Table 1 – Baseline characteristics of study participants according to dietary GI quintiles

	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	P[b]
GI	<66.2	66.2-68.5	68.6-70.4	70.5-72.6	≥72.7	
Age (y)	45.7 ± 6.0	46.2 ± 6.0	45.7 ± 6.2	46.0 ± 6.1	46.3 ± 5.8	.286
Height (cm)	169.7 ± 6.0	169.7 ± 6.1	170.0 ± 5.9	169.3 ± 5.9	169.1 ± 6.1	.113
Weight (kg)	68.2 ± 9.6	67.5 ± 9.5	67.0 ± 9.0	67.3 ± 9.5	67.3 ± 9.3	.178
BMI (kg/m^2)	23.6 ± 2.9	23.4 ± 2.9	23.1 ± 2.8	23.4 ± 2.8	23.5 ± 2.9	.541
Total cholesterol (mg/dL)	207.5 ± 34.0	208.6 ± 33.5	208.4 ± 35.1	210.8 ± 33.8	201.9 ± 31.5	.101
Triglycerides (mg/dL)[a]	106 (68-157)	103 (69-151)	114 (78-168)	103 (66-156)	97 (67-143)	.073
HDL cholesterol (mg/dL)	57.9 ± 14.9	57.3 ± 13.2	58.7 ± 15.4	57.9 ± 15.1	58.4 ± 14.6	.522
Fasting plasma glucose (mg/dL)	92.5 ± 10.1	92.8 ± 9.4	92.5 ± 9.6	93.4 ± 10.4	93.0 ± 9.6	.300
Fasting insulin (μU/mL)[a]	5.1 (3.0-7.3)	4.9 (3.0-7.0)	4.7 (3.0-7.0)	5.0 (3.0-8.0)	4.7 (3.0-7.0)	.129
HOMA-IR[a]	1.15 (0.73-1.74)	1.10 (0.70-1.67)	1.06 (0.73-1.62)	1.13 (0.69-1.76)	1.07 (0.68-1.53)	.212
HOMA-B[a]	66.2 (43.5-94.1)	60.9 (40.0-92.8)	60.6 (40.0-90.0)	61.4 (41.5-93.9)	59.6 (39.8-90.0)	.026
HbA_{1c} (%)	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.5	5.0 ± 0.4	.954
Systolic blood pressure (mm Hg)	120.5 ± 18.0	119.8 ± 17.4	120.4 ± 15.1	121.9 ± 18.8	120.2 ± 20.9	.668
Diastolic blood pressure (mm Hg)	77.9 ± 12.9	76.9 ± 12.1	78.0 ± 11.1	78.6 ± 13.4	77.6 ± 14.6	.765
Family history of diabetes (%)	13.9	12.6	14.0	14.7	12.2	.837
Smoking status						.001
Nonsmoker (%)	33.3	32.1	29.7	30.8	28.2	
Ex-smoker (%)	16.2	15.2	14.5	16.4	11.7	
Current smoker (%)	50.5	52.8	55.9	52.7	60.2	
Alcohol intake						.333
Nondrinker (%)	21.4	24.5	24.4	27.1	21.6	
Light drinker (<20 g/d; %)	36.3	34.6	33.7	32.3	30.7	
Moderate/heavy drinker (≥20 g/d; %)	42.3	40.9	41.9	40.5	47.7	
Habitual exercise, yes (%)	33.6	30.8	25.4	25.9	25.1	.021
Prevalence of high blood pressure[c] (%)	8.7	8.8	6.3	10.4	7.9	.302
Prevalence of dyslipidemia[c] (%)	10.2	10.1	9.0	9.0	6.6	.402
GI	63.4 ± 2.8	67.5 ± 0.7	69.5 ± 0.5	71.5 ± 0.6	74.2 ± 1.3	<.001
GL (/1000 kcal)	76.0 ± 16.2	85.1 ± 15.0	87.7 ± 17.0	92.9 ± 16.6	97.7 ± 19.9	<.001
Total energy intake (kcal/d)	2383 ± 695	2270 ± 631	2198 ± 586	2096 ± 518	2044 ± 559	<.001
Total fiber intake (g/1000 kcal)	5.7 ± 1.5	5.3 ± 1.3	4.9 ± 1.3	4.7 ± 1.2	4.0 ± 1.2	<.001
Protein (% energy)	12.5 ± 2.3	12.1 ± 2.2	11.6 ± 2.0	11.6 ± 2.0	10.8 ± 2.1	<.001
Fat (% energy)	24.1 ± 6.7	22.4 ± 6.1	21.6 ± 6.3	20.8 ± 5.9	18.4 ± 6.3	<.001
Carbohydrates (% energy)	54.9 ± 9.1	57.3 ± 8.0	57.3 ± 8.9	58.9 ± 8.2	59.7 ± 9.2	<.001

Values are mean ± standard deviation or percentage.

[a] Values are geometric means (interquartile range).

[b] Linear regression was used for continuous variables based on ordinal variables containing the median value for each quintile, and a χ^2 test was used for categorical variables.

[c] High blood pressure and dyslipidemia were defined using the Japanese criteria for metabolic syndrome.

interaction between GI and HOMA-IR (P = .005), and the influence of GI was more pronounced in the lowest HOMA-IR tertile subgroups. On the other hand, participants in the lowest HOMA-B tertile with the highest GI had the highest risk of diabetes (Fig. 1C). We observed no interaction between GI and BMI or HOMA-B.

4. Discussion

This study investigated the association between dietary GI and GL and the incidence of type 2 diabetes mellitus in middle-aged Japanese men. The results indicated that GI, but not GL, had a significant positive association with the incidence of diabetes. The analyses of insulin resistance and dietary GI indicated that the association between high dietary GI and type 2 diabetes mellitus was stronger in the lowest HOMA-IR subgroup. Furthermore, GI and pancreatic B-cell function were independently associated with incidence of type 2 diabetes mellitus; and the participants with low HOMA-B and the highest GI had the highest risk of diabetes.

The results of previous studies that evaluated the association between dietary GI and incidence of diabetes were controversial [8]. Although some reports showed no association between GI and diabetes, other reports and a recent meta-analysis showed positive associations. Differences in these results are probably due to differences in participant characteristics such as age, sex, ethnicity, and lifestyle. All previous studies of the association between GI and GL and the risk of diabetes have been conducted in Western countries [7-9], with the exception of one Chinese study of women [12]. The present study is the first report on an association between GI and GL and the risk of diabetes in Asian men. We found that the HR for the highest GI quintiles was 1.80 (model 1) to 1.96 (model 3); these values are somewhat higher than those reported in previous studies (0.89-1.59 for multivariate-adjusted models) [8].

The GL was not associated with the incidence of diabetes in our study; and our findings agree with those of previous

Table 2 – Baseline characteristics of study participants according to dietary GL quintiles

	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	P[b]
GL (/1000 kcal)	<72.8	72.8-83.1	83.2-91.5	91.6-103.3	≥103.4	
Age (y)	45.4 ± 6.0	46.5 ± 6.0	45.9 ± 6.2	45.9 ± 5.9	46.2 ± 6.1	.264
Height (cm)	169.7 ± 5.9	169.9 ± 6.0	169.6 ± 5.8	169.4 ± 5.8	169.2 ± 6.4	.102
Weight (kg)	67.9 ± 9.4	67.8 ± 9.3	67.3 ± 9.6	66.8 ± 8.6	67.4 ± 9.9	.178
BMI (kg/m^2)	23.5 ± 2.8	23.4 ± 2.8	23.3 ± 2.8	23.2 ± 2.8	23.5 ± 3.1	.650
Total cholesterol (mg/dL)	206.8 ± 33.4	205.8 ± 34.7	206.4 ± 35.2	208.6 ± 31.6	209.8 ± 33.4	.101
Triglycerides (mg/dL)[a]	108 (69-161)	100 (66-150)	109 (71-160)	99 (67-147)	106 (71-157)	.772
HDL cholesterol (mg/dL)	61.5 ± 15.5	58.8 ± 13.7	57.3 ± 15.3	57.7 ± 14.5	54.9 ± 13.4	<.001
Fasting plasma glucose (mg/dL)	93.6 ± 9.9	93.2 ± 9.6	93.1 ± 10.6	92.3 ± 9.7	92.0 ± 9.3	.010
Fasting insulin (μU/mL)[a]	4.5 (3.0-7.0)	4.8 (3.0-7.0)	5.0 (3.0-7.3)	4.9 (3.0-7.0)	5.1 (3.0-8.0)	.003
HOMA-IR[a]	1.03 (0.66-1.64)	1.09 (0.69-1.66)	1.14 (0.75-1.76)	1.11 (0.72-1.60)	1.15 (0.73-1.76)	.015
HOMA-B[a]	55.3 (37.9-81.3)	59.8 (40.0-83.1)	64.1 (44.7-96.0)	63.7 (41.5-93.9)	66.4 (43.2-102.9)	<.001
HbA_{1c} (%)	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.4	.747
Systolic blood pressure (mm Hg)	123.1 ± 16.7	120.6 ± 18.7	121.1 ± 17.6	119.4 ± 17.1	118.6 ± 20.2	<.001
Diastolic blood pressure (mm Hg)	79.9 ± 12.0	78.4 ± 13.4	78.1 ± 12.2	76.5 ± 12.1	76.1 ± 14.3	<.001
Family history of diabetes (%)	12.0	13.5	16.1	13.8	12.2	.451
Smoking status						.021
Nonsmoker (%)	23.0	29.9	30.9	34.3	36.1	
Ex-smoker (%)	17.8	15.5	14.6	16.5	9.6	
Current smoker (%)	59.3	54.6	54.5	49.3	54.3	
Alcohol intake						<.001
Nondrinker (%)	6.5	12.7	16.3	33.3	50.5	
Light drinker (<20 g/d; %)	17.5	29.9	42.5	40.8	37.1	
Moderate/heavy drinker (≥20 g/d;%)	76.0	57.4	41.2	26.0	12.4	
Habitual exercise, yes (%)	28.8	31.7	29.4	29.5	21.5	.018
Prevalence of high blood pressure[c] (%)	11.8	8.0	8.8	7.0	6.6	.070
Prevalence of dyslipidemia[c] (%)	8.7	7.8	10.1	9.5	8.9	.833
GI	67.1 ± 4.7	68.3 ± 3.7	69.2 ± 3.3	70.0 ± 3.3	71.4 ± 3.0	<.001
GL (/1000 kcal)	62.7 ± 8.8	78.0 ± 3.0	87.2 ± 2.5	97.1 ± 3.3	114.4 ± 9.6	<.001
Total energy intake (kcal/d)	2394 ± 616	2299 ± 581	2183 ± 578	2104 ± 556	2011 ± 653	<.001
Total fiber intake (g/1000 kcal)	4.9 ± 1.6	5.1 ± 1.5	5.0 ± 1.3	4.9 ± 1.4	4.6 ± 1.3	.001
Protein (% energy)	12.7 ± 2.8	12.3 ± 2.1	11.8 ± 1.9	11.5 ± 1.6	10.3 ± 1.6	<.001
Fat (% energy)	25.7 ± 7.7	23.7 ± 5.7	22.1 ± 5.3	20.1 ± 4.2	15.7 ± 4.4	<.001
Carbohydrates (% energy)	46.0 ± 5.6	53.3 ± 3.2	57.5 ± 2.8	62.0 ± 2.9	69.4 ± 4.5	<.001

Values are mean ± standard deviation or percentage.

[a] Values are geometric means (interquartile range).

[b] Linear regression was used for continuous variables based on ordinal variables containing the median value for each quintile, and a χ^2 test was used for categorical variables.

[c] High blood pressure and dyslipidemia were defined using the Japanese criteria for metabolic syndrome.

studies showing that GI, but not GL, was associated with the incidence of diabetes [15,19]. Although some studies have reported that dietary GL was associated with the risk of diabetes [12,16], a meta-analysis comparing the highest and lowest GI and GL quintiles showed that the HR for developing diabetes was more highly associated with GI than GL [8]. Thus, dietary GI is a better predictor of the risk of diabetes than is dietary GL.

High-GI foods are thought to increase insulin resistance, impair pancreatic B-cell function, and eventually lead to type 2 diabetes mellitus [20]. The adverse effects of a high-GI diet have been reported to be more evident in overweight or obese people who, presumably, were insulin resistant at baseline [17,40]. However, evidence of an effect of insulin resistance on the association between GI and diabetes is inconsistent. Some studies have shown that high GI was associated with a higher relative risk of diabetes in people who had a high BMI [12,19], whereas other studies have indicated that high GI was more strongly associated with incidence of diabetes in people with a low BMI [9,15]. These studies used obesity as a marker of insulin resistance; but in our study, insulin resistance was directly measured by HOMA-IR. Thus, we were able to compare the association between GI and the incidence of diabetes according to the degree of insulin resistance. We found a significant interaction between GI and HOMA-IR and also found a significant association between GI and the incidence of diabetes only in participants who were in the lowest tertile of HOMA-IR. Insulin resistance is a strong risk factor for type 2 diabetes mellitus, and it may be difficult to detect the effect of other risk factors in participants with higher insulin resistance.

In our study, GI and pancreatic B-cell function were independently associated with the incidence of diabetes; and participants with the lowest pancreatic B-cell function and the highest dietary GI were at the highest risk of diabetes. Dietary GI is higher in Asian populations than in Western populations. For example, the present study showed mean GI values of 69.2, which were similar to

Table 3 – Adjusted HR for type 2 diabetes mellitus according to quintiles of GI, GL, total energy intake, and total fiber intake in 1995 Japanese men

	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)
GI					
n	402	396	401	402	394
Total person-years	1786	1778	1766	1796	1862
Incident cases (n)	18	28	24	29	34
Rate per 1000 person-years	10.1	15.7	13.6	16.1	18.3
Adjusted HR (95% CI) model 1	1.00 (reference)	1.62 (0.89-2.93)	1.50 (0.81-2.77)	1.68 (0.93-3.03)	1.80 (1.01-3.18)
Adjusted HR (95% CI) model 2	1.00 (reference)	1.68 (0.92-3.04)	1.56 (0.84-2.89)	1.73 (0.96-3.13)	1.88 (1.06-3.35)
Adjusted HR (95% CI) model 3	1.00 (reference)	1.71 (0.94-3.10)	1.66 (0.89-3.10)	1.86 (1.01-3.44)	1.96 (1.04-3.67)
GL					
n	400	401	398	400	396
Total person-years	1733	1735	1739	1856	1924
Incident cases (n)	23	26	34	23	27
Rate per 1000 person-years	13.3	15.0	19.5	12.4	14.0
Adjusted HR (95% CI) model 1	1.00 (reference)	1.07 (0.61-1.88)	1.48 (0.87-2.52)	0.95 (0.53-1.70)	0.98 (0.56-1.72)
Adjusted HR (95% CI) model 2	1.00 (reference)	1.14 (0.65-2.02)	1.54 (0.89-2.65)	1.07 (0.58-1.96)	1.23 (0.67-2.28)
Adjusted HR (95% CI) model 3	1.00 (reference)	1.16 (0.66-2.06)	1.56 (0.89-2.71)	1.07 (0.57-1.99)	1.24 (0.65-2.34)
Total energy intake (range, kcal/d)	(<1703)	(1703-1971)	(1972-2246)	(2247-2641)	(>2641)
n	399	399	399	399	399
Total person-years	1790	1776	1748	1758	1917
Incident cases (n)	24	24	32	24	26
Rate per 1000 person-years	13.4	14.6	18.3	14.2	13.6
Adjusted HR (95% CI) model 1	1.00 (reference)	1.13 (0.65-1.96)	1.49 (0.88-2.54)	1.11 (0.63-1.95)	1.00 (0.57-1.74)
Adjusted HR (95% CI) model 2	1.00 (reference)	1.10 (0.63-1.92)	1.44 (0.84-2.48)	1.06 (0.60-1.87)	0.97 (0.55-1.71)
Adjusted HR (95% CI) model 3	1.00 (reference)	1.12 (0.64-1.97)	1.45 (0.84-2.49)	1.07 (0.60-1.91)	0.97 (0.55-1.72)
Total fiber intake (range, g/1000 kcal)	(<3.7)	(3.8-4.5)	(4.6-5.2)	(5.3-6.0)	(>6.0)
n	400	450	391	370	384
Total person-years	1938	2016	1781	1590	1663
Incident cases (n)	35	26	17	23	32
Rate per 1000 person-years	18.1	12.9	9.5	14.5	19.2
Adjusted HR (95% CI) model 1	1.00 (reference)	0.73 (0.44-1.22)	0.56 (0.31-1.01)	0.80 (0.47-1.35)	0.99 (0.61-1.60)
Adjusted HR (95% CI) model 2	1.00 (reference)	0.73 (0.44-1.23)	0.59 (0.32-1.05)	0.83 (0.48-1.43)	0.98 (0.59-1.64)
Adjusted HR (95% CI) model 3	1.00 (reference)	0.72 (0.43-1.21)	0.59 (0.33-1.06)	0.84 (0.49-1.45)	0.99 (0.59-1.66)

Model 1: adjusted for age and BMI; model 2: adjusted for age, BMI, family history of diabetes, smoking, alcohol intake, habitual exercise, and presence of hypertension and hyperlipidemia at baseline; model 3: adjusted for variables used in model 2 and dietary total energy (for the GI, GL, and total fiber intake) and dietary total fiber intake (for the GI, GL, and total energy intake). CI indicates confidence interval.

those previously reported in Japan [10,14] and higher than the values (range, 48-60) reported in US and European studies [15-19]. Furthermore, both obese and lean Asians who have lower B-cell function are at high risk for developing type 2 diabetes mellitus [4-6]. Our study indicates that the high prevalence of type 2 diabetes mellitus in Asian populations may be explained by high-GI diets in people with lower B-cell function. Thus, an evaluation of the risk of type 2 diabetes mellitus in Asian people must consider lifestyle and food intake as well as genetic background.

Individuals at high risk for diabetes are encouraged to increase their dietary fiber intake and to eat foods containing whole grains [41]. The consumption of such foods is associated with decreased dietary GI. However, the use of GI is recommended as an additional method for management of diabetes in an American Diabetes Association position statement [41] and a recommendation of the American Dietetic Association [42] because the effects of lower-GI diets on glucose metabolism were conflicting [42]. In our study, total fiber intake was not associated with the incidence of diabetes. Furthermore, a higher GI was associated with a higher risk for diabetes, despite a lower total energy intake; and there was no association between total energy intake and the incidence of diabetes. The appropriate energy intake of each person is important for maintaining body weight and preventing obesity and diabetes. However, appropriate energy intake is influenced by many factors, including body composition and physical activity. It is difficult to evaluate the association between total energy intake itself with diabetes; and indices of the quality of food intake such as GI, rather than the quantity of food intake, would be more useful for a population approach.

The strengths of this study include a large sample size, foods contributing to the dietary GI that differed from those in US and European populations, and the fact that it was the first study of the relationship between GI and the incidence of diabetes conducted in Japanese men. Moreover, several previous cohort studies used information collected from self-administered questionnaires, whereas our conclusions are based on more reliable data obtained from medical examinations and fasting blood glucose and insulin levels, HOMA-IR, and HOMA-B. In addition, GI and GL were calculated using responses to a validated questionnaire [11]. A limitation of the present study is that the sample included only people who

Table 4 – Incidence and adjusted HRs[a] for type 2 diabetes mellitus according to GI tertiles of BMI, HOMA-IR, and HOMA-B in 1995 Japanese men

	GI tertiles (range)			*P* for trend[b]
	T1 (<68.0)	T2 (68.0-71.0)	T3 (≥71.1)	
BMI (kg/m^2)				
<22.0				
Incident cases n/N	3/203	11/227	15/206	
Crude rate per 1000 person-years	3.2	10.4	15.1	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	4.09 (1.13-14.9)	5.78 (1.63-20.5)	.005
22.0-24.9				
Incident cases n/N	14/278	14/257	18/272	
Crude rate per 1000 person-years	11.5	12.4	14.4	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.10 (0.52-2.34)	1.20 (0.59-2.44)	.608
≥25.0				
Incident cases n/N	19/196	20/169	19/187	
Crude rate per 1000 person-years	21.9	28.8	22.5	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.41 (0.75-2.66)	1.11 (0.58-2.11)	.719
HOMA-IR tertiles				
<0.85				
Incident cases n/N	4/217	8/207	16/219	
Crude rate per 1000 person-years	4.1	8.5	15.4	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	2.07 (0.61-6.95)	3.67 (1.21-11.2)	.015
0.85-1.43				
Incident cases n/N	10/222	9/232	21/240	
Crude rate per 1000 person-years	10.2	8.6	18.6	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	0.78 (0.31-1.94)	1.58 (0.73-3.41)	.221
≥1.44				
Incident cases n/N	22/238	28/214	15/206	
Crude rate per 1000 person-years	20.5	31.4	16.3	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.73 (0.98-3.05)	0.83 (0.43-1.62)	.472
HOMA-B tertiles				
<48.4				
Incident cases n/N	16/227	23/230	31/226	
Crude rate per 1000 person-years	16.1	23.0	30.0	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.64 (0.86-3.13)	1.86 (1.01-3.44)	.049
48.4-79.3				
Incident cases n/N	10/218	11/205	12/224	
Crude rate per 1000 person-years	10.3	11.8	11.5	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.34 (0.56-3.20)	1.26 (0.53-3.00)	.600
≥79.4				
Incident cases n/N	10/232	11/218	9/215	
Crude rate per 1000 person-years	9.4	11.6	8.9	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.39 (0.58-3.31)	0.93 (0.37-2.34)	.922

[a] Adjusted for age, BMI, family history of diabetes, smoking, alcohol intake, habitual exercise, and presence of hypertension and hyperlipidemia at baseline.

[b] Linear regression was used for continuous variables based on ordinal variables containing the median value for each GI tertile.

were employed. Poor health may exclude some individuals from working; thus, the prevalence of obesity may be lower in our sample than in the general Japanese population. Another limitation is that we did not measure waist circumference at baseline, which might have provided more information about abdominal fat accumulation and insulin resistance than measuring BMI did. A further limitation of the present study is that we did not determine whether the diabetes mellitus that developed was type 1 or type 2. However, the study participants were middle-aged men; and as the condition was detected in an annual medical checkup, with relatively mild diabetes mellitus being found, it is most likely that the cases were type 2.

In conclusion, our results indicate that dietary GI is associated with the incidence of diabetes in middle-aged Japanese men. Dietary GI and pancreatic B-cell function were independently associated with the incidence of diabetes. Dietary GI is higher and pancreatic B-cell function is lower in Asian people, as compared with Western people; and these may result in a higher prevalence of diabetes in Asian populations. Our findings suggest that a low-GI diet may be beneficial in preventing type 2 diabetes mellitus in Asian people.

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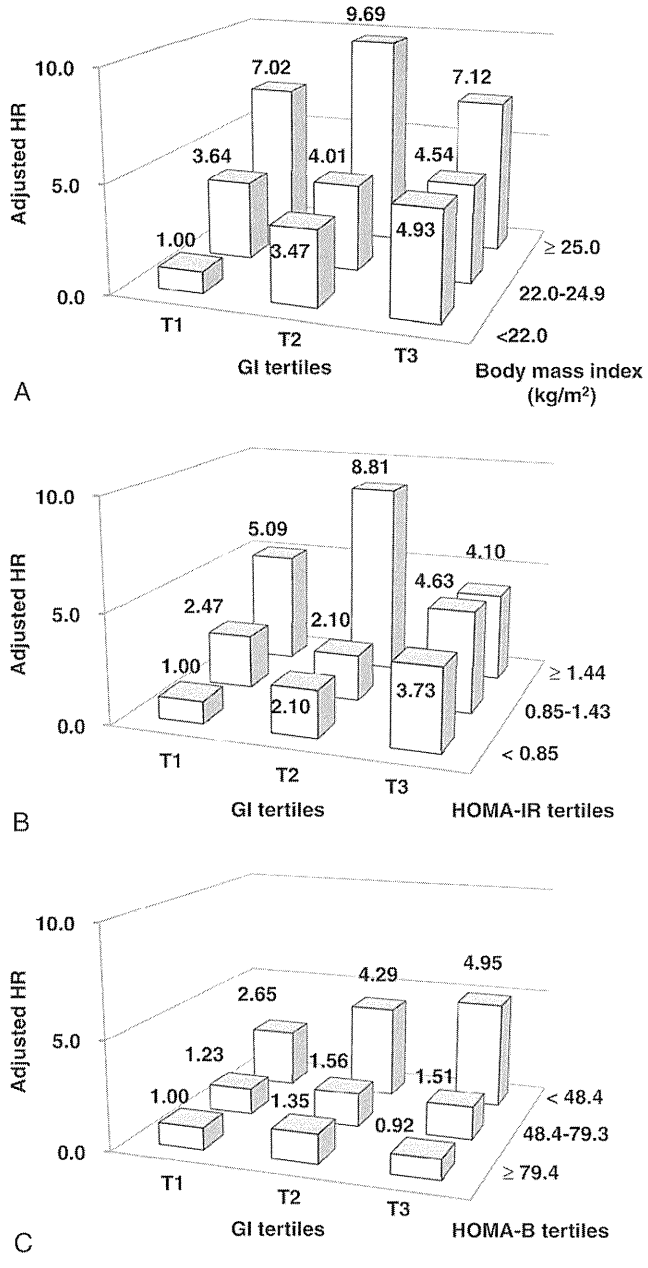

Fig. 1 – Adjusted HRs for type 2 diabetes mellitus by different levels of GI and BMI (A), HOMA-IR (B), and HOMA-B (C) in 1995 Japanese men. The HRs were adjusted for age, BMI, family history of diabetes, smoking, alcohol intake, habitual exercise, and presence of hypertension and hyperlipidemia at baseline.

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Conflict of interest disclosure

None.

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Original Article: Laboratory Investigation

Increased urethral resistance by periurethral injection of low serum cultured adipose-derived mesenchymal stromal cells in rats

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Objectives: To evaluate the effects of a periurethral injection of low serum cultured adipose tissue-derived mesenchymal stromal cells (LASC) and to develop a new autologous cell therapy for stress urinary incontinence.

Methods: F344 rats were divided into three groups as based on the periurethral injection of LASC, GAX collagen or vehicle (control). At 2 and 4 weeks after injection, leak point pressure (LPP) was measured before and after transection of the pelvic nerves. For cell tracking, LASC of green fluorescent protein transgenic rats were injected into nude rats.

Results: At 2 weeks, both the LASC and collagen groups showed significantly higher LPP than the control group. At 4 weeks, the increase in LPP in the LASC group remained, whereas LPP in the collagen group decreased to baseline levels. In the absence of the urethral closure reflex after transection of the pelvic nerves, LPP in the LASC group was significantly higher than that in the other two groups. Histologically, the size of the urethral lumen was smaller in the LASC group than the collagen group. At 4 weeks, most of the LASC were positive for myogenic antigens including α-smooth muscle actin, desmin and calponin I.

Conclusions: Periurethral injection of autologous LASC capable of myogenic differentiation made a greater contribution to the increase in urethral resistance than did the conventional collagen bulk injection. Thus, its use for treatment of stress urinary incontinence can be postulated.

Key words: mesenchymal stromal cells, muscle, regeneration.

Introduction

Injecting bulking agents is considered a less invasive procedure to treat stress urinary incontinence (SUI) compared with sling or open surgical procedures.[1] However, the therapeutic effects of injectable bulking agents, such as bovine collagen, are diminished through biodegradation over a period of 1 or 2 years and the patients often require repeated follow-up treatments.[2]

Cell therapy for the regeneration of injured tissues has recently been extensively investigated at the experimental level, and exploration of its clinical application in a variety of fields is also in progress. Mesenchymal stem cells (MSC) are multipotent adult stem cells that can proliferate in culture and differentiate into a variety of mesenchymal cell phenotypes.[3–7] Thus far, MSC have primarily been harvested from the bone marrow, a tissue source that has many limitations. These include donor-site morbidity in the bone marrow, which limits the amount of marrow that can be obtained.[8,9] In contrast, adipose tissue contains multipotent cells that are similar to MSC,[10,11] and the number of stem cells in adipose tissue is 100-fold higher than that in the bone marrow. This finding has generated significant interest, because unlike bone marrow cells, adipose tissue can be easily and safely harvested in large quantities with minimal morbidity. We have recently developed a system for expanding a unique preparation of adipose derived stem cells (ASC) by culturing them in a low serum medium.[12] ASC cultured in low serum medium (LASC) proliferate rapidly and secrete high levels of cytokines, including hepatocyte growth factor (HGF), which is known to promote muscle regeneration.

It was suggested that processed lipoaspirate (PLA) cells injected into the urinary tract show morphological and phenotypic evidence of smooth muscle incorporation and differentiation over time.[13] Imamura *et al.* have reported that bone marrow derived MSC differentiated into smooth muscles in the rat urinary bladder.[14,15] More recently, Lin *et al.* showed the therapeutic potential of adipose-derived stem cells in SUI, but whether or not the effects were greater

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than the conventional collagen injection remains to be clarified.[16] In the present study, we assessed the effect of periurethral injection of LASC on urethral function and compared the effects with collagen injection.

Methods

Animals

Seven-week-old male F344 rats ($n = 3$) and female F344 rats ($n = 46$), 7-week-old female F344/N-rnu/rnu rats (nude rat; $n = 6$) and 7-week-old male SD Tg (CAG-EGFP) rats (green rat; $n = 3$) were purchased from CLER Japan, Tokyo, Japan. All experiments were carried out in accordance with the Animal Experimentation Guidelines of Nagoya University Graduate School of Medicine.

Preparation of LASC and injection of vehicle, collagen and LASC

The isolation and expansion of LASC were carried out as previously described.[12] Briefly, inguinal adipose tissue was taken from male F344 rats or green rats, and stromal vascular fraction was prepared. LASC were cultured under a low serum medium containing 2% fetal bovine serum (FBS; ICN Biomedical, Aurora, OH, USA) and 10 ng/mL human FGF-2 (Peprotech, Rocky Hill, NJ, USA). LASC at passage 5 were used in the present study.

The female F344 rats ($n = 3$) were anesthetized with pentobarbital. A low midline incision was made to expose the urethra, and 20 μL of LASC (obtained from male F344 rats) was suspended in Dulbecco's modified Eagle's medium (DMEM; 3×10^6 cells per 20 μL), 20 μL of DMEM (vehicle) or 20 μL of collagen (GAX-collagen 10 μL + DMEM 10 μL) was injected into the proximal urethra of female F344 rats (each $n = 14$) using a microsyringe through a 29-gauge needle. Urethral resistance was measured at 2 weeks (each $n = 6$) and at 4 weeks (each $n = 6$), followed by urethral sample collection. Similarly, 3×10^6 green fluorescent protein (GFP)-positive LASC obtained from male green rats (GFP transgenic rats; $n = 3$) were suspended in DMEM and injected into the proximal urethral wall of nude rats ($n = 3$). Nude rats injected with DMEM only ($n = 3$) served as the control. Urethral tissues were taken for histological study at 4 weeks.

Measurement of urethral resistance

Urethral resistance was evaluated by measuring leak point pressure (LPP) according to the method previously reported.[17] After rats were anesthetized with urethane (Sigma-Aldrich, Tokyo, Japan) inhalation, the spinal cord was transected at the T8–T9 level. Under these conditions, it has been reported that supraspinal reflex voiding is eliminated, whereas urethral reflexes induced by bladder distention are preserved. The LPP was measured before and after bilateral transection of the pelvic nerves near the internal iliac vessels, which eliminated vesicourethral reflexes.

Histology and immunohistochemistry

After measuring LPP, tissue samples were taken from the F344 rats injected with LASC, collagen or medium only. They were fixed in 10% formalin, embedded in paraffin, cut along the transverse plane, and hematoxylin–eosin (HE) and Masson's Trichrome (MT) staining were carried out. The areas of bulking mass were measured using MetaMorph version 6.3 (Universal Imaging, West Chester, PA, USA).

In order to obtain samples with better preservation, LPP measurement was not carried out when the urethra samples were taken from another set of four female F344 rats at 4 weeks after injection of male F344 LASC. The tissue was cut along the sagittal plane, fixed in 10% formalin and embedded in paraffin. The serial sections were stained for α-smooth muscle actin (α-SMA; 1:100, mouse monoclonal; Progen Biotechnik GmbH, Heidelberg, Germany), desmin (1:50, rabbit monoclonal; Progen Biotechnik GmbH), calponin I (1:100, goat polyclonal; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and Ki67 (1:500, mouse monoclonal; Santa Cruz Biotechnology), as described.[18] The secondary antibodies were peroxidase-conjugated rabbit anti-mouse IgG, goat anti-rabbit IgG or rabbit anti-goat IgG (Nichirei, Tokyo, Japan).

In the cell tracking experiments, the urethral samples were taken from nude rats ($n = 6$) injected with GFP-positive LASC at 4 weeks, and cut along the transverse plane. One part of the sample was frozen in OCT compound (Sakura Finetek USA, Torrance, CA, USA), and the sections were stained with anti-α-SMA antibody (Progen Biotechnik) followed by rhodamin-conjugated rabbit anti-mouse IgG antibody (Sigma-Aldrich) or with anti-desmin antibody (Abcam, Cambridge, MA, England) followed by rhodamin-conjugated goat anti-rabbit IgG (Sigma-Aldrich) or with mouse antiproliferating cell nuclear antigen (PCNA) antibody (Thermo, Tokyo, Japan) followed by rhodamin-conjugated rabbit anti-mouse IgG (Sigma-Aldrich). The other part of the tissue was fixed in 10% formalin, embedded in paraffin and the serial sections were stained with anti-GFP antibody (Abcam) followed by peroxidase-conjugated rabbit anti-goat IgG (Nichirei) or with anti-α-SMA antibody followed by peroxidase-conjugated goat anti-mouse IgG antibody. Paraffin embedded tissue samples were also stained with mouse anti-PCNA antibody (Thermo) followed by peroxidase-conjugated goat anti-mouse IgG (Nichirei).

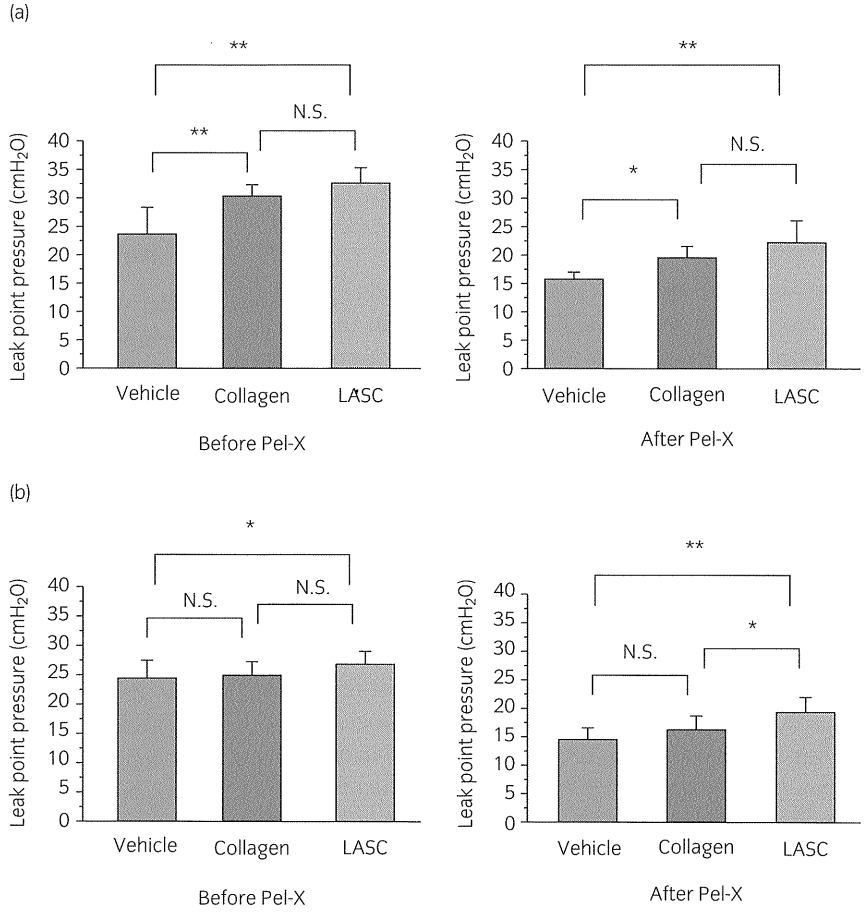

Fig. 1 Leak point pressure (LPP) measured at 2 and 4 weeks. LPP were measured in three groups of rats injected with vehicle, collagen or low serum cultured adipose tissue-derived mesenchymal stromal cells (LASC). (a) At 2 weeks, LPP in the collagen and LASC groups were significantly higher than that in the vehicle group, both before and after pelvic nerve transection (Pel-X). LPP in the LASC group tended to be higher than that in the collagen group, although the difference was not significant. (b) At 4 weeks, LPP in the collagen group decreased to a level not different from that in the vehicle group, both before and after pelvic nerve transection (Pel-X), whereas LPP in the LASC group remained higher. $*P < 0.05$; $**P < 0.01$. N.S., not significant.

Statistical analyses

Statistical analyses were carried out using Stat View 5.0 (SAS Institute, Cary, NC, USA). The Student's *t*-test was carried out to determine significant differences among the two groups. One-way analysis of variance (ANOVA) was used to determine significant differences among the three groups. When a statistical difference was shown by ANOVA, further analysis was carried out using Fisher's exact test to determine differences between a pair of groups. Significant differences were defined as *P*-values of <0.05. All values provided are means ± SEM.

Results

Effects of periurethral injection of collagen and LASC on urethral sphincteric function

LPP were measured 2 and 4 weeks after injection of the vehicle, collagen or LASC into the proximal urethra. At 2 weeks (Fig. 1a), before transection of the pelvic nerve, LPP in the collagen and LASC groups were significantly higher than that in the vehicle group. The mean LPP in the LASC group was higher than that in the collagen group; however, the difference was not statistically significant. LPP were repeatedly measured after cutting the pelvic nerves bilaterally in order to clarify urethral basal resistance without urethral closing reflexes.[17] Without the urethral reflex, LPP in the collagen and the LASC groups were higher than that in the vehicle group. Again, LPP tended to be higher in the LASC group than in the collagen group, although no significant differences were observed between the two groups.

At 4 weeks (Fig. 1b), both before and after pelvic nerve resection, LPP did not differ between the collagen and vehicle groups, whereas LPP in the LASC group remained significantly higher than that in the vehicle group both before and after the pelvic nerve resection. Without the urethral closure reflex, LPP in the LASC group were significantly higher than that in the collagen group.

We then compared the LPP measured after pelvic nerve transection at 2 weeks and those measured at 4 weeks. In the vehicle group, the value was not different (14.8 cmH_20 at 2 weeks and 13.9 cmH_20 at 4 weeks, $P = 0.239$). Although the difference was not significant, the LPP decreased by 15.7% (18.5 cmH_20 at 2 weeks and 15.6 cmH_20 at 4 weeks, $P = 0.059$) in the collagen group and by 11.8% (21.1 cmH_20 at 2 weeks and 18.6 cmH_20 at 4 weeks, $P = 0.137$) in the LASC group (Fig. 1).

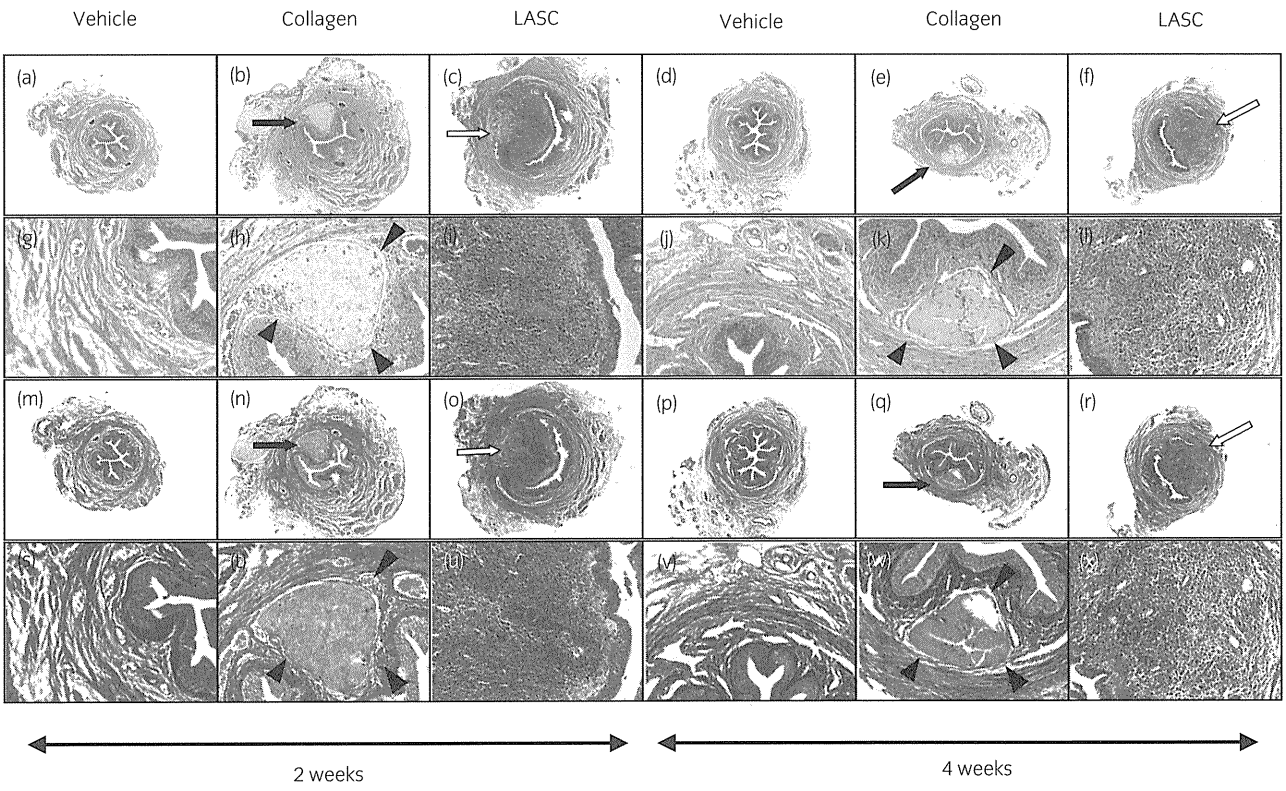

Fig. 2 Histology of the urethra. Representative photographs of the urethra taken at (a–c, g–i, m–o, s–u) 2 weeks and (d–f, j–l, p–r, v–x) 4 weeks after periurethral injection of (a,d,g,j,m,p,s,v) a vehicle, (b,e,h,k,n,q,t,w) collagen or (c,f,i,l,o,r,u,x) low serum cultured adipose tissue-derived mesenchymal stromal cells (LASC). (a–l) Hematoxylin–eosin and (m–x) Masson's Trichrome staining were carried out. Black arrows indicate collagen and white arrows indicate the mass composed of LASC. Arrow heads indicate absorption of the collagen. (a–f, m–r) Magnification: ×50. (g–l, s–x) Magnification: ×200.

In order to examine the mechanisms contributing to the increased urethral resistance, subtraction of the LPP after the pelvic nerve transection from those before the transection was made in each group of rats. We found that there was no significant difference among the subtracted values of LPP when we compared the three groups (Table 1), showing that the major factor of LASC injection is the bulking effect.

Despite the increase in urethral resistance, urinary excretion was observed and uroschesis did not develop in any group of rats.

Table 1 Subtraction of leak point pressures

	Vehicle	Collagen	LASC
2 Weeks	8.93 ± 5.38	11.8 ± 3.72	11.44 ± 5.18
4 Weeks	10.94 ± 3.55	9.39 ± 2.08	8.86 ± 3.13

The subtraction of leak point pressures after the pelvic nerve transection from those before the transection are shown (mean ± SD, cmH2O). No difference was observed among the three groups at 2 and 4 weeks. Vehicle indicates control rats treated with vehicle, collagen indicates rats injected with collagen, and LASC indicates rats injected with low serum cultured adipose tissue-derived mesenchymal stromal cells.

Histology of the urethra

Figure 2 shows the histological findings at 2 and 4 weeks after periurethral injection of the vehicle, collagen or LASC. In the rats that were injected with collagen, the urethral lumen was compressed by the collagen mass located between the transitional epithelium and the muscle layer at 2 weeks (Fig. 2b,h,n,t). At 4 weeks, the volume of the collagen mass decreased (Fig. 2e,q), showing its absorption (Fig. 2k,w). In the rats that were injected with LASC, the urethral lumen was highly compressed by a bulking mass protruding into the urethral lumen at 2 weeks (Fig. 2c,i,o,u); this persisted even at 4 weeks (Fig. 2f,l,r,x).

Size of the bulking mass in the urethra

The size of the bulking mass was analyzed by measuring the area of collagen or nodule composed of LASC on HE sec-

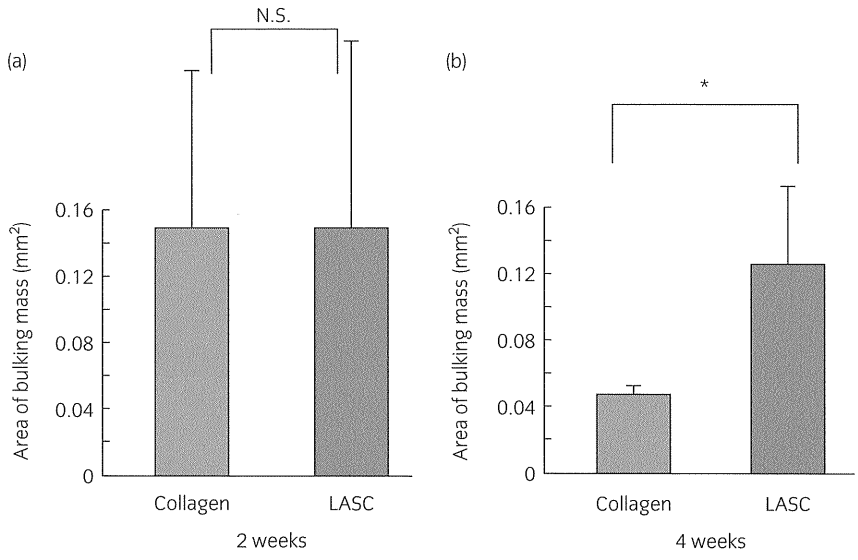

Fig. 3 The size of the mass in the urethra. The area of the bulking mass in the collagen and low serum cultured adipose tissue-derived mesenchymal stromal cells (LASC) groups was measured using MetaMorph. At 2 weeks, no significant difference was observed between the two groups. (a) At 2 weeks, the size of the mass in the collagen group did not differ from that in the LASC group. (b) At 4 weeks, the mass in the LASC group was larger than that in the collagen group. $*P < 0.05$. N.S., not significant.

tions using MetaMorph. At 2 weeks, the size of the mass in the collagen group did not differ from that in the LASC group (Fig. 3a). At 4 weeks, the size of the bulking mass in the collagen group was significantly decreased compared with that in the LASC group (Fig. 3b).

From F344 rats

LASC taken from male F344 rats were injected into the urethra of female F344 rats, and the staining for myogenic antigens was carried out on the urethral tissues taken at 4 weeks. Most of the cells at injected sites were positive for α-SMA (Fig. 4c,f), desmin (Fig. 4g,j) and calponin I (Fig. 4h,k). In contrast, staining for Ki67 was not observed (Fig. 4i,l), suggesting no proliferation of injected LASC.

Fate of LASC from green rats after injection into the urethra of nude rats

In order to clarify the fate of the transplanted cells, the LASC taken from GFP transgenic rats were injected into the proximal urethra of the nude rats, and the tissues were analyzed by immunohistochemistry. At 4 weeks after the periurethral injection, GFP positive cells were observed in the bulking mass in the urethra (Fig. 5a,c). α-SMA positive cells were also detected in the bulking mass (Fig. 5b,d), and double staining showed that a significant number of GFP positive cells had merged with α-SMA positive cells (Fig. 5e,f,g), suggesting that the LASC had differentiated into cells in the myogenic lineage. PCNA staining showed that almost no GFP positive cells were proliferating (data not shown).

Discussion

Using the low serum culture system,[12] 1×10^9 LASC, an amount sufficient for more than 10 cell therapies, can be obtained from 1 g of adipose tissue within 4 weeks of culture. Recently, Lin *et al.* reported the results of a cell therapy for SUI using adipose tissue-derived stem cells (ADSC).[16] However, they did not compare the effects of ADSC with the conventional collagen injection. Furthermore, LASC might have an advantage over ADSC, as LASC secrete higher levels of cytokines, such as vascular endothelial growth factor and HGF, which promote tissue regeneration, than ASC cultured in a high serum medium (HASC).[12]

In the present study, we examined the effects of periurethral injections of LASC cultured in low serum medium compared with GAX collagen and explored the potential of LASC as an alternative stem cell source for the treatment of SUI. The results showed that LASC increased urethral resistance during elevation of intravesical pressure. Of note, the effects of LASC persisted for a longer period than those of the GAX collagen injection. Our data are consistent with previous histological findings of Yokoyama *et al.*[12] that only a minimal volume of collagen was detectable in the urethra at 30 days after injection.[19] The present study shows that the bulking effect provided by cultured LASC persists unlike that of collagen, which was absorbed over time.

When intravesical pressure rises during an increase in intra-abdominal pressure, the urethral reflex normally closes the sphincter to prevent urine leakage.[20] Therefore, two mechanisms to increase urethral resistance by periurethral LASC injection are conceived:[21] the elevation of baseline urethral tone, and/or[22] the enhancement of urethral closure through the urethral reflex. To clarify this point, the effects of LASC injection were examined after transection of the pelvic nerves (to create reflex-free conditions) based on the phenomenon that bilateral transection of pelvic nerves prevents urethral closure caused by the urethral reflex.[17] As a result, LASC injection significantly increased LPP, showing increased baseline urethral resistance in the absence of the

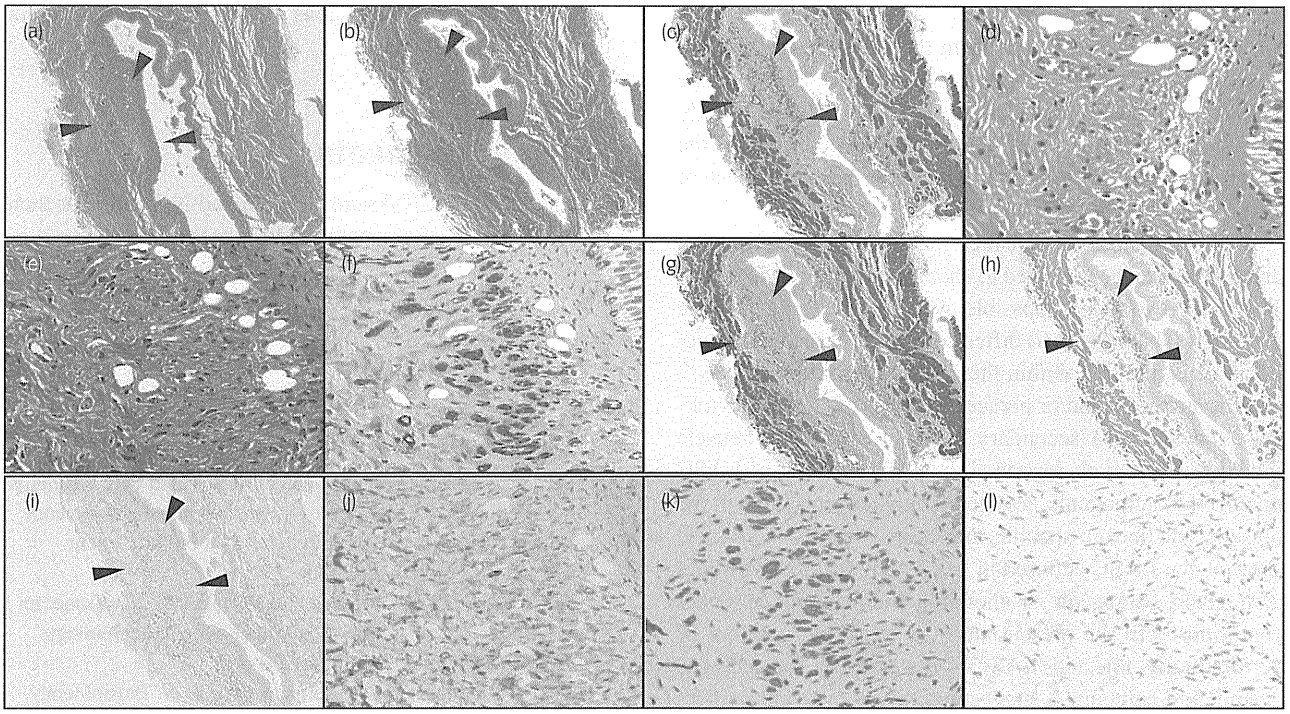

Fig. 4 Immunohistochemistry of the urethra from F344 rats. Representative photographs of immunohistochemistry using the urethral tissues of female F344 rats taken at 4 weeks after periurethral injection of male F344 low serum cultured adipose tissue-derived mesenchymal stromal cells (LASC). (a,d) Hematoxylin–eosin and (b,e) Masson's Trichrome staining show the mass composed of LASC. Positive staining was myogenic antigens, including (c,f) α-SMA, (g,j) desmin and (h,k) calponin I. (i,l) No staining was observed in the sections stained for Ki67. Arrow heads indicate the mass composed of LASC. (a–c, g–i) Magnification: ×50. (d–f, j–l) Magnification: ×200.

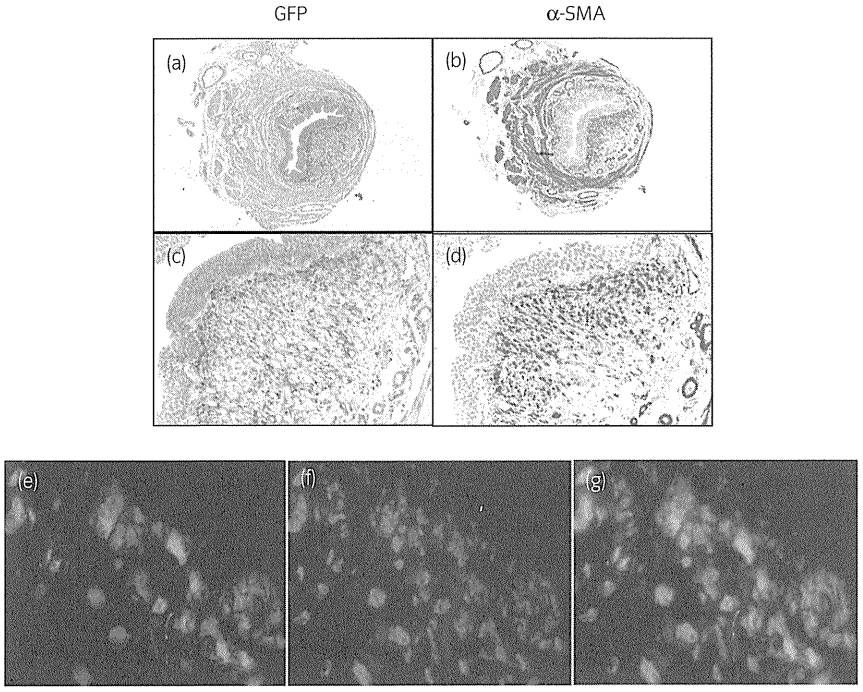

Fig. 5 Green fluorescent protein (GFP) positive low serum cultured adipose tissue-derived mesenchymal stromal cells (LASC) injected into the urethra of nude rats. Representative photographs of urethras injected with LASC taken from GFP transgenic rats at 4 weeks. Serial sections from paraffin-embedded tissues show positive staining for (a,c) GFP and (b,d) α-SMA. Frozen sections from rats injected with LASC show cells positive for (e) GFP, positive for ()f α-SMA and (g) both. (a,b) Magnification: ×50. (c,d) Magnification: ×200. (e–g) Magnification: ×400.

urethral reflex. We then subtracted the values of LPP after the pelvic nerve transection from those before the transection, and found that no difference was observed between the LASC group and vehicle group. Although we cannot fully rule out the minor effects of urethral reflex enhancement, the major factor in the present study is the bulking effect of LASC.

It is reasonable to consider that elevation of baseline urethral resistance is related to changes in the urethral tissue. There are two major possible ways that LASC promote histological changes:[21] (i) differentiation of LASC into functional cells that stay within the urethra as a bulking mass;[22] and (ii) autocrine and/or paracrine effects of secreted cytokines that induce a secondary increase in urethral muscle thickness. Zuk *et al.* described the differentiation of LASC *in vitro* into adipogenic, myogenic or osteogenic cells in the presence of lineage-specific induction factors.[11,13] We also showed that LASC cultured in low serum differentiated into adipogenic, osteogenic or chondrogenic cells under proper conditions.[12] In the present study, a significant number of periurethrally injected LASC were positive for α-SMA, desmin and calponin I, suggesting *in vivo* myogenic differentiation. Further study is necessary to confirm that injected LASC had turned into smooth muscle cells and functioned as muscles in the urethral sphincter. Nevertheless, it is likely that cells differentiated from LASC are firmly fixed in the proximal urethra, thus increasing urethral resistance.

Concerning cytokine secretion, we have shown that LASC cultured in low serum medium secrete a higher level of HGF than LASC cultured in high serum medium.[12] In addition, HGF is known to be associated with promoting both proliferation and differentiation of muscle satellite cells.[23–26] Therefore, it is possible that LASC induce the regeneration of the urethral sphincter as a result of the effects of secreted HGF. However, clear evidence showing this possibility has not been obtained in the present study. A limitation of the present study was that the urethral sphincter was not damaged before LASC injection. Because it has been reported that tissue damage is necessary to promote the regeneration process, the effect of secreted HGF to stimulate growth of satellite cells in an intact sphincter will be a matter of debate. As healthy rats were used in the present study, further studies of SUI models with impaired sphincters are necessary to clarify this issue.

Concerning the possible adverse effects of LASC treatment, no rats showed urinary retention. However, there is a possibility that partial urethral obstruction might occur, resulting in increased residual urine volume after ASC injection. Further studies focusing on the adverse effects of LASC therapy, preferably in larger animals, will be needed.

In conclusion, we found that periurethrally injected autologous LASC showed myogenic differentiation and that they showed a therapeutic advantage over GAX collagen. The present study suggests that ASC cultured in low serum medium have great potential as a novel stem cell source for the treatment of SUI.

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ORIGINAL ARTICLE

Peritonitis is still an important factor for withdrawal from peritoneal dialysis therapy in the Tokai area of Japan

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Abstract

Background In Japan, the population of patients on peritoneal dialysis (PD) is <4% of the total number of patients with end-stage renal disease. Few systemic analyses have examined why the number of PD patients has not increased in Japan. We organized a registry to analyze PD patients and retrospectively investigated 561 PD patients (about 5% of all Japanese PD patients) from 13 hospitals in the Tokai area for 3 years from 2005.

Methods We investigated background, physical status, laboratory data, status of PD therapy, and the occurrence of PD-related complications, and analyzed reasons for withdrawal from PD.

Results Nutrition did not change significantly during our observation. Urinary volume showed continued decreases after the introduction period. In contrast, PD fluid demand and ultrafiltration volume were significantly increased. For calcium metabolism, multiple phosphate binders were required after the second year of PD therapy. Early dropout within 3 years after starting PD therapy comprised 50.9% of total withdrawals, with PD-related peritonitis as the most common reason, mainly caused by Gram-positive organisms. Incidence of peritonitis was 42.8 months/patient. Culture-negative results were obtained for 32% of peritonitis cultures. Diabetes affects the prognosis of PD therapy, but not the incidence of peritonitis.

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Conclusion We examined clinical status over 3 years in the Tokai area. The results suggest that the incidence of peritonitis needs to be decreased to prevent early withdrawal of PD patients. Education systems to decrease the incidence of peritonitis and techniques to decrease culture-negative results might be important for improving the prognosis of peritonitis.

Keywords Complication · Peritoneal dialysis · Peritonitis

Introduction

Although more than 290,000 patients with end-stage renal disease (ESRD) were on dialysis therapy in Japan as of the end of 2009, only 3.4% were on peritoneal dialysis (PD) [1]. To explain this small proportion of patients on PD in Japan as compared with other countries [2, 3], Kawaguchi et al. [4, 5] suggested reasons such as unwillingness and insufficient information and education among patients, a lack of education about PD among nephrologists, and a fear of encapsular peritoneal sclerosis (EPS). The total number of PD patients in Japan has not increased since 1998; however, the number of patients initiating PD annually increased to more than 1950 in 2009 [1], suggesting that approximately 2000 patients were withdrawing from PD annually. No detailed information has been available regarding why the PD population has not increased or explaining the large number of patients discontinuing PD in Japan. In addition, the incidence of PD-related peritonitis in Japan remains obscure. Previous reports have mentioned PD-related peritonitis as one of the three main reasons for withdrawal from PD therapy in Japan [6, 7]. In contrast, the latest Japanese survey of adult PD-related peritonitis in 2005 showed an extremely low rate of 1 episode in 73.5 patient-months at medical centers with more than 20 PD patients [5].

We started a registry of PD patients to clarify problems associated with PD therapy in the Tokai area. This report summarized laboratory status and showed the incidence of PD-related complications over the course of 3 years. The total number of PD patients in our database was 561, representing approximately half of the PD patients in the Tokai area, and approximately 5% of all PD patients in Japan. Surprisingly, we observed that over 50% of withdrawals from PD therapy occurred within 3 years after PD introduction, even though the total number of PD patients was gradually increasing year by year in this area. We therefore focused on reasons for withdrawal, particularly regarding peritonitis, in our area. The present survey may provide insights into why the Japanese ESRD population on PD has not increased in line with the increase in the total number of ESRD patients in Japan.

Methods

Preparation to construct the database

Data for this cohort study were collected retrospectively. Thirteen of 18 institutes with PD patients and affiliated with the Nagoya University Renal Replacement Therapy Association accepted the invitation to join a registry. This registration system for PD patients was thus constructed for 13 institutions, comprising a university hospital, city hospitals, dialysis centers and satellite clinics in the Tokai area of Japan. The registration process to construct the database and analyze data was approved by the ethics committees from each of these institutions. All patients in each approved institution agreed to join the registry. Institutions and numbers of PD patients are shown in Table 1. In our registration system, patients on PD therapy starting between 2005 and the end of 2007 were registered. Of note, only one specialist PD nurse was working full-time at Nagoya University Hospital to support PD patients at any of the 13 institutions.

Details of the database

Collection of data from PD patients was performed in the introduction period (1–3 months after starting PD therapy) and every 12 months thereafter. The profile list for our database system is shown in Table 2. We also investigated causative microorganisms for PD-related peritonitis and analyzed risk factors such as age, device, gender, use of continuous ambulatory PD (CAPD) or automated PD (APD), presence of diabetes mellitus (DM), and use of the classical insertion technique (non-embedding) or Moncrief–Popovich technique (embedding) [8] as methods of PD catheter placement.

In the 317 patients who started PD between 2005 and 2007 (incidence of PD therapy), we investigated technical survival between DM ($n = 135$) and non-DM ($n = 173$)

Table 1 Number of peritoneal dialysis (PD) patients in the 13 institutions from 2005 to 2007

Year	Number of patients			
	<24	25–49	50–99	≥ 100
2005	9	3	1	0
2006	7	4	2	0
2007	5	6	2	0

The 13 institutions (11 institutions in Aichi Prefecture, 1 institution in Mie Prefecture, and 1 institution in Gifu Prefecture) were as follows: Nagoya University Hospital, Handa City Hospital, Minami Seikyo Hospital, Ogaki Kita Clinic, Yokkaichi Municipal Hospital, Anjo Kosei Hospital, Nagoya Kyoritsu Hospital, Tosei General Hospital, Chubu Rosai Hospital, Toyota Kosei Hospital, Daiyukai Daiichi Hospital, Kasugai Municipal Hospital, and Konankousei Hospital

Table 2 Profile lists of the database system in the present study

Major contents	Subgroups
1. Background of patients	
	1. Age
	2. Gender
	3. Renal disease causing end-stage renal disease
2. Incidence and prevalence of peritoneal dialysis (PD) patients	
3. Reasons for withdrawal from PD therapy,	
4. Duration of PD therapy and differences between diabetes mellitus (DM) and non-DM patients	
5. Nutritional condition	
	1. Body mass index
	2. Serum albumin
	3. Total cholesterol level
6. Anemia state	
	1. Blood hemoglobin level
	2. Blood hematocrit
	3. Monthly erythropoietin dose
7. Renal function	
	1. Urine volume
	2. Blood urea nitrogen level
	3. Serum creatinine level
8. PD status	
	1. PD catheter insertion technique[a]
	2. Continuous ambulatory PD or automated PD
	3. PD with sterile connecting devices to change PD fluid (PDF) bags or without (manual)
	4. Daily PDF doses
	5. Dialysate-to-plasma creatinine concentration ratio[b]
8. Cardiovascular status	
	1. Systemic blood pressure
	2. Cardiothoracic ratio
9. Relationship of calcium metabolism	
	1. Serum calcium
	2. Serum phosphate
	3. Intact parathyroid hormone
	4. Doses of phosphate binders ($CaCO_3$ and/or sevelamer hydrochloride)
10. Complications associated with PD	
	1. Occurrence of infectious peritonitis
	2. Occurrence of obstruction of the catheter wrapped in omentum
	3. Occurrence of hydrothorax due to defects in the diaphragm
	4. Occurrence of inguinal hernia (diameter, >3 cm), or abdominal wall herniation including umbilical hernias (diameter, >3 cm) [27]

[a] PD catheter insertion technique is categorized into the traditional PD technique (non-embedding) or the Moncrief–Popovich technique for embedding the PD catheter (embedding)

[b] Dialysate-to-plasma creatinine concentration ratio as determined by a fast peritoneal equivalent test [28], as a marker of peritoneal membrane function

for 3 years, after excluding withdrawals due to renal transplantation ($n = 8$) or religious reasons ($n = 1$). We also analyzed differences in factors such as age, device, gender or usage of icodextrin-based PD fluid (PDF) within 1 year, from 1 to 2 years, and from 2 years to 3 years after starting PD therapy between DM and non-DM patients.

Peritonitis was diagnosed according to the guidelines of the International Society for Peritoneal Dialysis (ISPD) [9, 10].

Notably, the number of patients receiving combined PD therapy in the present study was 12 (only 2.1% of the total PD population), substantially below the value of around 20% reported in the recent JSTD survey. The mean period of combined PD therapy was limited to 16.8 months/patient in these 12 patients. Given this low number of PD patients receiving combined therapy, we could not perform separate statistical analyses of those data in this study.

Statistical analysis

Occurrence of peritonitis was compared by logistic regression analysis between genders, DM or non-DM, device, insertion methods of PD catheter, and CAPD or APD. The Kaplan–Meier method was used to analyze differences between DM and non-DM for withdrawal from PD and patient survival, following log-rank analysis. Among incidental PD patients, the F test was performed for age and chi-squared tests were performed for factors of gender, device, and usage of icodextrin-based PDF within 1 year after starting PD therapy, for comparisons between DM and non-DM patients. All values are shown as mean ± standard deviation (SD). Values of $p < 0.05$ were considered significant. Analyses were performed using SPSS II for Windows software (SPSS, Tokyo, Japan).

Results

Background and basal characteristics of PD patients

The total number of PD patients, mean age, gender and pathology underlying ESRD are shown in Table 3. When we categorized methods of PD catheter placement, non-embedding was seen in 66.3% and embedding in 33.7%. Both the incidence of PD patients and prevalence gradually increased from 2005 to 2007 (Fig. 1a). In total, withdrawal was seen in 174 of the 561 PD patients. The number of withdrawals from PD increased in the second year, but was slightly decreased in the third year compared with the first year (Fig. 1a). Reasons for withdrawal included transfer to hemodialysis (HD) in 51.1%, death in 39.7%, renal transplantation in 6.9%, and other reasons in 2.3%. Mean period of PD therapy until withdrawal was 46.7 ± 38.4 months. Details for causes of death and transfer to HD are shown in Tables 4 and 5, respectively.

During our survey, over half of withdrawals withdrew within 3 years of starting PD ('early withdrawal'; Fig. 1b). The number of withdrawals for each duration of PD therapy is shown in Fig. 1c. The most common reason for early withdrawal was PD-related peritonitis (20.7%), followed by sudden death (12.6%), and social problems such as lack of family support and dissatisfaction with the PD therapy procedure (11.5%). Other reasons for early withdrawal included infection (10.3%), cerebrovascular disease (8.0%), renal transplantation (6.9%), and malignancy (4.6%).

As reasons for withdrawal from PD therapy, frequency of peritonitis was still high 5 years after PD introduction. Frequency of dialysis failure (or inadequate dialysis)/ ultrafiltration failure increased with the duration of PD therapy (Fig. 1d).

Table 3 Basic characteristics of 561 patients on peritoneal dialysis

Contents	*n* (%)
Total number of patients	561
Gender	
Male	360 (64.2)
Female	201 (35.8)
Age (years)	60.0 ± 13.1
Cause of end-stage renal disease	
Chronic glomerulonephritis	230 (41.0)
Diabetic nephropathy	191 (34.0)
Nephrosclerosis	88 (15.7)
Polycystic kidney	9 (1.6)
Chronic pyelonephritis	4 (0.7)
Post renal failure	3 (0.5)
Interstitial nephritis	3 (0.5)
Fabri disease	2 (0.4)
Post acute renal failure	2 (0.4)
Malignant hypertension	2 (0.4)
Other	9 (1.6)
Unknown	18 (3.2)

When we focused on withdrawals among the 308 incidental PD patients from the start of January 2005 to the end of December 2007, 13.8% withdrew within 12 months after PD introduction, 24.7% within 2 years, and 39.3% within 3 years. Withdrawal of incidental PD patients was significantly worse among DM patients than among non-DM patients (Fig. 2), although gender, age and device did not differ significantly between DM and non-DM patients among the incidental PD patients (data not shown). Within 2 years after starting PD therapy, the necessity for icodextrin-based PDF was significantly higher in DM patients than in non-DM patients ($p < 0.0001$ for year 1, $p < 0.005$ for year 2). From 2 to 3 years after starting PD therapy, the necessity for icodextrin-based PDF in DM patients tended to remain higher than in non-DM patients, but this result was not significant ($p = 0.095$).

Laboratory status, urine volume and PD status in the registry

Most aspects of laboratory status did not differ from those of the introduction period for PD therapy (Fig. 3). Usage for erythropoietin was slightly increased after the seventh year compared with the introduction period (Fig. 3f). Blood urea nitrogen and serum potassium levels showed little difference over the course of 8 years compared with the PD introduction period (Fig. 4). Serum creatinine level increased significantly year by year. In contrast, urine

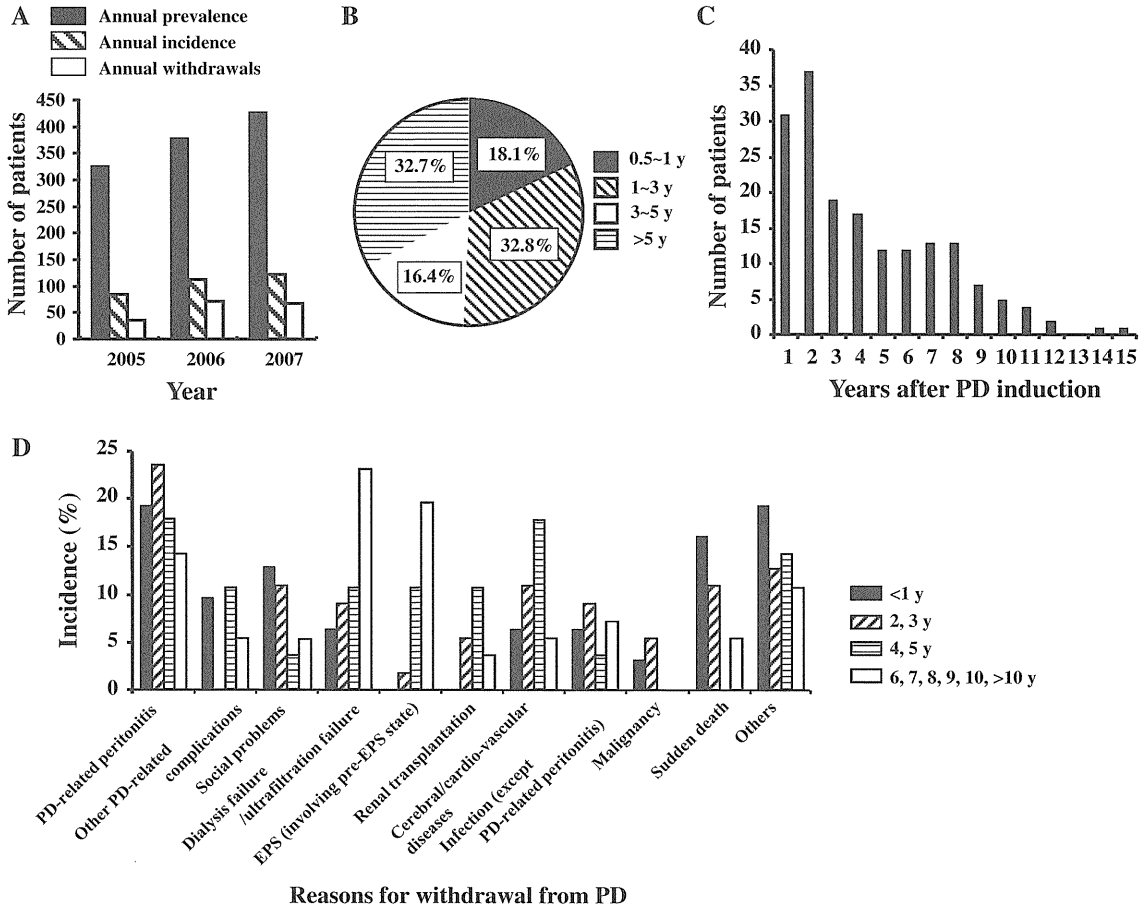

Fig. 1 Prevalence, incidence, and withdrawal in peritoneal dialysis (*PD*) patients and reasons for withdrawal from 2005 to 2007. **a** Prevalence, incidence and withdrawal in PD patients from 2005 to 2007. **b**, **c** Duration of PD therapy until withdrawal. **d** Reasons for withdrawal from PD therapy

Table 4 Cause of death among peritoneal dialysis (PD) patients from 2005 to 2007

Cause	*n* (%)
Infection (without PD-related peritonitis)	14 (20.3)
Cardiac disease	9 (13.0)
Sudden death	14 (20.3)
PD-related peritonitis	7 (10.1)
Intestinal bleeding perforation	4 (5.8)
Cerebrovascular disease	7 (10.1)
Malignancy	4 (5.8)
Others	10 (14.5)
Total	69

Table 5 Reason for transfer to hemodialysis for 89 patients on peritoneal dialysis (PD) from 2005 to 2007

Reason	*n* (%)
Peritonitis	24 (27.0)
PD-related complications other than peritonitis	9 (10.1)
Social problems	14 (15.7)
Dialysis failure/UF failure	19 (21.3)
EPS/prevention of EPS	17 (19.1)
Others	6 (6.7)

UF failure ultrafiltration failure, *EPS* encapsular peritoneal sclerosis

volume (UV) decreased year by year. Significant increases in ultrafiltration volume and usage of PDF were observed over the course of the 8 years. Dialysate-to-plasma creatinine concentration ratio (D/P Cre) showed no significant changes over 8 years.

Analysis of calcium metabolism and treatment with activated vitamin D and phosphate binders

Each year, serum levels of calcium and phosphate tended to show mild increases (Fig. 5a, b). Significant decreases in serum intact parathyroid hormone (iPTH) levels were observed in the second, third and fourth years compared

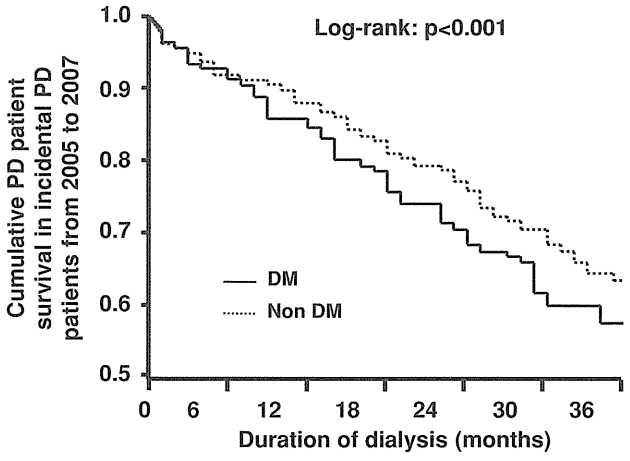

Fig. 2 Patient technical survival on peritoneal dialysis (*PD*) therapy. Cumulative patient survival in incidental PD patients (excluding withdrawal due to renal transplantation, movement to other medical institutions or religious reasons) from 2005 to 2007

with the introduction period (Fig. 5c). Dose of activated vitamin D showed no significant changes during the 8 years compared with the introduction period (Fig. 5d). In terms of the usage of phosphate binders, dose of calcium carbonate was increased in the first, second and third years. In contrast, dose of sevelamer hydrochloride was increased after the second year (Fig. 5f).

Incidental PD-related peritonitis and causative microorganisms

Since peritonitis represented a major reason for withdrawal from PD therapy, we conducted further analyses of PD-related peritonitis. For the 3 years of the study, mean incidence of peritonitis was 1 episode every 42.8 patient-months. Incidence of PD-related peritonitis varied among the 13 institutions, with a minimum incidence of once in 129.3 patient-months and a maximum of once in 28.2 patient-months.

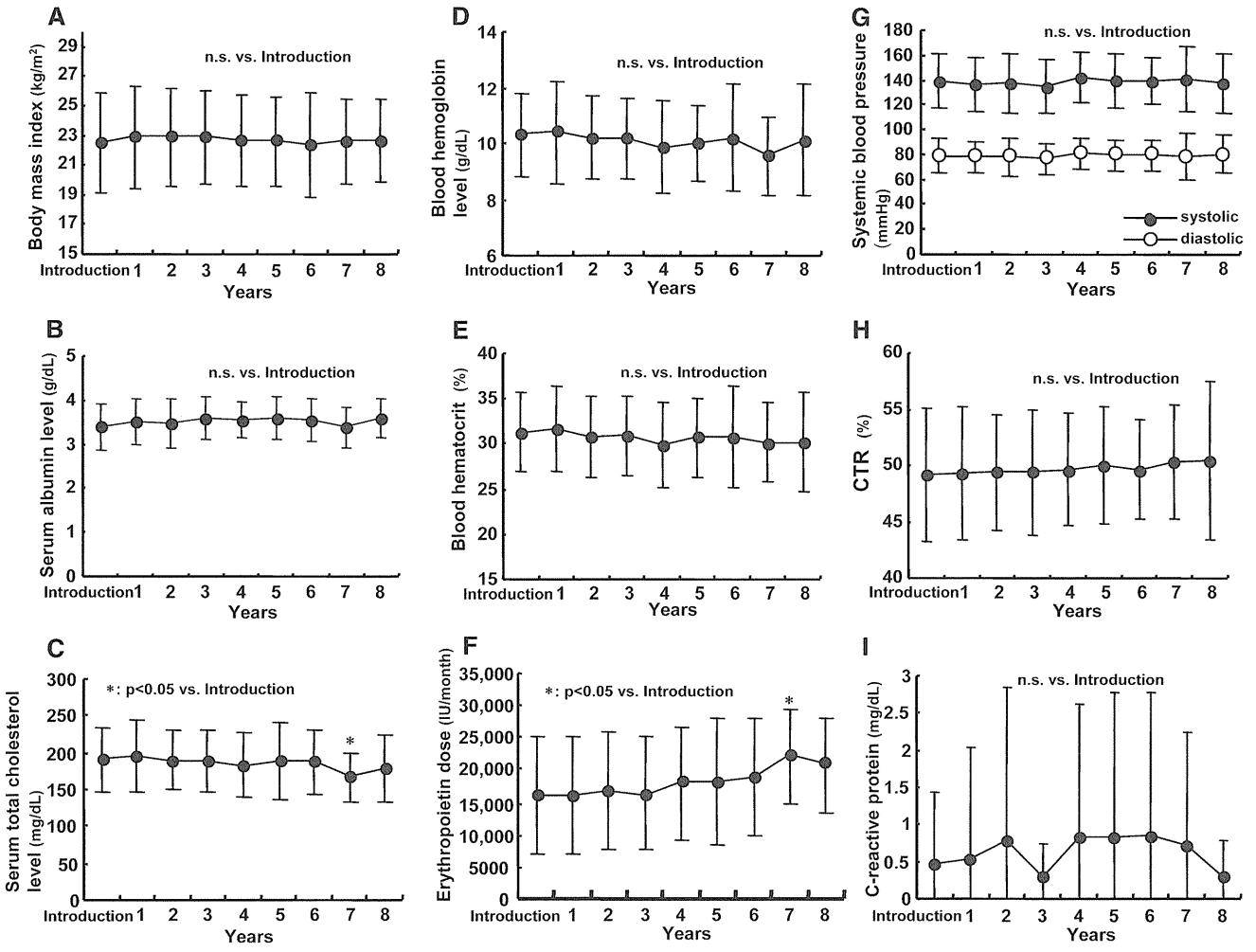

Fig. 3 Nutritional status, anemia, systemic blood pressure, cardiothoracic ratio (*CTR*), and C-reactive protein (*CRP*) level in peritoneal dialysis (*PD*) patients from 2005 to 2007. **a** Body mass index , **b** serum albumin level, and **c** cholesterol level are shown as markers of nutritional status. **d** Blood hemoglobin level, **e** hematocrit level, and **f** demand for erythropoietin are shown as markers of anemia status. **g** Systemic blood pressure. **h** CTR. **i** Serum CRP level is shown as an inflammatory marker. Each value represents mean ± SD