% (n = 135:yes 53, no 82)	
Fall fracture	24.4% (n = 135:yes 33, no 102)	
Dementia	9.6% (n = 135:yes 13, no 122)	
Infection	9.6% (n = 135:yes 13, no 122)	
Smoking	24.0% (n = 104:yes 25, no 79)	

ADL, activity of daily life; Alb, serum albumin; BMI, body mass index; CVD, cerebrovascular disease; DBP, diastolic blood pressure; DM, diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; HDS-R, Hasegawa dementia scale – Revised; IMT, intima-media thickness; LDL-C, low-density lipoprotein cholesterol; NDM, non-diabetic; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

patients and by the outpatients themselves. This study was approved by each local ethics committee. This study was performed from April 2005 to March 2008.

With regard to independent living of the disabled, we used the classification of the Japanese long-term care insurance, patients were classified as chair-bound (B) (39.3%) and the others were classified as bed-bound (C) (60.7%).[11]

According to medical record information provided by the families, the causes of decreased ADL were categorized into cerebrovascular diseases, bone fracture by fall, dementia, infection and others. Current age, age at the start of ADL decrease, intima-media thickness (IMT) measured by carotid artery ultrasonography, medical history, body mass index (BMI), blood pressure, blood glucose, HbA1c, lipid profiles (total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C], small dense LDL cholesterol [sLDL-C], triglyceride [TG]) and serum albumin were investigated. History of cigarette smoking was also taken. The definition of "smoking habit" indicates patients who had experienced smoking. All patients with impaired ADL were not current smokers because smoking was prohibited in the hospital.

The severity of dementia was evaluated using the Hasegawa Dementia Scale – Revised (HDS-R). Blood sugar, HbA1c, lipid profiles and sLDL-C were measured using the hydrogen peroxide electrode method, high-performance liquid chromatography, an automated lipid analyzer and the method reported by Hirano *et al.*,[12] respectively.

Simple regression analysis for age at the start of ADL reduction was performed with respective risk factors as independent variables (sex, diabetes mellitus, BMI, cerebrovascular diseases and serum albumin levels). Thereafter, multivariate regression analysis was performed using StatView ver. 5.0 for Windows in order to find the independent association of lifestyle risk factors with the age of becoming bedridden. Hypertension and dyslipidemia were entered as covariates besides variables that were shown to have significant simple correlation with the age at the start of ADL reduction. ANOVA followed by Scheffe's multiple comparison test was used for analysis between four study groups. The χ^2-test was also employed for comparison of frequency of bone fracture between the non-diabetic and diabetic groups of decreased ADL. Data were expressed as mean ± standard deviation.

Results

In the groups with decreased ADL, current age and the age at the start of ADL decrease of the diabetic group were lower than non-diabetic patients ($P < 0.05$ by ANOVA, Table 2). BMI and serum albumin tended to be higher in diabetic patients with decreased ADL. The levels of LDL-C and TG were higher in groups with favorable ADL and without diabetes mellitus. Blood pressure was not significantly different between any group. In the decreased ADL group, lipid parameters (except for TG) and IMT on carotid artery ultrasonography did not show any significant differences between

Table 2 Characteristics of four study groups

	Decreased ADL		Favorable ADL	
	Non-diabetics ($n = 155$)	Diabetics ($n = 69$)	Non-diabetics ($n = 22$)	Diabetics ($n = 27$)
Age (years)	84.7 ± 7.9†	80.3 ± 7.5‡	77.0 ± 5.9‡	76.7 ± 4.9‡
Age of decreased ADL (years)	82.7 ± 8.3† ($n = 95$)	77.6 ± 8.0‡ ($n = 40$)		
BMI (kg/m^2)	17.9 ± 3.3†	19.6 ± 3.2‡	20.5 ± 3.0‡	21.4 ± 2.8‡
Alb (g/dL)	3.3 ± 0.4†	3.5 ± 0.4‡	4.1 ± 0.3§	4.1 ± 0.3§
HbA1c (%)		6.4 ± 1.1		7.2 ± 1.2
TC (mg/dL)	170.1 ± 37.0†	174.1 ± 37.4†	212.8 ± 39.5‡	193.9 ± 27.9‡
TG (mg/dL)	88.0 ± 41.0†	95.9 ± 40.6‡	133.5 ± 64.0§	121.4 ± 75.5§
HDL-C (mg/dL)	54.6 ± 17.2†	52.3 ± 16.2†	70.0 ± 17.1‡	60.1 ± 14.0§
LDL-C (mg/dL)	99.1 ± 30.7†	102.6 ± 31.3†	119.3 ± 31.6‡	110.0 ± 23.9‡
SBP (mmHg)	120.5 ± 20.5†	125.7 ± 20.3†	133.6 ± 19.3†	130.2 ± 16.0†
DBP (mmHg)	67.6 ± 12.0†	66.4 ± 11.4†	65.3 ± 12.5†	69.1 ± 9.6†
IMT (mm)	1.2 ± 0.3†	1.2 ± 0.6†		
HDS-R	9.8 ± 7.6† ($n = 66$)	12.2 ± 8.6‡ ($n = 51$)		

†‡§There are significant differences between the groups not sharing the same symbol by ANOVA ($P < 0.05$). ADL, activity of daily living; Alb, serum albumin; BMI, body mass index; DBP, diastolic blood pressure; DM, diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; HDS-R, Hasegawa Dementia Scale – Revised; IMT, intima-media thickness; LDL-C, low-density lipoprotein cholesterol; NDM, non-diabetic; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

Table 3 Simple regression analysis to explore the determinant of disabled age of decreased ADL patients

	Regression coefficient	95% CI, upper	95% CI, lower	*P*-value
Sex (M 1, F 0)	−4.78	−1.33	−8.22	<0.05
BMI	−0.54	−0.12	−0.96	<0.05
Alb	−5.13	−1.89	−8.36	<0.05
DM (yes 1, no 0)	−5.19	−2.13	−8.25	<0.05
CVD (yes 1, no 0)	−3.16	−0.23	−6.09	<0.05
Fall fracture (yes 1, no 0)	1.72	5.24	−1.80	<0.05
Smoking (yes 1, no 0)	−0.01	−0.001	−0.02	0.07
HT (yes 1, no 0)	1.78	4.68	−1.12	0.23
DL (yes 1, no 0)	−1.84	−1.41	−5.09	0.26

ADL, activities of daily living; Alb, serum albumin; BMI, body mass index; CI, confidence interval; CVD, cerebral vascular disease; DL, dyslipidemia; DM, diabetes mellitus; HT, hypertension.

non-diabetic and diabetic groups (Table 1). sLDL-C level was measured in 24 non-diabetic and 18 diabetic patients, which were significantly higher in the diabetic group (16.7 ± 11.2 vs 26.2 ± 15.5 mg/dL, $P < 0.05$). Cognitive function was evaluated by HDS-R in 66 of 155 (42.5%) and 51 of 69 (73.9%) patients in non-diabetic and diabetic patients of the decreased ADL group, respectively, which is significantly higher in the diabetic patients of the decreased ADL group. However, the scores were 9.8 ± 7.6 and 12.2 ± 8.6, respectively, and the difference was not significant.

In the group with decreased ADL, 95 (20 male, 75 female) and 40 (11 male, 29 female) patients were non-diabetic and diabetic, respectively, at the age of becoming bedridden, while 22 patients were non-diabetic (seven male, 15 female) and 27 patients had diabetes mellitus (17 male, 10 female) in the favorable ADL group.

In the decreased ADL group, the coronary risk levels were categorized according to the number of risk factors (hypertension, dyslipidemia, diabetes mellitus) into three groups: the group with three risk factors, the group with two risk factors, the group with a single risk factor and the group with no risk factor. The age at the start of ADL decrease of the group with three risk factors was 77.2 ± 10.5 years, the group with two risk factors, with a single risk factor and with no risk factor were 80.5 ± 8.6, 81.3 ± 9.0 and 82.5 ± 7.3 years, respectively.

Causes of decreased ADL were clarified in 95 non-diabetic and 40 diabetic patients. The incidence of cerebrovascular diseases was 47.5% and 35.5% in diabetic and non-diabetic participants, respectively, and diabetic bedridden patients after cerebrovascular diseases were younger than non-diabetic individuals (75.1 ± 8.0 vs 82.0 ± 10.0 years, $P < 0.05$). The frequency of patients with bone fracture by fall in the diabetic group was higher than in the non-diabetic (32.5% vs. 21.1%) but the difference was not significant by the χ^2-test. The prevalence of dementia as a reason for ADL reduction was 7.5% and 16.1% in the diabetic and non-diabetic groups, respectively. While 10.5% of non-diabetic patients were bedridden after some serious infection such as pneumonia, no bedridden case after infection was found in the diabetic group.

Simple regression analysis for the age at the start of ADL reduction were performed with respective risk factors as independent variables. Male sex ($P = 0.01$), presence of diabetes mellitus ($P = 0.01$), higher BMI ($P = 0.01$), cerebrovascular diseases ($P = 0.03$) and higher levels of serum albumin ($P = 0.002$) were significantly associated with younger age of becoming bedridden (Table 3). To find the independent association of lifestyle risk factors with the age of becoming bedridden, hypertension and dyslipidemia were entered as covariates besides variables that were shown to have significant correlation ($P < 0.05$) in subsequent multivariate regression analysis. As a result, male sex, higher BMI, higher levels of serum albumin and presence of diabetes mellitus were the independent factors determining the age of becoming bedridden, while hypertension and dyslipidemia were not selected as an independent determinant (Table 4). These results showed the pronounced effects of diabetes on the severe impairment of ADL.

Because smoking habit seemed to have a substantial impact on the age at the start of ADL reduction, we further compared the additive effects of diabetes and smoking on the age of becoming bedridden. As shown in Figure 1, diabetic patients with smoking habit were significantly younger than diabetic and non-diabetic patients without smoking habit.

Discussion

As already mentioned, the population requiring daily living care in Japan has been steadily increasing. The

Table 4 Multiple regression analysis to explore the determinant of disabled age of decreased ADL patients

	Regression coefficient	95% CI, upper	95% CI, lower	*P*-value
Sex (M 1, F 0)	−4.40	−1.10	−7.69	<0.05
BMI	−0.43	−0.02	−0.84	<0.05
Alb	−4.16	−1.05	−7.27	<0.05
DM (yes 1, no 0)	−3.69	−0.68	−6.70	<0.05
CVD (yes 1, no 0)	−2.00	−0.86	−4.86	0.17
HT (yes 1 no 0)	2.00	4.64	−0.72	0.15
DL (yes 1, no 0)	−0.05	3.12	−3.22	0.98

ADL, activities of daily living; Alb, serum albumin; BMI, body mass index; CI, confidence interval; CVD, cerebrovascular disease; DL, dyslipidemia; DM, diabetes mellitus; HT, hypertension.

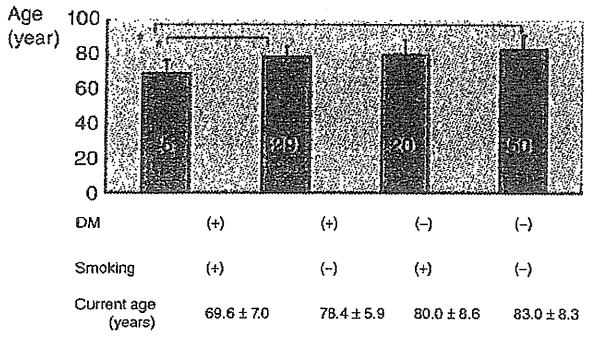

Figure 1 Mean age of bedridden of diabetic and non-diabetic patients with and without smoking habit. The number in the column represents the number in each group. *There are significant differences between each group by ANOVA ($P < 0.05$). DM, diabetes mellitus.

causes of this status are considered to include stroke, dementia and bone fracture by falls, all of which are closely associated with the progression of cerebral atherosclerosis. We conducted this study to investigate the causes of decreased ADL and the bedridden status, as well as to determine precautionary measures for shortening the bedridden period.

In the two groups with decreased ADL, the mean ages of the diabetic group were significantly younger than those of the non-diabetic groups. In other words, elderly diabetics will reach bedridden status approximately 5 years earlier than non-diabetics. Indeed, it is obvious that aging is one of the most important factors for decreased ADL. However, another group of old outpatients with comparable age to that of the decreased ADL group was not available this time. The diabetic patients with impaired ADL were under strict energy control in the hospital. On the other hand, the diabetic patients without impaired ADL were all outpatients and therefore they had free access to any food. Thus, diabetic patients without impaired ADL showed a higher HbA1c level than that of those with impaired ADL.

Roliz-Cruz *et al.* have reported that metabolic syndrome carries a 2.2-times higher risk for decreased ADL than the non-metabolic elderly population.[13] Because hyperglycemia is a major component of metabolic syndrome, the results of our study support this estimate.

The numbers of patients who were able to be evaluated using HDS-R were 51 of 69 (73.9%) and 66 of 155 (42.5%), which was obviously higher in the diabetic group. Furthermore, the mean HDS-R score did not differ between the two groups. It was suggested that the diabetic group was younger and the their periods after becoming bedridden were shorter than the non-diabetic group, and consequently, patients with more severe dementia were fewer in the diabetic group. The frequency of cerebrovascular diseases in the diabetic group was higher than that of the non-diabetic group. Also, diabetic patients who had decreased ADL by cerebrovascular disease were significantly younger than non-diabetics. From these results, it can be concluded that diabetics have a higher risk of becoming bedridden due to stroke. In support of this, it has been widely reported that diabetics have a higher mortality, with cerebrovascular diseases being an independent risk factor.[14–18]

Dementia is known to be one of the complications of the cerebrovascular disease. According to the Copenhagen Stroke Study, it was proven that the mean age of patients with cerebrovascular disease complications was younger in the diabetic group than in the non-diabetic group by 3.2 years.[16] On the other hand, in this study the frequency of dementia was not higher in the diabetic group than the non-diabetic group. The influence of older mean age in the non-diabetic group than in the diabetic group was suggested with regard to dementia.

A recent Taiwanese study on diabetes mellitus and bone fracture has reported a higher risk of femoral fracture in diabetic patients.[19] Functional impairment of osteoblasts[20] and apoptosis of osteoblasts induced by enhanced gluconeogenesis[21] have been suggested as the underlying mechanisms. Menz *et al.* have reported that diabetic individuals with peripheral neuropathy had

impaired peripheral sensation and reaction time, and had impaired ability to stabilize their body when walking on irregular surfaces.[22] They also had reduced walking speed and step length, and less rhythmic acceleration patterns at the head and pelvis compared with controls.[23] In this study, the experience of bone fracture in diabetic subjects with decreased ADL was more frequent than that of non-diabetics, but the difference was not significant. Further study will contribute to better understanding of the influence of bone fracture on decreased ADL of diabetic patients.

Infection was considered to be the cause of decreased ADL in 12.9% and 0% of the patients in the non-diabetic and diabetic groups, respectively. This is contrary to the fact that the defense mechanism against infection is weakened in diabetics. We believe that further research is needed to clarify this finding. With regard to sex, men showed an odds ratio of 2.11 on diabetes and fracture, which are both associated with decreased ADL.[24] Furthermore, because increased BMI may lead to failure of independent living, men over 50 years should particularly be paid attention to in this index.[25]

In this study, the levels of sLDL-C were significantly higher in the diabetic group than in the non-diabetic group (17.0 ± 11.4 vs 25.2 ± 10.6 mg/dL; $P < 0.05$ by ANOVA). The atherogenic phenotype, which refers to a tendency to demonstrate a predominant sLDL-C, has been reported to have a higher risk of myocardial infarction.[26] Increased sLDL-C has also been reported in diabetics.[27] Increased sLDL-C in the diabetic group suggests susceptibility to cerebrovascular diseases in elderly diabetics, and consequently, lower age at becoming bedridden than in the non-diabetic group.

In spite of the overt higher risk in diabetics, plaque scores on carotid artery ultrasonography were not significantly different between the two groups. This may be due to the younger mean age of patients in the diabetic group.

Simple regression analysis on age of becoming bedridden suggested a correlation with sex, BMI, diabetes mellitus and serum albumin. Multiple regression analysis revealed that sex, BMI, serum albumin and the presence of diabetes mellitus were the factors determining the age of becoming bedridden. However, because BMI scores used in this study were determined from weights measured during the observation period, which might differ from those measured at hospitalization from decreased ADL, BMI cannot be considered as one of the causes of the bedridden status. In addition, multiple regression analysis using age at the bedridden status as a dependent variable and the presence of diabetes mellitus and smoking history as independent variables suggested that both diabetes mellitus and smoking history were correlated with the age of becoming bedridden. Therefore, it can be concluded that diabetic men with a smoking history among the elderly become bedridden at the youngest age.

The limitations of the present study are as follows: First, because this investigation is a cross-sectional study of a number of severely demented patients with a mean HDS-R score of 10.8, the causes of decreased ADL were estimated from medical records instead of being directly obtained from the patients. Second, because the range of the subjects examined was limited to patients hospitalized in a nursing ward, it was difficult to compare the examined groups to a healthy elderly group. Third, with regard to diabetes mellitus, interpretation was not performed regarding types (two type I vs 67 type II patients) and treatments (32% with insulin vs 36% with oral hypoglycemic agents). Fourth, the number of bedridden diabetics with smoking habit was only five. Because this patient group was very young, it is possible that many of them might have been dead earlier in a nursing ward. This conjecture warrants retrospective analysis using deceased patient records. Fifth, complications of diabetes mellitus, especially retinopathy, were not considered as a significant factor. Because complications of diabetes mellitus such as visual disturbance, peripheral neuropathy and nephropathy have been reported to be risk factors of falls in the elderly,[28] this area needs further studies. Finally, causes of dependence should be multi-factorial and heterogeneous. However, undernutrition cannot be the main cause of dependence in our wards although undernutrition can be the results of bedridden status.

In conclusion, among diabetes mellitus, hypertension and dyslipidemia, this study showed that diabetes mellitus is an independent risk factor of becoming bedridden. In the diabetic groups, cerebrovascular diseases were the major causes of becoming bedridden at a younger age. Also smoking habit was an independent determinant of becoming bedridden at a younger age. Therefore, the major targets of medical care among elderly should be diabetic male patients with a smoking habit in order to lower the risk of becoming bedridden at a younger age.

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ORIGINAL ARTICLE: BIOLOGY

Effects of insulin and amyloid β_{1-42} oligomers on glucose incorporation and mitochondrial function in cultured rat hippocampal neurons

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Aim: The molecular basis for impaired glucose metabolism in patients with Alzheimer's disease (AD) has not been fully clarified. We tested whether insulin and amyloid (A)β_{1-42} oligomers would regulate glucose metabolism and energy homeostasis directly in cultured rat hippocampal neurons and evaluated possible interactions between insulin signaling and $A\beta_{1-42}$ oligomers.

Methods: Dissociated hippocampal neurons were prepared from Wistar rat embryos at day 21 of gestation and cultured for 14 days. Cultured neurons were exposed to insulin (1 μM) for 30 min, and $A\beta_{1-42}$ oligomers (1 μM) were added to culture media for 10–30 min. The glucose uptake of cultured neurons was measured by enzymatic fluorescence assay using 2-deoxy-d-glucose (2DG), and adenosine triphosphate (ATP) contents were quantified using a luciferin/luciferase luminescence assay.

Results: $A\beta_{1-42}$ oligomers did not suppress 2DG uptake, reflecting the activities of glucose transporters and/or hexokinase, but led to disrupted ATP contents in the presence and absence of monocarboxylates (lactate/pyruvate). Insulin and C-peptide did not change glucose uptake or ATP concentrations.

Conclusion: The primary target of $A\beta_{1-42}$ oligomers might be mitochondria, which could explain the reduced cerebral glucose levels in patients with AD. Moreover, insulin signaling was not directly linked to glucose metabolism or energy homeostasis in cultured rat hippocampal neurons. **Geriatr Gerontol Int 2011; 11: 517–524.**

Keywords: 2-deoxyglucose, Alzheimer's disease, adenosine triphosphate, insulin, mitochondria.

Introduction

Alzheimer's disease (AD) is a late-onset, progressive, age-dependent neurodegenerative disorder, characterized clinically by the impairment of cognitive functions, and changes in behavior and personality.[1–4] Clinical studies using the positron emission tomography tracer fluoro-2-deoxy-d-glucose (2DG) in patients with AD have demonstrated that glucose metabolism is impaired in the cerebral cortex. The hypometabolism is most prominent in the posterior cingulate and parietotemporal regions in early stages, and spreads to the prefrontal cortex as the disease progresses.[5–8]

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To date, the molecular basis for reduced glucose uptake in AD has not been fully clarified. Biochemical analyses of autopsied brains from patients with AD indicate impaired activities of enzymes involved in anaerobic glycolysis, such as hexokinase and glucose-6-phosphate dehydrogenase (G6P-DH), and reduced expression of several isoforms of the glucose transporter.[9] Pharmacological experiments have demonstrated that amyloid-β (Aβ) in the millimolar range prohibited glucose uptake in primary cultured neurons,[10,11] suggesting decreased levels of nonoxidative glycolysis. On the other hand, Parihar *et al.* have shown that mRNA expression levels of mitochondrial-encoded genes (subunit 5 of complex I and cytochrome oxidase) are reduced in the temporal cortex of patients with AD, suggesting the importance of altered mitochondrial function for reduced glucose incorporation in this disease.[12]

Recently, Aβ oligomers have attracted considerable attention for their pivotal roles in the development and progression of cognitive decline in the early stage of AD. They have been implicated as primary candidates for initiating the deterioration of synaptic function, composition and structure.[13,14] However, little is known about the impacts of Aβ oligomers on glucose metabolism and energy homeostasis.

It has been postulated that insulin might be involved in regulating glucose and energy metabolism, synthesis of neurotransmitters and modulation of synaptic plasticity as well as metabolism of Aβ and tau in AD pathogenesis. Deficiency of insulin's effects in the brain is closely associated with cognitive dysfunction. Several epidemiological studies have indicated that insulin-resistant disorders, including obesity, metabolic syndrome and diabetes, are risk factors for developing AD.[15–17] However, the effects of insulin on glucose and energy metabolism in the brain are still controversial. Zhao *et al.* have reported a molecular link between insulin resistance and AD pathogenesis in that insulin receptor signaling in neurons is strikingly sensitive to disruption by Aβ oligomers.[18] However, the pathways involved in glucose utilization and adenosine triphosphate (ATP) synthesis by insulin and Aβ oligomers have not been investigated.

The current study addressed whether insulin and $A\beta_{1-42}$ oligomers would regulate glucose metabolism and energy homeostasis in cultured rat hippocampal neurons. We wished to evaluate whether possible interactions of insulin signaling and $A\beta_{1-42}$ could cause impaired glucose metabolism in patients with AD. We found that neuronal glucose incorporation was not altered after stimulation with insulin and $A\beta_{1-42}$ oligomers, whereas mitochondrial function was significantly affected by $A\beta_{1-42}$ oligomers.

Methods

Preparation and characterization of Aβ1–42 oligomers

$A\beta_{1-42}$ was purchased from Peptide Institute (Osaka, Japan). The oligomers were prepared as described.[19] Briefly, the $A\beta_{1-42}$ peptide was dissolved in dimethylsulfoxide (DMSO) at 2 mM and then diluted 1:10 in sterile phosphate-buffered saline (PBS), vortexed for 30 min at room temperature and centrifuged at 15 000 *g* for 1 h at 4°C. The supernatant (200 μM) was aliquoted (25 μl) and snap frozen at −20°C.[19] Unless stated differently, aliquots were diluted in culture medium to a final concentration of 1 μM immediately before use. $A\beta_{42-1}$, a reverse amyloid beta peptide, was also obtained from Peptide Institute, treated similarly and used for the control experiments.

Oligomerization of $A\beta_{1-42}$ was confirmed by gel electrophoresis. Samples (18 μL) were mixed with a 4 × Nu Page sample buffer (Invitrogen, San Diego, CA, USA) and incubated at 70°C for 10 min. The sample was briefly centrifuged at room temperature for 2 min and applied to a 12% Nu-Page Bis-Tris gel (Invitrogen). The samples were electrophoresed at 100 V for 2 h, transferred to a nitrocellulose membrane at 100 V for 1 h and blocked with 5% milk in Tris-buffered saline containing 0.05% Tween-20 for 1 h at room temperature. The blots were probed with 6E10 (1:1000; Chemicon International, Temecula, CA, USA) – a monoclonal antibody against amino acid residues 1–16 of Aβ – overnight at room temperature, followed by incubation with horseradish peroxidase-conjugated antimouse antibody (1:5000) for 2 h at room temperature. Then, membranes were developed with enhanced chemiluminescence reagents (GE Healthcare Japan, Tokyo, Japan)[20] and visualized using a Kodak X-Omat 1000 processor (Kodak Japan, Tokyo, Japan). Molecular mass was estimated using Rainbow molecular weight markers (Bio-Rad, Tokyo, Japan).

Primary hippocampal neuronal cultures

All animals were treated according to the guidelines for animal experimentation of Kobe University School of Medicine. Dissociated embryonic hippocampal neurons were prepared from Wistar rat embryos at day 21 of gestation. Neurons were dissociated from fetal hippocampi in Hank's balanced salt solution containing 0.5% glucose, 2% sucrose, 1 mM sodium pyruvate and 15 mmol/L 2-(4-[2-hydroxyethyl]-1-piperazinyl) ethanesulfonic acid, and then centrifuged for 1 min at 15 000 *g*. Cells were plated onto poly-d-lysine-coated dishes at a density of 5×10^5 cells/mL in B27-neurobasal medium (B27-NBM). Cultured neurons were incubated with B27-NBM for 14 days.[21]

2DG uptake study

The glucose uptake of cultured neurons was measured by enzymatic fluorescence assay as described.[22] Incorporated 2DG is phosphorylated to form 2DG-6-phosphate, which is not further metabolized and can be used for quantifying glucose uptake. At day 14 after plating the hippocampal cells, neurons were washed twice with Locke's buffer (154 mM NaCl, 5.6 mM KCl, 2.3 mM CaCl, 3.6 mM NaHCO, 3.5 mM HEPES) without serum or glucose. Neurons were incubated in Locke's buffer for 1 h at 37°C in 5% CO_2 for insulin starvation with and without glucose, followed by exposure to insulin (1 μM) for 30 min. During exposure to insulin, $A\beta_{1-42}$ oligomers (1 μM) were added to culture media for 10 min, 20 min and 30 min (Fig. 2a).

After terminating the exposure to insulin and $A\beta_{1-42}$ oligomers, 2DG was added to cultures for 5 min at 1 μM. The incorporation of 2DG was terminated by three washes with ice-cold 0.1 mol PBS and the cells were digested with 0.1 N NaOH. To degrade NAD(P)H, $NAD(P)^+$ and any glycolytic enzymes derived from the digested neurons, the culture plate was incubated at 85°C for 60 min in a temperature-controlled bath. The components in the wells were then neutralized by the addition of 0.1 N HCl and then solubilized in 150 mM triethanolamine hydrochloride (TEA) buffer (pH 8.1). Then, incorporated 2DG was quantified by fluorescence assay of resorufin converted from resazurin.[22] A standard curve was generated by placing 2DG standard solutions in wells of a culture plate lacking cells but prepared similarly.

Measurement of ATP levels

At day 14 after plating the hippocampal cells, neurons were washed and incubated with $A\beta_{1-42}$ oligomers for 30 min and 60 min in the presence and absence of insulin (1 μM) and C-peptide (1 μM). They were immediately homogenized in 0.5 mM perchloric acid with 1 mM ethylenediamine-N,N,N′,N′-tetraacetic acid and centrifuged for 15 min at 300 *g*. The supernatant was neutralized with 2 mol $KHCO_3$, recentrifuged and stored at −30°C until assayed. ATP was quantified using a luciferin/luciferase luminescence assay kit (Invitrogen). The protein content of the cells was determined by the method of Lowry and Passonneau.[23]

Materials

2-Deoxy-d-glucose, recombinant G6P-DH, phloretin, C-peptide, sodium pyruvate and sodium lactate were obtained from Sigma-Aldrich (Tokyo, Japan), and all other chemicals were from Wako (Tokyo, Japan) or Nacalai Tesque (Kyoto, Japan).

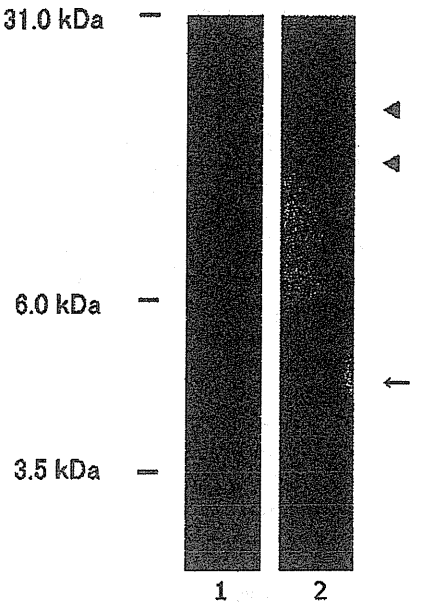

Figure 1 Immunoblotting of $A\beta_{1-42}$ monomer and oligomers. $A\beta_{1-42}$ oligomers were prepared as described.[19] The blots were probed with the 6E10 anti-Aβ antibody, followed by horseradish peroxidase-conjugated antimouse antibody and developed with ECL reagents. Representative blots of $A\beta_{1-42}$ monomers (arrow) and oligomers (arrowheads) are shown (lanes 1 and 2, respectively).

Statistical analysis

The data were analyzed using two-tailed Student's *t*-tests, and $P < 0.05$ was considered to be statistically significant.

Results

Immunoblotting of $A\beta_{1-42}$ monomer and oligomers

Figure 1 shows representative blots of $A\beta_{1-42}$ monomers and oligomers. The monomeric form of Aβ was observed at 5 kDa, whereas several oligomeric Aβ forms were at 10–20 kDa (lanes 1 and 2). Furthermore, biological activity of the $A\beta_{1-42}$ oligomers was tested by electrophysiological experiments using hippocampal slice cultures.[24] We found that these oligomers effectively inhibited the induction of long-term potentiation (LTP) in the CA1 region of hippocampal slices after tetanic stimulation (100 Hz, 1 s), while application of $A\beta_{1-42}$ monomer at the identical concentration did not inhibit LTP (data not shown). These results confirmed that the 5–6 mers of $A\beta_{1-42}$ peptides used in this study were biologically active.

Specific uptake of 2DG in cultured hippocampal neurons

In these experiments, cultured neurons were exposed to glucose-free medium for more than 90 min before the 2DG uptake study (Fig. 2a), which could influence neuronal viability. Therefore, the rate of glucose incorporation was compared in neurons kept in glucose-free media and in medium containing 10 mM glucose. The 2DG uptake tended to increase in the absence of glucose, suggesting that cultured neurons were being maintained in relatively healthy conditions (data not shown). Based on this observation, glucose-free medium was used for following several sets of experimental conditions.

To confirm specific incorporation of 2DG in neurons, cultured hippocampal neurons were treated with phloretin, an inhibitor for glucose transporters, during 2DG uptake studies.[11] Administration of phloretin inhibited neuronal 2DG uptake significantly, indicating that 2DG was incorporated in a glucose transporter-mediated manner, but not by simple diffusion (data not shown).

Effects of insulin and $A\beta_{1-42}$ oligomers on 2DG uptake in cultured hippocampal neurons

To investigate the effects of insulin on neuronal 2DG uptake, hippocampal neurons were exposed to insulin (1 μM) for 30 min after insulin starvation (Fig. 2a). Because abundant distribution of insulin receptors and insulin-sensitive glucose transporter 4 has been identified in the hippocampus,[25] we expected an insulin-stimulated 2DG uptake in the cultured neurons. However, insulin-dependent increase of neuronal 2DG was not demonstrated after repeated experiments (Fig. 2b).

The impact of $A\beta_{1-42}$ oligomers on 2DG uptake was tested next. Incubation with $A\beta_{1-42}$ oligomers for 10–30 min did not change neuronal 2DG uptake. In addition, no synergistic effects of insulin and $A\beta_{1-42}$ oligomers could be demonstrated. Prolonged treatment with $A\beta_{1-42}$ oligomers for more than 24 h showed no changes in 2DG uptake (data not shown). These results indicated that neither insulin nor $A\beta_{1-42}$ oligomers had any effects on neuronal glucose incorporation.

Changes in intracellular ATP levels by $A\beta_{1-42}$ oligomers and insulin/C-peptide

Adenosine triphosphate levels in the hippocampal neurons were determined for evaluating mitochondrial function. Because Wang *et al.* reported that Aβ toxicity depends on extracellular glucose concentrations,[24] cultured cells were incubated in media containing a variety of glucose concentrations (0–10 mM) with and without $A\beta_{1-42}$ oligomers. ATP concentrations in the cultured neurons were quantified by the luciferin/luciferase luminescence method. In the presence of 10 mM glucose, $A\beta_{1-42}$ oligomers gradually decreased the ATP levels, and a significant reduction in ATP concentration was observed after 60 min ($P < 0.05$), while $A\beta_{42-1}$ did not affect ATP contents (Fig. 3a). When the glucose concentration was less than 5 mM, $A\beta_{1-42}$ oligomers decreased ATP contents more rapidly but to similar final levels.

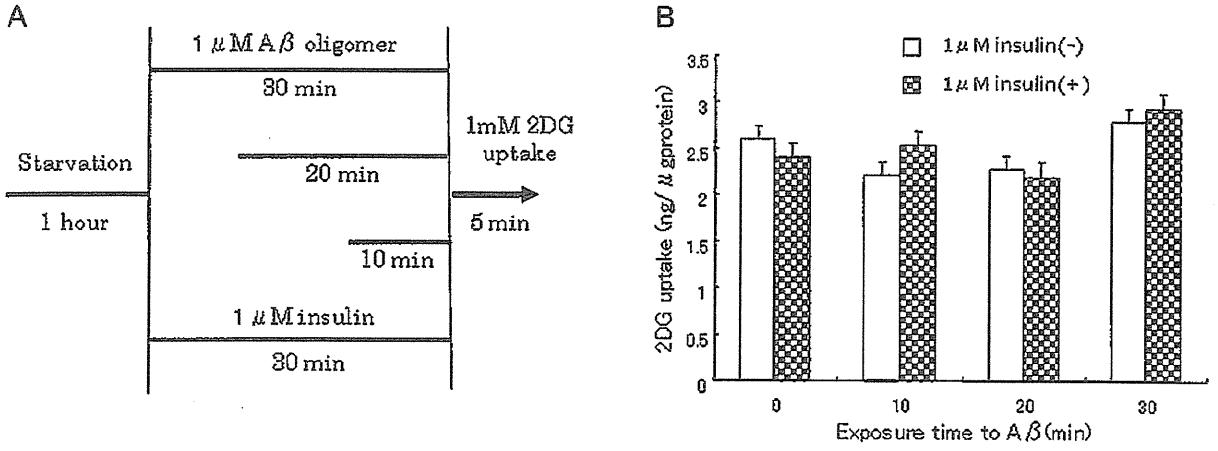

Figure 2 Effects of $A\beta_{1-42}$ oligomers and insulin on glucose incorporation in the hippocampal neurons. (a) Experimental protocols to investigate the effects of $A\beta_{1-42}$ oligomers and insulin on glucose uptake. After insulin starvation, hippocampal neurons were incubated with and without insulin (1 μM), and 2-deoxy-d-glucose (2DG) uptake was determined as described. (b) No insulin-dependent increase of neuronal 2DG could be demonstrated (Aβ exposure time 0). Incubation with $A\beta_{1-42}$ oligomers for 10–30 min did not change neuronal 2DG uptake. No synergistic effects of insulin and $A\beta_{1-42}$ oligomers were observed.

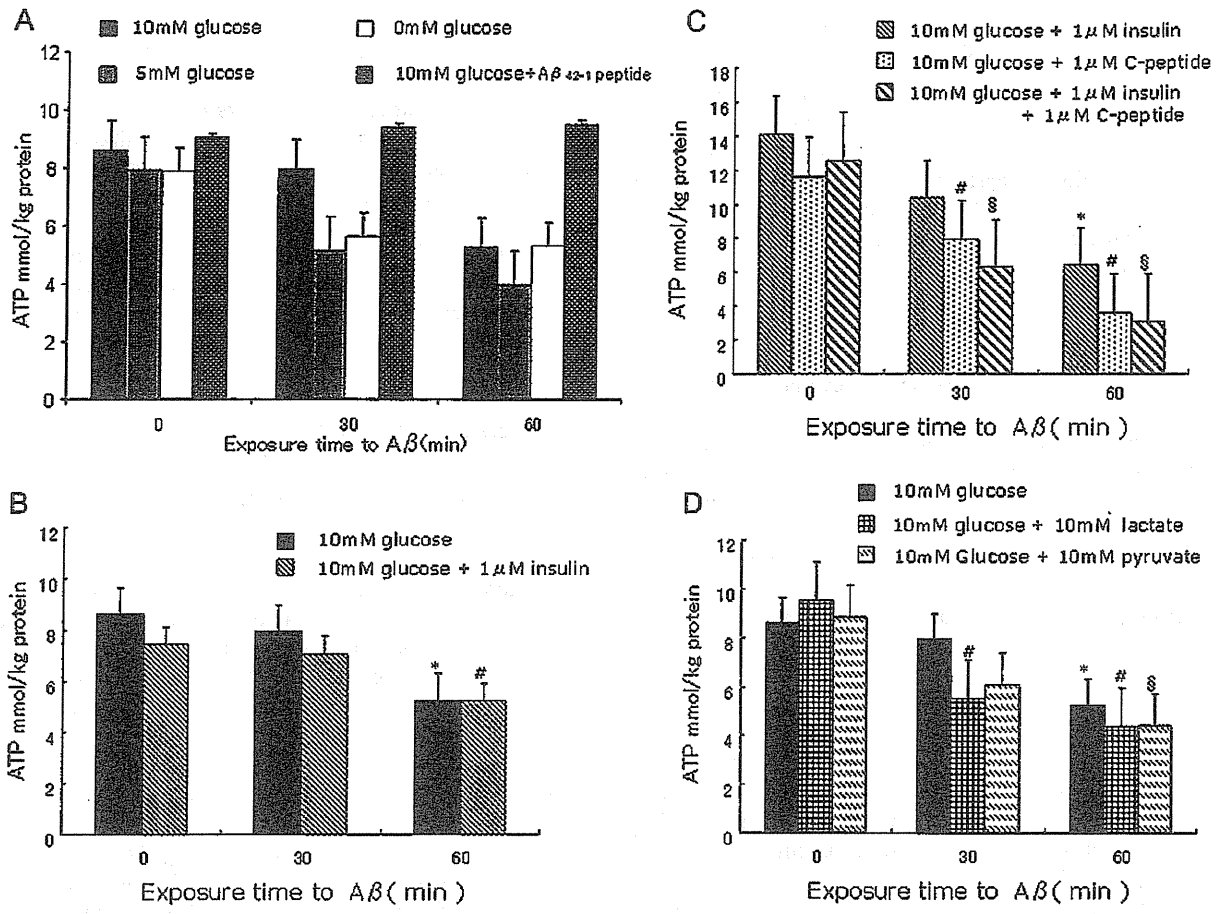

Figure 3 Intracellular adenosine triphosphate (ATP) levels after application of $A\beta_{1-42}$ oligomers, insulin/C-peptide and monocarboxylates. At day 14 post-plating, hippocampal neurons were incubated with $A\beta_{1-42}$ oligomers for 30–60 min in the presence and absence of insulin, C-peptide and lactate/pyruvate. ATP concentration was quantified using a luciferin/luciferase luminescence assay. (a) Cultured cells were incubated in media containing a variety of glucose concentrations (0–10 mM) with and without $A\beta_{1-42}$ oligomers. Each bar represents the mean ± standard error of the mean ($n = 4$). In the presence of 10 mM glucose, $A\beta_{1-42}$ oligomers gradually decreased ATP levels, and significant reduction of ATP was observed after 60 min ($^{\S}P < 0.05$ compared with time 0). $A\beta_{42-1}$ peptide did not decrease neuronal ATP levels. When glucose concentration was less than 5 mM, $A\beta_{1-42}$ oligomer decreased ATP contents more rapidly: $^{\#}P < 0.05$ (5 mM glucose) and $^{*}P < 0.05$ (0 mM glucose) compared with time 0. (b) Insulin did not change ATP contents in the cultured hippocampal neurons over 60 min of incubation. Concomitant application of $A\beta_{1-42}$ oligomers significantly reduced ATP concentration. Each bar represents the mean ± SEM ($n = 4$). (c) In the presence of insulin and/or C-peptide, addition of $A\beta_{1-42}$ oligomers similarly reduced intracellular ATP levels. Each bar represents the mean ± SEM ($n = 4$). $^{*}P < 0.05$ (10 mM glucose + 1 μM insulin), $^{\#}P < 0.05$ (10 mM glucose + 1 μM C-peptide) and $^{\S}P < 0.05$ (10 mM glucose + 1 μM insulin + 1 μM C-peptide) compared with time 0. (d) $A\beta_{1-42}$ oligomers decreased intracellular ATP concentrations in hippocampal neurons in the presence of lactate and pyruvate plus glucose. Each bar represents the mean ± SEM ($n = 4$). $^{*}P < 0.05$ (10 mM glucose), $^{\#}P < 0.05$ (10 mM glucose + 10 mM lactate) and $^{\S}P < 0.05$ (10 mM glucose + 10 mM pyruvate), compared with time 0.

The effect of insulin on ATP production was examined next (Fig. 3b). Insulin did not change ATP contents in the cultured hippocampal neurons during 60 min of incubation, and concomitant application of $A\beta_{1-42}$ oligomers significantly reduced the concentration. Because C-peptide, another derivative of proinsulin, has been shown to have neuroprotective effects,[26] we also examined the effects of C-peptide on the intracellular ATP concentrations. The addition of $A\beta_{1-42}$ oligomers also reduced intracellular ATP levels in the presence of insulin and/or C-peptide ($P < 0.05$, Fig. 3c).

Finally, the impacts of $A\beta_{1-42}$ oligomers on ATP concentrations in hippocampal neurons were determined in the presence of lactate and pyruvate plus 10 mM glucose, because these monocarboxylates are incorporated into neuronal cells via monocarboxylate transporters and

are metabolized in mitochondria to produce ATP directly without using anaerobic glycolytic pathways.[27] It has been shown that pyruvate serves as a scavenger of reactive oxygen species (ROS) induced by $A\beta_{1-42}$ oligomers.[28] However, incubation of hippocampal slices in Locke's buffer containing glucose plus lactate or pyruvate did not influence the reduction of neuronal ATP levels by $A\beta_{1-42}$ oligomers ($P < 0.05$, Fig. 3d).

Discussion

This study has provided evidence for the impacts of $A\beta_{1-42}$ oligomers and insulin on glucose metabolism and energy homeostasis *in vitro*. Although an inhibitory effect of high concentrations of Aβ peptides on glucose uptake has been suggested,[10,11] here we clearly demonstrate that Aβ oligomers did not suppress the 2DG uptake reflecting the activities of glucose transport and/or hexokinase but disrupted ATP contents in the presence and absence of monocarboxylates (lactate/pyruvate). These results imply that the primary target of $A\beta_{1-42}$ oligomers could be mitochondria but not anaerobic glycolysis. This could result in the reduced cerebral glucose metabolism observed in patients with AD. On the other hand, insulin and C-peptide were not directly linked to glucose uptake or ATP production, although insulin receptors and GLUT 4, an insulin-sensitive glucose transporter, are known to be distributed abundantly in the hippocampal neurons.[25]

In the present study, we have demonstrated that Aβ oligomers can cause reduction in neuronal ATP levels. In this connection, Saraiva *et al.* have recently reported decreased ATP levels and impaired mitochondrial enzymes in mature cortical neurons by exposure to Aβ oligomers.[29] Aβ oligomers have now been identified in mitochondrial membranes in the neurons of postmortem brain specimens from patients with AD, in brain neurons of mice with AD and in neuronal cells expressing mutant amyloid precursor protein (APP).[30] In mitochondria, Aβ oligomers induce elevated levels of ROS, deterioration of mitochondrial enzymes and failure of Ca^{2+} homeostasis *in vivo*.[30] However, the mechanism by which Aβ is transported to the mitochondrial membrane is not fully understood. Besides the plasma membrane, APP generated in neurons localizes to the Golgi apparatus, to the endoplasmic reticulum and to the endosomal, lysosomal and mitochondrial membranes. Aβ is produced by the sequential cleavage of APP by β-secretase and γ-secretase. In addition, previously secreted Aβ can be also taken up by cells and internalized into intracellular Aβ pools through various receptors and transporters.[30]

Furthermore, extracellular Aβ oligomers activate nerve growth factor receptors to induce apoptotic cell death and N-methyl-d-aspartate (NMDA)-type glutamate receptors to cause abnormal calcium homeostasis, leading to increased oxidative stress and synapse loss.[31] Aβ oligomers cause a rapid and substantial loss of neuronal surface insulin receptors specifically on dendrites, with increased receptor immunoreactivity in the cell body, indicating the redistribution of insulin receptors. Aβ oligomers reduce autophosphorylation of the insulin receptor mediated by the NMDA receptor in both hippocampal and cortical neurons, and impair LTP-associated kinase activity.[18,32] Involvement of the Frizzled (Fz) receptor, an acceptor of the Wnt protein, has been proposed.[33] Wnt signaling promotes progenitor cell proliferation and directs cells into a neuronal phenotype during brain development. This inactivates glycogen synthase kinase-3β (GSK-3β) and increases β-catenin levels. Inhibition of Wnt signaling by Aβ oligomers through the Fz receptor could cause tau phosphorylation and neurofibrillary tangles, which supports the idea of a Wnt/β-catenin toxicity pathway.[33] Aβ oligomers can impair presynaptic P/Q-type calcium currents, which are related to neurotransmission and synaptic plasticity.[34] Direct or indirect metabolic signals originating from Aβ binding sites on the neuronal surface could lead to alterations in mitochondrial structure and function.

It should be noted that Aβ was used at 1 μM in this study. Most estimates for its concentration in the human brain have been in the low nanomolar range.[35] To span this large concentration gap, several potential mechanisms have been proposed. Thus, Hu *et al.* reported that low, physiologically-relevant concentrations of extracellular Aβ can be taken up by neurons and then concentrated into endosomes/lysosomes (>2.5 μM).[36] Our experimental conditions might have accelerated this process by using higher concentrations of Aβ, leading to increased Aβ neurotoxicity. Detailed studies are needed to reveal how Aβ oligomers induce mitochondrial insufficiency.

Interactions of insulin signaling and Aβ oligomers have been proposed.[37] De Felice *et al.* have reported that Aβ oligomers cause major downregulation of plasma membrane insulin receptors in hippocampal cortical cultures.[37] Aβ oligomer-induced oxidative stress and synaptic spine deterioration could be completely prevented by insulin at 1 μM. Therefore, we used 1 μM insulin in the present experiment.

It has been postulated that insulin resistance plays a critical role in the development and progression of AD.[15] The beneficial effects of insulin in the central nervous system include improved glucose metabolism and energy homeostasis. Increased synaptic plasticity and modulation of Aβ and tau metabolism have also been proposed. Acute administration of insulin with sustained blood glucose concentrations facilitates memory in healthy elderly people as well as in patients with AD. This effect occurs at lower insulin doses for the healthy elderly group than for patients with AD and is suppressed by insulin-induced elevations of Aβ in

cerebrospinal fluid.[38] However, our results here clearly indicate that insulin signaling was not directly linked to glucose metabolism or energy homeostasis in hippocampal cultured neurons with and without Aβ oligomers. This suggests that insufficient insulin action might contribute to progression of the AD pathology by accelerating impaired synaptic activity and metabolic changes in the Aβ cascade. Because insulin could not ameliorate the disruption of energetic homeostasis in neurons induced by Aβ oligomers, it seems plausible that therapeutic strategies to prevent or correct insulin resistance should be conducted in the earlier stages of AD when synaptic abnormalities are irreversible. Further intensive studies are needed to develop new drugs for improving mitochondrial impairment in patients developing AD.

Acknowledgments

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