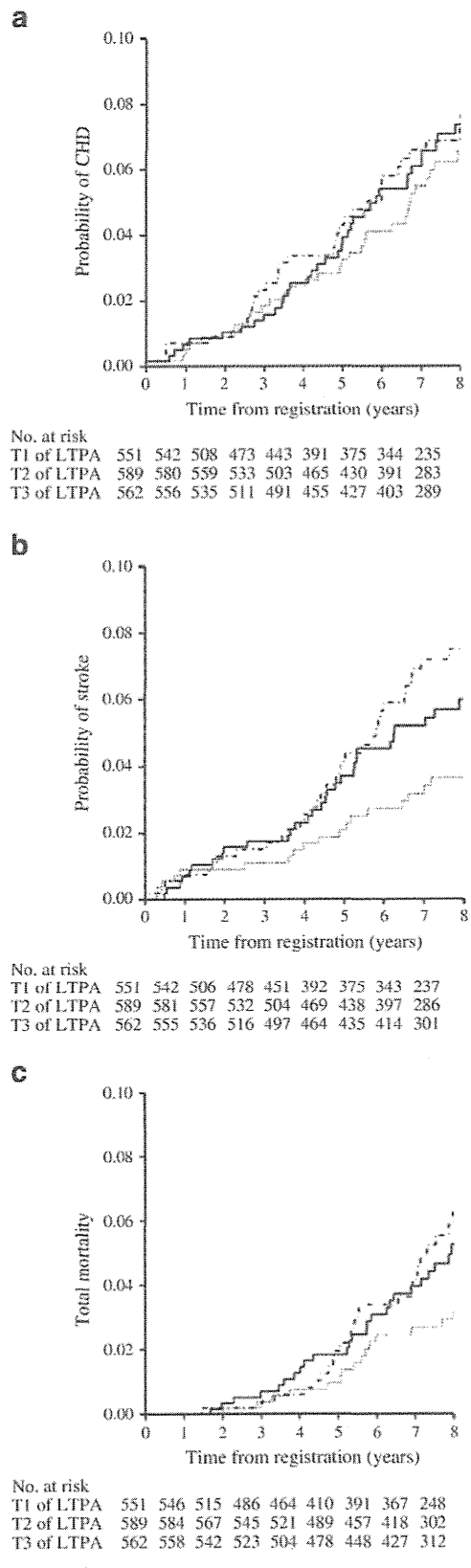

◀ **Fig. 1** Probability of coronary heart disease (**a**), stroke (**b**) or total mortality (**c**) according to tertiles of LTPA determined by the Kaplan–Meier method. Broken line, tertile 1 (T1) of LTPA (≤3.7 MET h/week); solid line, tertile 2 (T2) of LTPA (3.8–15.3 MET h/week); dotted line, tertile 3 (T3) of LTPA (≥15.4 MET h/week)

evaluated and scored was significantly associated only with cerebrovascular events and not with cardiac events [4].

The precise mechanisms for these findings cannot be clarified merely from epidemiological studies. Although statistically nonsignificant, since a weak tendency for a decrease in HR for CHD across LTPA tertiles was observed in our cohort, it is possible that the relationship between CHD and LTPA would become significant with a longer period of observation. Because the biological mechanisms for stroke prevention by physical activity in patients with type 2 diabetes are only partially understood [34], the possibility exists that exercise ameliorates undetermined cardiovascular risk factors, such as quality of life [2] or other health behaviours [3], which more strongly affect stroke risk than CHD risk. This possibility should be investigated in the future.

The significant risk reduction in stroke by LTPA was weakened after stepwise adjustment for lifestyle factors and clinical variables, which suggested that some of the involved elements confounded the association although these adjustments did not compromise the beneficial effects of LTPA. However, these findings could be helpful in understanding the mechanisms behind the associations. Individual adjustments for each lifestyle factor instead of simultaneous adjustment for all lifestyle factors suggested that dietary factors (intake of energy, saturated fat and dietary fibre) had a relatively larger effect than the other lifestyle factors, suggesting that individuals who exercised more also had a tendency to pay more attention to the amount and content of meals (see ESM Table 1). On the other hand, individual adjustments for each clinical variable instead of simultaneous adjustment for all clinical variables suggested that triacylglycerol and LDL-cholesterol had a larger effect than the other clinical variables, indicating that LTPA might have exerted its effect on stroke reduction partly through ameliorating serum lipids (see ESM Table 1). Even though it became nonsignificant, the fully adjusted HR and its p value for stroke did not alter dramatically from those when adjusted only by age, sex and diabetes duration. This suggests that undetermined risk factors associated with physical activity should exist. This is also supported in part by the results of subgroup analysis of the risk of stroke and total mortality, which indicated that greater LTPA was not necessarily associated with lower risk in those who had typical cardiovascular risk factors.

The current results suggested that the effect of exercise on total mortality was independent of lifestyle factors and clinical variables, which included typical cardiovascular risk

determine physical activity related to occupation and commuting since we did not survey working hours per week or commuting methods. However, Hu and colleagues [37] reported that physical activity related to occupation and commuting significantly affected the results in their cohort of individuals with diabetes [10], although adjustment by individual or dichotomous (physically active/sedentary) classifications of occupations did not affect our results. It might be meaningful to show the effects of LTPA independently of occupation since an occupation per se is not an easily 'modifiable' element for many individuals. Only baseline data, including those using medication, were considered in this analysis; however, adjustment by baseline use of medication did not fundamentally change our results, therapeutic management during the follow-up period could have influenced these results. In fact, we found a substantial increase in usage of antihypertensive and hypolipidaemic agents during the follow-up period, as previously reported [38]. However, it would not be appropriate to adjust for the influence of medications during follow-up in our analysis, as these variables can be outcomes of low LTPA at baseline. We could only show that clinical variables according to tertiles of LTPA remained quite stable during the observational period (see ESM Fig. 1).

Loss to follow-up is an inevitable problem in most cohort studies. In this study, the 8-year follow-up rate was not necessarily high, and LTPA, total energy intake and treatment with insulin were associated with follow-up status (see ESM Table 2). Theoretically, in such cases, a valid analysis requires inclusion of all observed prognostic factors associated with follow-up status into the model, even under the assumption of 'missing at random'. Taken together, HRs should be estimated with adjustment for LTPA, total energy intake and treatment by insulin, and Model 4 in Table 2 is the least likely to be biased according to this theoretical consideration. We did not assess cardiorespiratory fitness, which is known to be closely related to cardiovascular events in general [39] and in diabetic populations [40, 41], although the beneficial effects of LTPA are not fully explained only by cardiorespiratory fitness [42, 43].

In conclusion, in our cohort of Japanese individuals with type 2 diabetes, an LTPA level of 15.4 MET h/week of more was associated with a significantly lower risk of stroke through, at least partially, ameliorating the effects of combinations of known cardiovascular risk factors. Higher LTPA was also associated with significantly reduced total mortality but independent of cardiovascular risk factors or events. These findings, which imply differences from Western diabetic populations, should be considered in the clinical management of East Asians with diabetes.

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Contribution statement All authors contributed to the conception and design of the study, acquisition, analysis and interpretation of data and drafting and editing the manuscript. All of the authors approved the final version of the manuscript. H. Sone had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Comparison of Various Lipid Variables as Predictors of Coronary Heart Disease in Japanese Men and Women With Type 2 Diabetes

Subanalysis of the Japan Diabetes Complications Study

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OBJECTIVE—To determine the best lipid variable to predict coronary heart disease (CHD) in Japanese patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS—Eligible Japanese men and women (1,771) aged 40–70 years with type 2 diabetes from 59 institutes nationwide were followed for a planned 8-year period. The performance of eight conventional lipid variables, i.e., total cholesterol (TC), LDL-cholesterol (LDLC), HDL-cholesterol (HDLC), triglycerides (TGs), non-HDLC, TC/HDLC ratio, LDLC/HDLC ratio, and TG/HDLC ratio, as predictors of incident CHD were evaluated by four methods: hazard ratio (HR) per one SD increment by multivariate Cox analysis, χ^2 likelihood ratio test, area under the receiver operating characteristic curve (AUC), and tertile analysis.

RESULTS—Although all variables significantly predicted CHD events in men, non-HDLC (HR per one SD 1.78 [95% CI 1.43–2.21]; AUC 0.726) and TC/HDLC (HR 1.63 [1.36–1.95]; AUC 0.718) had the better predictive performances among the variables, including LDLC. In women, TGs (log-transformed; HR 1.72 [1.21–2.43]; AUC 0.708) were the best predictor according to results of tertile analysis (HR of the top tertile versus the bottom tertile 4.31 [1.53–12.16]). The associations with incident CHD were linear and continuous.

CONCLUSIONS—For Japanese diabetic men, non-HDLC and TC/HDLC were the best predictors, whereas TGs were most predictive for women. These findings, which included prominent sex differences, should be considered among clinical approaches to risk reduction among East Asians with diabetes.

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Type 2 diabetes is characterized by an excessive incidence of coronary heart disease (CHD), and serum lipid values are among the strongest predictors of CHD (1,2). Although serum LDL-cholesterol (LDLC) has been conventionally used as a therapeutic marker and/or target in many guidelines based on trials using statins (1,2), characteristic features of diabetic dyslipidemia, which are closely associated with insulin resistance, are elevated levels of triglycerides (TGs) and small, dense LDLC (independent of LDLC level) as well as decreased levels of HDL-cholesterol (HDLC) (1,2). The use of LDLC alone for assessment of cardiovascular risk would ignore these TG-rich lipoproteins (TRLs, i.e., VLDL and intermediate-density lipoprotein) and low HDLC, all of which affect the risk of a CHD event independently of LDLC (1–4). Moreover, LDLC values, as estimated by the Friedewald formula, become progressively less accurate as the TG level increases.

Based on this background, it has been established that other lipid parameters, typically non-HDLC (determined by subtracting the HDLC concentration from the total cholesterol [TC] concentration in plasma) or apolipoprotein B (apoB), both of which reflect TRLs and small, dense LDLC, can be considered better predictors of CHD than LDLC and have been introduced into some guidelines as a secondary target for therapy (5–7). Furthermore, the ratios of TC to HDLC (TC/HDLC), which has clinical significance equivalent to non-HDLC/HDLC, LDLC to HDLC (LDLC/HDLC), and TGs to HDLC (TG/HDLC) are also used for assessing cardiovascular risk (3,4). It should be mentioned that non-HDL/HDL is always one unit lower than TC/HDLC.

Despite these considerations, these fundamental lipid measures (TC, HDLC, and TGs) and their calculated indices (LDLC, non-HDLC, TC/HDLC, LDLC/HDLC, and TG/HDLC) have not been completely and directly compared as predictors of CHD by

multiple analytical methods in past prospective studies in diabetic subjects (8–19). Results obtained have been inconsistent, and only one study (19) analyzed men and women separately. Therefore, whether LDLC performs better than the other indices or, if not, which variable is the best predictor of a CHD event has not been fully determined in diabetic subjects. Furthermore, all previous examinations of the performance of lipid variables as predictors of CHD in diabetic subjects (8–19) were performed in Western countries or in Caucasians. It is uncertain whether their results can be extrapolated to East Asian diabetic subjects, who have substantially different profiles regarding CHD and its risk factors, including a much lower incidence of CHD and degree of obesity (20–22).

In this analysis of data from a long-term follow-up of Japanese patients with type 2 diabetes, we compared eight conventional lipid variables, all of which are routinely measured or can be easily calculated in clinical care settings, as predictors of CHD events. To directly and quantitatively compare variables having different average values as well as variations in quantities and ratios, we used four different analytical methods to determine the best predictor of CHD. These were the multivariate-adjusted hazard ratio (HR) per one SD increment in the Cox hazard model, χ^2 likelihood ratio test, area under the receiver operating characteristic (ROC) curve (AUC), and tertile analysis.

RESEARCH DESIGN AND METHODS

Recruitment of patients

The present analysis was conducted as part of the Japan Diabetes Complications Study, a multicenter prospective study of the incidence of and risk factors for macro- and microvascular complications among 2,033 Japanese patients with type 2 diabetes aged 40–70 years with HbA_{1c} levels >6.5% who were registered from January 1995 to March 1996 from outpatient clinics in 59 university and general hospitals nationwide that specialize in diabetes care. For this analysis of macrovascular complications, of those 2,033 individuals, 940 men (mean age 57.8 ± 7.1 years) and 831 women (mean age 58.7 ± 6.8 years) were selected for the current study after consideration of the exclusion criteria prespecified in the study protocol (23). Excluded were patients with impaired glucose tolerance, a history of angina pectoris, myocardial infarction, stroke, peripheral artery disease, familial hypercholesterolemia, type III hyperlipidemia (diagnosed by broad β band on electrophoresis), nephrotic syndrome (urine protein >3.5 g per day and serum total protein <6.0 mg/dL), and serum creatinine levels >1.3 mg/dL (120 μmol/L). In the 8-year planned observation period, the median follow-up for the 1,771 patients was 7.86 years (final follow-up rate was 75%; 1,332/1,771 patients). The total person-years studied was 11,743 (6,106 for men and 5,637 for women). Diabetes and impaired glucose tolerance were diagnosed according to the Report of the Committee of the Japan Diabetes Society on the Classification and Diagnostic Criteria of Diabetes Mellitus, which is almost identical in terms of thresholds for glucose levels to those of the World Health Organization. The study protocol, which is in accordance with the Declaration of Helsinki and the Ethical Guidelines for Clinical/Epidemiological Studies of the Japanese Ministry of Health, Labor, and Welfare, received ethical approval from the institutional review boards of all participating institutes. All enrolled patients provided written informed consent.

Clinical and laboratory measurements

Patients were assessed yearly after the baseline evaluation. Mean values of at least two measurements each year were obtained for HbA_{1c}, fasting plasma glucose, and fasting serum lipids. HbA_{1c} assays were performed according to procedures outlined by the Laboratory Test Committee of the Japan Diabetes Society (JDS), which is known to be converted by the formula HbA_{1c} (JDS)(%) = HbA_{1c} (National Glycohemoglobin Standardization Program [NGSP])(%) − 0.4%. All other laboratory tests were performed at each participating institute. Serum LDLC was calculated using the Friedewald equation, except where TGs exceeded 400 mg/dL, in which case LDLC data were treated as "missing". This was applicable to 20 subjects. All other measurements, including those for body weight, blood pressure, and a 12-lead electrocardiogram, were performed at least once yearly. A baseline dietary survey, which was validated and is widely used in Japan (24) and was comprised of food records and a food frequency questionnaire that included alcohol consumption, was undertaken. Information on cigarette smoking was collected using a self-administered questionnaire. Smoking status was classified into one of three categories: current smokers, ex-smokers, and never smokers (25).

Outcome measures

The outcomes analyzed were a fatal or first nonfatal manifestation of CHD comprised of angina pectoris and myocardial infarction, both of which were diagnosed according to criteria defined by the Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA; World Health Organization) project. A patient with a first percutaneous coronary intervention or coronary artery bypass graft was also counted as having a CHD event. Information regarding primary outcome and other clinical variables for each subject was collected through an annual report that included detailed findings at the time of the event from each participating diabetologist who was providing care to those patients. The adjudication of end points was performed by central committees comprised of experts who were masked to risk factor status and was based on additional data such as a detailed history, sequential changes in electrocardiogram and serum cardiac biomarkers, and results of coronary angiography. The rate of concordance in diagnosis between participating diabetologists and committee experts was 93%.

Statistical analysis

All statistical analyses and data management were conducted at the central data center. Patient characteristics were described as mean ± SD, median and interquartile range, or percentage. We compared a CHD group with a no-CHD group by Student *t* test and Fisher exact test for numerical and categorical variables, respectively. Multivariate Cox regression analysis was used to calculate the adjusted HRs and 95% CIs for risk factors. The strength of associations of each lipid variable was assessed using the χ^2 likelihood ratio test, and the corresponding *P* values were estimated from the regression coefficient based on the Cox proportional hazards model. In addition, the relationships between tertiles of each baseline lipid variable and HR for CHD risks were assessed by the Cox proportional hazards model using the first tertile of each variable as the reference group. The discriminatory powers for CHD of the lipid variables were also compared by ROC curve analysis with application of various thresholds to the predicted probability obtained from the logistic regression model. The AUC was calculated by integrating the area between the ROC curve and the diagonal line where sensitivity

is equal to one specificity based on the trapezoidal rule. Multivariate-adjusted generalized additive models with a spline function of three degrees of freedom were used to explore potential nonlinear relationships. All *P* values are two sided and the significance level is 0.05. All statistical analyses were conducted using SAS packages version 9.2 (SAS Institute Inc., Cary, NC).

RESULTS

Baseline clinical variables according to occurrence of CHD events

Table 1 summarizes clinical baseline variables for men and women who had or had not experienced a CHD event during the follow-up period. In comparison with men without CHD, those with CHD had significantly higher levels of all lipid variables (but lower HDLC values) determined except for TGs, which was higher with borderline significance. Women with CHD had significantly higher systolic blood pressure and significantly higher levels of lipid variables with the exception of LDLC/HDLC, which was of borderline significance, and HDLC. In addition, women with, rather than without, CHD were significantly more likely to use an insulin sensitizer and agents for hypertension and dyslipidemia.

Relationships between various lipid variables and CHD outcome

Multivariate-adjusted HRs per one SD, χ^2 values, and AUCs for CHD events for each lipid variable at baseline are shown in Table 2. In men, all lipid variables significantly predicted a CHD event with HRs per one SD ranging between 1.42 and 1.78. The largest HR value per one SD, χ^2 statistics, and AUCs were found for non-HDLC followed by TC/HDLC, which had findings very close to non-HDLC results.

In women, the largest HR per one SD was found for TGs (log-transformed) followed by non-HDLC and TC. These three indices had substantially larger χ^2 values and slightly larger AUCs than the other indices, whereas non-HDLC had the largest χ^2 value and TC had the largest AUC value (Table 2). Since subjects with elevated TGs are likely have higher glycemic or weight levels, we performed stratified analysis to categorize women according to values equal to or above or below the median of HbA_{1c} or BMI, which were 7.6% and 22.8 kg/m^2, respectively. As a result, a significantly larger multivariate-adjusted HR per one SD of log-transformed TGs was observed only in those whose HbA_{1c} or BMI level was equal to or greater than the median, i.e., HbA_{1c} ≥7.6%, HR 1.78 (95% CI 1.21–2.63), and $P = 0.005$ versus HbA_{1c} <7.6%, 1.37 (0.76–2.47), and $P = 0.27$ (Supplementary Table 1); BMI ≥22.8, 1.75 (1.17–2.62), and $P = 0.008$ versus BMI <22.8, 1.51 (0.86–2.65), and $P = 0.14$ (Supplementary Table 2).

In the combined analysis of men and women, non-HDLC identified patients at greater risk of CHD than the other lipid variables and had an HR of 1.69 (95% CI 1.41–2.01), χ^2 statistic of 29.4 ($P < 0.001$), and AUC of 0.713 (95% CI 0.663–0.762) followed by TC/HDLC, for which results were 1.55 (1.33–1.81), 23.9 ($P < 0.001$), and 0.703 (0.651–0.754), respectively. These were better predictors than LDLC, for which results were 1.51 (1.26–1.80), 18.2 ($P < 0.001$), and 0.690 (0.641–0.738), respectively.

Table 3 shows HRs for CHD according to tertiles of lipid variables. In men, HRs were significantly elevated in the top compared with the bottom tertile (bottom compared with the top in case of HDLC) in all variables determined. Subjects in the top tertile of TC/HDLC and LDLC/HDLC had a four times or greater risk of CHD than those in the respective bottom tertile, followed by non-HDLC and LDLC, both of which had relatively high HRs of ~3.5 between extreme tertiles. In women, significantly elevated HRs in the top tertile compared with the bottom tertile were observed only for TGs, TG/HDLC ratio, and LDLC. Among those, the highest HR was noted for TGs, and was 4.31, which was considerably higher than that for the other lipid variables. Even subjects in the middle tertile for TGs, which indicated the normal level of 0.90–1.36 mmol/L, had a significantly higher risk of CHD than those in the bottom tertile. On the other hand, the HR for the TG/HDLC ratio was not higher than that for TGs alone either in men or women. If we again stratified women with values below and equal to or above the median for HbA_{1c} or BMI, which were 7.6% and 22.8 kg/m^2, respectively, significantly elevated HRs for TGs in the top tertile compared with the bottom tertile were observed only in those whose HbA_{1c} or BMI was at or greater than the median, i.e., HbA_{1c} ≥7.6%, HR 6.74 (95% CI 1.43–31.67), and $P = 0.016$ versus HbA_{1c} <7.6%, 2.95 (0.65–13.47), and $P = 0.163$ (Supplementary Table 3); BMI ≥22.8, 3.95 (1.08–14.54), and $P = 0.039$ versus BMI <22.8, 5.13 (0.90–29.30), and $P = 0.066$ (Supplementary Table 4).

Dynamic change in risk association of important lipid variables

To explore dynamic changes in risk association, including possible thresholds for lipid variables that were found to be good predictors, sex-stratified spline analysis was performed for non-HDLC, TC/HDLC, and TGs (Fig. 1). In each variable, the relationship was on a continuum, indicating difficulty in determining a clear cutoff value. When risks for men and women whose non-HDLC was 3.88 mmol/L (150 mg/dL) were set as a reference, risks of those with a non-HDLC value of ~4.3 mmol/L (170 mg/dL) became significant with HRs of ~1.5 in both men and women. When the TC/HDLC level of 5.0 was set for reference, risks in those whose TC/HDLC levels were ~6.3 became significant in both men and women but the HR was greater in women (~2.0) than in men (~1.5).

CONCLUSIONS—The current analysis of our Japanese subjects with type 2 diabetes revealed distinct sex differences in lipid variables that predict a CHD event. Although large sex differences in incidence and risk profiles (such as smoking) of CHD are well known, most previous studies on lipid variables as predictors of CHD (8–15,17,18) did not separately analyze men and women with diabetes. Our previous investigation to clarify risk factors (involving nonlipid parameters) for cardiovascular complications in Japanese diabetic subjects, which also analyzed men and women together, demonstrated that the serum TG level was a potent risk factor, unlike findings for Western diabetic subjects (23). Our current results further clarified that this effect of TGs was exclusively derived from its effect in women (23).

In our Japanese men with diabetes, non-HDLC and TC/HDLC, which are calculated from TC and HDLC, were the two best predictors of CHD and were superior to LDLC. These results confirmed the validity in Japanese diabetic men of the previously reported superiority of non-HDLC (9–11,13) or TC/HDLC (or non-HDLC/HDLC) (9,10,12,17,18) over LDLC as CHD predictors among Western diabetic populations. Also supported is that lipoproteins other than LDL, such as VLDL and chylomicron remnants, provide predictive power in addition to that of LDLC and could

Table 1—*Patient characteristics at baseline*

	Men			Women		
	No-CHD	CHD	*P*	No-CHD	CHD	*P*
n	870	70		786	45	
Age (years)	57.9 ± 7.1	60.0 ± 6.3	0.027	58.8 ± 6.8	59.9 ± 6.7	0.28
Diabetes duration (years)	11.4 ± 7.6	12.2 ± 7.7	0.35	10.2 ± 6.6	11.2 ± 4.9	0.053
BMI (kg/m^2)	22.8 ± 2.7	22.7 ± 2.4	0.90	23.2 ± 3.4	24.2 ± 3.1	0.060
Blood pressure (mmHg)	131 ± 16/ 77 ± 10	134 ± 16/ 79 ± 9	0.40/0.19	132 ± 17/ 76 ± 10	139 ± 15/ 78 ± 8	0.004/0.16
Fasting plasma glucose (mmol/L)	8.5 ± 2.6	8.4 ± 3.4	0.33	8.6 ± 2.8	9.2 ± 3.1	0.23
HbA_{1c} (%)	7.7 ± 1.2	8.0 ± 1.5	0.17	8.1 ± 1.4	8.2 ± 1.3	0.36
Serum lipid variables						
TC (mmol/L)	5.00 ± 0.89	5.37 ± 0.77	<0.001	5.38 ± 0.86	5.81 ± 0.93	0.004
HDLC (mmol/L)	1.36 ± 0.42	1.25 ± 0.38	0.008	1.49 ± 0.46	1.43 ± 0.49	0.29
TGs (mmol/L)*	1.19 (0.82)	1.35 (0.91)	0.076	1.10 (0.81)	1.45 (0.51)	<0.001
LDLC (mmol/L)	2.99 ± 0.84	3.40 ± 0.81	<0.001	3.31 ± 0.79	3.64 ± 0.79	0.014
Non-HDLC (mmol/L)	3.64 ± 0.92	4.12 ± 0.85	<0.001	3.88 ± 0.89	4.39 ± 0.97	0.002
TC/HDLC ratio	3.97 ± 1.30	4.63 ± 1.36	<0.001	3.89 ± 1.19	4.49 ± 1.59	0.023
LDLC/HDLC ratio	2.41 ± 1.07	2.96 ± 1.07	<0.001	2.43 ± 0.95	2.91 ± 1.34	0.056
Therapeutic measures						
Diabetes						
Diet only (%)	21	17	0.54	16	9	0.29
Insulin (%)	20	23	0.65	23	33	0.15
Sulfonylureas (%)	55	61	0.32	60	60	1.00
α-Glucosidase inhibitors (%)	21	21	0.88	20	20	1.00
Biguanides (%)	6	2	0.72	5	4	1.00
Insulin sensitizer (%)	2	1	1.00	2	9	0.014
Others						
Antihypertensive agents (%)	21	21	0.88	30	58	<0.001
Agents for dyslipidemia (%)	14	16	0.72	34	53	0.010
Diet						
Energy intake (kJ/day)*	1,776 (567)	1,703 (508)	0.82	1,597 (491)	1,568 (394)	0.94
Fat intake (g/day)*	53 (22)	53 (17)	0.45	50 (21)	49 (16)	0.94
Exercise (kJ/day)*	140 (302)	145 (264)	0.73	118 (229)	95 (254)	0.35
Current/past smoker (%)	44/39	54/36	0.20	9/6	7/5	1.00
Alcohol intake: never, three drinks or less, more than three drinks (%)**	40/48/12	45/46/9	0.61	87/13/0	87/13/0	1.00

Data are mean ± SD or *median (interquartile range). **One drink is equivalent to 12.6 g of ethanol based on the U.S. Department of Agriculture definition.

explain part of the residual cardiovascular risk characterized by the LDLC level alone (3,4). It also has been suggested that non-HDLC is superior as a predictor to LDL-C because non-HDLC is an indirect estimate of LDL particle number, and LDL particle number relates more closely to risk than LDL-C (6). Although studies have attempted to determine whether non-HDLC or TC/HDLC best identifies patients at greater risk of CHD, the statistical differences between the two were relatively small (10,12). For example, in the UK Prospective Diabetes Study (12), although TC/HDLC was a significantly stronger predictor of CHD than non-HDLC, HRs per one SD increment for those two variables were very close (1.36 and 1.35, respectively), and differences in results of ROC analysis were not clinically important, which was supported by the results of another study (10).

Although our results for men were quite close to those in Western studies that analyzed men and women together, our findings in female subjects differed from those findings or results in Japanese men with diabetes. Among our female subjects, TGs, TC, and non-HDLC were the best predictors of CHD risk as assessed by HRs for one SD increment, χ^2 statistics, or AUCs. However, tertile analysis indicated that TGs were the best variable examined, and that it was a significant predictor beginning at values as low as 0.90 mmol/L. That value was lower than reported in Western countries (14,17) but was close to the optimal upper limit in the newest U.S. guidelines (4).

Although the role of TGs in CHD is known to be influenced by ethnicity, especially in Asians (26), the specific reasons why TGs were a leading predictor of CHD in Japanese diabetic women but not in men have yet to be clarified. However, our results in women are similar to those in other studies of East Asian diabetic subjects (27–29), which showed that TGs had stronger associations with cardiovascular morbidity (27,29) and mortality (28) than LDLC, although these studies were either cross-sectional (27,29) or relatively small-scale and short-term (28). In particular, a cross-sectional study in Hong Kong (27) revealed that TGs were strongly associated with ischemic heart disease in women but not in men with type 2 diabetes. A meta-analysis of cohort studies in Asian-Pacific general populations also revealed that TGs were the best predictor of CHD death among single lipid variables, although

Table 2—*Multivariate-adjusted HRs per one SD increment with 95% CI, χ^2 (likelihood ratio test) statistics, and the AUC*

	Men			Women		
	HR (95% CI)	χ^2 (*P* value)	AUC (95% CI)	HR (95% CI)	χ^2 (*P* value)	AUC (95% CI)
TC	1.57 (1.25–1.99)	13.4 (<0.001)	0.697 (0.636–0.758)	1.58 (1.20–2.06)	9.6 (0.002)	0.721 (0.644–0.798)
LDLC	1.59 (1.28–1.98)	14.8 (<0.001)	0.694 (0.629–0.758)	1.41 (1.06–1.86)	5.3 (0.021)	0.705 (0.626–0.784)
HDLC	1.47 (1.09–1.98)	6.9 (0.009)	0.669 (0.604–0.734)	1.03 (0.72–1.48)	0.03 (0.85)	0.667 (0.577–0.756)
TGs (log-transformed)	1.42 (1.08–1.85)	6.4 (0.011)	0.664 (0.595–0.733)	1.72 (1.21–2.43)	9.2 (0.002)	0.708 (0.630–0.786)
Non-HDLC	1.78 (1.43–2.21)	22.0 (<0.001)	0.726 (0.664–0.787)	1.60 (1.21–2.12)	9.7 (0.002)	0.715 (0.634–0.796)
TC/HDLC ratio	1.63 (1.36–1.95)	19.7 (<0.001)	0.718 (0.656–0.780)	1.48 (1.11–1.95)	6.8 (0.009)	0.696 (0.609–0.782)
LDLC/HDLC ratio	1.52 (1.29–1.79)	16.1 (<0.001)	0.709 (0.646–0.772)	1.44 (1.09–1.91)	6.2 (0.013)	0.695 (0.608–0.781)
TG/HDLC ratio	1.49 (1.20–1.85)	10.4 (0.001)	0.680 (0.615–0.746)	1.36 (1.01–1.85)	3.4 (0.066)	0.683 (0.597–0.769)

Each lipid variable for CHD events at baseline adjusted by age, diabetes duration, BMI, systolic blood pressure, HbA_{1c}, smoking, and alcohol intake.

men and women were not separately analyzed (30). Interestingly, in our female subjects, TC was a better predictor than LDLC by all four analytical methods, suggesting that TLRs involving remnant or small, dense LDL strongly affect the etiology of CHD in this population.

It is well known that the serum level of TGs, which is closely associated with insulin resistance, is influenced by a number of metabolic factors, typically including glycemic and weight status. Insulin resistance is believed to contribute to the atherogenic dyslipidemia seen in diabetes by increasing the hepatic secretion of VLDL and other apoB-containing lipoprotein particles as a result of increased free fatty acid flux to the liver (31). This raises the long-standing debate as to whether the association of the TG level to CHD is a direct effect of the TRLs themselves or is a biomarker of accompanying disorders (32). Our results in stratified, multivariate-adjusted analysis suggested that at least the serum level of TGs is a significant and independent predictor in women whose HbA_{1c} or BMI was equal to or above the median. Although the precise mechanisms of these phenomena cannot be derived from epidemiological observations, improving glycemic and weight status could be beneficial to avoid the harmful influence of hypertriglyceridemia. Conversely, HDLC was not a significant predictor of CHD in women although it was moderately predictive in

Table 3—*HRs with 95% CIs for each lipid variable according to tertiles*

	Men			Women		
	Ranges	HR (95% CI)	*P*	Ranges	HR (95% CI)	*P*
TC (mmol/L)	4.63–5.40	1.81 (0.95–3.44)	0.069	5.02–5.69	1.23 (0.45–3.38)	0.687
	5.41–	2.98 (1.61–5.51)	0.001	5.70–	2.23 (0.90–5.56)	0.084
LDLC (mmol/L)	2.66–3.33	1.81 (0.93–3.52)	0.081	2.97–3.62	2.31 (0.82–6.54)	0.114
	3.34–	3.45 (1.83–6.48)	0.0001	3.63–	3.02 (1.12–8.12)	0.029
HDLC (mmol/L)	1.14–1.40	1.74 (0.82–3.67)	0.147	1.27–1.55	0.83 (0.38–1.84)	0.652
	–1.13	2.48 (1.23–5.00)	0.011	–1.26	1.31 (0.61–2.79)	0.487
TGs (mmol/L)	0.94–1.48	1.09 (0.55–2.13)	0.810	0.90–1.36	3.35 (1.21–9.23)	0.020
	1.49–	2.01 (1.07–3.78)	0.031	1.37–	4.31 (1.53–12.16)	0.006
Non-HDLC (mmol/L)	3.25–3.98	1.42 (0.70–2.86)	0.328	3.49–4.19	1.14 (0.44–2.94)	0.791
	3.99–	3.67 (1.97–6.83)	<0.0001	4.20–	2.02 (0.84–4.86)	0.118
TC/HDLC ratio	3.4–4.3	1.95 (0.91–4.19)	0.088	3.3–4.2	1.17 (0.50–2.73)	0.724
	4.4–	4.13 (2.05–8.33)	<0.0001	4.3–	1.50 (0.67–3.35)	0.329
LDLC/HDLC ratio	1.9–2.7	1.66 (0.78–3.53)	0.185	2.0–2.7	1.11 (0.48–2.58)	0.810
	2.8–	4.11 (2.09–8.08)	<0.0001	2.8–	1.57 (0.71–3.48)	0.265
TG/HDLC ratio	0.70–1.26	1.38 (0.66–2.90)	0.399	0.56–1.05	2.60 (1.04–6.46)	0.041
	1.27–	2.86 (1.44–5.69)	0.003	1.06–	3.27 (1.30–8.25)	0.012

HRs with 95% CIs for each lipid variable according to tertiles (HRs for the lowest tertile as a reference are shown except for HDLC where the top tertile is the reference) for CHD risk analyzed by Cox multivariate models adjusted by age, sex, diabetes duration, BMI, HbA_{1c}, systolic blood pressure, smoking status, and alcohol intake.

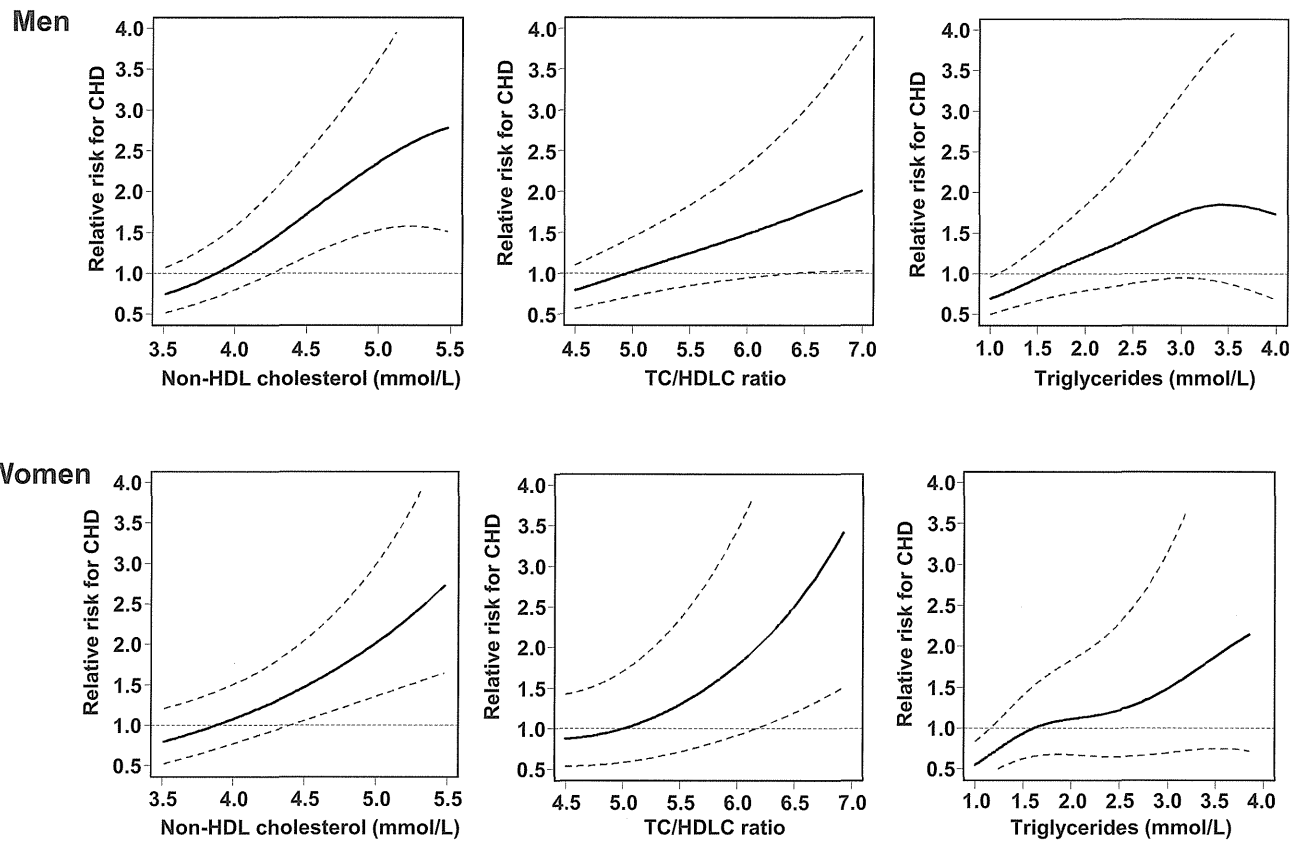

Figure 1—*Relative risk (solid line) and 95% CIs (broken line) of the incidence of CHD in relation to non-HDLC, TC/HDLC ratio, and TGs estimated by generalized additive models.*

men. The serum level of HDLC is naturally higher in East Asians than in Western populations, especially women (33,34), as in our cohort. Therefore, it is possible that the clinical impact of low HDLC was not apparent and, instead, that of TGs was enhanced in East Asians. Accordingly, TG/HDLC did not add useful information to that provided by TGs alone either in men or women. TG/HDLC was also reportedly not superior to non-HDLC in Spanish patients with type 2 diabetes (35).

This investigation has several strengths, including the nationwide sampling from nearly 60 institutes. We also used four different analytical methods and analyzed men and women separately, which was not done in past studies. Nevertheless, some limitations of our study deserve consideration. Variability in laboratory measurements could be present among participating hospitals (36). However, such an influence is virtually negligible because laboratory testing in Japan is well standardized. In fact, a nationwide precision control survey (37) demonstrated that coefficients of variation of tests of TC, HDLC, and TGs were <5%. Only baseline data were used for this analysis; therefore, therapeutic management during the follow-up period could have influenced results. Baseline proportions of women receiving therapy with insulin sensitizers or agents for hypertension or dyslipidemia were higher in the CHD group than in the no-CHD group, probably because of treatment selection bias. The large difference in the proportion of subjects taking agents for dyslipidemia (mainly statins) between men and women also might have influenced the results.

That we did not measure apolipoproteins in this study was another limitation. Although some studies of subjects with (14,15) and without (38,39) diabetes have provided relatively small support for replacement of conventional variables with measurements of apolipoproteins, recent meta-analysis (7) demonstrated that the use of apoB, a measure of the number of atherogenic lipid particles, could be more beneficial to prevent cardiovascular events than that of non-HDLC in clinical settings because there might be substantial discordance between apoB and non-HDLC levels depending on individual differences in composition of the apoB lipoproteins. In addition, apoB is a better predictor of cardiovascular risk especially when cholesterol-enriched remnants or cholesterol-enriched LDL is present; therefore, apoB is not necessarily interchangeable with non-HDLC for evaluation of individual patients in clinical settings (40). Finally, in this analysis, we did not use detailed dietary data, including data on saturated fat, carbohydrates, and the ratio of energy requirements to ingested calories, which could influence serum lipid profiles. This should be clarified in a future study.

In conclusion, the present analysis shows that for Japanese subjects with diabetes, non-HDLC and TC/HDLC for men and TGs for women were the best predictors of CHD. These findings should be considered in the clinical approach to risk reduction among East Asians with diabetes, and using these variables as management markers for dyslipidemia among this population has potential value.

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Risk of Cardiovascular Diseases Is Increased Even with Mild Diabetic Retinopathy

The Japan Diabetes Complications Study

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Objective: Diabetic retinopathy (DR) is linked to cardiovascular risk in diabetic patients. This study examined whether mild-stage DR is associated with risk of coronary heart disease (CHD) and stroke in type 2 diabetic patients of the Japan Diabetes Complications Study (JDCS).

Design: Prospective cohort study.

Participants: In the JDCS, there were 2033 Japanese persons with type 2 diabetes free of cardiovascular diseases at baseline.

Methods: Diabetic retinopathy was ascertained from clinical and photographic grading (70%) following the international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Incident CHD and stroke were followed up prospectively annually up to 8 years.

Main Outcome Measures: Eight-year incidence of CHD and stroke compared between persons with or without DR.

Results: After adjusting for traditional cardiovascular risk factors, persons with mild to moderate nonproliferative DR had a higher risk of CHD (hazard ratio [HR], 1.69; 95% confidence interval [CI], 1.17–2.97) and stroke (HR, 2.69; 95% CI, 1.03–4.86). Presence of retinal hemorrhages or microaneurysms was associated with risk of CHD (HR, 1.63; 95% CI, 1.04–2.56) but was not associated with stroke (P = 0.06). Presence of cotton-wool spots was associated with risk of incident stroke (HR, 2.39; 95% CI, 1.35–4.24) but was not associated with CHD (P = 0.66). When information about DR was added in the prediction models for CHD and stroke based on traditional cardiovascular risk factors, the area under the receiver operating curve improved from 0.682 to 0.692 and 0.640 to 0.677, and 9% and 13% of persons were reclassified correctly for CHD and stroke, respectively.

Conclusions: Type 2 diabetic patients with even a mild stage of DR, such as dot hemorrhages, are already at higher risk of CHD and stroke independent of traditional risk factors.

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Diabetic retinopathy (DR) is estimated to affect approximately 100 million people worldwide when extrapolated to the world diabetes population in 2010.[1] Increasing DR severity is associated with an increased risk of vision loss and risk of vision-threatening proliferative disease over time.[2,3] Presence of DR is not only one of the most common microvascular complications of diabetes, it also is an established predictor of cardiovascular diseases (CVDs). Diabetic patients with DR have been reported to be at higher risk of incident stroke[4–7] and coronary heart disease (CHD).[4,5,8] Kramer et al[9] reported that persons with any degree of DR are at 61% higher risk of CVD events and all-cause mortality independent of traditional risk factors based on the meta-analysis data of 20 epidemiologic studies.

However, there is limited knowledge regarding whether this association is observed consistently in Asian populations.[10,11] Sasaki et al[10] reported an association between the presence of any stage of DR and all-cause mortality in a Japanese type 2 diabetic cohort; detailed association between DR severity and specific CVD outcomes of stroke and CHD is unclear. Considering that duration of diabetes and glucose control or other risk factors are associated with severity of DR,[1] it is reasonable to speculate that people with a severe stage of microvascular complications such as advanced DR have macrovascular complications of CVD. What remains less understood is whether milder stage DR is associated with increased risk of CHD and stroke. There have been limited data reporting associations of early stage of DR and CVD and, if such as association exists, whether there is a continuous association between severity of DR and risk of CVD.[7,12]

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Table 1. Baseline Characteristic of the 1620 Patients Included in the Analysis Compared with Those Who Were Excluded

Characteristic	Included (n = 1620)		Excluded (n = 413)		P Value
	Mean	*Standard Deviation*	*Mean*	*Standard Deviation*	
Age (yrs)	58.3	7.0	59.5	6.8	<0.01
Women (%)	46.4		47.0		0.84
HbA1c (%)	7.9	1.3	7.9	1.2	0.92
Fasting blood sugar (mg/dl)	160.2	43.7	159.6	41.8	0.81
Years after diagnosis	10.6	7.0	11.9	8.0	<0.01
Weight (kg)	58.6	9.4	59.2	9.6	0.20
BMI, kg/m^2 (%)	23.0	3.0	23.3	3.0	0.11
<18.5	5.5		4.4		0.36
≥25	24.3		27.2		0.23
Systolic blood pressure (mmHg)	131.2	16.3	133.7	16.2	<0.01
Diastolic blood pressure (mmHg)	76.7	9.9	77.4	10.3	0.23
Total cholesterol (mg/dl)	200.9	33.9	203.6	38.7	0.16
LDL cholesterol (mg/dl)	122.3	31.8	123.3	34.6	0.58
HDL cholesterol (mg/dl)	54.7	16.7	54.1	17.1	0.51
Triglyceride* (mg/dl)	101.5	73.0	109.0	83.0	0.02
Treated by insulin (%)	20.0		24.3		0.06
Current smoker (%)	28.3		25.8		0.35

BMI = body mass index; HbA1c = glycosylated hemoglobin; HDL = high-density lipoprotein; LDL = low-density lipoprotein.
*Geometric mean.

Whether the presence or severity of DR is associated with CVD independent of traditional cardiovascular factors also is important to understand the potential usefulness of DR information as additional information to improve CVD prediction. Therefore, this study examined associations between the presence and severity of DR and risk of 8-year incident CHD, stroke, and combined outcome of any CVD in the Japanese Diabetes Complications Study (JDCS).

Table 2. Baseline Clinical Characteristics of Type 2 Diabetes Patients in the Japan Diabetes Complications Study

Characteristics	Persons without Diabetic Retinopathy (n = 1141)	Persons with Mild Nonproliferative Diabetic Retinopathy (n = 412)	Persons with Moderate Nonproliferative Diabetic Retinopathy (n = 67)	P Value (for Trend)
Age (yrs)	58.2 (6.9)	58.6 (7.0)	58.0 (7.0)	0.54
Women (%)	44.9	50.5	47.8	0.10
HbA1c (%)	7.8 (1.3)	8.0 (1.2)	8.2 (1.3)	<0.01
Fasting glucose (mmol/l)	8.9 (2.4)	8.9 (2.5)	8.9 (2.2)	0.90
Duration of diabetes (yrs)	9.7 (6.8)	12.7 (7.0)	13.1 (6.5)	<0.01
Insulin treated (%)	15.5	28.9	43.9	<0.01
Oral hypoglycemic agents (%)	64.1	69.9	68.7	0.05
BMI (kg/m^2)	23.0 (3.0)	23.1 (3.0)	22.8 (3.1)	0.85
<18.5 (%)	5.8	4.6	6.0	0.55
≥25 (%)	24.9	23.1	22.4	0.41
Systolic blood pressure (mmHg)	130.4 (16.2)	132.7 (16.5)	136.4 (16.4)	<0.01
Diastolic blood pressure (mmHg)	76.8 (10.1)	76.3 (9.3)	77.4 (10.3)	0.82
LDL cholesterol (mmol/l)	3.19 (0.82)	3.09 (0.81)	3.19 (0.94)	0.12
HDL cholesterol (mmol/l)	1.40 (0.42)	1.45 (0.43)	1.51 (0.52)	<0.01
Triglycerides (mmol/l)*	1.15 (0.82)	1.09 (0.81)	1.12 (0.50)	0.02
Current smoker (%)	29.8	23.7	32.8	0.18
Physical exercise (kilocalories/day)	143.5 (267.5)	117.4 (265.8)	91.9 (288.0)	0.16
Spot urine ACR (mg/gCr)	15.3 (25.0)	19.2 (42.3)	25.2 (75.7)	<0.01
Retinopathy lesions				
Dot/blot retinal hemorrhages (%)	*	88.1/32.9	93.8/78.1	—
Hard exudates (%)	*	0	1.0	—
Cotton-wool spots (%)	*	32.6	62.5	—

ACR = albumin-to-creatinine ratio; BMI, body mass index; HbA1c = glycosylated hemoglobin; HDL = high-density lipoprotein; LDL, low-density lipoprotein.
Data shown as mean ± standard deviation unless otherwise indicated.
*Geometric mean (1 standard deviation).

Table 3. Cox Regression Analysis of the 1620 Type 2 Diabetic Japanese Patients for Diabetic Retinopathy and Cardiovascular Diseases

	Coronary Heart Disease			Stroke			Any Cardiovascular Disease		
	*Hazard Ratio**	95% *Confidence Interval*	P *Value*	*Hazard Ratio**	95% *Confidence Interval*	P *Value*	*Hazard Ratio**	95% *Confidence Interval*	P *Value*
Age (+1 yr)	1.03	1.00–1.07	0.08	1.07	1.03–1.11	<0.01	1.06	1.03–1.09	<0.01
Women (vs. men)	0.60	0.37–0.96	0.03	0.73	0.42–1.25	0.25	0.57	0.38–0.85	0.01
HbA1c (+1%)	1.10	0.93–1.30	0.27	1.15	0.96–1.38	0.14	1.16	1.02–1.33	0.03
Duration of diabetes (+1 yr)	1.02	0.99–1.05	0.12	0.97	0.94–1.01	0.15	1.00	0.97–1.02	0.84
BMI (+1 kg/m^2)	1.03	0.95–1.12	0.48	1.01	0.92–1.10	0.86	1.02	0.95–1.09	0.67
Systolic blood pressure (+1 mmHg)	1.02	1.00–1.03	0.04	1.02	1.00–1.03	0.04	1.02	1.00–1.03	0.01
LDL cholesterol (+0.025 mmol/l)	1.01	1.01–1.02	<0.01	1.00	0.99–1.01	0.53	1.01	1.00–1.01	<0.01
HDL cholesterol (+0.025 mmol/l)	1.00	0.99–1.02	0.61	1.00	0.98–1.02	0.84	1.00	0.99–1.01	1.00
Log triglycerides (+1 unit)	2.41	1.52–3.83	<0.01	1.22	0.72–2.07	0.46	1.94	1.32–2.84	<0.01
Log ACR (+1 unit)	0.97	0.80–1.16	0.72	0.97	0.79–1.19	0.78	0.93	0.79–1.08	0.32
Current or past smoker (vs. never smoked)	1.86	1.17–2.97	0.01	1.42	0.81–2.47	0.22	1.67	1.13–2.46	0.01
Presence of DR	1.69	1.09–2.63	0.02	1.69	1.03–2.80	0.04	1.92	1.33–2.75	<0.01

ACR = albumin-to-creatinine ratio; BMI = body mass index; DR = mild to moderate nonproliferative diabetic retinopathy; HbA1c = glycosylated hemoglobin; HDL = high-density lipoprotein; LDL = low-density lipoprotein.
*Hazard ratios of multivariate model with listed variables.

Patients and Methods

Study Participants

The detailed study design and protocol of the JDCS have been described elsewhere.[13,14] In brief, the JDCS is a multicenter, open-labeled, randomized trial of type 2 diabetic patients examining the impact of lifestyle intervention on diabetic complications. In 1996, 2033 adults Japanese persons (age range, 40–70 years) with type 2 diabetes whose hemoglobin A1c (HbA1c) levels were 6.5% or more were enrolled and randomized; primary outcome analyses have been reported elsewhere.[13] After excluding patients with a known history of CVD and familial hypercholesterolemia and those without baseline assessment of DR, 1620 patients were included in this analysis. Persons included in this analysis were younger, had a shorter duration of diabetes, had a lower systolic blood pressure, and had a lower triglyceride level compared with those who were excluded from the current analysis (Table 1). This study was performed in accordance with the Declaration of Helsinki and received ethical approval from the institutional review boards; all participants gave written informed consent. This study is a subanalysis of the JDCS, which has been registered with identifier C000000222 in a trial registry (www.umin.ac.jp; accessed February 13, 2012).

Assessment of Diabetic Retinopathy

Ophthalmologists who have a subspecialty and experience in retinal diseases at each study site determined the pathologic features related to DR by mydriatic indirect ophthalmoscopic examination and slit-lamp biomicroscopic fundus examination using a precorneal lens. Supplemental information from fundus photography and fluorescein angiography were allowed to be used as needed. A standardized paper-based grading form was used to record individual lesions of DR (e.g., microaneurysms or dot hemorrhages, blot hemorrhages, hard exudates, cotton-wool spots, venous beading, intraretinal microvascular abnormalities, retinal neovascularization, and other proliferative changes). At each visit, ophthal-

Table 4. Cox Regression Analysis of the 1620 Type 2 Diabetic Japanese

	Coronary Heart Disease			
	Crude Incidence Rate per 1000 Person-Years	*Hazard Ratio**	95% *Confidence Interval*	P *Value*
Severity of DR				
No DR	7.54	1	Reference	
Mild nonproliferative DR	12.46	1.62	1.02–2.58	0.04
Moderate nonproliferative DR	13.61	2.18	0.92–5.17	0.08
P value for trend			0.01	
DR lesions				
Retinal hemorrhages (dot or blot) or microaneurysms (present vs. absent)		1.63	1.04–2.56	0.03
Hard exudates (present vs. absent)		1.83	0.78–4.25	0.16
Cotton-wool spots (present vs. absent)		1.15	0.62–2.14	0.66

DR = diabetic retinopathy.
*Adjusted for age, sex, hemoglobin A1c, duration of diabetes, body mass index, systolic blood pressure, low-density lipoprotein cholesterol,

mologists filled in the grading form and sent them with retinal images (macula-centered and disc-centered image or centered between the fovea and disc if wide photographic angle was 45° or 50°). Standardized images could be obtained from 1424 of 2033 patients (70%). However, because standardized retinal images could not be obtained (e.g., different camera type, different format of film or digital, and different photographic angles) in the remaining 30% of the participants, a clinical diagnosis of the presence and severity of DR based on the standardized form provided by ophthalmologists was used when retinal images were not available. Severity of DR was categorized following the international clinical diabetic retinopathy severity scales into 5 categories: no retinopathy (equivalent to the Early Treatment of Diabetic Retinopathy Study [ETDRS] scale level 10), mild nonproliferative DR (equivalent to ETDRS level 20), moderate nonproliferative DR (equivalent to ETDRS levels 35, 43, and 47), severe nonproliferative DR (equivalent to ETDRS levels 53A–53E), and proliferative DR (PDR; equivalent to ETDRS levels 61 or higher).[15] To assess consistency in detecting and classifying DR solely based on clinical examination, a random sample was selected and the assessment was cross-validated by ophthalmologists on site using a centralized assessment. The grading agreement on the status of DR was cross-validated; the weighted κ statistics for the agreement of DR severity of 5 categories was 0.59 (95% confidence interval [CI], 0.54–0.65) and was considered to be more than moderate.[14] Persons with severe nonproliferative DR or worse were excluded from this study because the primary outcome of this study was to investigate the occurrence of DR and progression of DR from mild nonproliferative DR to severe nonproliferative DR or nonproliferative DR.[10,11] A history of ocular diseases and surgeries also was surveyed; persons with significant cataract or other ocular diseases confounding the diagnosis of DR were excluded.[14]

Patients were assessed for CHD and stroke at baseline and annually for up to 8 years. Information regarding CVD outcomes was collected from death certificates, hospital admission or discharge records, community health centers, medicolegal records, general practitioners, and interviews with patients and relatives, in addition to electrocardiogram records and laboratory records. Fatal and nonfatal CHD and stroke events were identified during follow-up and were certified by at least 2 members of the experts' committee who were masked to subjects' characteristics and the other member's diagnosis. Myocardial infarction and CHD were defined according to the World Health Organization (WHO) Monitoring of Trends and Determinants in Cardiovascular Disease criteria.[16] In brief, the diagnosis of CHD was based on clinical symptoms, electrocardiography electrocardiography findings, cardiac enzymes, necropsy findings, and history of CHD. In all subjects at risk, a 12-lead electrocardiogram was recorded at each assessment. Angina pectoris was defined as typical effort-dependent chest pain or oppression relieved at rest or by using nitroglycerin, as validated by exercise-positive electrocardiography, angiography, or both. A patient with a first percutaneous coronary intervention or coronary artery bypass graft also was considered to have a CHD event.

Stroke events were defined as a constellation of focal or global neurologic deficits of sudden or rapid onset and for which there was no apparent cause other than a vascular accident, as determined by a detailed history, a neurologic examination, and ancillary diagnostic procedures such as computed tomography, magnetic resonance imaging, cerebral angiography, and lumbar puncture. Stroke events were classified as cerebral infarction (including embolus), intracranial hemorrhage (including subarachnoid hemorrhage), transient ischemic attack, or stroke of undetermined type in accordance with WHO criteria.[17] Cases of asymptomatic lesions detected by brain imaging (i.e., silent infarction) were not included. Only a first-ever event during the study period was counted for the analysis; "any CVD" was defined as "either CHD or stroke," or as "one having developed earlier event if patients had experienced both events."

Statistical Analysis

The Kaplan-Meier method was used to plot a survival curve for incidence of CVD. The Cox proportional hazard model was used to estimate hazards ratios (HRs) associated with the presence or absence of DR at baseline examination adjusting for age, sex, HbA1c level, duration of diabetes, body mass index, systolic blood pressure, low-density lipoprotein (LDL) cholesterol level, high-density lipoprotein cholesterol level, log triglycerides, log albumin-to-creatinine ratio, and smoking. The same adjustment factors were used to estimate HRs for the severity of DR (i.e., no DR vs. mild nonproliferative DR and no DR vs. moderate nonproliferative DR) and the presence or absence of individual DR lesions, namely dot hemorrhages, blot hemorrhages, hard exudates, and cotton-wool spots.

Then, changes in predictive accuracy were examined by adding DR information onto prediction by the traditional car-

Patients for Diabetic Retinopathy and Cardiovascular Diseases

Stroke				Any Cardiovascular Disease			
Crude Incidence Rate per 1000 Person-Years	*Hazard Ratio**	*95% Confidence Interval*	P *Value*	*Crude Incidence Rate per 1000 Person-Years*	*Hazard Ratio**	*95% Confidence Interval*	P *Value*
5.72	1	Reference		11.03	1	Reference	
9.50	1.64	0.98–2.76	0.06	18.70	1.86	1.28–2.71	<0.01
9.15	2.15	0.75–6.21	0.16	18.86	2.34	1.11–4.93	0.03
		0.03				<0.01	
	1.63	0.97–2.73	0.06		1.78	1.23–2.58	<0.01
	1.76	0.62–4.97	0.28		1.83	0.88–3.80	0.10
	2.39	1.35–4.24	<0.01		1.87	1.20–2.91	0.01

log triglycerides, log albumin-to-creatinine ratio, and smoking.

diovascular risk factors in the United Kingdom Prospective Diabetes Study (UKPDS) risk engine.[18,19] Changes in the area under the receiver operating characteristic curve (AUC) were examined by integrating the presence or absence of DR lesions with logistic regression models based on the UKPDS risk factors. Changes in reclassification capacity also were assessed by plotting a risk of CVD predicted by the UKPDS risk factors plus information regarding the presence or absence of DR lesions against the results predicted by the UKPDS risk factors alone. All *P* values were 2 sided. A *P* value less than 0.05 was considered statistically significant. Statistical analyses were carried out using the SAS software package version 9.2 (SAS Institute, Cary, NC).

Results

Of the 1620 patients, 412 (25.4%) and 67 (4.1%) had mild or moderate nonproliferative DR, respectively (Table 2). The cumulative number of CHD events in persons with mild nonproliferative DR and moderate nonproliferative DR were 35 (8.5%) and 6 (9.0%), respectively; the cumulative number of stroke events in persons with mild nonproliferative DR and moderate nonproliferative DR were 27 (6.6%) and 4 (6.0%), respectively.

Older age, male sex, higher HbA1c level, systolic blood pressure, LDL cholesterol level, triglycerides level, and smoking status were associated significantly with any CVD. Male sex, higher systolic blood pressure, LDL cholesterol level, triglycerides level, and smoking status were associated with a higher risk of CHD in the multivariate model. Older age and higher systolic blood pressure were associated with a higher risk of stroke (Table 3). Persons with any DR had a 1.69 times higher risk of both CHD and stroke ($P = 0.02$ and $P = 0.04$) and a 1.92 times higher risk of any CVD compared with persons without DR ($P<0.01$) after adjusting for age, sex, HbA1c level, duration of diabetes, body mass index, systolic blood pressure, LDL cholesterol level, high-density lipoprotein cholesterol level, log triglycerides, log albumin-to-creatinine ratio, and smoking status (Table 3). When the analyses were repeated to the confined subsample that had standardized retinal images with confirmed diagnosis based on central grading for DR, the associations between DR and stroke were consistently significant (adjusted HR, 1.86; 95% CI, 1.00–3.45; $P = 0.049$). However, the association with CHD was diminished to a nonsignificant level (adjusted HR, 1.34; 95% CI, 0.76–2.34; $P = 0.31$).

Persons with a mild or moderate stage of DR had higher risk of CHD, stroke, and any CVD ($P<0.01$ for trend, $P = 0.03$ for trend, and $P<0.01$ for trend, respectively; Table 4). Presence of retinal hemorrhages or microaneurysms was associated with up to approximately a 60% to 80% higher risk of CHD developing ($P = 0.03$) and any CVD ($P<0.01$; Table 4). Persons with hard exudates seem to have a higher risk of CHD, stroke, and any CVD, but these associations did not reach statistical significance. Presence of cotton-wool spots was associated with a more than 2-fold higher risk of incident stroke and an 87% higher risk of any CVD but not with CHD (Table 4).

With the model estimating risk of CHD based on traditional cardiovascular risks factors proposed by the UKPDS,[18] the AUC analysis improved from 0.682 in the model without DR (95% CI, 0.626–0.737; shown with light blue, Fig 1A) to 0.697 in the model with DR (95% CI, 0.641–0.752; shown with dark blue, Fig 1A). This difference did not reach statistical significance ($P = 0.22$). Figure 2A shows how adding DR information on the model with UKPDS risk factors reclassified CHD cases (n = 100, red dot) and noncases (n = 1520, blue dot). Reclassified correctly in the model including DR information were 6 cases (6%) and 53 noncases (3%); however, 6 cases (6%) and 63 noncases (4%) were reclassified in the model with DR incorrectly.

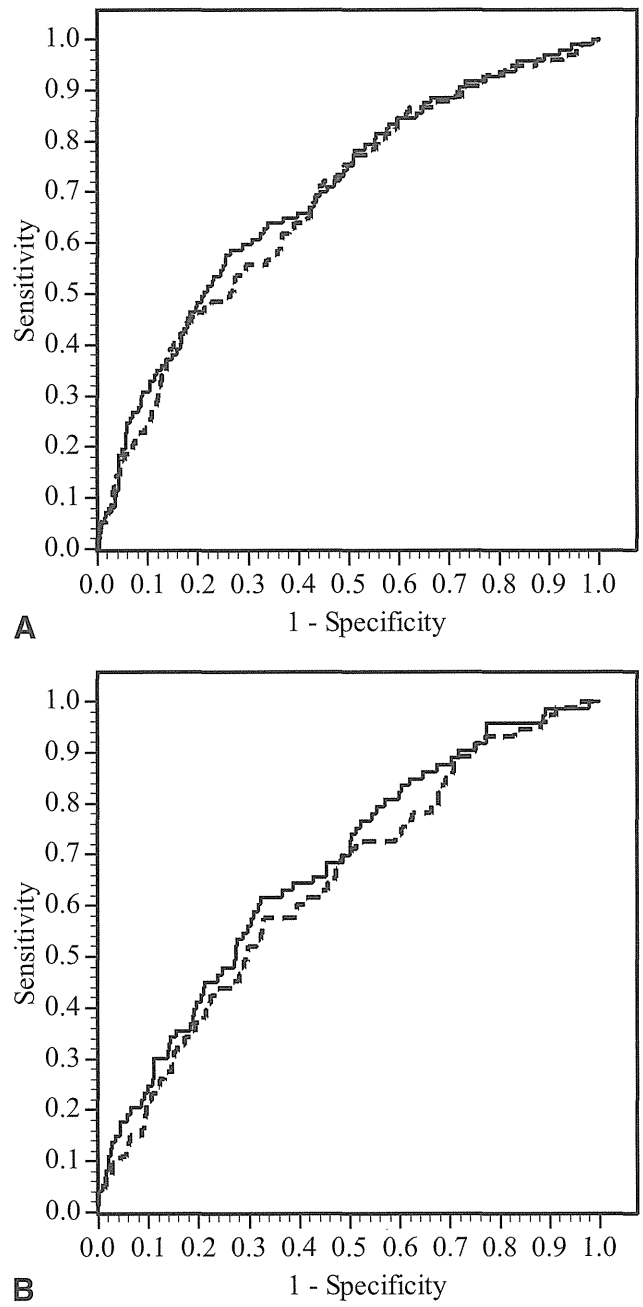

Figure 1. **A**, Graph showing a comparison of the receiver operating curves for coronary heart disease based on the United Kingdom Prospective Diabetes Study (UKPDS) risk factors with (solid line) or without (dashed line) diabetic retinopathy. **B**, Graph showing a comparison of receiver operating curves for stroke based on the UKPDS risk factors with (solid line) or without (dashed line) diabetic retinopathy.

For prediction of stroke, the AUC analysis improved from 0.640 (95% CI, 0.576–0.704; shown with dark blue, Fig 1B) to 0.677 (95% CI, 0.615–0.739; shown with light blue, Fig 1B) by adding DR information; again, this difference was not statistically significant ($P = 0.12$). As shown in Figure 2B, reclassification of stroke cases (n = 76, red dot) and noncases (n = 1544, blue dot)

was in favor of prediction with the model including DR; 9 cases (12%) and 1 noncase (1%) were reclassified correctly by adding DR to the prediction model, whereas 1 case (1%) and 55 noncases (4%) were reclassified by DR incorrectly.

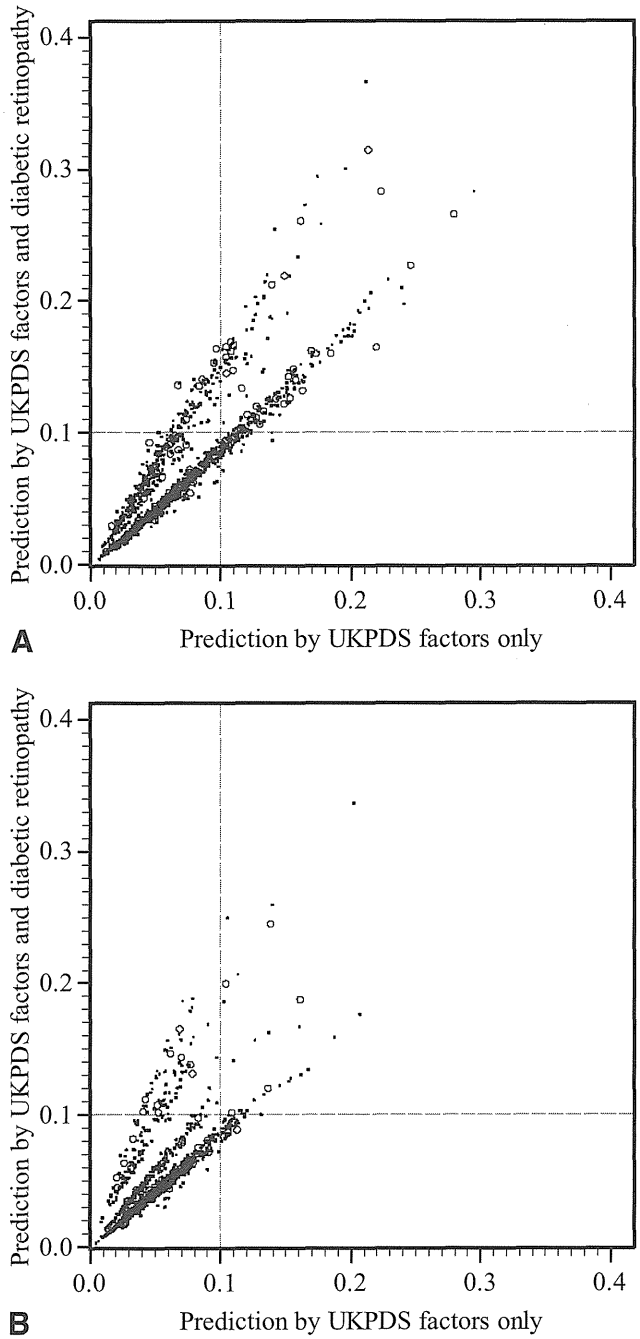

Figure 2. **A**, Graph showing the risk of coronary heart disease plotted for predicted risk by United Kingdom Prospective Diabetes Study (UKPDS) models with diabetic retinopathy against predicted risk by the UKPDS model without diabetic retinopathy. Reclassification of coronary heart disease is shown for cases (circles) and noncases (black dots). **B**, Graph showing reclassification of stroke cases (circles) and noncases (black dots) based on the UKPDS risk factors with or without diabetic retinopathy.

Discussion

This analysis of adult Japanese persons with type 2 diabetes found persons with even a mild stage DR already are at approximately at a 70% higher risk of developing CHD and stroke independent of cardiovascular risks. There were significant increasing trends for CHD, stroke, and any CVD by increasing severity stage of DR. Most importantly, these associations were confirmed to be significant after adjusting for traditional cardiovascular risk factors. The association between diabetic retinopathy and risk of developing CVD has been reported in multiple cohort studies.[4,5,7,8,12,20–23] In the Wisconsin Epidemiological Study of Diabetic Retinopathy, the presence of PDR was associated with incident stroke in both younger-onset and older-onset diabetes, independent of duration of diabetes, glucose level, and other risk factors.[4,12,23] In the WHO Multinational Study of Vascular Disease in Diabetes, this not only was confirmed, but also the findings showed that any level of DR was associated with incident stroke both in men and women with type 2 diabetes.[5] Severity of DR also was associated with risk of stroke in persons with type 1 diabetes.[12] Associations between milder stage of DR and stroke are controversial. In the Atherosclerosis Risk in Communities (ARIC) Study, the presence of nonproliferative DR was associated with a 2-fold increased risk of developing stroke in persons with type 2 diabetes.[7] Similarly, the ARIC study reported that the presence of DR is associated with a 2-fold increased risk of CHD and that the severity of DR was associated with increasing CHD risk.[8]

However, there has been limited knowledge from Asian populations.[10,11] This is important because there exist differences in the epidemiologic and risk associations of CVD between a white population and an Asian Japanese population.[24–26] For example, the incidence of stroke is much higher in Japanese persons than American Japanese persons in Hawaii.[27] For risk associations of CVD, this study showed a discrepancy in body mass indices between white and Japanese patients with type 2 diabetes (approximately 29 kg/m^2 in white patients from the UKPDS vs. 23 kg/m^2).[28] Another example is lipid profile and its association with CVD. Low-density lipoprotein cholesterol was the most important risk factor for CHD in both white and Asian populations, and the second most important risk in the cohort of the JDCS was the serum triglyceride level,[24,26] whereas lower high-density lipoprotein cholesterol was considered to be the second most important risk factor in the UKPDS.[24,26] Based on these differences in risk associations of CVD, there is a potential need for an ethnicity-specific risk prediction model of CVD (e.g., such as the ethnicity-specific metabolic syndrome and its component guideline as risk of CVD).[25,29] This study confirmed that the presence of DR is found consistently to be associated with an increased risk of stroke and CHD in Japanese persons with type 2 diabetes. Sasaki et al[10] reported an association between any stage of DR and all-cause mortality in a Japanese type 2 diabetic cohort; the present findings further elucidated that even a mild stage of DR is associated with a higher risk of both CHD and stroke.

Although a strong and consistent association between PDR and CVD has been reported, it is still controversial whether a milder stage of DR (i.e., nonproliferative DR) is associated with an increased risk of CVD. Mild nonproliferative DR was not associated with an increased risk of stroke in persons with older-onset diabetes in 16 years of follow-up,[23] and any level of DR was not associated with stroke in persons with type 1 diabetes in the WHO Multinational Study of Vascular Disease in Diabetes.[5] An association between nonproliferative DR and risk of CHD was not significant in a Finnish study.[30] This study found a significant increasing trend in risk of CVD by increasing severity of DR. The observed strength of association between the presence of relatively mild stage DR and CVD seems to be in concordance with previous epidemiologic studies. In the present study, risk of stroke and CHD were approximately 1.7 times higher in persons with mild to moderate nonproliferative DR than in those without DR, which was slightly weaker than that found with PDR. This supports indirectly that there is an increasing association between severity of DR and higher risk of CVD even at a milder stage of DR. There have not been many studies reporting detailed associations of DR level and risk of CVD. Klein et al[12] reported an increasing association between severity of DR and CVD in people with type 1 diabetes. They categorized DR severity into 4 groups of no DR, early nonproliferative DR, moderate to severe nonproliferative DR, and PDR, and risk of mortality including any heart disease outcome was increased by 30% for each higher severity of DR. In the diabetic participants of the ARIC study, which is assumed to be mainly type 2 diabetic patients, there were no increasing associations observed between retinopathy grade and risk of ischemic stroke.[7]

This study found that the presence of retinal hemorrhages (dot or blot) or retinal microaneurysms was associated with a 60% to 80% increased risk of CHD and any CVD. Retinal hemorrhages and microaneurysms are well recognized as early signs of DR. In the international severity scale for DR, presence of dot hemorrhages per se are categorized into mild nonproliferative DR, that is, the mildest stage in the classification.[15] In the ARIC study,[7] the presence of microaneurysms was associated significantly with incident ischemic stroke after adjusting for cardiovascular risks, whereas the presence of retinal hemorrhages did not increase the risk. This study also found an association between cotton-wool spots and increased risk of stroke. Pathologically, cotton-wool spots in the retina constitute a focal retinal capillary obstruction[31]; ischemic change in the retina observed as cotton-wool spots may reflect similar pathologic changes in the cerebral microcirculation related to stroke. Retinal and cerebral vasculatures share similarities in embryologic, anatomic, and physiologic characteristics[32,33]; retinal microvasculature may provide a window to observe vascular health directly in vivo.[32,33] Patton et al[33] pointed out that constituents of both retinal and cerebral microvasculatures are common (i.e., endothelial cells surrounded by pericytes, supported by basement membranes, and further surrounded by glial cells), and they have so-called barrier endothelia for mechanical and metabolic activities. An autoregulated mechanism to maintain constant blood flow is another common property of both retinal and cerebral circulation.[33] Assessing cerebral vasculature, especially for microcirculation, remains challenging; we speculate that simple direct visualization of retinal microvasculature may provide information of concurrent pathologic features in the cerebral vasculature. Supporting this finding and this concept, the ARIC study reported that the presence of cotton-wool spots was associated with a 2-fold risk of having subclinical cerebral infarction detected by magnetic resonance imaging scans,[34] and the presence of cotton-wool spots was associated with a 3-fold risk of incident stroke in a nondiabetic population.[35] In a diabetic population in the ARIC study, the association between cotton-wool spots and ischemic stroke was attenuated to nonsignificance after adjusting for other cardiovascular risk factors.[7] This is partially in keeping with the present findings, which suggest that potential variation in the association may exist for specific subtypes of stroke (i.e., ischemic infarction or hemorrhagic stroke).

Significant associations were found between mild stage DR and CVD independent of cardiovascular risk factors. Furthermore, whether integrating DR status into the CVD risk prediction models contributes to better prediction was examined. Although changes in the AUC were not statistically significant when adding DR lesions onto UKPDS proposed cardiovascular risk factors for CHD and stroke, there were moderate improvements. The most beneficial effect in reclassifying cases and noncases by adding DR information was observed for the stroke prediction model where the model with DR reclassified 12% of stroke cases and 1% of noncases correctly compared with the model without DR, with minimal tradeoffs of reclassifying 1% of cases and 4% of noncases incorrectly. The clinical relevance of incorporating DR assessment in a risk prediction model for CHD or stroke in addition to traditional cardiovascular markers may need further investigation. Although there are many attempts to refine CVD risk prediction using newer risk markers, such as high-sensitivity C-reactive protein or a combination of multiple markers, there are modest improvements in their performance on CVD risk prediction and they are now established as robust markers.[36–40] Kim et al[36] reported that when 18 new potential biomarkers were added to a traditional risk factor model, there was significant improvement in the AUC (+0.02) and net reclassification of 6.45%. Observed in this study was a +0.037 improvement in AUC for stroke and a +13% net reclassification when adding DR information to traditional risk factors of UKPDS risk engine; the usefulness of DR assessment as a biomarker of stroke prediction warrants further study to explore its potential. The strength of using DR assessment as a biomarker of CVD may include its long-term stability. Based on a 4-year observation in the older-onset diabetic patients in the Wisconsin Epidemiological Study of Diabetic Retinopathy, 15% to 19% of eyes with DR improved more than 2 step in the ETDRS severity scale.[41] However, no improvement was observed in persons with a level of DR of less than 21/21, which corresponds to mild nonproliferative DR.[41] This is keeping with the clinical impression that it is not likely to see complete natural resolution as soon as diabetic patients demonstrate any level of DR. When con-

sidering the presence of DR as a biomarker to predict CVD, this characteristic is beneficial because it is stable over time. Also, given that assessment of DR already is performed routinely by ophthalmologists, sharing this information and using it proactively in CVD risk assessment will benefit both clinicians and patients for achieving better prediction of CVD with minimum additional effort and cost; additional cost could be one of the concerns for adopting a new biomarker for CVD in clinical practice.[40]

The implications of these study findings in daily clinical practice should be emphasized. The data suggest that even with the most mild form of DR, patients already are at approximately a 70% higher risk of CVD developing, independent of cardiovascular risks. Furthermore, when ophthalmologist see progression of DR, this suggests increasing risk of CVD at the same time. Ophthalmologists need to inform the patients and physicians or diabetologists who are managing diabetes to optimize modifiable cardiovascular risk factors immediately.

Limitations of this study should be mentioned. First, DR was not confirmed by centralized grading of the fundus photographs. Although the agreement between ophthalmologists in each site was confirmed, it was moderate and misclassification was possible. This may result in overlooking the pathologic features of DR and underestimating the number of patients with DR. Misclassification for milder stage DR also is possible. These in turn may result underestimation of the association between DR and CVD. Second, persons with more severe stages of DR were not included because the study aimed to examine the incidence and progression from mild to severe stages of DR as the primary outcomes. External validity of this study also may be compromised because the participants of this study were a relatively well-managed type 2 diabetic cohort. The association between DR and increased risk of CVD in an Asian population should be confirmed further in a larger longitudinal study with a broader spectrum of potential confounding factors.

In conclusion, this study found that risk of CVD is increased even with a mild stage DR in type 2 diabetic Japanese persons over the 8-year follow-up of the JDCS. Further studies are required to validate the role of DR assessment for CVD risk stratification in clinical contexts.

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Footnotes and Financial Disclosures

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Fruit Intake and Incident Diabetic Retinopathy with Type 2 Diabetes

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Background: Antioxidants and dietary fiber are postulated to have preventive effects on diabetic retinopathy, but evidence is lacking. We investigated this association in a cohort with type 2 diabetes 40–70 years of age with hemoglobin (Hb)A_{1C} ≥6.5%, originally part of the Japan Diabetes Complications Study.

Methods: After excluding people who did not respond to a dietary survey and patients with diabetic retinopathy or a major ocular disease at baseline, we analyzed 978 patients. Baseline dietary intake was assessed by a food frequency questionnaire based on food groups and 24-hour dietary records. Primary outcome was incident diabetic retinopathy determined using international severity scales.

Results: Mean fruit intake in quartiles ranged from 23 to 253 g/day, with increasing trends across quartiles of fruit intake for vitamin C, vitamin E, carotene, retinol equivalent, dietary fiber, potassium, and sodium. Mean energy intake ranged from 1644 to 1863 kcal/day, and fat intake was approximately 25%. HbA_{1C}, body mass index, triglycerides, and systolic blood pressure were well controlled. During the 8-year follow-up, the numbers of incident cases of diabetic retinopathy from the first through the fourth quartiles of fruit intake were 83, 74, 69, and 59. Multivariate-adjusted hazard ratios for the second, third, and fourth quartiles of fruit intake compared with the first quartile were 0.66 (95% confidence interval = 0.46–0.92), 0.59 (0.41–0.85), and 0.48 (0.32–0.71) (test for trend, $P < 0.01$). There was no substantial effect modification by age, sex, HbA_{1C}, diabetes duration, overweight, smoking, and hypertension. Risk for diabetic retinopathy declined with increased intake of fruits and vegetables, vitamin C, and carotene.

Conclusion: Increased fruit intake in ranges commonly consumed was associated with reduced incident diabetic retinopathy among patients adhering to a low-fat energy-restricted diet.

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Diabetic retinopathy accounts for 5% of blindness in the world and 15–17% of blindness in developed countries.[1] Strict glycemic and blood pressure control reduces the incidence and progression of diabetic retinopathy, and the importance of lipid control is emerging, although the possible value of other treatments remains unclear.[2] In the management of diabetic patients, a diet rich in fruit is encouraged by guidelines in the United States,[3–5] Europe,[6] and Canada,[7] mainly based on its benefits for the prevention of hypertension and cardiovascular disease.[8–10] However, the direct effects of a high-fruit diet on diabetic retinopathy are not well understood. In contrast, guidelines of the Japan Diabetes Society (JDS) recommend fruit intake of only up to one unit.[11]

Several studies have examined nutrients that are abundant in fruits, such as vitamins C and E, carotene, and dietary fiber.[12–15] The pathogenesis of diabetic retinopathy is closely linked to oxidative stress and the antioxidants mentioned above are potential agents for preventing diabetic retinopathy.[16] The associations between antioxidants and diabetic retinopathy have been examined only cross-sectionally and remain unclear,[12] but some antioxidants have been shown to reduce risk of age-related macular degeneration.[17] Fruits are low-glycemic-index foods rich in dietary fiber[18] that can slow glucose response, and a few studies have reported an inverse association between increased intake of dietary fiber and prevalence of diabetic retinopathy.[13–15] These lines of evidence prompted us to investigate the association between intake of fruits and related nutrients and incident diabetic retinopathy in a cohort of patients with type 2 diabetes.

METHODS

Study Cohort

This study is part of the Japan Diabetes Complications Study, an open-labeled randomized trial originally designed to evaluate the efficacy of a long-term therapeutic intervention

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