Effects of Metformin Dose on Cancer Risk Reduction in Patients with Type 2 Diabetes Mellitus: A 6-Year Follow-up Study

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Study Objective To explore the effects of metformin dose on cancer risk reduction in patients with type 2 diabetes.

Design Population-based cohort study.

Data Source National Health Insurance program Longitudinal Health Insurance Database.

Patients A total of 65,754 age- and gender-matched patients without diabetes and no previous cancer diagnosis were extracted from the database.

Measurements and Main Results We compared cancer risk among the subjects who had no diabetes, had type 2 diabetes but were not on diabetes drugs, used metformin only, used antidiabetic drugs other than metformin, or used metformin in combination with other antidiabetic drugs. Our results revealed dose-dependent effects of metformin on cancer risk and cancer onset times. A significant decrease in cancer risk was found in the monotherapy group who received more than 360 defined daily doses (DDDs) of metformin (hazard ratio [HR] 0.40, 95% confidence interval [CI] 0.24–0.66). The greatest decrease in cancer risk was observed in patients who took more than 1080 DDDs (HR 0.27, 95% CI 0.09–0.84). Significantly greater dose-dependent effects were seen in patients who used metformin in combination with other antidiabetic drugs.

Conclusion The magnitude of cancer risk reduction and prolonged cancer onset times produced by metformin in patients with type 2 diabetes depended on the dose of metformin, regardless of whether metformin was used alone or combined with other antidiabetic drugs.

Key Words metformin, cancer, risk, dose effect, type 2 diabetes.

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Metformin, a biguanide antidiabetic agent, has long been effectively used for the treatment of type 2 diabetes. It acts by increasing the uptake of glucose into cells, inhibiting gluconeogenesis in the liver, and increasing insulin sensitivity, with a resulting reduction of blood glucose and insulin levels. One of the advantages of metformin is that it does not cause hypoglycemia.[1] In addition to its glycemic- and insulin-lowering effect, the use of metformin is also associated with favorable antineoplastic effects, such as reduced risk and favorable prognosis in certain cancers.[2, 3]

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An association has been found between diabetes and cancer,[4–8] with diabetes mellitus shown to increase the risk of cancer.[5, 8, 9] Data also suggest that antidiabetes medications can affect the risk and prognosis of cancer.[10–14] For example, metformin is associated with a reduced cancer risk compared with other glucose-lowering agents in patients with type 2 diabetes.[15, 16]

The actions of metformin as an anticancer agent are mediated through several mechanisms including a decrease in insulin resistance that indirectly reduces insulin levels, an effect thought to be beneficial because insulin promotes cancer cell proliferation.[2, 17, 18] Metformin also produces numerous cellular activities that have potential antineoplastic activity including adenosine monophosphate kinase (AMPK) pathway activation,[19, 20] p53 activation,[21] the downregulation of cyclin D1,[20, 22] and the suppression of HER2 oncoprotein expression,[19] with resultant inhibition of cancer cell growth and proliferation[2, 23] and apoptosis.[2]

Epidemiologic studies[3, 24–28] suggest that the use of metformin in patients with type 2 diabetes reduced cancer risk and produced a favorable prognosis in several cancers, but most of these studies were conducted in white populations. Furthermore, these studies did not provide a clear analysis of the effect of metformin dose on cancer risk reduction, and they provided few details about the dose of metformin taken by the subjects involved in the study. Because there is a paucity of studies among Asian populations, we conducted the present study to investigate the effect of metformin dose on reducing cancer risk in patients with type 2 diabetes.

Methods

Database

The main objective of this study was to examine the effects of metformin dose on cancer risk in Asian patients in Taiwan with type 2 diabetes mellitus who did and did not receive metformin. Clinical data from a random 5% of Taiwan's 25.68 million residents were obtained from the National Health Insurance Research (NHRI) program Longitudinal Health Insurance Database 2005 (LHID 2005). No significant differences in the distribution of age and gender were found between the patients in the sample group and the original population. The medical claims data of the LHID 2005 include diagnosis codes, drug prescriptions, details of inpatient orders, details of ambulatory care orders, and ambulatory care expenditures by visit. As a result, the LHID 2005 offered unrestricted access to examining the effects of metformin on the risk of cancer among patients with type 2 diabetes mellitus. Because the identification numbers of all of the individuals in the NHRI database were encrypted to protect the privacy of the individuals, this study was exempt from full review by the institutional review board.

Study Sample

The present study used LHID 2005 records for the years 1997–2002. We identified 63,942 patients with a principal diagnosis of type 2 diabetes (*International Classification of Diseases, 9th edition, Clinical Modification* [ICD-9-CM], code 250.XX) between January 1, 1998, and December 31, 2002. Each patient was individually tracked for 6 years from their index outpatient visit to identify all of the patients who developed cancer (ICD-9-CM codes 140.XX–208.XX, 209.XX–239.XX). Of the remaining subjects without diabetes in the 2002 administrative data set, we identified two control patients per case patient. A total of 65,754 patients without diabetes mellitus and with no previous cancer diagnosis were randomly chosen using an SAS software program and extracted as an age- and gender-matched control group.

Exposure to Metformin

Information on all metformin prescriptions was extracted from the NHRI prescription database. We collected the date of prescription, daily dose, and number of days supplied. The defined daily dose (DDD) recommended by the World Health Organization of 2000 mg/day was used to quantify metformin usage. The cumulative usage of metformin was calculated based on all of the prescriptions dispensed during the follow-up period. The total DDDs were estimated as the dispensed dose of metformin during the follow-up period. The DDDs were calculated by dividing the total dose of metformin taken by the patient during the study period by 2000 mg (1 DDD of metformin). For example, if a patient took 400 g of metformin during the study period, the DDD taken by that particular patient during this period was calculated by dividing 400,000 mg (400 g) by 2000 mg, yielding 200 DDDs.

Statistical Analysis

The SAS v.9.1 statistical package (SAS Institute, Cary, NC, USA) was used to perform all of the analyses in this study. The Student *t* test and Pearson χ^2 test were performed to examine differences in sociodemographic characteristics (i.e., age, gender, and income-related insurance payments) and potential confounding factors (i.e., Charlson score, sulfonylurea use, thiazolidinedione use, and insulin use) among all four groups of patients with type 2 diabetes and the control group of patients without diabetes during the follow-up period.

Cox proportional hazard regression analyses were performed to estimate the hazard of cancer for the study and make comparisons between groups after adjusting for age, gender, income, Charlson score, sulfonylurea use, thiazolidinedione use, and insulin use.

Income data were collected from the LHID 2005 as income-related premium payments made by the patients because the Taiwan National Health Insurance program calculates insurance premiums according to the patient's income (i.e., salary received from companies or institutions). The Charlson score was calculated using the Charlson Comorbidity Index (CCI). The CCI rates the total burden of illnesses unrelated to the principal diagnosis of the patient (i.e., comorbidities). The CCI assigns a whole-number integer that is proportional to the relative risk of an outcome, such as death or cancer, at 1 year. We aggregated all of the integers for the relevant comorbidities in both the study group (type 2 diabetes) and control group (patients without type 2 diabetes) to determine the Charlson scores we used to adjust for confounding comorbid conditions. In the present study, the mean and standard deviation of the total scores in each particular patient group were used to adjust for confounding comorbid conditions.

Furthermore, we calculated the 6-year cancer-free survival rate and examined differences in the risk of cancer among the five groups. In the present study, the differences were determined to be statistically significant if the two-sided *p* value was ≤ 0.05.

Results

The selected study sample was composed of 35,758 patients who had at least a consensus of three diagnosed episodes of diabetes to increase the validity of the diagnosis. Additionally, we excluded 2881 patients who were diagnosed with type 2 diabetes before January 1, 1998, to ensure that only new cases were included in the study. Ultimately, our study sample included 32,877 patients with type 2 diabetes. Of these patients, 23,217 had received metformin treatment, and 5400 had received no antidiabetic drug treatment during the follow-up period. The 32,877 patients in the type 2 diabetes group were further divided into four groups. Group 1 included 5400 patients with type 2 diabetes not taking any antidiabetic drugs. Group 2 consisted of 1024 patients who took metformin only. Group 3 consisted of 4160 patients who took antidiabetic drugs other than metformin including sulfonylureas, thiazolidinediones, acarbose, and insulin analogs, among others. Group 4 consisted of 22,193 patients who took metformin plus other antidiabetic drugs (Figure 1).

Table 1 presents the characteristics of the study patients including age, gender, income, Charlson score, sulfonylurea use, thiazolidinedione use, and insulin use. The gender and age distributions were similar in the diabetes and nondiabetes groups. In these two groups, the male-to-female ratio and mean age were 0.95 and 57.5 ± 12.9 (SD) years, respectively. Patients without type 2 diabetes and those with type 2 diabetes but on no antidiabetes medication served as the control groups.

Significant differences were found between the study groups and control group with regard to age, gender, income, Charlson score, sulfonylurea use, thiazolidinedione use, and insulin use. The use of insulin by the patients without diabetes for other indications, such as septic shock, was of short duration and not considered to affect significantly the incidence of cancer in this group.

We calculated crude and adjusted hazard ratios (HRs) of being diagnosed with cancer in patients with and without type 2 diabetes (Tables 2 and 3). The analysis includes patients with diabetes who took no antidiabetic drugs, patients on metformin monotherapy, patients who took antidiabetic drugs other than metformin, and patients who took metformin combined with other antidiabetic drugs. Compared with patients who did not have type 2 diabetes, two groups had adjusted HRs greater than 1.0: the group who took no antidiabetic drugs and the group who received metformin monotherapy. The adjusted HRs were 1.58 (95% confidence interval [CI] 1.47–1.68) and 1.50 (95%

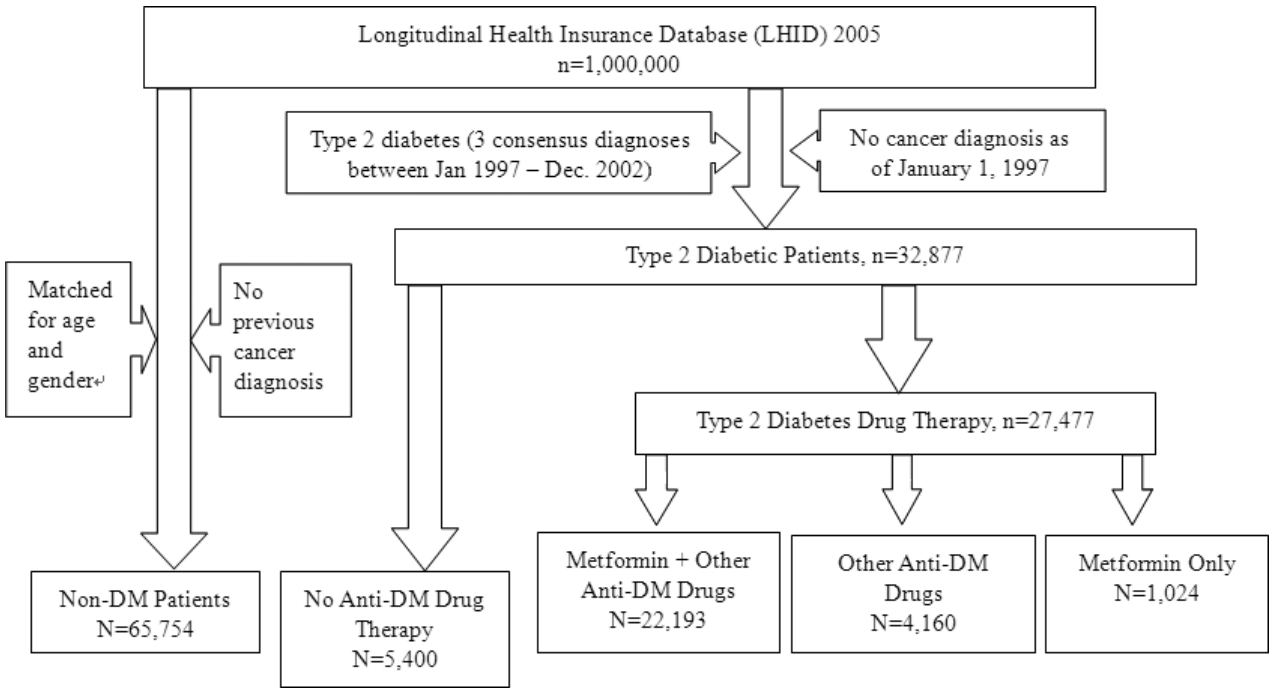

Figure 1. Study flow chart.

Table 1. Characteristics of Study Patients According to Diabetes and Medication Use Status

		DM patients (n=32,877)				
Variable	Non-DM Patients (n=65,754)	No Anti-DM Drug Therapy (n=5400)	Metformin Monotherapy (n=1024)	Other Anti-DM Drugs Without Metformin (n=4160)	Metformin Combined With Other Anti-DM Drugs (n=22,193)	
Age, yrs (mean ± SD)	57.5 ± 12.9	55.7 ± 14.9	58.4 ± 14.1	58.9 ± 14.4	57.6 ± 12.0	$p^a < 0.001$ $p^b < 0.05$
Gender male (*n*, %)	32,104 (48.8)	2449 (45.4)	501 (44.6)	2150 (51.7)	10,947 (49.3)[c]	$p^a < 0.01$ $p^b < 0.001$
Income (mean ± SD)	16,279 ± 20,086	15,451 ± 20,292[c]	13,618 ± 20,749[c, d]	11,718 ± 16,871	12,733 ± 17,053	$p^a < 0.001$ $p^b < 0.001$
Charlson score (mean ± SD)	2.29 ± 1.42	4.38 ± 1.93	4.84 ± 2.0[d]	5.02 ± 1.95[d]	5.11 ± 1.79	$p^a < 0.001$ $p^b < 0.001$
Insulin use Yes (*n*, %)	427 (0.6)	0	47 (4.6)	744 (17.9)	4,565 (20.6)	$p^a < 0.001$
Sulfonylurea use Yes (*n*, %)	0	0	0	3861 (92.81)	21,995 (99.11)	$p^a < 0.001$ $p^b < 0.001$
Thiazolidinedione use Yes (*n*, %)	0	0	0	754 (18.13)	9647 (43.47)	$p^a < 0.001$ $p^b < 0.001$

DM=diabetes mellitus.
p^a=non-DM patients as control group.
p^b=DM patients with no anti-DM drugs as control group.
[c]Non-DM patients as control group, p>0.05.
[d]DM patients with no anti-DM drugs as control group, p>0.05.

CI 1.32–1.71), respectively. Patients who took antidiabetic drugs other than metformin had an adjusted HR of 0.95 (95% CI 0.81–1.12). These results suggest that type 2 diabetes increased the risk of cancer in the study patients. A significant decrease in cancer risk was noted in the combination group (i.e., metformin with other antidiabetic drugs), the adjusted HRs of which were

Table 2. Comparison of Crude and Adjusted Hazard Ratios for Cancer Risk Between All Study Groups and Non-DM Patients

		DM patients (n=32,877)			
Outcome	Non-DM Patients (n=65,754)	No Anti-DM Drug Therapy (n=5400)	Metformin Monotherapy (n=1024)	Other Anti-DM Drugs Without Metformin (n=4160)	Metformin Combined With Other Anti-DM Drugs (n=22,193)
Cancer (*n*, %)	8400 (12.77)	1313 (24.31)	272 (24.19)	948 (22.79)	2688 (12.11)
Crude HR (95% CI)	1.00	2.09*** (1.98–2.22)	2.08*** (1.84–2.54)	1.93*** (1.80–2.06)	0.94** (0.90–0.98)
Adjusted HR (95% CI)	1.00	1.58*** (1.47–1.68)	1.50*** (1.32–1.71)	0.95 (0.81–1.12)	0.51*** (0.42–0.61)
Cancer onset time (days, mean ± SD)	1073 ± 636	824 ± 644	856 ± 677	890 ± 661	1163 ± 626
p value		0.54	0.14	0.11	0.30

DM = diabetes mellitus; HR = hazard ratio; CI = confidence interval.
The HRs were adjusted for age, gender, income, Charlson score, sulfonylurea use, thiazolidinedione use, and insulin use.
***p<0.001, **p<0.01.

Table 3. Comparison of Crude and Adjusted Hazard Ratios for Cancer Risk Between Diabetic Patients Who Received or Did Not Receive Antidiabetes Medications

	DM patients (n=32,877)			
Outcome	No Anti-DM Drug Therapy (n=5400)	Metformin Monotherapy (n=1024)	Other Anti-DM Drugs Without Metformin (n=4160)	Metformin Combined With Other Anti-DM Drugs (n=22,193)
Cancer (*n*, %)	1313 (24.31)	272 (24.19)	948 (22.79)	2688 (12.11)
Crude HR (95% CI)	1.00	0.99 (0.87–1.13)	0.92 (0.85–1.02)	0.45** (0.42–0.48)
Adjusted HR (95% CI)	1.00	0.88 (0.77–1.01)	0.41*** (0.37–0.46)	0.18*** (0.16–0.20)
Cancer onset time (days, mean ± SD)	824 ± 644	856 ± 677	890 ± 661	1,163 ± 626
p value		0.28	0.39	0.22

DM = diabetes mellitus; HR = hazard ratio; CI = confidence interval.
The HRs were adjusted for age, gender, income, Charlson score, sulfonylurea use, thiazolidinedione use, and insulin use.
***p<0.001, **p<0.01.

0.94 (95% CI 0.90–0.98, p<0.01) and 0.51 (95% CI 0.42–0.61, p<0.001), respectively (Table 2). These results suggest that type 2 diabetes patients who received metformin combined with other antidiabetic drugs had a lower cancer risk than patients without diabetes. Furthermore, compared with the group who took no antidiabetic drugs, the adjusted HRs for the group who took antidiabetic drugs other than metformin and the combination group were 0.41 (95% CI 0.37–0.46, p<0.001) and 0.18 (95% CI 0.16–0.20, p<0.001), respectively. In the metformin monotherapy group, the crude and adjusted HRs were 0.99 (95% CI 0.87–1.13, p=0.89) and 0.88 (95% CI 0.77–1.01, p=0.06), respectively (Table 3). The metformin monotherapy group showed no reduction of cancer risk. However, a dose-response analysis of the groups on metformin monotherapy and metformin combined with other antidiabetic drugs showed a dose-dependent decrease in the risk of cancer in both groups (Tables 4 and 5).

The dose-response analysis of the patients on metformin monotherapy showed a reduction of cancer risk starting with patients who received 180–360 DDDs. These patients had the least reduction of cancer risk (adjusted HR 0.77, 95% CI 0.54–1.10, p=0.15), with a greater risk reduction as the dose of metformin increased. A significant reduction in cancer risk was seen starting with patients who took 360–720 DDDs (adjusted HR 0.40, 95% CI 0.24–0.66, p<0.001). Patients who received more than 1080 DDDs had the greatest reduction in cancer risk (adjusted HR 0.27, 95% CI 0.09–0.84, p=0.02; Table 4). An analysis of the group who received metformin combined with other antidiabetic drugs showed a similar dose-dependent decrease in the risk of cancer. Patients who received more than 1080 DDDs of metformin showed the largest decrease (adjusted HR 0.06, 95% CI 0.05–0.07, p<0.001). However, these patients also showed a reduction of cancer risk after taking only 90 DDDs of metformin (adjusted HR 0.32,

Table 4. Dose-Response Analysis of Diabetic Patients Who Received Metformin Monotherapy

	DM Patients With no Anti-DM Drug Therapy (n=5400)	DM Patients on Metformin Monotherapy (n=1024)					
		< 90 DDD (n=590)	90–180 DDD (n=153)	180–360 DDD (n=140)	360–720 DDD (n=128)	720–1080 DDD (n=77)	> 1080 DDD (n=36)
Cancer (*n*, %)	1,313 (24.31)	167 (28.31)	48 (31.37)	31 (22.14)	15 (11.72)	8 (10.39)	3 (8.33)
Crude HR (95% CI)	1.00	1.22* (1.04–1.43)	1.29 (0.97–1.73)	0.88 (0.61–1.25)	0.44*** (0.26–0.73)	0.38** (0.19–0.46)	0.30* (0.09–0.94)
Adjusted HR (95% CI)	1.00	1.09 (0.92–1.28)	1.09 (0.82–1.46)	0.77 (0.54–1.10)	0.40*** (0.24–0.66)	0.37*** (0.18–0.74)	0.27*** (0.09–0.84)
Cancer onset time (days, mean ± SD)	824 ± 644	683 ± 662	1058 ± 646	1007 ± 526	1186 ± 569	1690 ± 444	1785 ± 349
p value		0.63	0.93	0.17	0.61	0.29	0.51

DM = diabetes mellitus; DDD = defined daily dose; HR = hazard ratio; CI = confidence interval.
The hazard ratios were adjusted for age, gender, income, Charlson score, and insulin use.
***p<0.001, **p<0.01, *p<0.05.

Table 5. Dose-Response Analysis of Diabetic Patients Who Received Metformin Combined with Other Antidiabetic Drugs

	DM Patients With no Anti-DM Drug Therapy (n=5400)	DM Patients on Metformin Combined With Other Antidiabetic Drugs (n=22,193)					
		< 90 DDD (n=4272)	90–180 DDD (n=2287)	180–360 DDD (n=3401)	360–720 DDD (n=4864)	720–1080 DDD (n=3441)	> 1080 DDD (n=3928)
Cancer (*n*, %)	1313 (24.31)	858 (20.08)	410 (17.93)	502 (14.76)	517 (10.63)	222 (6.45)	179 (4.56)
Crude HR (95% CI)	1.00	0.79*** (0.73–0.87)	0.69*** (0.62–0.78)	0.56*** (0.50–0.62)	0.39*** (0.35–0.43)	0.23*** (0.20–0.27)	0.16*** (0.14–0.19)
Adjusted HR (95% CI)	1.00	0.32*** (0.28–0.36)	0.26*** (0.23–0.30)	0.21** (0.19–0.24)	0.15*** (0.13–0.17)	0.08*** (0.07–0.09)	0.06*** (0.05–0.07)
Cancer onset time (days, mean ± SD)	824 ± 644	909 ± 649	1032 ± 597	1162 ± 565	1360 ± 518	1580 ± 503	1680 ± 449
p value		0.83	0.06	0.0005	< 0.0001	< 0.0001	< 0.0001

DM = diabetes mellitus; HR = hazard ratio; DDD = defined daily dose; CI = confidence interval.
The hazard ratios were adjusted for age, gender, income, Charlson score, sulfonylurea use, thiazolidinedione use, and insulin use.
***p<0.001, **p<0.01.

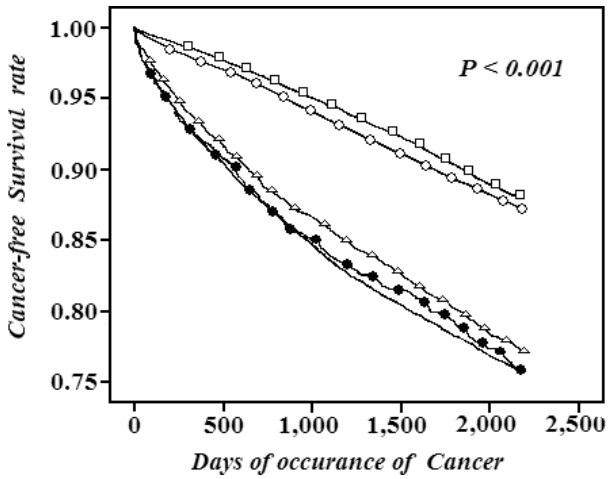

Figure 2. Cancer free survival rates at 6 years for non-DM and DM patients in Taiwan DM = diabetes mellitus. —□— DM with metformin and other anti-DM drugs; —○— Non-DM patients; —△— DM with other anti-DM drugs; —●— DM with metformin monotherapy; – – DM without medication.

95% CI 0.28–0.36, p<0.001; Table 5). Additionally, patients who received a higher DDD of metformin, whether in combination with other antidiabetic drugs or as monotherapy, had the longest time to onset of cancer (1680.68 ± 449 days and 1785.33 ± 349 days, respectively). The survival plots in Figure 2 show that patients who took metformin with other antidiabetic drugs had the longest cancer-free time.

Discussion

To our knowledge, this is the first retrospective study to analyze the effects of metformin dose on cancer incidence in patients with type 2 diabetes. Our study showed a reduced incidence of cancer in type 2 diabetic patients treated with metformin monotherapy and an even greater reduction in patients treated with metformin combined with other antidiabetic drugs. One prospective study conducted in Taiwan suggested that metformin reduced the risk of cancer.[28] They concluded that a significant decrease in cancer risk was achieved with doses lower than 500 mg/day. However, this dose is probably unrealistic because the recommended daily dose of metformin in Taiwan is 500 mg twice/day or 500 mg 3 times/day, up to 1000 mg 3 times/day if necessary, with a maximum of 3000 mg/day. Another study showed that the cancer risk associated with metformin was lower in patients who took a higher dose of metformin.[29] However, the authors' dose-response analysis was crude because it classified the doses taken by patients only as low, medium, and high, depending on the highest dose prescribed. In the present study, we defined the DDD of metformin as 2000 mg, according to World Health Organization Collaborating Centre for Drug Statistics methodology, and the doses taken by the patients were classified into six categories, from less than 90 DDDs to more than 1080 DDDs. This provided a clear picture of the effects of different doses taken by the patients and their relationship to cancer incidence.

Although no other studies have analyzed the dose-dependent effects of metformin in patient populations, several cancer cell culture[20, 30, 31] and animal[32] studies of the action of metformin showed that the effects of metformin on cancer are dose dependent. A 2010 study demonstrated that the decrease in tobacco-induced lung tumor volume and burden in A/J mice was greater with a metformin concentration of 5 mg/ml than with a concentration of 1 mg/ml.[33] A 2009 study also showed that metformin combined with other anticancer medications produced a greater reduction of tumor cells than metformin alone.[31] Hyperglycemia and hyperinsulinemia in type 2 diabetes are well known to increase the risk of cancer.[34, 35] Antidiabetic drugs including metformin increase insulin sensitivity and decrease hyperinsulinemia and hyperglycemia, potentially exerting an anticancer effect. In fact, some antidiabetic agents have been shown to have antiproliferative activity mediated by several mechanisms in addition to decreasing hyperinsulinemia and hyperglycemia.[36–38] Some studies[7, 15, 16] have shown that insulin and insulin analogs amplify cancer risk by significantly increasing the levels of insulin in the systemic circulation. One group of authors showed that metformin, acarbose, gliclazide, glitazones, insulin, and repaglinide did not increase the risk of cancer after 36 months of use in patients with type 2 diabetes.[12] Even the combination of metformin and insulin secretagogues, such as sulfonylureas, has been shown to decrease cancer risk.[16] One of the drugs used in combination with metformin by the patients in the present study was thiazolidinedione. This medication has been shown to produce anticancer effects through a similar mechanism of action as metformin, such as acting on the Akt/mammalian target of rapamycin (mTOR) pathway and activating adenosine monophosphate-activated protein kinase, among others.[31, 32, 37]Additionally, the anticancer actions of thiazolidinediones

appear to be mediated by the upregulation of plasminogen activator inhibitor (PAI)-1, which inhibits cell invasion. PAI-1 is thought to be inversely related to glucose disposition; it is significantly higher and positively correlated with abdominal fat in Asians compared with whites.[39, 40] South Asians in particular have higher rates of central obesity, which is associated with high insulin resistance.[41, 42] A 2012 study showed a significant decrease in overall cancer risk in type 2 diabetes patients from Hong Kong who used thiazolidinedione.[43] Therefore, insulin-lowering therapy might have a significant effect on cancer, either as a monotherapy or combined with other drugs that have anticancer properties, such as metformin. Thus the larger decrease in cancer risk in the group who took metformin with other antidiabetic medications in the present study may be attributable to a synergistic effect achieved via metformin's ability to decrease cancer risk and the action of the other antidiabetic medications on hyperglycemia and hyperinsulinemia that decreased (or did not increase) cancer risk in patients with type 2 diabetes.

In the present study, the increase in cancer onset time was dose dependent with higher doses of metformin resulting in a longer time to the onset of cancer. Increases in onset times became evident at more than 360 DDDs in the metformin monotherapy group. In the group of patients who took metformin combined with other antidiabetic drugs, the increase in onset became evident at 90 DDDs or less. In a 2009 study,[29] the median cancer onset times were 3.5 years and 2.6 years for metformin users and nonusers, respectively. Although the dose definition in the study in the study was imprecise, other factors might be responsible for the large difference in cancer onset times in patients on metformin in the two studies. These two studies were performed in two racially different populations, but the results showed similar patterns of cancer risk reduction.

Metformin does not appreciably accumulate in the body after multiple dosing.[44] This means that the significantly greater effects observed at larger doses in the present study are attributable to the DDD given to the patients and not to metformin accumulation. Metformin is thought to exert its antiproliferative effect through the activation of AMPK, an intracellular regulator of cellular energy metabolism. The activation of AMPK results in the arrest of cellular growth and proliferation. AMPK is activated by intracellular changes in the AMP-to-adenosine triphosphate (ATP) ratio and various cellular kinases, such as liver kinase B1 (LKB1) and calcium-dependent kinase (CaMKKβ).[2]

Metformin is thought to activate AMPK in two ways: by activating LKB1,[29] which phosphorylates AMPK, and through an inhibitory effect of metformin on mitochondrial complex 1 protein in the mitochondrial electron transport chain, leading to changes in the AMP-to-ATP ratio.[2] Activated AMPK activates tuberous sclerosis 2 protein, which has inhibitory effects on mTOR. mTOR plays a central role in regulating cellular growth and proliferation by controlling protein synthesis in response to the availability of energy and nutrients. AMPK activation also leads to a decrease in cyclin D1, an important regulator of the cell cycle. Cyclin D1 regulates the progression of proliferating cells from the G0 phase to S phase of the cell cycle.[2, 30]

The present study has several strengths, the first of which is the large database of one million people that was available to us. Our study also considered several confounding factors, including age, gender, income, Charlson score, sulfonylurea use, thiazolidinedione use, and insulin use, all of which may influence the development of cancer. The second strength is that the diagnosis of type 2 diabetes was confirmed on at least three occasions in each of the patients included in our study, which could increase the accuracy of the diagnosis.[45] The third strength is the further classification of metformin users according to the dose used by the patients, demonstrating an association between larger doses of metformin and a greater reduction of cancer risk and longer cancer onset times. A fourth strength is that the dose of metformin used by the patients included in the study was precisely defined.

The limitations of our study include the use of an administrative database that lacked records of patient lifestyles, such as smoking, body mass index, and hemoglobin A_{1c}, which may be considered confounding factors. Nevertheless, our results are consistent with another study[29] that adjusted for these factors. We showed that metformin use is associated with a dose-dependent reduction of cancer incidence and prolongation of cancer onset times. However, the significant dose-dependent reduction of cancer incidence was associated with the use of metformin (at all of the DDD ranges) in combination with other antidiabetic drugs and the use of more than 360 DDDs of metformin monotherapy. Significantly longer cancer onset times were only associated

with the use of metformin in combination with other antidiabetic drugs.

In conclusion, we showed that the magnitude of cancer risk reduction and prolonged cancer onset times produced by metformin in patients with type 2 diabetes patients depended on the dose of metformin, regardless of whether metformin was used alone or combined with other antidiabetic drugs. We also showed that metformin used in combination with other antidiabetic drugs produced a significant reduction of cancer incidence and prolongation of cancer onset times than metformin monotherapy. These results may indicate synergism in the reduced cancer risk and onset times achieved by the combination of metformin with other antidiabetic drugs. Metformin is a relatively safe drug. It does not cause hypoglycemia, except in patients with severe renal impairment (i.e., with serum creatinine levels higher than 1.5 mg/dl in males or higher than 1.4 mg/dl in females). Therefore, metformin may be an appropriate chemopreventive agent in patients with type 2 diabetes, with maximum anticancer effects achieved at daily doses of 2000 mg/day for patients without severe renal impairment. For patients who cannot tolerate this, doses of metformin less than 2000 mg/day can be used. However, the use of less than 2000 mg metformin per day will likely delay the anticancer effects because the anticancer effects of the drug depend on the cumulative number of DDDs taken by the patient.

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References

1. **Nathan DM, Buse JB, Davidson MB, et al.** Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Association and the European Association for the Study of Diabetes. Diabetes Care 2009;32:193–203.
2. **Sahra IB, Le Marchand-Brustel Y, Tanti J–F, Bost F.** Metformin in cancer therapy: a new perspective for an old antidiabetic drug? Mol Cancer Ther 2010;9:1092–9.
3. **Evans JMM, Donnelly LA, Esmile–Smith AM, Alessi DR, Morris AD.** Metformin and reduced risk of cancer in diabetes patients. BMJ 2005;330:1304–5.
4. **Giovannucci E, Harlan DM, Archer MC, et al.** Diabetes and cancer: a consensus report. Diabetes Care 2010;33:1674–85.
5. **Helminki K, Li X, Sundquist J, Sandquist K.** Risk of cancer following hospitalization for type 2 diabetes. Oncologist 2010;15:548–55.
6. **Vigneri P, Frasca F, Sciacca L, Pandini G, Vigneri R.** Diabetes and cancer. Endocr Relat Cancer 2009;16:1103–23.
7. **Suh S, Kim KW.** Diabetes and cancer: is diabetes causally related to cancer? Diabetes Metab J 2011;35:193–8.
8. **Cannata D, Fierz Y, Vijayakumar A, LeRoith D.** Type 2 diabetes and cancer: what is the connection? Mt Sinai J Med 2010;77:197–213.
9. **Larsson SC, Orsini N, Brismar K, Wolk A.** Diabetes mellitus and risk of bladder cancer: a meta–analysis. Diabetologia 2006;49:2819–23.
10. **Smith U, Gale EAM.** Does diabetes therapy influence the risk of cancer? Diabetologia 2009;52:1699–708.
11. **Monami M, Colombi C, Balzi D, et al.** Metformin and cancer occurrence in insulin-treated type 2 diabetic patients. Diabetes Care 2011;34:129–31.
12. **Monami M, Lamanna C, Balzi D, Marchionni N, Mannucci E.** Sulphonylureas and cancer: a case-control study. Acta Diabetol 2009;46:279–84.
13. **Govindarajan R, Ratnasinghe L, Simmons DL, Siegel ER, Midathada MV, Kim L.** Thiazolidinediones and the risk of lung, prostate, and colon cancer in patients with diabetes. J Clin Oncol 2007;25:1476–81.
14. **Dai Y, Wang WH.** Peroxisome proliferator-activated receptor γ and colorectal cancer. World J Gastrointest Oncol 2010;2:159–64.
15. **Bowker SL, Yasui Y, Veugelers P, Johnson JA.** Glucose-lowering agents and the cancer mortality in type 2 diabetes assessing effects of time-varying exposure. Diabetologia 2010;53:1631–7.
16. **Currie CJ, Poole CD, Gale EAM.** The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. Diabetologia 2009;52:1766–77.
17. **Lipscombe LL, Goodwin PJ, Zinman B, Mclaughlin JR, Hux JE.** Increased prevalence of prior breast cancer in women with newly diagnosed diabetes. Breast Cancer Res Treat 2006;98:303–9.
18. **Pollak M.** Insulin and insulin-like growth factor signaling in neoplasia. Nat Rev Cancer 2008;8:915–28.
19. **Vazquez-Martin A, Oliveras-Ferraros C, Menendez JA.** The antidiabetic drug metformin suppresses HER2 (erbB–2) oncoprotein overexpression via inhibition of the mTOR effector p70S6K1 in human breast carcinoma cells. Cell Cycle 2008;8:88–96.
20. **Zhuang Y, Miskimins WK.** Cell cycle arrest in metformin treated breast cancer cells involves activation of AMPK, down–regulation of cyclin D1, and requires p27Kip1 or p21Cip1. J Mol Signal 2008;3:18–28.
21. **Buzzai M, Jones RG, Amaravadi RK, et al.** Systemic treatment with antidiabetic drug metformin selectively impairs p53-deficient tumour cell growth. Cancer Res 2007;67:6745–53.
22. **Ben Sahra IB, Laurent K, Loubat A, et al.** The antidiabetic drug metformin exerts an antitumoural effect in in-vitro and in-vivo through a decrease of cyclin D1 level. Oncogene 2008;27:3576–86.
23. **McFarland MS, Cripps R.** Diabetes mellitus and increased risk of cancer: focus on metformin and the insulin analogs. Pharmacotherapy 2010;30:1159–78.
24. **Wright JL, Stanford JL.** Metformin use and prostate cancer in Caucasian men: results from a population-based case-control study. Cancer Causes Control 2009;20:1617–22.
25. **Jiralerspong S, Palla SL, Giordano SH, et al.** Metformin and complete pathologic response to neoadjuvant chemotherapy in diabetic patients with breast cancer. J Clin Oncol 2009;27:3297–302.
26. **Li D, Yeung SC, Hassan MM, Konopleva M, Abbruzzese JL.** Antidiabetic therapies affect the risk of pancreatic cancer. Gastroenterology 2009;137:482–8.
27. **Bo S, Ciccone G, Rosato R, et al.** Cancer mortality reduction and metformin. Diabetes Obes Metab 2012;14:23–9.
28. **Lee MS, Hsu CC, Wahlqvist LM, Tsai HN, Chang YH, Huang YC.** Type 2 diabetes increases and metformin reduces total, colorectal, liver and pancreatic cancer incidences in Taiwanese: a representative population prospective cohort study of 800,000 individuals. BMC Cancer 2011;11:20. doi:10.1186/1471-2407-11.

29. **Libby G, Donnelly LA, Donnan PT, Alessi DR, Morris AD, Evans JM.** New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. Diabetes Care 2009;32:1620–5.
30. **Zakikhani M, Dowling R, Fantus IG, Sonenberg N, Pollak M.** Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. Cancer Res 2006;66:10269–73.
31. **Hirsch HA, Iliopoulos D, Tsichlis PN, Struhl K.** Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. Cancer Res 2009;69:7507–11.
32. **Fierz Y, Novosyadlyy R, Vijayakumar A, Yakar S, LeRoith D.** Insulin-sensitizing therapy attenuates type 2 diabetes–mediated mammary tumor progression. Diabetes 2010;59:686–93.
33. **Memmott RM, Mercado JR, Maier CR, Kawabata S, Fox SD, Dennis PA.** Metformin prevents tobacco carcinogen-induced lung tumorigenesis. Cancer Prev Res (Phila) 2010;3:1066–76.
34. **Jalving MG, Gietema JA, Lefrandt JD, et al.** Metformin: taking away the candy for cancer? Eur J Cancer 2010;46:2369–80.
35. **Engelman JA, Cantley LC.** Chemoprevention meets glucose control. Cancer Prev Res (Phila) 2010;3:1049–52.
36. **Youssef J, Badr M.** Peroxisome proliferator-activated receptors and cancer: challenges and opportunities. Br J Pharmacol 2011;164:68–82.
37. **Oliveria SA, Koro CE, Yood MU, Sowell M.** Cancer incidence among patients treated with antidiabetic pharmacotherapy. Diabetes Metab Syndr 2008;2:47–57.
38. **Chowdhury TA.** Diabetes and cancer. QJM 2010;103:905–15.
39. **Okumura T.** Mechanisms by which thiazolidinediones induce anti-cancer effects in cancers in digestive organs. J Gastroenterol 2010;45:1097–102.
40. **Raji A, Seely EW, Arky RA, Simonson DC.** Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. J Clin Endocrinol Metab 2001;86:5366–71.
41. **Weber MB, Oza-Frank R, Staimez LR, Ali MK, Narayan KM.** Type 2 diabetes in Asians: prevalence, risk factors, and effectiveness of behavioral intervention at individual and population levels. Annu Rev Nutr 2012;32:417–39.
42. **Ramachandran A, Ma RC, Snehalatha C.** Diabetes in Asia. Lancet 2010;375:408–18.
43. **Yang X, So WY, Ma RC, et al.** Use of thiazolidinedione and cancer risk in type 2 diabetes: the Hong Kong diabetes registry. Diabetes Res Clin Pract 2012;97:e13–7.
44. **Graham GG, Punt J, Arora M, et al.** Clinical pharmacokinetics of metformin. Clin Pharmacokinet 2011;50:81–98.
45. **Lin CC, Lai MS, Syu CY, Chang SC, Tseng FY.** Accuracy of diabetes diagnosis in health insurance claims data in Taiwan. J Formos Med Assoc 2005;104:157–63.