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Short communication

Are serum cholesterol levels associated with silent brain infarcts? The Seiryo Clinic Study

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ABSTRACT

Objective: High levels of serum cholesterol are associated with the risk of stroke. However, the association of serum cholesterol with silent brain infarcts (SBIs) is unclear. We investigated the association between SBI and various clinical factors.
Methods: We conducted a cross-sectional study that included 324 apparently healthy Japanese men (mean age 53.8 ± 9.2 years). Combinations of three types of scan (T1-weighted, T2-weighted and FLAIR images) were used to detect and discriminate SBI.
Results: Serum cholesterol was significantly associated with SBI [total cholesterol, odds ratio (OR) 3.75 (95% confidence interval (CI) 1.45–9.68); LDL-cholesterol, OR 2.54 (95% CI 1.03–6.27), and non-HDL-cholesterol, OR 2.54 (95% CI 1.03–6.27)] after adjustment for age, smoking status, serum triglycerides, maximal-intima-media thickness, obesity, hypertension, diabetes mellitus, hyperuricemia, coronary heart disease and lipid-lowering agent use.
Conclusion: Our cross-sectional data suggest that serum cholesterol levels are associated with SBI independently of known confounders.

1. Introduction

The significance of detecting silent brain infarction (SBI) and its associated factors is increasing in the primary prevention of stroke and dementia, which threaten quality of life [1]. Although increased total cholesterol (TC) levels are known to be associated with a higher risk of ischemic stroke [2], it remains unclear whether SBI is associated with serum cholesterol [1]. The aim of this study was to investigate factors associated with the development of SBI.

2. Methods

Subjects of the Seiryo Clinic Study were a cohort of apparently healthy Japanese men, aged 30–80 years who consecutively had undergone MRI of the brain as part of a health check-up between May 2000 and April 2008 in northern Japan. From the 483 persons who had MRI, measurements of maximal-intima-media thickness (maximal-IMT) and blood pressure, as well as blood tests, we excluded women ($n = 137$) and all men who had symptomatic stroke ($n = 2$), epilepsia ($n = 1$), a space-occupying lesion shown on brain MRI due to brain tumor ($n = 2$), pituitary adenomas ($n = 2$), vascular malformations ($n = 2$), venous angioma ($n = 1$), meningioma ($n = 1$), microbleeding ($n = 1$), brain contusion ($n = 1$), brain injury ($n = 1$) or serum triglyceride (TG) ≥4.52 mmol/L ($n = 8$). Ultimately, 324 men were analyzed.

Blood pressure was measured in a resting state while sitting, and body mass index (BMI) was calculated by weight [kg]/(height [m])^2. The blood test was performed under fasting conditions. Serum TC and HDL-cholesterol, trygricerides, glucose and uric acid were measured by an autoanalyzer. Serum LDL-cholesterol was calculated by the Friedewald equation [3], and non-HDL-cholesterol was determined by subtracting HDL-cholesterol from TC.

Carotid arteries were evaluated independently by two trained technicians using high-resolution B-mode ultrasonography SSA-550A (Toshiba, Tokyo, Japan). The technicians were also masked to clinical information. Four segments of the common carotid artery and of the internal carotid artery at the near and far wall on both left and right sides were examined [4] and maximal-intima-media thickness (maximal-IMT) was obtained.

The following data were obtained by a questionnaire completed by the participant: smoking habit, medication and medical history.

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Table 1
Clinical characteristics of the 324 study men according to the presence of silent brain infarcts.

	All (n=324)	With SBI (n=17)	Without SBI (n=307)	P
Age, year	53.8±9.2	63.1±7.2	53.3±9.0	<0.001
Body mass index, kg/m^2	24.6±3.1	25.4±3.4	24.5±3.1	0.28
Obesity (body mass index ≥25)	130 (40%)	8 (47%)	122 (40%)	0.55
Systolic blood pressure, mmHg	121±15	132±11	120±15	0.001
Diastolic blood pressure, mmHg	75.4±9.9	79.6±7.7	75.1±10.0	0.07
Hypertension	102 (31%)	12 (71%)	90 (29%)	<0.001
Fasting blood sugar, mmol/L	5.5±1.2	5.6±0.6	5.5±1.2	0.89
Diabetes mellitus	38 (12%)	2 (12%)	36 (12%)	1.00
Total cholesterol, mmol/L	5.19±0.80	5.32±0.81	5.18±0.80	0.50
LDL-cholesterol, mmol/L	3.48±0.77	3.57±0.73	3.47±0.78	0.59
Non-HDL-cholesterol, mmol/L	3.77±0.83	3.84±0.74	3.76±0.83	0.71
HDL-cholesterol, mmol/L	1.43±0.37	1.48±0.37	1.42±0.36	0.54
Triglycerides, mmol/L	1.45±0.75	1.33±0.56	1.45±0.76	0.50
Use of lipid-lowering agents	26 (8%)	1 (6%)	25 (8%)	0.73
Uric acid, μmol/L	357±70	378±62	356±71	0.20
Hyperuricemia	82 (25%)	7 (41%)	75 (24%)	0.14
Maximal-IMT, mm	1.44±0.75	2.03±0.63	1.41±0.74	<0.001
Maximal-IMT, top tertile (≥1.6 mm)	112 (35%)	14 (82%)	98 (32%)	<0.001
Current smoker	100 (31%)	6 (35%)	94 (31%)	0.69
Coronary heart disease	8 (2%)	2 (12%)	6 (2%)	0.06

Values are means ± S.D. or numbers (percentages). P values were obtained using the analysis of variance for continuous variables and chi-square test for categorical variables.

Table 2
Logistic regression model to investigate the clinical factors associated with the presence of silent brain infarcts according to each cholesterol levels in Japanese men.

Variables	Odd ratio (95% confidence interval)			
	Model 1	Model 2	Model 3	Model 4
Total cholesterol, per mmol/L	3.75 (1.45–9.68)**			
LDL-cholesterol, per mmol/L		2.54 (1.03–6.27)*		
Non-HDL-cholesterol, per mmol/L			2.54 (1.03–6.27)*	
HDL-cholesterol, per mmol/L				4.52 (0.97–21.0)
Triglycerides, per mmol/L	0.58 (0.19–1.73)	0.67 (0.23–1.97)	0.56 (0.18–1.76)	1.36 (0.48–3.84)
Use of lipid-lowering agent (Yes vs No)	0.54 (0.05–6.27)	0.42 (0.04–4.38)	0.42 (0.04–4.38)	0.52 (0.04–5.77)
Age, per year	1.15 (1.05–1.25)**	1.12 (1.04–1.21)**	1.12 (1.04–1.21)**	1.12 (1.03–1.21)**
Obesity (body mass index ≥25 kg/m^2 vs <25 kg/m^2)	0.98 (0.29–3.29)	0.81 (0.25–2.70)	0.81 (0.25–2.70)	1.12 (0.32–3.91)
Maximal-IMT (≥1.6 mm vs <1.6 mm) (highest tertile vs middle or lowest tertiles)	6.48 (1.33–31.6)*	5.03 (1.14–22.3)*	5.03 (1.14–22.3)*	5.51 (1.31–23.1)*
Hypertension (Yes vs No)	7.03 (1.77–28.0)**	5.46 (1.51–19.8)**	5.46 (1.51–19.8)**	3.98 (1.19–13.3)*
Diabetes mellitus (Yes vs No)	0.46 (0.07–2.83)	0.55 (0.09–3.30)	0.55 (0.09–3.30)	0.38 (0.05–2.60)
Hyperuricemia (Yes vs No)	3.46 (0.97–12.3)	3.01 (0.87–10.4)	3.01 (0.87–10.4)	2.66 (0.80–8.88)
Coronary heart disease (Yes vs No)	3.40 (0.38–30.5)	3.33 (0.42–26.2)	3.33 (0.42–26.2)	3.46 (0.38–31.5)
Current smoker (vs never and former smoker)	4.13 (1.07–15.9)*	3.15 (0.87–11.4)	3.15 (0.87–11.4)	2.60 (0.76–8.91)

Odds ratios were adjusted for all of the other covariates listed in Table 2.
* P<0.05.
** P<0.01.

No subject had a previous transient ischemic attack nor was atrial fibrillation (AF) shown by electrocardiogram.

Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg, a previous diagnosis of hypertension, or treatment with antihypertensive medications agents. Diabetes mellitus was defined as fasting blood sugar ≥126 mg/dL (7 mmol/L), a previous diagnosis of diabetes mellitus or the use of antidiabetic agents. Hyperuricemia was defined as uric acid ≥7 mg/dL (416 μmol/L), use of medication for hyperuricemia or a history of hyperuricemia. Smoking status was defined as current smoker and past or never smoker. Coronary heart disease was defined as a history or the presence of myocardial infarction or angina pectoris.

SBI and other brain lesions were assessed by a SIGNA 1.5T MRI scanner (GE Healthcare, Waukesha, WI) with T1-weighted, T2-weighted and FLAIR (fluid attenuated inversion recovery) scans with an axial AC-PC line, 5-mm slice thickness and a 2-mm interslice gap. We defined SBI according to the criteria of the Japanese Society for the Detection of Asymptomatic Brain Disease [5]: namely, a focal hyperintensity area ≥2 mm in diameter on T2-weighted images and low intensity on T1-weighted images. We also used a combination of the three types of scan to discriminate SBI from white matter lesions [6] or dilated perivascular spaces. Two board-certified radiologists who were masked to clinical data independently assessed the images. Final decisions were made by including two or more radiologists for consensus.

To examine the potential risk factors associated with SBI, logistic regression analysis was performed by entering all possible confounders determined by biological measurements and results of the questionnaire as explanatory variables. A two-sided P value of less than 0.05 was statistically significant. All analyses were performed with SPSS (Ver.15.0J for Windows, Chicago, IL) software. The institutional review board of this institution approved this study.

3. Results

Seventeen participants (5.2%) had SBI. A total of 31 infarcts were detected, with locations in the basal ganglia (77%,) subcortical area (17%), and cerebellum (6%). No infarcts were noted in the cortical area.

Table 1 shows baseline characteristics of participants with and without SBI. Participants with SBI were significantly older and had higher blood pressure and maximal-IMT values than those without SBI. Odds ratios (OR) of increased risk of SBI per unit IMT, SBP

and DBP were 2.09 (95% confidence interval (CI) 1.30–3.35, *P* value 0.002), 1.05 (95% CI 1.02–1.09, *P* value 0.002), and 1.07 (95% CI 0.99–1.09, *P* value 0.074), respectively, by univariate analysis.

Results of logistic regression analyses to examine the possible factors associated with SBI are shown in Table 2. Serum cholesterol (TC, LDL and Non-HDL) levels were significantly associated with SBI (*P* value, 0.006 for TC; 0.042 for LDL-cholesterol; 0.042 for non-HDL-cholesterol) after adjustment for age, smoking status, triglycerides, obesity, highest tertile of maximal-IMT (≥1.6 mm) and history of hypertension, diabetes mellitus, hyperuricemia, coronary heart disease and use of lipid-lowering agents in the multivariate analysis (Models 1, 2 and 3). There was a tendency for a positive association of HDL-cholesterol with SBI (*P* value, 0.06) (Model 4).

4. Discussion

Our results showed an independent association between serum cholesterol levels and SBI after controlling for classical risk factors and IMT. This supports results of a recent study [7]; however, most previous studies have not indicated a possible association between cholesterol values and the presence of SBI [1,8,9].

Hypertension is known to be strongly associated with SBI [1]. We also found significant association between hypertension and SBI. However, we simultaneously found significant association between serum cholesterol levels and SBI. Results of a meta-analysis [10] indicated that TC was significantly associated with ischemic stroke in subjects with normal blood pressure (SBP ≤125 mmHg). Consequently, this might account for our findings, because the average SBP of our subjects was 121 mmHg. In contrast, the association between SBI and IMT has been established [4,8,9].

In population studies in the United States [8] and Europe [9], SBI included not only non-cortical but also cortical lesions. In addition, those study participants were older than those in the present study and a few percent had AF. In contrast, our subjects, who had undergone health screening, had neither cortical infarcts nor AF. Most of the infarcts detected were in the basal ganglia. We speculate that the inconsistency between our results and those of others was due to differences in the brain infarction site, as well as in age and race, which could influence the association of serum cholesterol with SBI.

Uehara [11] suggested dyslipidemia as a potential associated factor for SBI in the basal ganglia, not in white matter. In subjects who use antihypertensive agents, LDL-3 levels are associated with the prevalence of silent lacunar infarcts in basal ganglia not in white matter [12]. Therefore, cholesterol is possibly that serum cholesterol is associated more with non-cortical infarcts, especially in basal ganglia.

In this study, HDL-cholesterol tended to be positively associated with SBI. However, the TC-to-HDL-cholesterol ratio (TC/HDL-C) was not significantly associated with SBI [OR 0.92 (95% CI 0.42–2.03), *P* value 0.84]. Although a recent study recommended the usefulness of TC/HDL-C for predicting stroke [2], SBI was not associated with TC/HDL-C.

Our study has one strong point: SBI was evaluated by a combination of three images involving FLAIR to ensure a higher reliability of discrimination [6]. In most previous studies, a lack of proton density or FLAIR images for diagnosis of SBI might have reduced the specificity [13].

We should address several limitations. First, the relatively small number of participants resulted in a small number of cases with SBI. Second, our results cannot be applied to women, as women were excluded because too few attended the health screening for an investigation by multivariate analysis. Third, our subjects may not have been representative of the general population because of selection bias. These participants might have been more concerned about their health than the general male population, thus allowing more early detection of hypertension and diabetes mellitus. Fourth, although obstructive sleep apnoea is a risk factor for SBI, we did not investigate its association with SBI. Fifth, we could not elucidate risk factors because of the cross-sectional study design.

Additionally, we unfortunately did not evaluate serum lipoprotein (a) [Lp (a)], although Lp (a), which has a structure like LDL-cholesterol, has been reported to be associated with lacunar infarcts defined by MRI [14]. Therefore, we cannot predict the extent to which Lp (a) confounded the relationship between cholesterol and SBI and it is possible that the observed significant association between cholesterol and SBI could be partially explained by Lp (a).

In conclusion, our cross-sectional data suggest that serum cholesterol is a key factor in the development of SBI. From these findings, evaluating serum cholesterol levels together with brain MRI might be recommended in the primary prevention of SBI as well as prevention of symptomatic infarction. Further studies are necessary to clarify what characteristics in a population and what infarction sites would indicate that serum cholesterol is strongly associated with SBI.

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Contributors: Dr. Sone had full access to all of the data in the study and takes responsibility for the accuracy of the data analysis. Study concept and design is by Asumi, Yamaguchi, Saito, Kodama, Fukuda, Sone. Acquisition of data is by Asumi, Yamaguchi, Miyazawa, Matsui. Analysis and interpretation of data is by Asumi, Yamaguchi, Saito, Kodama, Miyazawa, Matsui, Suzuki, Fukuda, Sone. Drafting of the manuscript is done by Asumi, Yamaguchi, Sone. Critical revision of the manuscript for important intellectual content is by Asumi, Yamaguchi, Saito, Kodama, Miyazawa, Matsui, Suzuki, Fukuda, Sone. Statistical analysis is by Asumi, Yamaguchi, Saito, Kodama, Sone. Administrative, technical, or material support is by Asumi, Yamaguchi, Saito, Kodama, Miyazawa, Matsui, Fukuda, Sone and study supervision is by Fukuda, Sone.

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ARTICLE

Low transition rate from normo- and low microalbuminuria to proteinuria in Japanese type 2 diabetic individuals: the Japan Diabetes Complications Study (JDCS)

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Abstract
Aims/hypothesis The aim of the study was to determine the transition rate and factors associated with the progression of normo- and low microalbuminuria to diabetic nephropathy (overt proteinuria).
Methods For 8 years we prospectively observed 1,558 Japanese patients with type 2 diabetes mellitus whose basal urinary albumin:creatinine ratio (UACR) had been measured as <17.0 mg/mmol at entry. The incidence of nephropathy (UACR >33.9 mg/mmol) was determined by measuring UACR twice a year.
Results Progression to nephropathy occurred in 74 patients. The annual transition rate was 0.67%, and was substantially higher for the low-microalbuminuric group than for the normoalbuminuric group (1.85% and 0.23%, respectively; hazard ratio for the low-microalbuminuric group 8.45, $p<0.01$). The hazard ratio for an HbA_{1c} of 7–9% or ≥9% was 2.72 ($p<0.01$) or 5.81 ($p<0.01$) relative to HbA_{1c} <7.0%, respectively. In comparison with individuals with a systolic blood pressure (SBP) of <120 mmHg, the hazard ratios for patients with an SBP of 120–140 mmHg or ≥140 mmHg were 2.31 ($p=0.06$) and 3.54 ($p<0.01$), respectively. Smoking also affected progression to proteinuria (hazard ratio 1.99, $p<0.01$). In contrast, 30.3% of the low-microalbuminuric group returned to normoalbuminuria (i.e. were in remission).
Conclusions/interpretation These results suggest that if patients with type 2 diabetes mellitus are receiving

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treatment from diabetologists for hyperglycaemia and hypertension when they are in the early stages of nephropathy (i.e. normo- or low microalbuminuria), their rate of transition to proteinuria is considerably lowered, and that differentiating patients with low microalbuminuria from those with high microalbuminuria might be clinically useful.

Trial registration UMIN Clinical Trials Registry C000000222
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Keywords Blood pressure · Diabetic nephropathy · Glycaemic control · Progression · Remission · Smoking

Abbreviations

ACE	Angiotensin-converting enzyme
ARB	Angiotensin receptor blocker
eGFR	Estimated glomerular filtration rate
ESRD	End-stage renal disease
INNOVATION	Incipient to Overt: Angiotensin II Receptor Blocker, Telmisartan, Investigation on Type 2 Diabetic Nephropathy
JDCS	Japan Diabetes Complications Study
SBP	Systolic blood pressure
UACR	Urinary albumin/creatinine ratio
UKPDS	UK Prospective Diabetes Study

Introduction

Diabetic nephropathy is the most common cause of end-stage renal disease (ESRD) in many countries, including Japan [1–3]. In the UK Prospective Diabetes Study (UKPDS), 24.9% of patients developed microalbuminuria within 10 years of diagnosis of type 2 diabetes, but only 0.8% developed ESRD, as assessed by an elevated plasma creatinine level (>250 μmol/l) or the need for renal replacement therapy [4]. Annual rates of transition between successive stages within the classic paradigm of normoalbuminuria to microalbuminuria to macroalbuminuria to ESRD were 2–3% per year [4].

In Japan, the number of patients requiring renal replacement therapy has increased threefold in less than 15 years [3]. Among 36,017 patients who started haemodialysis in 2007, the number of diabetic patients has reached 15,663 (43.5%) [3]. In Hong Kong, the overall number of people receiving renal replacement therapy increased by 50% between 1995 and 1999, and in the diabetic group, a 100% increase was observed [5]. Thus, Asians have a predisposition to diabetic nephropathy and ESRD. In fact, the recent Japanese Incipient to Overt: Angiotensin II Receptor Blocker, Telmisartan, Investigation on Type 2 Diabetic Nephropathy (INNOVATION) trial revealed that about 50% of diabetic individuals with high microalbuminuria (urinary albumin/creatinine ratio [UACR] between 11.3 and 33.9 mg/mmol [100–300 mg/g]) progressed to proteinuria within 2 years [6], indicating that progression is very rapid once high microalbuminuria develops. On the other hand, intervention using angiotensin receptor blockers (ARBs) such as losartan or telmisartan seems to be very effective in Asians in comparison with Europeans [6, 7]. The Japan Diabetes Complications Study (JDCS) is a nationwide randomised controlled study of type 2 diabetic patients focusing on lifestyle modification [8, 9]. Although the status of control of most classic cardiovascular risk factors, including body weight, glycaemia, serum lipids and blood pressure, did not differ between the two groups during the study period, the incidence of stroke in the intensive lifestyle intervention group (0.55/100 patient-years) was significantly lower than in the control group (0.95/100 patient-years) by Kaplan–Meier analysis, while the incidence of nephropathy did not differ significantly between the groups [9]. Here, we report the rate of transition and factors associated with the development and/or progression of normo- and low microalbuminuria to diabetic nephropathy (overt proteinuria) in this JDCS cohort.

Methods

In 1996, 2,205 patients aged 40–70 years with previously diagnosed type 2 diabetes and HbA_{1c} levels of >6.5% were recruited and registered from 59 hospitals specialising in diabetes care. The protocol for the study, which was in accordance with the Declaration of Helsinki and the Ethical Guidelines for Clinical/Epidemiological Studies of the Japanese Ministry of Health, Labour and Welfare, received ethics approval from the institutional review boards of all the participating institutions. Written informed consent was obtained from all the patients enrolled. The inclusion criteria for participating patients have been described previously by Sone et al. [8]. A final total of 2,033 patients aged 58.5±6.9 years (mean ± SD) were included in the study, and their diabetes duration was 10.9±7.2 years.

The recruited patients were randomly allocated to either an intensive lifestyle intervention group or a conventional treatment group. Details of the intervention have been described previously by Sone et al. [8, 9]. We selected a cohort of 1,558 patients in whom the mean value of the two-spot UACR was <17.0 mg/mmol (150 mg/g) without microscopic haematuria or other clinical findings indicating other renal diseases. We followed this cohort for 8 years, and measured their body weight, waist/hip circumference

and blood pressure at least twice a year. Fasting plasma glucose, HbA_{1c}, serum lipids and serum creatinine levels were also determined twice a year. Spot UACR was also determined at least twice a year using the turbidimetric immunoassay to measure the urinary albumin concentration. We defined normoalbuminuria as a UACR of <3.4 mg/mmol (30 mg/g), and low microalbuminuria as a UACR of 3.4 to 17.0 mg/mmol (30 to 150 mg/g). Estimated glomerular filtration rate (eGFR) was calculated using serum creatinine levels according to the modification of diet in renal disease (MDRD) formula modified for Japanese populations [10].

Statistical analyses The primary endpoint for the nephropathy analysis was transition from normo- or low microalbuminuria to proteinuria (>33.9 mg/mmol [300 mg/g]) in two consecutive urine samples. Transition to proteinuria was summarised by the annual rate of transition to proteinuria and the remission proportion was defined as those patients whose mean UACR at the final two visits was <3.4 mg/mmol. Risk factors for proteinuria were explored by the following survival analysis methods. Univariate analyses were performed by the Kaplan–Meier method, logrank test, and univariate Cox regression with a 95% CI. Multivariate Cox regression was also used. The SAS software package (version 9.2, SAS Institute, Cary, NC, USA) was used for all analyses, with the level of significance set at $p<0.05$.

Results

Tables 1 and 2 give the baseline characteristics and glycaemic and blood pressure control at baseline, and at 4 and 8 years after the start of observation. As shown in Table 2, the proportion of patients who were receiving insulin injections increased from 20.7% to 41.9% over 8 years. The use of antihypertensive agents also increased over this period from 28.2% to 42.0%. In particular, usage of renin–angiotensin system inhibitors such as angiotensin-converting enzyme (ACE) inhibitors and/or ARBs increased from 12.3% to 28.4% over 8 years. The use of statins also increased from 20.5% to 31.1%. Over a median follow-up period of 7.98 years, 74 patients developed proteinuria. The annual transition rate was 0.67 per 100 person-years (95% CI 0.53–0.84). For the low-microalbuminuric group, the annual transition rate per 100 person-years was substantially higher than for the normoalbuminuric patients (1.85 [95% CI 1.43–2.41] and 0.23 [95% CI 0.14–0.36]), respectively. On the other hand, remission (i.e. normalisation) occurred in 137 (30.3%) of the 452 individuals with low microalbuminuria (Table 3).

Table 1 Baseline characteristics of 1,558 patients included in the nephropathy analysis

Variable	Mean ± SD[a]
n (men/women)	1,558 (813/745)
Age (years)	58.5±6.9
BMI (kg/m^2)	23.0±2.9
Waist (cm)	79.4±9.2
SBP (mmHg)	132.4±15.8
DBP (mmHg)	76.6±9.5
Fasting plasma glucose (mmol/l)	8.9±2.4
HbA_{1c} (%)	7.8±1.3
Duration of diabetes (years)	10.7±7.1
Serum total cholesterol (mmol/l)	5.19±0.89
Serum triacylglycerols (mmol/l)[b]	1.15±0.82
Serum HDL-cholesterol (mmol/l)	1.41±0.43
UACR (mg/mmol)[b]	1.8±3.0
eGFR (ml min^{-1} 1.73 m^{-2})[b]	81.3±32.1
Current/past/never smoker (%)	27/24/49
Ethanol intake: 0/1–38/≥38 g/day (%)	62/31/7

DBP, diastolic blood pressure

[a] Unless otherwise stated

[b] Median±interquartile range

Figure 1 shows the Kaplan–Meier curves for progression to overt nephropathy on the basis of UACR (Fig. 1a), HbA_{1c} level (Fig. 1b), systolic blood pressure (SBP, Fig. 1c) and smoking status (Fig. 1d). As can be seen, patients with higher UACR, higher HbA_{1c}, higher SBP or current smokers had a higher risk for progression to proteinuria. The hazard ratio for the low-microalbuminuric group was 8.45 ($p<0.01$) relative to the normoalbuminuric group. Stratification of eGFR to >90, 60–90 and <60 ml min^{-1} 1.73 m^{-2} did not predict progression to proteinuria. The hazard ratio of HbA_{1c} for a range of 7–9% or for ≥9% was 2.72 ($p<0.01$) or 5.81 ($p<0.01$) relative to an HbA_{1c} of <7%, respectively. In comparison with individuals with an SBP of <120 mmHg, the hazard ratio for patients with an SBP of 120–140 mmHg or ≥140 mmHg was 2.31 ($p=0.06$) and 3.54 ($p<0.01$), respectively. Smoking also affected progression to proteinuria, with a hazard ratio of 1.99 ($p<0.01$).

Table 4 shows risk factors for the development of proteinuria based on multivariate Cox regression analysis. All the factors shown to be significant by univariate analysis—UACR, HbA_{1c} level, SBP level and smoking status—were significantly associated with the development of proteinuria after adjustment for other clinical factors. Multivariate Cox regression analysis showed that the hazard ratio for use of ACE inhibitors and/or ARBs was 1.49 (95% CI 0.83–2.69, $p=0.19$) and that the hazard ratio for use of statins was 0.73 (95% CI 0.38–1.41, $p=0.35$) in relation to the progression to proteinuria.

Table 2 Measures of glycaemic and blood pressure control at the baseline and at 4 and 8 years after the start of intervention

Variable	Baseline	4 years after start of intervention	8 years after start of intervention
BMI (kg/m^2)	23.0±2.9	23.0±3.0	23.0±3.1
SBP (mmHg)	132.4±15.8	132.5±15.4	132.5±15.9
DBP (mmHg)	76.6±9.5	75.9±9.1	74.0±10.0
Fasting plasma glucose (mmol/l)	8.9±2.4	8.9±2.6	8.6±2.5
HbA_{1c} (%)	7.8±1.3	7.7±1.2	7.7±2.0
Hypoglycaemic agent (%)			
Any use	84.4	89.3	86.6
Insulin	20.7	30.1	41.9
Sulfonylurea	62.3	63.3	59.7
Alpha-glucosidase inhibitor	25.9	29.9	28.8
Biguanide	7.5	16.1	32.8
Insulin sensitiser	1.2	8.0	9.1
Antihypertensive agent (%)			
Any use	28.2	33.3	42.0
ACE inhibitor/ARB	12.3	16.6	28.4
Calcium-channel blocker	20.7	24.4	27.2
Diuretic	1.2	1.1	2.9
Other	6.0	7.1	8.6
Statin (%)	20.5	23.7	31.1

Each value is expressed as mean ± SD or percentage

DBP, diastolic blood pressure

Discussion

Based on the main result of the JDCS study, which was reported previously by Sone et al., the incidence of stroke in the intensive lifestyle intervention group was significantly lower, by 38%, than in the control group, while the incidence of nephropathy did not differ significantly between the groups [9]. Lifestyle intervention resulted in a small but significant temporary improvement of glycaemic control and only minimal changes in other known risk factors for diabetic complications, including blood pressure, indicating the difficulty of changing the lifestyle of patients with long-term diabetes. In this sense, patients who participated in this study could be considered as representative of the general population of patients with type 2 diabetes. This might explain why there was no difference in the incidence of diabetic nephropathy. The main finding of interest in this study was that the annual incidence of proteinuria was as low as 0.67% (0.67/100 person-years), in marked contrast to previous reports. In the UKPDS, the annual rates of transition from normoalbuminuria to microalbuminuria and from microalbuminuria to macroalbuminuria in newly diagnosed patients with type 2 diabetes were 2% and 2.8% per year, respectively [4]. Ravid et al. [11] reported higher progression rates in type 2 diabetic patients in Israel, i.e. 35% from normoalbuminuria to microalbuminuria and 16% from normoalbuminuria to macroalbuminuria during 7.8 years. In Pima Indians with normotensive type 2 diabetes, Nelson et al. [12] also reported that the rates of progression from normoalbuminuria to microalbuminuria and to macroalbuminuria during 4.7 years was 37.8% and 4.3%, respectively. In Japan, a clinic-based observational 6.8 year longitudinal study of 426 patients who developed diabetes before the age of 30 years revealed that the incidence of proteinuria developing from normoalbuminuria or microalbuminuria was 1.41/100 person-years [13]. In another Japanese clinic-based observational longitudinal study conducted for 6 years, 28% of 216 patients enrolled from 1996 to 1998 showed progression from microalbuminuria to proteinuria [14]. It is difficult to compare the annual incidence of proteinuria with that found in other studies because the stages of nephropathy

Table 3 Mean UACR measured at the final two visits stratified by the basal value

Basal UACR (mg/mmol)	Final UACR (mg/mmol)			
	<3.4	3.4–17.0	17.0–33.9	≥33.9
<3.4	817 (73.9)	244 (22.1)	27 (2.4)	18 (1.6)
3.4–17.0	137 (30.3)	203 (44.9)	56 (12.4)	56 (12.4)
Total	954 (61.2)	447 (28.7)	83 (5.3)	74 (4.8)

Data shown are *n* (%)

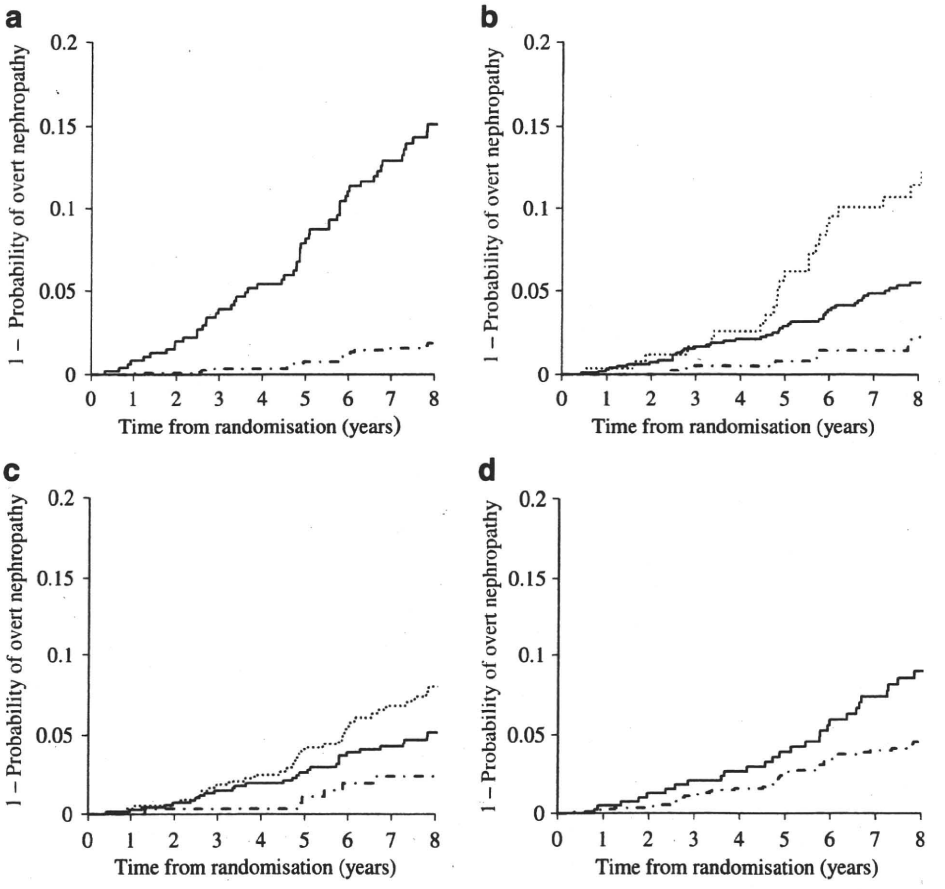

Fig. 1 Kaplan–Meier curves for progression to overt nephropathy according to: UACR (**a**), HbA_{1c} levels (**b**), SBP (**c**) and smoking status (**d**). **a** The hazard ratio for the low-microalbuminuric group (solid line) was 8.45 (95% CI 4.97–14.38, $p<0.01$) relative to the normoalbuminuric group (dashed–dotted line). **b** The hazard ratio of HbA_{1c} for a range of 7–9% (solid line) and for ≥9% (dotted line) was 2.72 (95% CI 1.22–6.03, $p<0.01$) and 5.81 (95% CI 2.49–13.55, $p<0.01$), respectively, relative to an HbA_{1c} of <7% (dashed–dotted line). **c** The hazard ratio for an SBP of 120–140 mmHg (solid line) or ≥140 mmHg (dotted line) was 2.31 (95% CI 0.96–5.54, $p<0.06$) and 3.54 (95% CI 1.50–8.40, $p<0.01$), respectively, relative to an SBP of <120 mmHg (dashed–dotted line). **d** The hazard ratio for current smoking (solid line) was 1.99 (95% CI 1.24–3.18, $p<0.01$) relative to past smoking or never smoked (dashed–dotted line)

differ from one study to another. However, the rate of transition to proteinuria in the JDCS seems to be very low. Of course, one of the reasons for this low incidence might be that two-thirds of the enrolled patients had normoalbuminuria and one-third had low microalbuminuria. In contrast, the placebo group in the INNOVATION trial showed a considerably higher transition rate, amounting to 50%, from high microalbuminuria to proteinuria within 2 years, with a UACR between 11.3 and 33.9 mg/mmol [6], although the UACR was determined using the first-voided

Table 4 Risk factors for progression to proteinuria demonstrated by multivariate Cox regression analysis

Risk factor	Hazard ratio	95% CI	*p* value
Conventional/intervention	1.01	0.63–1.61	0.98
Age, +10 years	1.03	0.71–1.49	0.87
Sex, woman/man	0.74	0.41–1.34	0.32
Duration, +10 years	1.16	0.80–1.68	0.44
BMI, +1 kg/m^2	1.01	0.93–1.10	0.73
SBP, 120–140/<120 mmHg	1.90	0.73–4.95	0.19
SBP, ≥140/<120 mmHg	2.55	0.98–6.63	0.05
HbA_{1c}, 7–9/<7%	2.22	1.00–4.96	0.05
HbA_{1c}, ≥9/<7%	4.16	1.73–10.04	<0.01
LDL-cholesterol, ≥4.0/<4.0 mmol/l	0.85	0.48–1.49	0.57
Triacylglycerol, ≥2.3/<2.3 mmol/l	1.60	0.88–2.89	0.12
HDL-cholesterol, ≥1.0/<1.0 mmol/l	1.43	0.79–2.61	0.24
UACR, ≥3.4/<3.4 mg/mmol	6.98	4.02–12.10	<0.01
Current smoker/past or never smoker	1.87	1.07–3.25	0.03
Ethanol intake, ≥38 g/<38 g/day	0.99	0.98–1.01	0.38

Missing values meant 126 patients were excluded

morning urine. Taken together with these studies, the data suggest that the current treatment by diabetologists along with administration of the usual hypoglycaemic and hypotensive drugs from the stage of normoalbuminuria or low microalbuminuria reduced the annual incidence of proteinuria to a level as low as 0.67/100 person-years. Ideally, however, the inclusion of a control group receiving placebo and matched to the drug-treated diabetic patients would be desirable in order to allow a firm conclusion to be drawn, although admittedly this would be ethically problematic. As the baseline UACR profoundly affected the cumulative incidence of proteinuria, it might be clinically useful to divide patients with microalbuminuria into low- and high-risk groups, i.e. those with low and high microalbuminuria, although the cut-off value remains to be determined.

In the present study, progression to proteinuria was independently associated with higher baseline HbA_{1c} and SBP levels in addition to an elevated baseline UACR. Furthermore, smoking was also a significant predictor of proteinuria. These results are consistent with previous studies [11, 15]. In the UKPDS, the risk factors most highly associated with proteinuria were reported to be urinary albumin, plasma creatinine, waist circumference, SBP, glycaemic control, LDL-cholesterol, and plasma triacylglycerol [15]. Indian-Asian ethnicity was also an independent risk factor for microalbuminuria and/or proteinuria [12, 15]. Smoking and male sex were reported to be independent predictors of proteinuria in addition to plasma cholesterol, mean blood pressure and HbA_{1c} [11]. Based on these epidemiological studies, tight glycaemic control has been reported to be effective for preventing the onset and/or progression of nephropathy in clinical trials such as the Diabetes Control and Complications Trial (DCCT), the Kumamoto study and the UKPDS [16–18]. Strict blood pressure control, especially with ACE inhibitors or ARBs, has also been demonstrated to be effective for delaying the progression of diabetic nephropathy [6, 7, 19–21]. However, in the present study, the initial usage of an ACE inhibitor and/or ARB, or statin was not significantly associated with the prevention of proteinuria. As this study was designed to clarify the effects of lifestyle intervention on subsequent occurrence of diabetic complications, it might have been difficult to recognise the effects of such drugs on the progression of diabetic nephropathy. In some studies, normalisation of microalbuminuria, i.e. remission/regression, has also been reported [6, 12]. In fact, in our study, 30.3% of 452 individuals with low microalbuminuria demonstrated normalisation.

However, following the advent of modern therapeutics, especially hypoglycaemic and antihypertensive agents, diabetic nephropathy is the most common cause of ESRD, and the number of patients being started on haemodialysis is still increasing dramatically in many countries, particularly in Asia. Our data have major clinical relevance because we have demonstrated that the initiation of hypoglycaemic and antihypertensive treatment from the early stage of nephropathy might lower the rate of transition to proteinuria even in the Japanese, who are highly susceptible to diabetic nephropathy. To reduce the number of patients who require haemodialysis, it is very important to measure UACR, make a diagnosis of diabetic nephropathy, define the stage of nephropathy and initiate strict glycaemic and blood pressure control as early as at the normo- or low-microalbuminuria stage.

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Duality of interest The authors declare that there is no conflict of interest associated with the manuscript.

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