Table 1 Basic patient profile

n = 4014	Total		<65 years		65-74 years		≥75 years		P1	Male		Female		P2
	n = 4014		n = 1267		n = 1731		n = 1016			n = 2078		n = 1936		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Gender (% male)	51.2	53.1	49.8	49.9	*	100.0	0	-						
Age (yrs,mean/median)	67.9/70	2.0	56.5/58	7.0	70.0/70	2.7	78.8/78	3.5	-	67.0/69	10.0	69.7/70	8.7	**
Duration of DM (months)	177.9	70.0	156.3	64.5	186.1	101.2	190.7	104.3	***	191.6	108.9	169.8	79.7	**
HbA1C (%)	7.70	0.80	7.74	1.38	7.72	1.18	7.62	1.14	0.17	7.61	1.25	7.79	1.20	**
FPG (mg/dl)	149.8	30.3	157.9	53.2	146.4	45.7	145.4	42.0	**	150.9	48.8	146.9	44.9	0.17
SBP (mmHg)	137.3	11.7	133.1	17.3	135.3	16.8	146.0	17.5	**	133.8	17.2	135.9	17.1	*
DBP (mmHg)	74.0	7.3	76.1	11.8	73.5	10.6	72.3	10.7	***	74.5	11.1	73.3	10.9	*
TG (mg/dl)	138.2	53.8	159.4	156.3	128.5	82.0	128.4	73.0	***	142.9	126.3	131.3	83.7	0.18
LDL-C (mg/dl)	118.2	21.3	121.6	33.8	117.6	32.1	115.0	29.3	*	115.0	31.8	121.2	31.4	**
HDL-C (mg/dl)	55.8	10.7	54.8	15.6	55.4	15.5	57.7	15.8	0.38	53.42	15.4	56.78	15.7	**
Non-HDL-C (mg/dl)	145.8	23.4	153.2	40.3	143.3	35.5	141.0	31.8	*	143.6	36.9	147.5	35.6	*
LDL-C/HDL-C	2.31	0.74	2.41	1.17	2.31	1.21	2.27	0.88	*	2.33	0.95	2.33	1.28	0.21
Agents for HT (%)	55.5		49.3		56.0		62.3		**	48.5		62.0		***
ACEI/ARB	39.7		36.9		40.4		41.9		0.65	35.8		43.4		*
CCB	41.2		32.4		43.7		52.6		0.31	36.0		46.5		*
Others	28.3		22.5		31.8		31.4		0.69	28.9		26.7		0.66
Agents for DL (%)	57.3		63.8		54.8		52.6		**	52.1		60.1		***
Strong statins	29.6		31.1		28.3		25.4		0.36	29.7		29.5		0.89
Classical statins	53.3		47.0		58.0		61.6		0.11	51.9		55.0		0.23
Fibrates	8.9		12.0		6.5		6.8		0.13	9.8		7.9		0.18
Others	8.2		9.9		7.2		6.2		0.10	8.6		7.6		0.22
Agents for DM (%)	86.6		76.9		91.6		90.3		**	85.1		88.6		*
Insulin	23.9		24.4		24.6		21.7		0.42	28.0		32.4		*
Sulfonylurea	49.5		41.5		51.3		53.7		0.21	50.0		48.9		0.29
Others	26.1		34.6		21.4		24.5		0.19	26.0		22.0		*
IHD (/1000 year)	9.68		8.84		10.04		9.87		0.97	10.26		9.47		0.32
CVA (/1000 year)	6.78		4.45		7.44		7.56		0.21	7.02		5.72		0.27

P1: Differences in each factor among ages. P2: Differences in each factor between genders. HbA1C:NGSP, *P < 0.05, **P < 0.01, ***P < 0.001.

to avoid the interactive effect on non-HDL-C, or excluding LDL-C and HDL-C levels for the LDL-C/HDL-C ratio). The non-HDL-C level was only correlated with IHD in patients younger than 65. The LDL-C/HDL-C ratio was significantly correlated with IHD in patients of all generations. Age and lower HDL levels were correlated with CVA in patients over 75 years old (Table 2, Figure 2). Subsequently, we evaluated the relationships with IHD and CVA according to the quartile categories for each age group by Kaplan-Meier estimator curves. The HDL-C level was inversely correlated with IHD and CVA, particularly in individuals over 75 (Figure 3). The LDL-C/HDL-C ratio tended to correlate with IHD in all individuals (Figure 3). For the variable current smokers, 6.8% of the total population of subjects smoked. By age category, 9.9, 6.7 and 3.8% of patients younger than 65, patients between 65 and 74, and patients older than 75 smoked, respectively. As the duration of diabetes is pretty long, number of present smokers is not many.

Discussion

Background and discussion points of the study

The numbers of diabetic elderly and their associated net medical costs have drastically increased in recent decades. The mean life expectancy is now approximately an additional 12 and 16 years at age 75 for males and females in Japan, respectively, although the average life span is 78.9 and 85.6 years, respectively. Consequently, the number of late elderly (individuals older than 75) exceeds 13 million, or 10% of the total Japanese population. Diabetes can either develop in the elderly or continue through old age after an earlier onset, and the numbers of diabetic elderly are increasing. In Japan, 55% of diabetic individuals were elderly in 2007, and

Table 2 Risk factors for IHD and CVA by Cox multivariate models in each age group (IHD, upper; CVA, lower)

n = 4014	Total (n = 4014)			<65 years (n = 1267)			65-74 years (n = 1731)			≥75 years (n = 1016)		
IHD	Adjusted HR	95% CI	P	Adjusted HR	95% CI	P	Adjusted HR	95% CI	P	Adjusted HR	95% CI	P
Gender (women vs. men)	1.103	0.972–1.268	0.197	1.044	0.967–1.073	0.456	1.085	0.978–1.210	0.101	**1.132**	**0.992–1.278**	**0.019***
Age (per 10 years)	1.013	0.972–1.066	0.328	1.022	0.977–1.079	0.229	**1.054**	**1.002–1.106**	**0.049***	1.005	0.871–1.139	0.682
Duration of Diabetes (months)	0.995	0.988–1.003	0.053	1.001	0.991–1.008	0.582	**0.993**	**0.985–0.999**	**0.033***	**0.992**	**0.982–0.999**	**0.023***
HbA1C (per 1%)	**1.171**	**1.001–1.356**	**0.047***	**1.327**	**1.025–1.686**	**0.032***	1.219	0.973–1.487	0.083	0.792	0.479–1.059	0.134
FPG (per 10 mg/dl)	1.004	0.997–1.008	0.432	1.005	0.996–1.013	0.355	1.004	0.997–1.009	0.592	0.999	0.987–1.007	0.761
SBP(per 10 mmHg)	1.008	0.995–1.021	0.186	**1.030**	**1.000–1.055**	**0.035***	1.014	0.994–1.037	0.175	0.986	0.954–1.014	0.331
DBP(per 10 mmHg)	0.995	0.978–1.015	0.618	0.982	0.948–1.024	0.386	0.980	0.950–1.011	0.206	1.027	0.986–1.073	0.202
TG (quartile)	1.005	0.889–1.166	0.555	1.002	0.996–1.006	0.502	1.108	0.997–1.220	0.065	1.001	0.961–1.046	0.454
LDL-C (quartile)	**1.318**	**1.103–1.585**	**0.023***	**1.571**	**1.128–2.524**	**0.016***	1.050	0.932–1.176	0.112	1.156	0.998–1.309	0.054
HDL-C (quartile)	**0.751**	**0.611–0.917**	**0.005****	0.828	0.646–1.017	0.072	0.987	0.966–1.008	0.204	**0.629**	**0.401–0.856**	**0.001****
Non-HDL-C (quartile)	1.023	0.981–1.072	0.075	**1.025**	**1.001–1.121**	**0.044***	1.073	0.982–1.161	0.086	0.941	0.791–1.102	0.621
LDL-C/HDL-C (quartile)	**1.583**	**1.298–1.945**	**0.001****	**2.324**	**1.516–3.795**	**0.001****	**1.359**	**1.028–1.824**	**0.021***	**1.407**	**1.015–2.592**	**0.029***
CVA	**Adjusted HR**	**95% CI**	**P**	**Adjusted HR**	**95% CI**	**P**	**Adjusted HR**	**95% CI**	**P**	**Adjusted HR**	**95% CI**	**P**
Gender	1.164	0.985–1.296	0.351	1.014	0.897–1.240	0.655	1.208	0.896–1.526	0.112	0.953	0.912–1.012	0.063
Age	1.015	0.986–1.039	0.282	1.002	0.957–1.076	0.754	1.007	0.916–1.166	0.537	**1.103**	**1.002–1.217**	**0.048***
Duration of Diabetes	0.998	0.992–1.001	0.206	1.003	0.987–1.017	0.709	0.996	0.989–1.001	0.096	0.999	0.991–1.005	0.818
HbA1C	1.001	0.790–1.214	0.128	1.019	0.691–1.401	0.814	0.997	0.855–1.222	0.569	0.928	0.822–1.010	0.059
FPG	1.005	0.995–1.005	0.803	1.003	0.990–1.018	0.741	1.002	0.995–1.008	0.592	0.998	0.986–1.008	0.711
SBP	1.009	0.993–1.024	0.276	1.024	0.988–1.055	0.185	1.015	0.992–1.037	0.206	0.989	0.957–1.018	0.458
DBP	0.998	0.978–1.020	0.846	0.995	0.958–1.046	0.831	0.981	0.948–1.016	0.278	1.024	0.978–1.074	0.317
TG	1.132	0.908–1.302	0.156	1.053	0.658–1.742	0.833	1.253	0.900–1.780	0.184	1.169	0.746–1.853	0.497
LDL-C	1.009	0.912–1.191	0.675	**1.005**	**1.001–1.100**	**0.047***	1.015	0.892–1.136	0.714	0.997	0.982–1.012	0.631
HDL-C	**0.742**	**0.596–0.901**	**0.003****	0.715	0.591–1.191	0.200	**0.750**	**0.494–1.000**	**0.049***	**0.536**	**0.320–0.851**	**0.007****
Non-HDL-C	0.981	0.945–1.019	0.206	**1.021**	**1.003–1.141**	**0.045***	0.942	0.872–1.013	0.172	1.012	0.954–1.077	0.226
LDL-C/HDL-C	1.180	0.951–1.477	0.132	1.271	0.819–2.232	0.263	1.114	0.853–1.582	0.356	1.209	0.803–1.847	0.364

The top panels show the analyses of IHD for subjects aged <65 years (left), 65–74 years (middle) and ≥75 years (right). The lower panels show the incidence of CVA. Bold indicate statistically significant factors. Hazard ratios and 95% CIs are shown. The ratio of males to females was 1. As LDL-C/HDL-C interacts strongly with LDL-C and HDL-C, and non-HDL-C interacts triglyceride and LDL-C, analysis of non-HDL-C and LDL-C/HDL-C were separately shown in methods section.

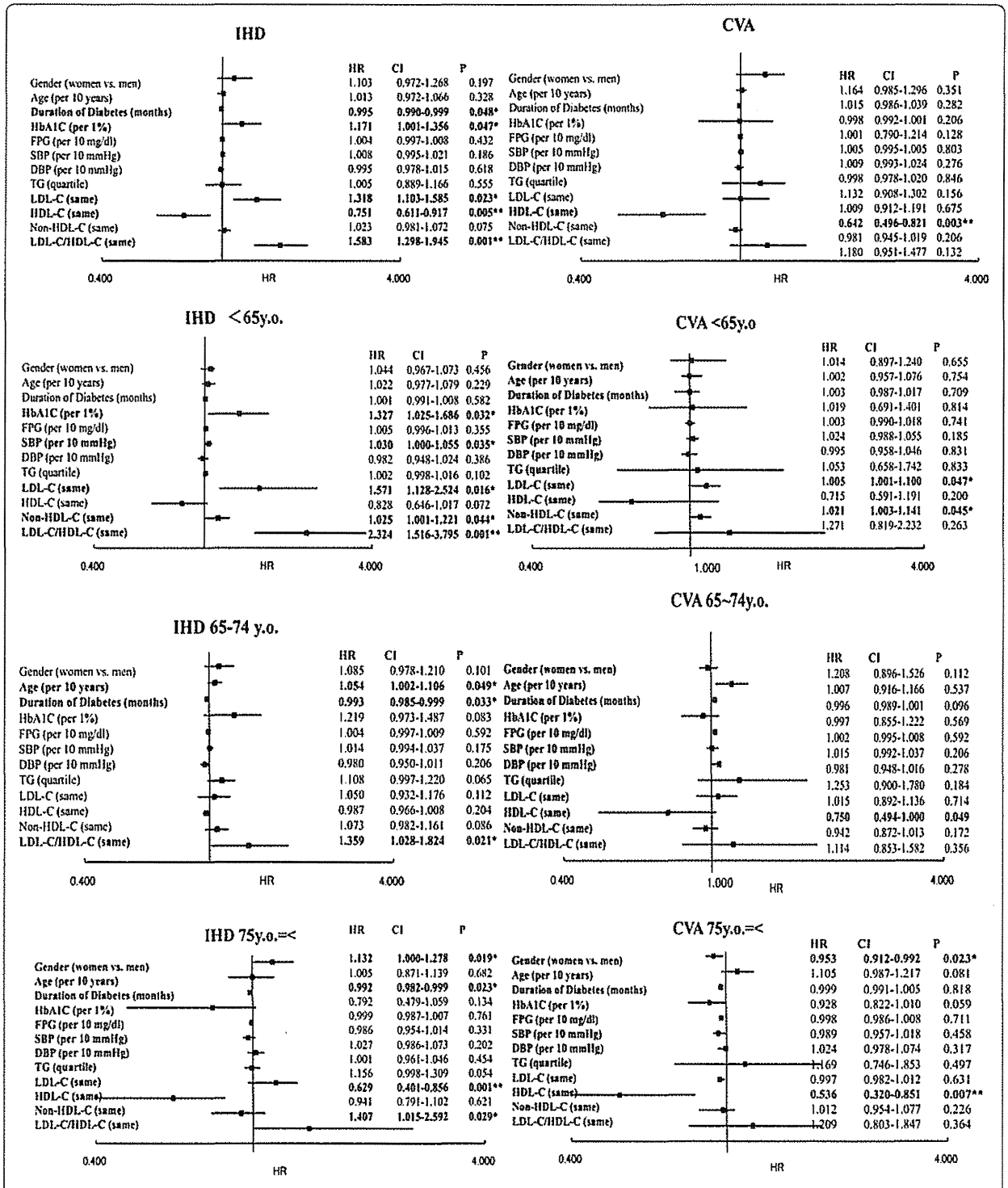

Figure 2 Risk factors for IHD and CVA by Cox multivariate models in representative age groups (IHD, Left; CVA, Right). The upper panels show the analyses of IHD for those younger than 65 years old (Left) and CVA for those younger than 65 years old (Right). The lower panels show the analyses of IHD (Left) and CVA (Right) for those equal to or older than 75 years. HR stands for Hazard Ratio (vertical bar shows 1). Bold characters indicate statistically significant factors. The right side of each figure shows Hazard Ratios and 95% CIs. Because LDL-C/HDL-C interacts strongly with LDL-C and HDL-C and because non-HDL-C interacts with triglyceride and LDL-C, we analyzed non-HDL-C and LDL-C/HDL-C separately. In other words, common factors (gender, age, duration of diabetes, HbA1C, FPG, systolic BP (SBP), and diastolic BP (DBP)), TG, LDL-C and HDL-C were analyzed. Then, non-HDL-C and common factors were analyzed. Finally, LDL-C/HDL-C, common factors and TG were analyzed.

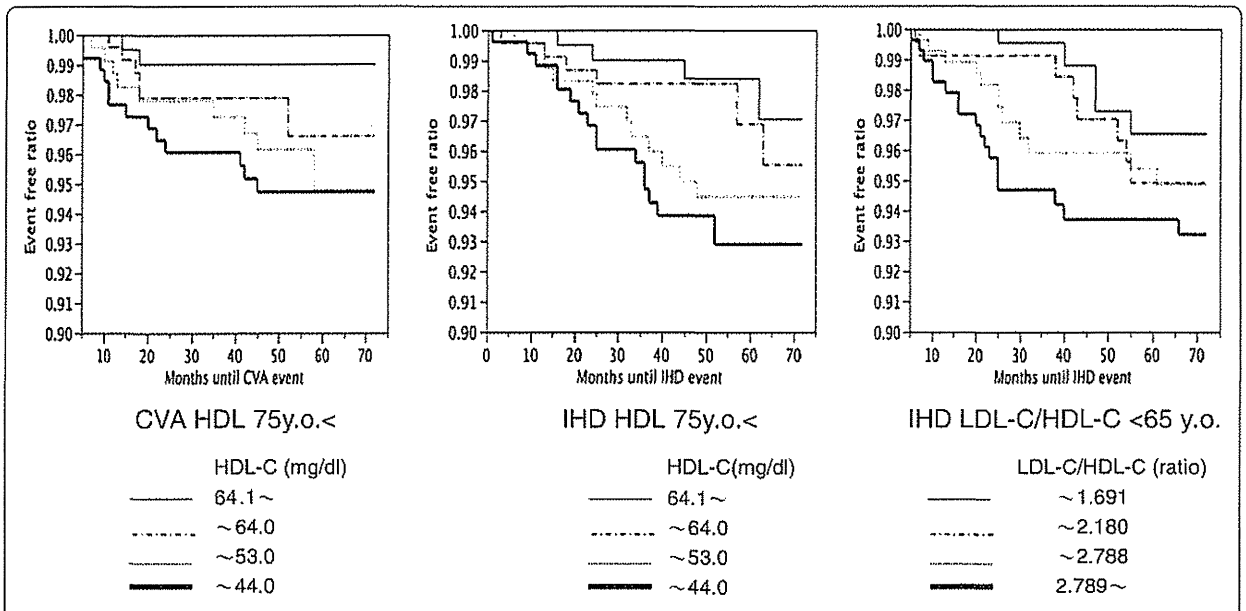

Figure 3 The relationships of HDL-C levels and the LDL-C/HDL-C ratio with IHD and CVA in quartile categories for each age group based on Kaplan-Meier estimator curves. Left: The LDL-C/HDL-C ratio correlates with IHD. The figure shows the data for individuals aged <65 years. **Center:** The HDL-C level was inversely correlated with IHD, particularly in individuals aged >75 years. **Right:** The association between CVA and HDL-C was more prominent in those individuals aged ≥ 75 years. Quartile categories HDL-C: <44.0, 44.1-53.0, 53.1-64.0, >64.1 mg/dl LDL-C/HDL-C: <1.691, 1.692-2.180, 2.181-2.787, >2.789.

approximately 25% were late elderly. These trends are spreading across the world, mainly in developed countries; however, the risk factors for IHD or CVA in late elderly diabetic individuals have not been identified. In the late elderly, atherosclerotic diseases, such as IHD and CVA, are a more frequent cause of death than malignancy. In Canada, diabetic patients are reported to suffer myocardial infarction approximately 14 years earlier than patients without diabetes [13]. However, there is little evidence on the risk and preventive factors for IHD or CVA in the diabetic elderly, and there are no reports on the late elderly [14,15].

Therefore, we organized this study as one of the largest attempts to examine IHD and CVA in middle-aged to elderly diabetic individuals. We defined the age categories as follows: 1) non-elderly: younger than 65, 2) early elderly: from 65 to 74, and 3) late elderly: equal to or older than 75. Sixty-five is usually defined as the threshold for being elderly worldwide [13,16], and 75 is the beginning of the late elderly age in Japan, as defined by health insurance and care insurance systems and the Japan Geriatric Society [12].

The effect of age on IHD and CVA risk factors

One hundred fifty-three cases of IHD and 104 CVAs occurred, which represents 7.8 and 5.7/1,000 people per year, respectively, over this 5.5-year study, although we defined stroke strictly and excluded cerebral and subarachnoid hemorrhages from this definition. IHD occurs 2 to 3 times more frequently in diabetic individuals compared to the normal Japanese population, and CVA also occurs more frequently in diabetic individuals [17]. The prevalence of IHD and CVA is slightly higher than reported in previous Japanese diabetic studies because we targeted relatively older diabetic individuals [16,17]. However, even in diabetic individuals, the combined frequency of IHD and stroke was slightly lower in the Japanese population than among Caucasians [18].

To look for the candidate metabolic markers that may predict IHD and CVA in various age groups, Cox regression analyses were performed. The analyses showed that higher HbA1C and LDL-C levels, SBP and non-HDL-C were significantly correlated with the occurrence of IHD in subjects <65 years old, which is similar to previous reports [14-16]. The ratio of males/females was not significantly different between patients < 65, patients between 65 and 74, and patients ≥75. A relation between diabetes and ischemic stroke was reported. Patients (59.8 ± 7.2 y.o.) having a history of coronary heart disease with diabetes mellitus exhibited a 2.29-fold increased risk for stroke or TIA during the 4.8- to 8.1-year follow-up period than patients without diabetes. Impaired fasting

glucose and hypertension were predictors, while HDL-C was not. These results are fairly consistent with those of the younger patients group (< 65 y.o.) in the present study [19].

In patients ≥75 y.o., a lower HDL-C level was correlated with IHD and CVA. This is a novel finding of the present study. Few data are available on the relationship between elderly type 2 diabetic patients and CVA, particularly among the late elderly [16-18,20]; therefore, the finding of the importance of HDL-C in CVA in the late diabetic elderly may be important. The Kaplan-Meier estimator curves, which are shown in Figure 1, support these findings.

Thus, a lower HDL-C level is an important risk factor for both IHD and CVA among the late elderly diabetic patients in this study. Although the protective effects of higher HDL-C on IHD in the non-elderly are known, the effects on IHD among late elderly diabetics are not known [21]. The CVA and IHD incidences in the late elderly may decrease to the levels found in middle-aged cohorts if higher HDL-C has protective effects on late elderly diabetic individuals and if their levels are easily increased. There are few agents available to increase HDL-C levels, except exercise, and adequate exercise or bodily movement may be necessary even in the elderly. The low HDL-C level may be related to low levels of physical activity in the elderly, which could influence a CVA in many ways that are separate from the HDL-C level. Atherosclerosis is an inflammatory disorder, and HDL-C may preserve endothelial function by increasing endothelial NO [22].

For LDL-C, three large-scale clinical studies on dyslipidemia, which included participants who were up to 75 or 80 years of age, are available [23-25]. Although these studies reported that the reduction in LDL-C by statins decreases IHD (including in diabetic people), the effects were weak in the elderly compared with those in the non-elderly (e.g., Prosper reported that pravastatin, a water-soluble statin, induced a 16% decrease in IHD without any effect on CVA in elderly patients compared to a 21% decrease in non-elderly patients). These data suggest that simply controlling LDL-C may not prevent IHD or CVA in the elderly. There are also no large observational studies on the diabetic elderly older than 75 [26,27]. For example, the international FIELD study analyzed approximately 10,000 patients up to the age of 75 years, with a mean age 63 years [26], and the Swedish NDR-study analyzed 18,673 patients up to 70 years old, with a mean age of 60 years [27]. These large observational studies, analyzing all patients, found LDL-C, non-HDL-C, HDL-C, triglycerides and ratios of LDL-C/HDL-C and total-cholesterol/HDL-C to be significant risk factors for IHD. These data are consistent with our data on participants younger than 65, although those observational studies did not include patients older than 75. To lower LDL-C levels, 57% of the patients in our study had already been prescribed anti-dyslipidemic agents, of which 83% were statins. The average LDL-C level was 120 mg/dl, which matches the guidelines of the Japan atherosclerosis society but not that of the American Heart Association or IDF (100 mg/dl). Although doses and types of anti-dyslipidemic agents were changed often during the study, their effects other than LDL reduction (pleiotropic effects) cannot be evaluated yet.

Our study shows the importance of the LDL-C/HDL-C ratio as well as HDL-C and LDL-C levels, although the strength of these effects is different based on age. The LDL-C/HDL-C ratio was associated with IHD, which may represent the effect of LDL-C levels in the non-elderly and HDL-C levels in the elderly [28]. The non-HDL-C level and the total cholesterol/HDL-C ratio are also proposed markers of atherosclerotic diseases [29,30]. The non-HDL-C level was associated with IHD only among those younger than 65, and the total cholesterol/HDL-C ratio was not significantly associated with IHD (data not shown). We believe that these data are consistent with previous data from non-elderly diabetic individuals because the non-HDL-C level is a reflection of the effect of triglyceride levels, and hyper-triglyceridemia, complicated with metabolic syndrome, occurs more often in non-elderly than in elderly people.

Emerging Risk Factors Collaboration analysis showed the association of non-HDL-C with IHD and CVA. However, in this study, it was associated with CVA only in those younger than 65. The two studies are different in that 1) our cohort consisted only of diabetic patients; 2) in the Collaboration analysis, the mean age was 56.6 y.o., compared to 67.4 y.o. in our study; and 3) in the Collaboration analysis, almost all of the patients were North American or European, whereas our study was Japanese patients only. In the elderly, triglycerides are usually lower than in younger individuals, and non-HDL-C represents triglyceride.

A 1-mg/dl change in HDL-C and/or a 2-mg/dl change in LDL-C reflect a 2% change in the risk for atherosclerotic diseases, and this may be partially consistent within our diabetic elderly study [31]. The LDL-C/HDL-C ratio may reflect the direct effects of both LDL-C and HDL-C levels, which may affect or interact with the progression of atherosclerosis and thrombosis formation more than other lipids, such as chylomicrons and chylomicron remnants, which are represented by the non-HDL-C level or the TC/HDL-C ratio. The fact that elderly individuals have different risk factors than younger individuals could be associated with genetic protection from such events or an accumulation of personal habits that may provide the elderly with protection. For example, differences in single nucleotide polymorphisms (SNP)

may be related to the severity of atherosclerosis and, subsequently, to the different effects of predictors by age and should be evaluated in the future [32].

Interestingly, impaired fasting glucose and hypertension were the strongest predictors of risk for ischemic stroke or TIA in metabolic syndrome, and HbA1c had positive associations with glycemia, TG, HDL-C, and TG/HDL-C but not LDL-C in the study of 118 older adults aged 65–95 years, of whom less than 6.5% had an HbA1c of 93% [19,33]. These data is consistent with our data in diabetic patients younger than 65 [33]. Another study evaluated the predictors of stroke stratified by age (at symptom onset: young; <50 years, older; 51–75 years, and oldest; 75 < years) using data collected over a 4-year period from 3,053 subjects with stroke. The metabolic syndrome was the only predictor among the older patients (OR 1.58) but not in the others. Although most patients were not diabetic, these types of studies should be accumulated to evaluate the effect of age on atherosclerotic diseases [34].

Conclusions

HbA1C, LDL-C, SBP and non-HDL-C in non-elderly diabetic individuals, HDL-C in late elderly diabetic individuals and the LDL-C/HDL-C ratio in all diabetic individuals were associated with IHD in this population. HDL-C was also associated with CVA in late elderly diabetic individuals. The differences in atherosclerotic risk by age must be considered in developing individualized strategies for the prevention of atherosclerotic diseases. Because this was an observational study, we could not analyze the detailed effects of treatment, such as the effect of statins on the risk of IHD or CVA. Although this study targets Japanese, these new findings on metabolic markers in the late elderly could provide additional data for the annotation of cardiovascular risk factors in the diabetic elderly across the world.

Abbreviations

CVA: Cerebrovascular attack; IHD: Ischemic heart disease; LDL-C: LDL-cholesterol; HDL-C: HDL-cholesterol; TG: Triglyceride; FPG: Fasting plasma glucose; HbA1C: Hemoglobin A1C; SBP: Systolic blood pressure; UKPDS: United Kingdom Prospective Diabetes Study.

All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TH and HN wrote the manuscript and researched the data. AA, SK, HS, HW, TO, KY, MT, KK, MN, HN, and KI contributed to the research and reviewed the manuscript. All authors read and approved the final manuscript.

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Cancer Risk in Diabetic Patients Treated with Metformin: A Systematic Review and Meta-analysis

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Abstract

Background: A growing body of evidence has suggested that metformin potentially reduces the risk of cancer. Our objective was to enhance the precision of estimates of the effect of metformin on the risk of any-site and site-specific cancers in patients with diabetes.

Methods/Principal Findings: We performed a search of MEDLINE, EMBASE, ISI Web of Science, Cochrane Library, and ClinicalTrials.gov for pertinent articles published as of October 12, 2011, and included them in a systematic review and meta-analysis. We calculated pooled risk ratios (RRs) for overall cancer mortality and cancer incidence. Of the 21,195 diabetic patients reported in 6 studies (4 cohort studies, 2 RCTs), 991 (4.5%) cases of death from cancer were reported. A total of 11,117 (5.3%) cases of incident cancer at any site were reported among 210,892 patients in 10 studies (2 RCTs, 6 cohort studies, 2 case-control studies). The risks of cancer among metformin users were significantly lower than those among non-metformin users: the pooled RRs (95% confidence interval) were 0.66 (0.49–0.88) for cancer mortality, 0.67 (0.53–0.85) for all-cancer incidence, 0.68 (0.53–0.88) for colorectal cancer (n = 6), 0.20 (0.07–0.59) for hepatocellular cancer (n = 4), 0.67 (0.45–0.99) for lung cancer (n = 3).

Conclusion/Significance: The use of metformin in diabetic patients was associated with significantly lower risks of cancer mortality and incidence. However, this analysis is mainly based on observational studies and our findings underscore the more need for long-term RCTs to confirm this potential benefit for individuals with diabetes.

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Introduction

Hyperinsulinemia and hyperglycemia are thought to promote carcinogenesis in patients with diabetes mellitus. Several meta-analyses have demonstrated that diabetes is associated with increased risks of site-specific cancers of the breast (1.2) [1], endometrium (2.1) [2], bladder (1.2) [3], liver (2.5) [4], colorectum (1.3) [5], and pancreas (1.8–2.1) [6,7], and also a decreased risk of prostate cancer (0.8–0.9) [8,9]. The evidence for non-Hodgkin's lymphoma remains inconclusive [10,11]. Our previous meta-analyses showed that patients with diabetes have an increased risk of total cancer (relative risk, 1.1–1.7) [12–14], whereas more recent studies did not [15,16]. Metformin is an insulin sensitizer that is the drug of first choice in the management of type 2 diabetes [17], given its safety profile and lower cost. Metformin reportedly has a potential anti-cancer effect by activating adenosine 5′-mono-phosphate-activated protein kinase (AMPK) in addition to alleviating hyperinsulinemia and hyperglycemia. Although other mechanisms for this risk reduction have been hypothesized, none have been elucidated entirely. Previous meta-analyses have suggested that metformin is associated with a reduced risk of cancer in diabetic subjects [18,19]. However, those analyses were based solely on a few observational studies and additional reports have been published recently.

In light of the worldwide diabetes epidemic and the higher mortalities in cancer patients with diabetes [20,21], explorations of effective cancer prevention are of clinical importance for the targeted management of diabetes in daily practice. Moreover, they are crucial in the areas of public health, since a modest increase in the risk of cancer translates into a substantial social burden. These circumstances prompted us to investigate, with greater precision, the preventive effect of metformin on cancer mortality and incidence by scrutinizing pertinent original reports including randomized controlled trials (RCTs), and combining their data in an attempt to obtain meaningful clues for the prevention of cancer in patients with diabetes [13].

Methods

Search

Searches of MEDLINE, EMBASE, ISI Web of Science, Cochrane Library, and ClinicalTrials.gov from their inception until October 12, 2011, were performed. Studies evaluating the risks of cancer mortality or incidence among diabetic patients

taking metformin, compared with those not taking metformin, were identified using a combination of the following medical subject heading terms: 'diabetes', 'metformin', 'cancer' or 'neoplasms', and 'risk' or 'risk factors'. The reference lists of the pertinent articles were also inspected.

Selection/Study Characteristics

We assessed all the identified RCTs, cohort studies, case-control studies, and cross-sectional studies on the risk of cancer based on original data analyses to determine their eligibility for inclusion in a qualitative analysis. The inclusion criteria in the meta-analysis are as follows: published full-text report in English-language, RCTs with parallel-design of metformin as a treatment of type 2 diabetes at least one year's follow-up period, observational studies of any duration in patients with type 2 diabetes, reporting relative risks, i.e. hazard ratios (HRs), RRs, or odds ratios, adjusted for possible confounders with confidence intervals (CIs). The comparators were defined as any treatment not including metformin.

Validity assessment

To ascertain the validity of the eligible studies, the quality of each report was appraised in reference to the CONSORT statement [22] and the STROBE statement [23].

Data abstraction

We reviewed each full-text report to determine its eligibility and extracted and tabulated all the relevant data independently. The extracted data included the characteristics of the subjects (including age, sex, and other treatment), study design, published year, follow-up period, and the methods used for ascertaining the diagnosis of cancer. Study authors were contacted as needed to obtain detailed data. Any disagreement was resolved by a consensus among the investigators.

Quantitative data synthesis

If more than one study was published for the same cohort, the report containing the most comprehensive information on the population was included to avoid overlapping populations. The reports were summarized both qualitatively and quantitatively. Three articles that did not specify the case numbers were not included in the calculation of the mortality and incidence. If the metformin comparator included more than one treatment, the oral monotherapy groups were included in the analysis because these groups were deemed to be at an equivalent stage of diabetes. If an article provided the relative risks for all cancer and site-specific cancers, the all cancer data were included in the primary qualitative and quantitative analyses and the site-specific data were used in the secondary analyses performed according to cancer site. The risks for site-specific cancers were appraised if three or more qualified reports were identified for a given cancer site. Response to metformin exposure was evaluated by using linear-regression analysis.

In the meta-analysis, each adjusted relative risk was combined and the pooled RRs with the 95% CI was calculated using the random-effects model with inverse-variance weighting. Heterogeneity among the studies was evaluated using I^2 statistics. The possibility of a publication bias, which can result from the non-publication of small studies with negative findings, was assessed visually using a funnel plot for asymmetry. RevMan (version 5.1) was used for these calculations. A sensitivity analysis was performed by separating the RCTs and the observational cohort / case-control studies and the equality of RRs between RCTs and observational studies were assessed by using z-statistic tests. All the procedures were in accordance with the guidelines for the Quality of Reporting of Meta-analyses [24], the meta-analysis of observational studies in epidemiology [25] and the PRISMA statement [26].

Results

Search Results

A total of 412 articles were identified during our search; of these, 32 were assessed with respect to their eligibility for inclusion in our review, which was aimed at determining the influence of metformin on cancer mortality and incidence in patients with diabetes (**Fig. 1**). Four articles [27–30] were excluded from the systematic review because of population overlapping and four other reports were excluded because they investigated the overall survival rate [31,32], cancer incidence exclusively in patients with hepatitis C [33], and biochemical recurrence [34]. Out of these 32 articles, a total of 24 (11 observational cohort studies [35–45], 3 randomized controlled trials [46–49], and 10 case-control studies [29,50–58]) were included in the systematic review and meta-analysis. The UK Prospective Diabetes Study (UKPDS) 34 [49] involved two independent investigational trials (metformin vs. conventional therapy and sulfonylurea vs. sulfonylurea plus metformin), and these trials were included in the meta-analysis as two separate data.

Table S1 shows the characteristics of each included study according to the study design. The 24 selected articles included in the systematic review were moderately heterogeneous in terms of population demographics, study design, and the assessment of confounding factors. The diabetes sample size in these studies ranged from 361 to 998,947 patients. Of the 21,195 diabetic patients in 6 studies, 991 (4.5%) cases of cancer death were reported. A total of 11,117 (5.3%) cases of incident cancer at any site were reported among 210,892 patients in 10 studies. Major confounding factors such as cigarette smoking, alcohol intake, and hyperglycemia were not reported in several studies.

The risk of bias and the adjustment factors among the studies are summarized in **Table S2**. Diabetes was diagnosed using blood tests (n = 8), prescription databases (n = 6), medical records (n = 4), self-reports (n = 3), and health insurance database (n = 4). All the diagnoses of cancer were confirmed using valid records or registries. All the studies, except for the RCTs, adjusted the estimates for potential confounding factors. The analysis of dose-response was performed in 3 studies [38–40]. Some studies excluded the data for metformin exposure less than 1 year [50,52] or 2 years [58] to minimize bias. The effect on the total cancer risk over the follow-up period was inspected in 3 studies [40,55,58]. Direct comparison of the effect between metformin and other specific medications were reported in 2 RCTs [46–48].

Qualitative Summary

The majority of the studies included were methodologically fair in quality. Among 10 case-control studies, six were nested ones [50–52,55,56,58]. All the four cohort studies [35,38,40,41] on cancer mortality revealed a significant decrease (range, 23%–75%), and the two RCTs showed no significant effect of metformin [49]. There was no study that directly compared the risk associated with metformin vs other medications or analyzed the correlation between the follow-up length and the effect of metformin on cancer mortality. The overall correlation of the follow-up period with the mortality was nonsignificant ($r = -0.04$, $p = 0.9$). One study revealed that the HR (95% CI) for cancer mortality with every increase of 1 g metformin was 0.58 (0.36–0.93) [38].

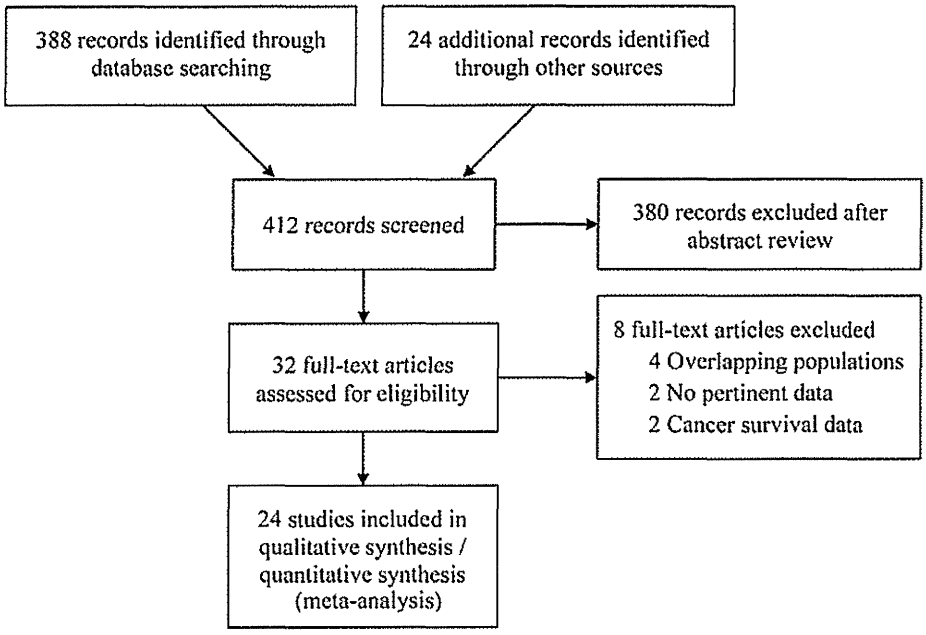

Figure 1. Flow diagram of study selection.
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Five studies (3 cohort studies [36,39,40] and 2 case-control studies [55,56]) reported a significant decrease (range, 26%–88%), the two RCTs showed no significant effect of association [46–48] and none demonstrated a statistically significant increase in the risk of all-cancer incidence among metformin users. The cancer risk for metformin users was not significantly different from that for rosiglitazone or sulfonylurea users in RCTs [46–48]. One cohort study showed a trend for metformin users to have a higher risk of cancer in the first 2 years of follow-up. The beneficial effect of metformin on the risk of total cancer incidence was exposure-dependent in 2 case-control studies [55,56]. The overall correlation of the follow-up period with the incidence was nonsignificant ($r = -0.32$, $p = 0.4$). One study reported that its effect on cancer incidence was dose-dependent (p for trend <0.05) [39] suggesting that the minimal effective dose can be 500 mg /day, while the other showed no significant differences among doses [40].

Among the studies evaluating the risks of site-specific incident cancers in patients with diabetes who were taking metformin, more than two studies (including subgroup analyses) recognized significantly reduced risks for cancers of the pancreas [36,39,54], colorectum [36,39,40], and liver [29,39,53], and none showed a significantly increased risk of a site-specific cancer. All these risk decrements were moderate (RR range, 0.06–0.60). Of note, no significant increases or decreases in the risk of cancers of the breast, prostate or stomach were reported, except for a significant decrease in the risk of prostate cancer in one report [42] and breast cancer in another [52]. The number of studies examining other cancer sites was two or fewer, and these studies were not reviewed in the present analysis.

Quantitative Summary (Meta-analysis)

Based on the quality appraisal in our systematic review, a total of 24 articles that provided sufficient information were included in the meta-analysis (**Fig. 1**). **Fig. 2** illustrates the significantly decreased risks of all-cancer mortality and incidence in metformin-users, compared with non-metformin users. In a sensitivity analysis, the pooled estimate (95% CI) for all-cancer mortality among the observational cohort studies was 0.62 (0.46–0.82), $I^2 = 56\%$, $p = 0.08$ and the estimate among the RCTs was 1.22 (0.36–4.11), $I^2 = 60\%$, $p = 0.12$. The difference in the RRs between the observational studies and the RCTs was not statistically significant ($p = 0.35$). The pooled RR (95% CI) for all-cancer incidence among the observational cohort studies was 0.66 (0.49-0.88), $I^2 = 96\%$, $p<0.00001$, the pooled RR among the case-control studies was 0.38 (0.23-0.61), $I^2 = 3\%$, $p = 0.31$ and the estimate among the RCTs was 1.03 (0.82–1.31), $I^2 = 30\%$, $p = 0.23$. The difference in the RRs between the observational studies and the RCTs was statistically significant ($p = 0.019$). As summarized in **Fig. 3 and Fig. 4**, the incident cancer risks were also significantly decreased for cancers of the colorectum, liver and lung. The RRs of prostate cancer, breast cancer, pancreatic cancer and gastric cancer were not statistically significant. Significant heterogeneity was observed in the majority of these analyses. No apparent publication bias was apparent, as assessed using a funnel plot (**Fig. S1**).

Discussion

Our systematic review and meta-analyses of worldwide reports demonstrated that metformin is associated with a substantially lower risk of all-cancer mortality and incidence, compared with other treatments for diabetes. They also showed that metformin significantly reduced the risks of cancers of the colorectum, liver and lung. These findings support the hypothesis that metformin potentially has an anti-cancer effect. In light of the fact that cancer is the second and diabetes the twelfth leading cause of death worldwide [59] and that the number of people with diabetes is rapidly increasing, our findings have substantial clinical and public implications on a global scale and point to the need for the further investigation of the anti-cancer mechanism of metformin and for long-term RCTs to confirm this clinical benefit.

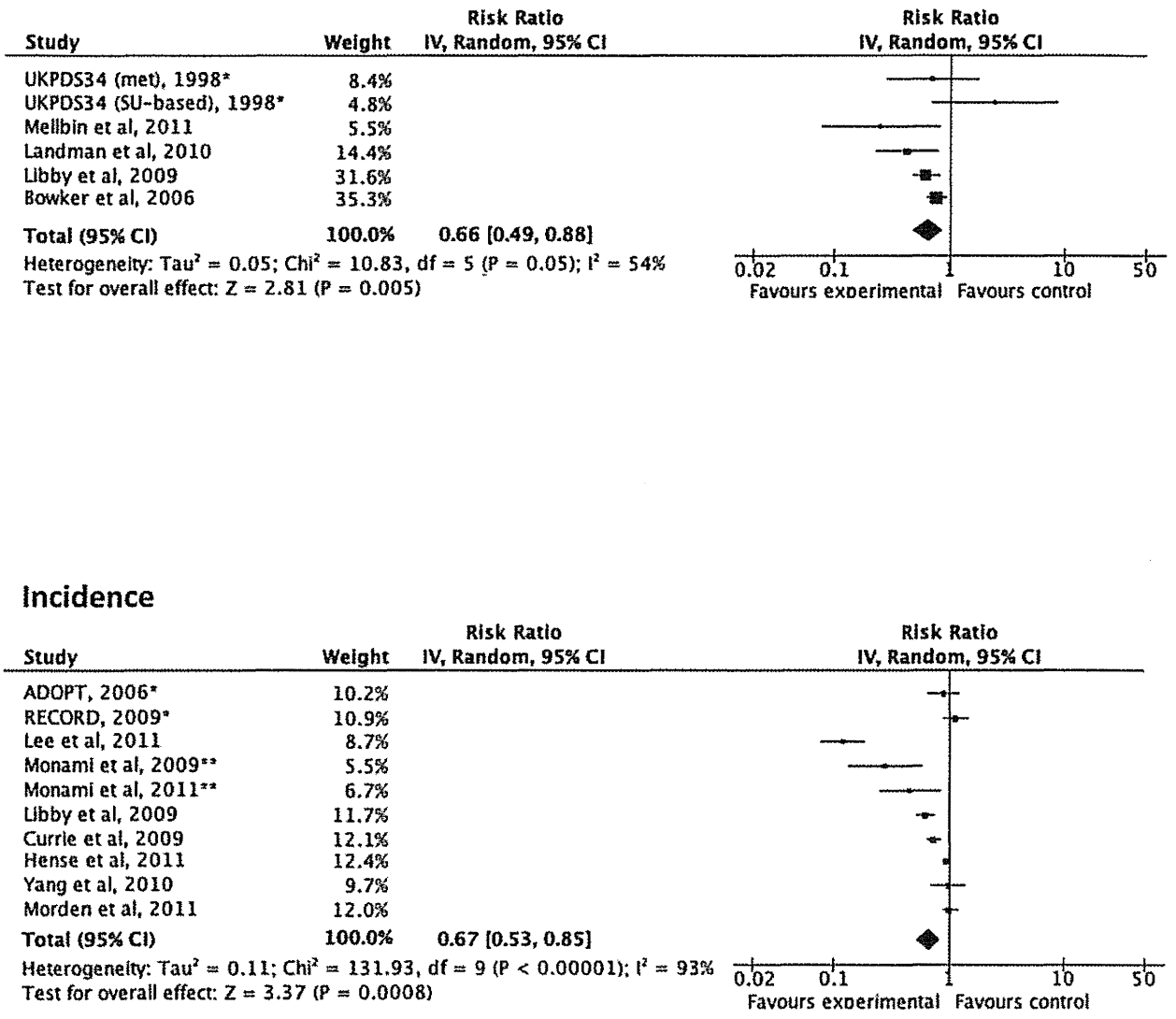

Figure 2. Adjusted risk ratios for all-cancer mortality and incidence among subjects with diabetes taking metformin. Boxes, estimated risk ratios (RRs); bars, 95% confidence intervals (CIs). Diamonds, random-effects model RRs; width of diamonds; pooled CIs. The size of each box is proportional to the weight of each study in the meta-analysis. *, randomized controlled trials; **, case-control studies; IV, inverse-variance.
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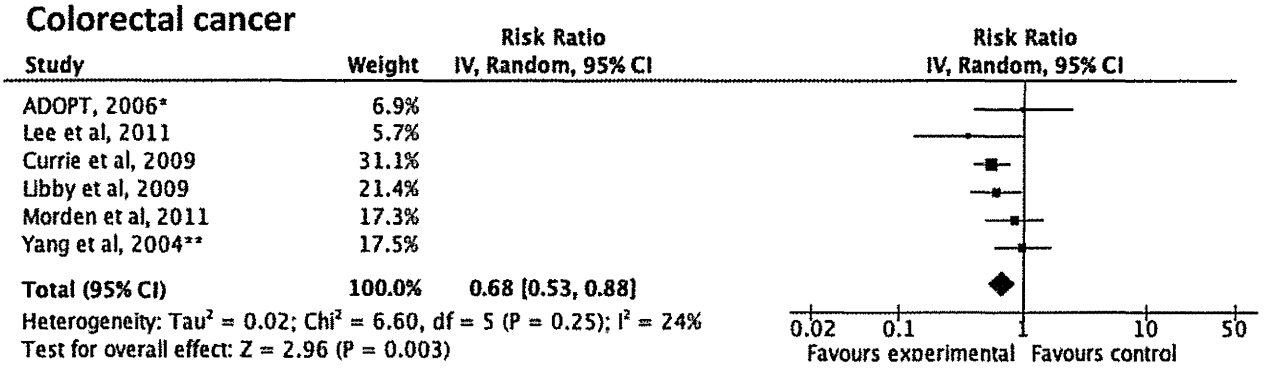
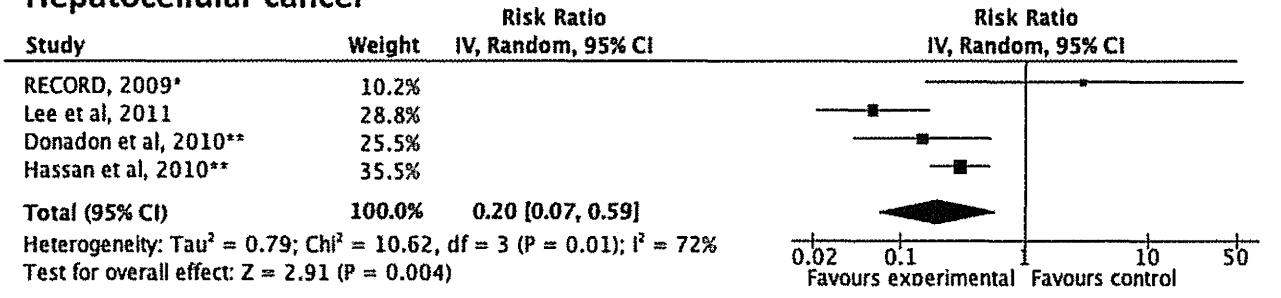
The strength of our present study is that the analysis was mainly based on large population-based data originating from multiple nations and was performed with a high level of precision. Compared with recently published studies [18,19], our updated study is novel in that data from RCTs were incorporated and cancer risks for substantially more sites were analyzed. Although the significantly decreased pooled RRs for all-cancer mortality / incidence and cancer at most sites were robust, the results of the component studies were statistically heterogeneous. Of note, all the individual and pooled results of the RCTs were neutral. It seems that each follow-up period in these RCTs is similar to many others in the observational studies and they have power enough to detect the differences in cancer risk. In the analysis of cancer mortality, there was no significant difference in RR between the RCTs and the observational studies. For cancer incidence, on the other hand, the overall RR was significantly reduced but the difference was statistically significant. This discordance may imply that the apparent anti-cancer effect of metformin in observational studies was affected by confounding biases and thus more RCTs are awaited to clarify the effect of metformin on cancer incidence. The large I^2 values indicated that the range of the plausible risk estimates was wide but no evidence in our analysis suggested that metformin may increase the risk of cancer. These findings may reflect the different mechanisms of cancer prevention at different sites and / or different epidemiological characteristics among the diverse populations included in our study.

Evidence has been accumulating to suggest that diabetic patients have a higher risk of cancer than non-diabetic people [12,13]. While the mechanisms are yet to be investigated, insulin resistance with secondary hyperinsulinemia is the most frequently proposed hypothesis, as insulin may have a possible mitogenic effect via its binding to the insulin-like growth factor-1 receptor





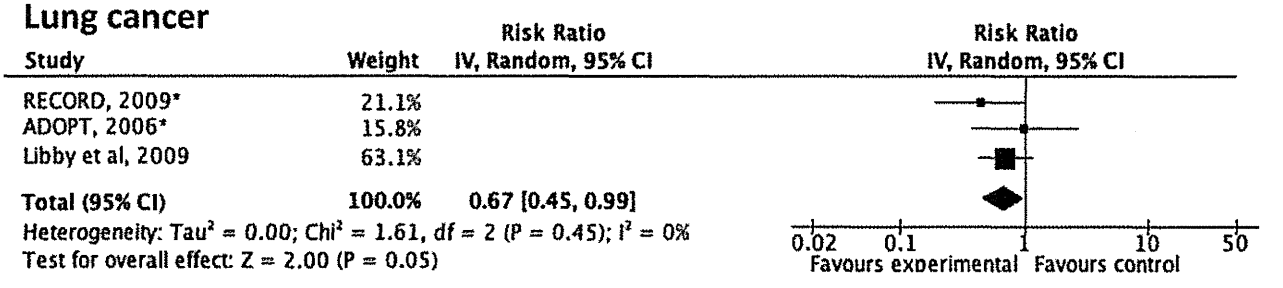


Figure 3. Adjusted risk ratios for site-specific cancer incidence among subjects with diabetes taking metformin. Boxes, estimated risk ratios (RRs); bars, 95% confidence intervals (CIs). Diamonds, random-effects model RRs; width of diamonds; pooled CIs. The size of each box is proportional to the weight of each study in the meta-analysis. *, randomized controlled trials; **, case-control studies; IV, inverse-variance.
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[60–70]. In addition, hyperglycemia itself may promote carcinogenesis directly [71,72] or indirectly by increasing oxidative stress [73–79]. However, these speculations are derived from retrospective observational studies and may not necessarily demonstrate causality because of possible biases and confounders, such as co-existing obesity and age [15,80,81]. In fact, more recent studies demonstrated no or minimal increments in cancer risk [15,16] and the data from insulin-treated patients are inconclusive [82]. Of interest, diabetes reportedly protects against the development of prostate cancer [8,9], since it is testosterone-dependent and testosterone deficiency is common among men with diabetes secondary to low levels of sex hormone-binding globulin (SHBG)

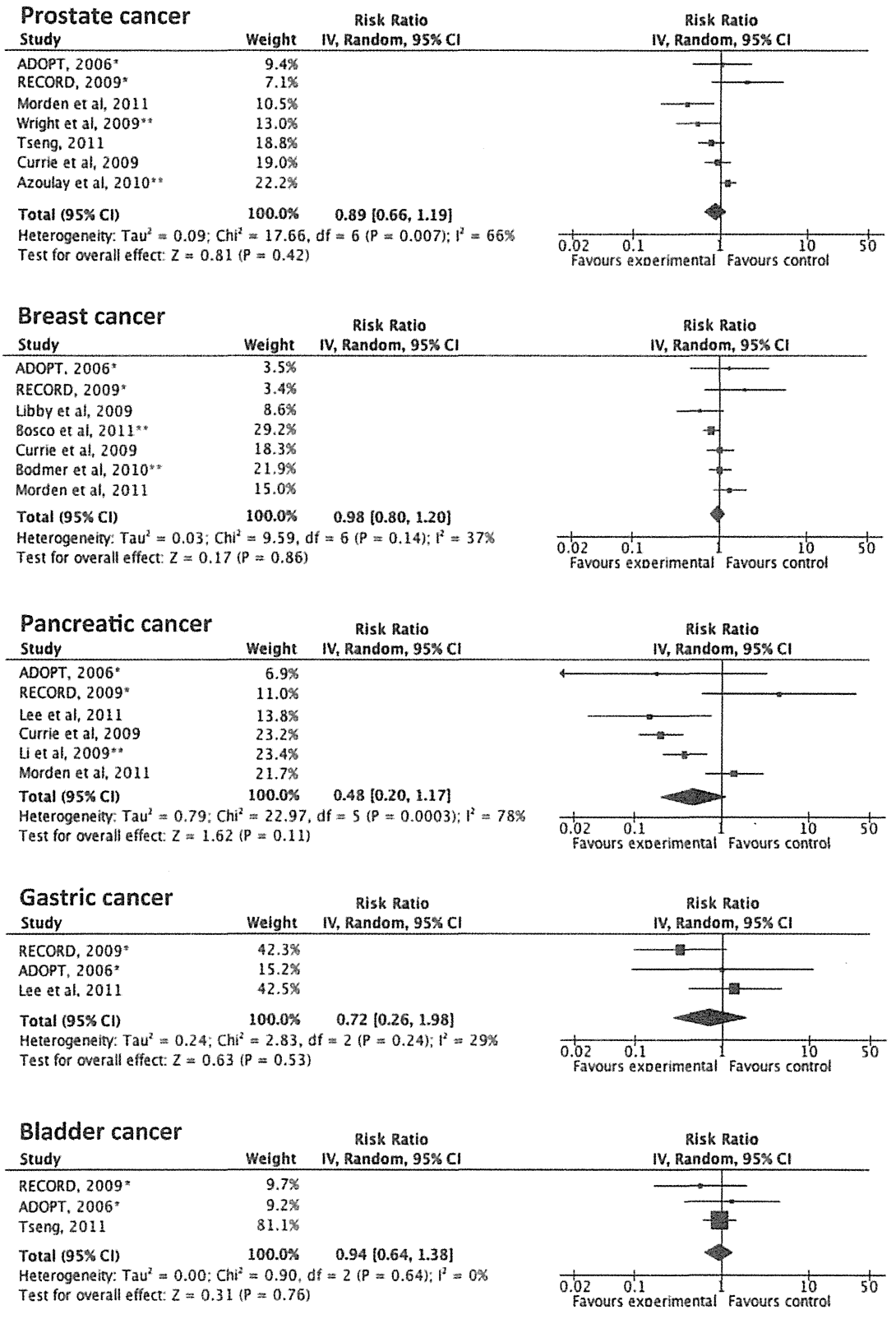

Figure 4. Adjusted risk ratios for other site-specific cancer incidence among subjects with diabetes taking metformin. Boxes, estimated risk ratios (RRs); bars, 95% confidence intervals (CIs). Diamonds, random-effects model RRs; width of diamonds; pooled CIs. The size of each box is proportional to the weight of each study in the meta-analysis. *, randomized controlled trials; **, case-control studies; IV, inverse-variance.
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and partially because of insulin resistance [83–85]. Low SHBG levels may facilitate the conversion of testosterone to estradiol, which in turn may result in an increased risk of hormone-dependent breast cancer.

Several mechanisms for the anti-cancer effect of metformin have been postulated, and several prospective clinical trials to evaluate its safety and efficacy are ongoing [82,86]. Indirect pathways include the prevention of weight gain and the amelioration of hyperinsulinemia, both of which may promote carcinogenesis. In addition, metformin activates AMPK through LKB-1, a tumor suppressor protein kinase. AMPK inhibits protein synthesis and gluconeogenesis during cellular stress and inhibits mammalian target of rapamycin (mTOR), a downstream effector of growth factor signaling, which is frequently activated in malignant cells. In human breast cancer cells, it reduces HER-2 protein expression by inhibiting mTOR. Metformin also induces cell cycle arrest and apoptosis and reduces growth factor signaling. Supporting the idea of these direct effects, metformin reportedly potentiated the effect of neoadjuvant chemotherapy in early-stage breast cancer [87], decreased the risk of colorectal cancer in a small randomized trial involving non-diabetic subjects [88], and was associated with a decreased cancer risk while another insulin-sensitizer, thiazolidinedione, were not [18,54, 89,90].

Our research revealed that metformin use is associated with reduced mortality and incidence of cancer at any site, supporting the general applicability of the proposed anti-cancer mechanisms. The anti-cancer effect of metformin may also be applicable to diabetic Asians, who are generally lean and insulinopenic [12], given the fact that they have a higher cancer risk than non-diabetic Asians [12–14] and the data for Asians [39] were in line with the results of our meta-analyses. On the other hand, the magnitude of the risk reduction varies among site-specific cancers. This variance in efficacy may result from differences in carcinogenesis at certain sites. For instance, elevated levels of insulin and glucose may exert an important influence in the development or growth of epithelial malignant tumors of the colon [91–93], pancreas [94,95], and breast [96], and metformin may prevent incident colon cancer in non-diabetic subjects [88]. An animal study suggested that metformin prevented smoking-related lung cancer in mice, probably by inducing some hormone from the liver [97]. With regard to sex hormone-dependent cancers, the effect of metformin on the development of prostate cancer and breast cancer in our analysis was neutral. Metformin improves insulin sensitivity, thereby possibly raising the testosterone level. This may have promoted prostate cancer development and may have diluted the beneficial effect of metformin. In fact, one cohort study reported no benefit of metformin in terms of the biochemical recurrence rate after radical prostatectomy in diabetic patients [34]. The nonsignificant pooled RR for breast cancer may have resulted from the diversity in confounder adjustments and follow-up periods: some analyses were not fully adjusted for risk factors, including the menopause status, and one study suggested that only long-term exposure to metformin reduced the risk of breast cancer [51]. The fact that one preliminary study suggested a promising effect of metformin on pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer [87] may point to the possibility that metformin simply augmented the efficacy of chemotherapy for breast cancer [18,86]. Further detailed studies to analyze the interaction between carcinogenesis and the action of metformin, and to evaluate its effect for nondiabetic people are eagerly awaited.

Limitations

Our analysis should be interpreted in the context of the following limitations. First, the relation may not necessarily be causal, particularly in the observational studies [80], because of possible confounding factors and biases that may not have been fully adjusted for in this study: some risk factors such as cigarette smoking, alcohol intake, and hyperglycemia were not specified in several studies, which may have rendered the results less valid. Few studies demonstrated the dose-response to support biological plausibility. Confounding by treatment indication [98], which may have been minimizes by using propensity-score matching analysis, might overestimate the effect of metformin: the presence of such pre-existing conditions as older age and liver disease precludes metformin usages and thus, metformin users may be generally younger and at lower risk of cancer than in those in comparator groups. Only a few observational studies analyzed the effects over time and thus protopathic bias (i.e. early cancer leading to unstable diabetes and hyperglycemia, with patients switching diabetes treatment) [15] may remain moderate. In fact, the individual and pooled estimates from the RCTs were all neutral; the estimates comparing with other medication were neutral, as well. For all these limitations, however, observational studies provide the good available evidence regarding potential treatment effects / harms and the overall pooled estimates were robust. Moreover, evidence has been accumulating to support causality, both clinically and biochemically, as discussed earlier. Secondly, it is also important to realize that the populations of the studies were heterogeneous, most likely because of the diversity of the study designs and ethnicities, and that the sensitivity of each site-specific cancer to metformin may vary. Lack of the standardized treatment protocol in the descriptive studies might explain the observed associations: the possibility that other diabetes treatments may increase the risk of cancer may have resulted in an overestimation of the effect of metformin. Lack of the standardized diagnostic procedures for cancer may have caused detection bias in some cases. Even with these limitations, our analysis supports oncogenic safety of metformin and it should provide physicians with an additional incentive to pay integrated clinical attention and elucidate the complex interactions between diabetes treatment and cancer.

Conclusions

Our meta-analysis favors the oncogenic benefit of metformin for diabetic patients. However, observational studies were moderately heterogeneous and biased, and RCTs did not show a significant effect. Our findings underscore the need for long-term randomized prospective studies to confirm this potential benefit.

Supporting Information

Figure S1 Funnel plot of the included studies.
(TIFF)

Table S1 Study characteristics.
(DOC)

Table S2 Quality assessments of the included studies.
(DOC)

Checklist S1 PRISMA Checklist.
(PDF)

Author Contributions

Conceived and designed the experiments: HN MN. Performed the experiments: HN AG TT. Analyzed the data: HN AG TT. Contributed reagents/materials/analysis tools: HN AG TT. Wrote the paper: HN. Reviewed/edited the manuscript: AG TT MN.

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PLOS ONE

Low-Carbohydrate Diets and All-Cause Mortality: A Systematic Review and Meta-Analysis of Observational Studies

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Abstract

Objective: Low-carbohydrate diets and their combination with high-protein diets have been gaining widespread popularity to control weight. In addition to weight loss, they may have favorable short-term effects on the risk factors of cardiovascular disease (CVD). Our objective was to elucidate their long-term effects on mortality and CVD incidence.

Data sources: MEDLINE, EMBASE, ISI Web of Science, Cochrane Library, and ClinicalTrials.gov for relevant articles published as of September 2012. Cohort studies of at least one year's follow-up period were included.

Review methods: Identified articles were systematically reviewed and those with pertinent data were selected for meta-analysis. Pooled risk ratios (RRs) with 95% confidence intervals (CIs) for all-cause mortality, CVD mortality and CVD incidence were calculated using the random-effects model with inverse-variance weighting.

Results: We included 17 studies for a systematic review, followed by a meta-analysis using pertinent data. Of the 272,216 people in 4 cohort studies using the low-carbohydrate score, 15,981 (5.9%) cases of death from all-cause were reported. The risk of all-cause mortality among those with high low-carbohydrate score was significantly elevated: the pooled RR (95% CI) was 1.31 (1.07–1.59). A total of 3,214 (1.3%) cases of CVD death among 249,272 subjects in 3 cohort studies and 5,081 (2.3%) incident CVD cases among 220,691 people in different 4 cohort studies were reported. The risks of CVD mortality and incidence were not statistically increased: the pooled RRs (95% CIs) were 1.10 (0.98–1.24) and 0.98 (0.78–1.24), respectively. Analyses using low-carbohydrate/high-protein score yielded similar results.

Conclusion: Low-carbohydrate diets were associated with a significantly higher risk of all-cause mortality and they were not significantly associated with a risk of CVD mortality and incidence. However, this analysis is based on limited observational studies and large-scale trials on the complex interactions between low-carbohydrate diets and long-term outcomes are needed.

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Introduction

A growing body of evidence has suggested that low-carbohydrate diets and their combination with high-protein diets are effective in weight loss. [1–3] In addition, they reportedly ameliorate the risk factors of cardiovascular disease (CVD) in the short term, [4–6] which would decrease incident CVD and mortality. However, recent cohort studies did not support this hypothesis [7–12] and their long-term health benefit and risk remain controversial. In fact, low-carbohydrate diets tend to result in reduced intake of fiber and fruits, and increased intake of protein from animal sources, cholesterol and saturated fat, all of which are risk factors for mortality and CVD. [13,14].

In light of the worldwide obesity epidemic and the widespread popularity of low-carbohydrate diets, explorations of their long-term health outcome are of clinical importance for the control of weight. Moreover, they are crucial in the areas of public health, since a modest increase in the risk of morbidity and mortality [15] translates into a substantial social burden. These circumstances prompted us to investigate, with greater precision, the effects of low-carbohydrate diets on mortality and CVD incidence by scrutinizing pertinent original reports and combining their data in an attempt to obtain meaningful clues for the evaluation of benefit and harm associated with dietary modification.

Methods

Search

Searches of MEDLINE, EMBASE, ISI Web of Science, Cochrane Library, and ClinicalTrials.gov from their inception until September 12, 2012, were performed. Studies evaluating the risks of mortality or CVD incidence among subjects with low-carbohydrate intake, compared with those with high-carbohydrate intake, were identified using a combination of the following keywords: 'low-carbohydrate diet' or 'carbohydrate-restricted diet', and 'mortality' or 'survival', and 'cardiovascular disease'. The reference lists of the pertinent articles were also inspected.

Selection

We assessed all the identified studies on the effects of low-carbohydrate diets on mortality and CVD risk based on original data analyses to determine their eligibility for inclusion in a qualitative analysis. The inclusion criteria in the meta-analysis were as follows: a published full-text report, randomized controlled trials (RCTs) or observational studies of at least one year's follow-up period, reporting relative risks, i.e. hazard ratios (HRs), risk ratios (RRs), or odds ratios with confidence intervals (CIs), adjusted for at least three of the following possible major confounders for CVD and death: age, gender, obesity, smoking status, diabetes, hypertension, hypercholesterolemia, prior history of CVD, and family history of CVD. Studies in which the low-carbohydrate/high-protein (LC/HP) score was utilized to evaluate the carbohydrate intake were also included.

Validity and Quality Assessment

To ascertain the validity of the eligible studies, the quality of each report was appraised in reference to the CONSORT statement [16] and the STROBE statement [17] as appropriate. The quality of the studies that were included in the meta-analysis were further evaluated using Newcastle-Ottawa Scale [18] with a score of 5 or less (out of 8) indicating a high risk of bias.

Data Abstraction

We reviewed each full-text report to determine its eligibility and extracted and tabulated all the relevant data independently. The extracted data included the characteristics of the subjects (including age, gender, and region), study design, published year, follow-up period, outcomes and the methods used for risk estimation. Any disagreement was resolved by a consensus among the investigators.

Quantitative Data Synthesis

If more than one study was published for the same cohort with identical endpoints, the report containing the most comprehensive information on the population was included to avoid overlapping populations. The reports were summarized both qualitatively and quantitatively.

In the computation of the low-carbohydrate diet score, percentages of energy from protein and carbohydrate were divided into deciles. [7] For carbohydrate, the lowest decile received 10 points and the highest received 0 points, inversely. We pooled the relative risk in the highest score (lowest-carbohydrate intake) group with the lowest score (highest-carbohydrate intake) group as a referent. If an original article classified diets by the carbohydrate intake amount rather than the proportion to the total energy intake, the inverse relative risk for the lowest intake group was calculated with the highest intake group as a referent. If a relative risk was given per score in the original study, the relative risk in the highest score (lowest-carbohydrate intake) group was estimated by calculating the relative risk per score to the ninth power with the lowest score (highest-carbohydrate intake) group as a referent. Sensitive analysis was done using a composite LC/HP score. For protein, participants in the highest decile received 10 points, participants in the ninth decile received 9 points, and so forth. The protein and carbohydrate scores were then summed to create the composite LC/HP score (ranging from 2 to 20), which simultaneously assessed the position of each participant in terms of protein and carbohydrate intake. [9] Thus, a participant with a score of 2 was one with very high consumption of carbohydrates and very low consumption of proteins, whereas a participant with a score of 20 was one with very low consumption of carbohydrates and very high consumption of proteins. We pooled the relative risks similarly.

In the meta-analysis, each adjusted relative risk with low-carbohydrate intake was combined and the pooled RR with a 95% CI was calculated using the random-effects model with inverse-variance weighting. If a study separately reported relative risks for men and women, an overall estimate for the study was calculated from the two relative risks using the fixed-effects model with inverse-variance weighting and these single estimates were used in the subgroup analysis evaluating the individual contribution of the gender. [19] The results based on the LC/HP score were pooled separately. Heterogeneity among the studies was evaluated using I^2 statistics. RevMan (version 5.1) was used for these calculations. All the procedures were in accordance with the guidelines for the meta-analysis of observational studies in epidemiology [20] and the PRISMA statement [21].

Results

Search Results

A total of 492 articles were identified during our search; of these, 18 were assessed with respect to their eligibility for inclusion in our review, which was aimed at determining the influence of low-carbohydrate diets on mortality and CVD incidence (**Fig. 1**). No RCTs were identified. One article [22] was excluded from the systematic review because of population overlapping. Out of these 18 articles, a total of 17 cohort studies [7–12,14,23–32] were included in the systematic review and meta-analysis.

Table 1 shows the characteristics of each included study according to the published year. The 17 selected articles included in the systematic review were moderately heterogeneous in terms of population demographics, carbohydrate intake parameter, and the assessment of confounding factors. The population sample size in these studies ranged from 647 to 129,716. The majority of the articles were published from Sweden and the United States (US).

The adjustment factors and the risk of bias among the studies are summarized in **Table 2** and **Table S1**, respectively. Major confounding factors such as total energy intake were not stated in two studies. [23,32] Few inspected any updates of the carbohydrate intake over the follow-up period. Protein source was added to analysis in 3 studies. [7,9,30] The risk of bias among the researches involved in the meta-analysis was low.

Qualitative Summary

The all of the studies included in our analysis were methodologically good in quality. Regression coefficients of the multiple logistic model were provided in two articles [23,24] and CI was not estimable in another report. [32] Five articles analyzed the risk by diet quality without quantifying carbohydrate intake. [14,25–28] These 8 articles were not included in the subsequent meta-analysis. Most of the studies included in the systematic review were conducted in the US and European countries and their follow-up

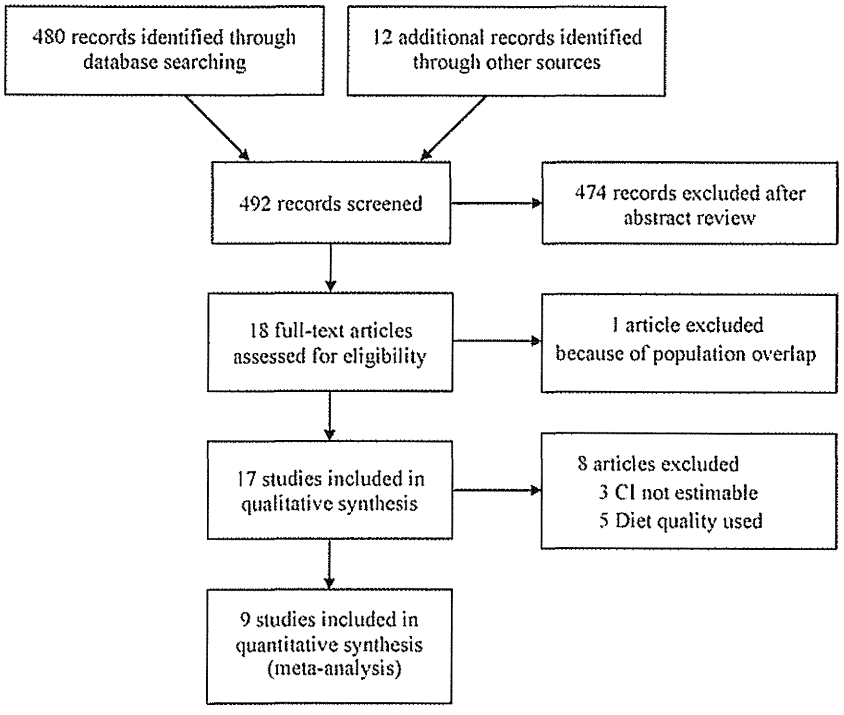

Figure 1. Flow diagram of study selection.
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durations were long enough for the outcomes to occur. Although the majority of the enrolled subjects were middle-aged and free of such chronic comorbidities as diabetes and coronary heart disease, healthcare professionals dominated in the US cohorts, who may not truly represented the average population in the community.

All-cause mortality was assessed in 7 reports. Four cohort studies using the low-carbohydrate score [7,10,11,32] and two using the LC-HP score [11,12] showed a significant increase associated with low-carbohydrate diets (relative risk range 1.12–25.0). One diet quality study suggested 0.27 shorter years of life in 10 years, which was statistically significant. [28] Only two out of five studies demonstrated a significantly elevated risk of CVD mortality (relative risk range 2.17–3.52) evaluated by the LC-HP score. [11,12] One article showed a significantly elevated risk of CVD incidence estimated by the low-carbohydrate score and the LC-HP score (relative risk range 1.42–1.55), [9] whereas three diet quality researches suggested a significantly increased risk of incident CVD (relative risk range 1.30–1.56). [14,26,27] Neither of the studies that calculated regression coefficients showed a significant correlation between low-carbohydrate diets and CVD. [23,24] Some studies suggested that low-carbohydrate diets might increase the risk of mortality and CVD in animal-based dietary patterns whereas they might decrease the risk in plant-based diets. [7,9,30].

The estimates in all the other analyses using either score were non-significant and none of these studies revealed that low-carbohydrate diets were associated with a significantly decreased risk of these outcomes.

Quantitative Summary (Meta-analysis)

A total of 9 articles that provided sufficient information using the low-carbohydrate score and/or the LC-HP score were included in the meta-analysis (**Fig. 1**). All the ascertainment of diagnosis was based on the valid registries but only a few specified the diagnostic criteria for CVD. [12,29,30] The follow-up rate was more than about 90% in each study. Carbohydrate intake was assessed by the residual method in 5 studies [8–12] and by the density method in 4 studies. [7,29–31] Of the 272,216 people in 4 cohort studies using the low-carbohydrate score, 15,981 (5.9%) cases of death from all-cause were reported. **Fig. 2** illustrates the significantly increased risk of all-cause mortality among those adherent to low-carbohydrate diets: the pooled RR (95% CI) 1.31 (1.07–1.59); $p = 0.007$; $I^2 = 53\%$ ($p = 0.09$). Analysis using the LC/HP score yielded a similar significant increase in the risk of all-cause mortality: RR 1.30 (1.01–1.68); $p = 0.04$; $I^2 = 65\%$ ($p = 0.04$). A dose-response was observed in 2 analyses. [7,12] Since heterogeneity among reports in the all-cause mortality using the low-carbohydrate score was statistically significant, we conducted a subgroup analysis according to the possible predictors. The pooled RRs of the studies conducted in Europe [10–12] and the United States [7] (RR 1.42 [1.18–1.72] vs 1.12 [1.01–1.24]) were both significantly elevated; and the diet assessment method (residual method [10–12] or density method [7]) coincided with these regions; the studies with follow-up length shorter than 10 years [10,12] were associated with a statistically high RR while those with follow-up length longer than 10 years [7,11] were not (RR 1.40 [1.12–1.74] vs 1.27 [0.88–1.84]); The pooled RR for men [7,10] was statistically elevated while that for women [7,9,10] was not (RR 1.19 [1.08–1.31] vs 1.34 [0.96–1.87]). We were unable to perform a subgroup analysis according to the body-mass index because the mean values were not stated or estimable in the majority of the reports.

A total of 3,214 (1.3%) cases of CVD death among 249,272 subjects in 3 cohort studies and 5,081 (2.3%) incident CVD cases among 220,691 women in different 4 cohort studies were reported. As summarized in **Fig. 3** and **Fig. 4**, the RRs of CVD mortality

Table 1. Study characteristics.

Source	Country, region/cohort	Follow-up, yr	N (women, %)	Age, yr	Diabetes, %	Coronary heart disease, %	Outcome, n
Garcia-Palmieri, 1980* [23]	USA, Puerto Rico	6	8218 (0)	45–64	NR	0	Myocardial infarction or coronary heart disease death 286
McGee, 1984* [24]	USA, Japanese ancestry	10	7088 (0)	45–68	NR	0	Coronary heart disease 456
McCullough, 2000* [25]	USA, NHS	12	67272 (100)	45–64	0	0	All CVD 1427
McCullough, 2000* [26]	USA, HPFS	8	51529 (0)	40–75	0	0	All CVD 1092
McCullough, 2002* [14]	USA,	8–12					
	a. NHS		a. 67271 (100)	a. 30–55	0	0	a. All CVD 1365
	b. HPFS		b. 38615 (0)	b. 40–75	0	0	b. All CVD 1092
Fung, 2001* [27]	USA, NHS	12	69017 (100)	38–63	0	0	Coronary heart disease 821
Diehr, 2003* [28]	USA, US Cardiovascular Health Study	10	5888 (58)	73	11	25	Coronary heart disease 2179
Oh, 2005 [29]	USA, NHS	18	78779 (100)	30–55	0	0	All stroke 1020
							Ischemic stroke 515
							Hemorrhagic stroke 279
Halton, 2006 [30]	USA, NHS	20	82802 (100)	30–55	0	0	Coronary heart disease 1994
Beulens, 2007 [31]	Netherland, Prospect-EPIC	Mean 9	15714 (100)	49–70	0	0	All CVD 799
							Coronary heart disease 556
							Stroke 243
Lagiou, 2007 [11]	Sweden, Scandinavian Women's Lifestyle and Health Cohort	Mean 12	42237 (100)	30–49	0	0	All-cause death 588
							CVD death 75
Massimino, 2007* [32]	Brazil, Japanese-Brazilians	8	647 (52)	Mean 63.5	20	NR	All-cause death 71
Trichopoulou, 2007 [12]	Greece, EPIC	Mean 4.9	22944 (59)	Adults	0	0	All-cause death 455
							CVD death 193
Fung, 2010 [7]	USA,						
	a. NHS	a. 26	a. 85168 (100)	a. 34–59	a. 0	a. 0	a. All-cause death 12555
							CVD death 2458
	b. HPFS	b. 20	b. 44548 (0)	b. 40–75	b. 0	b. 0	b. All-cause death 8678
							CVD death 2746
Sjögren, 2010 [8]	Sweden, Uppsala	Mean 10.1	924 (0)	Mean 71	0	0	All-cause death 215
							CVD death 88
Lagiou, 2012 [9]	Sweden, Uppsala Longitudinal Study of Adult Men cohort	Mean 15.7	43396 (100)	30–49	NR	0	All CVD 1268
							Ischemic heart disease 701