Low HDL Cholesterol, Metformin Use, and Cancer Risk in Type 2 Diabetes

The Hong Kong Diabetes Registry

XILIN YANG, PHD[1,2,3]
WING YEE SO, MD, FRCP[1,2,3]
RONALD C.W. MA, MBBCHIR, FRCP[1,2,3]
ALICE P.S. KONG, MBCHB, FRCP[1,2,3]
HEUNG MAN LEE, PHD[1,2,3]
LINDA W.L. YU, MBCHB, MRCP[1]
CHUN-CHUNG CHOW, MBCHB, FRCP, MD[1,2]
RISA OZAKI, MBCHB, MRCP[1,2]
GARY T.C. KO, MD, FRCP[1,2]
JULIANA C.N. CHAN, MD, FRCP[1,2,3]

OBJECTIVE—The AMP-activated protein kinase (AMPK) pathway is a master regulator in energy metabolism and may be related to cancer. In type 2 diabetes, low HDL cholesterol predicts cancer, whereas metformin usage is associated with reduced cancer risk. Both metformin and apolipoprotein A1 activate the AMPK signaling pathway. We hypothesize that the anticancer effects of metformin may be particularly evident in type 2 diabetic patients with low HDL cholesterol.

RESEARCH DESIGN AND METHODS—In a consecutive cohort of 2,658 Chinese type 2 diabetic patients enrolled in the study between 1996 and 2005, who were free of cancer and not using metformin at enrollment or during 2.5 years before enrollment and who were followed until 2005, we measured biological interactions for cancer risk using relative excess risk as a result of interaction (RERI) and attributable proportion (AP) as a result of interaction. A statistically significant RERI >0 or AP >0 indicates biological interaction.

RESULTS—During 13,808 person-years of follow-up (median 5.51 years), 129 patients developed cancer. HDL cholesterol <1.0 mmol/L was associated with increased cancer risk among those who did not use metformin, but the association was not significant among those who did. Use of metformin was associated with reduced cancer risk in patients with HDL cholesterol <1.0 mmol/L and, to a lesser extent, in patients with HDL cholesterol ≥1.0 mmol/L. HDL cholesterol <1.0 mmol/L plus nonuse of metformin was associated with an adjusted hazard ratio of 5.75 (95% CI 3.03–10.90) compared with HDL cholesterol ≥1.0 mmol/L plus use of metformin, with a significant interaction (AP 0.44 [95% CI 0.11–0.78]).

CONCLUSIONS—The anticancer effect of metformin was most evident in type 2 diabetic patients with low HDL cholesterol.

Diabetes Care 34:375–380, 2011

Patients with type 2 diabetes have increased cancer risk, although the risk association of cancer with antidiabetes drugs remains controversial (1). This is in part attributed to heterogeneity in causalities, phenotypes, and treatment responses. In addition to age and smoking status, abnormal lipids are strong predictors of cancer in type 2 diabetes (2). Our group has reported a V-shaped risk association of HDL cholesterol with cancer, with an optimal level of 1.22 mmol/L and a rapid increase above and below the optimal point (2). Low HDL cholesterol is a common feature of type 2 diabetes (3) and obesity, the latter often considered a linking factor for cancer risk because of insulin resistance (4). However, apolipoprotein (Apo) A-I, the main lipoprotein of HDL cholesterol, also can stimulate the phosphorylation of AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) to increase glucose uptake in muscle (5) and insulin sensitivity. In this light, the AMPK pathway is considered to be a master switch in sensing and regulating energy metabolism by balancing catabolism (lipolysis) and anabolism (protein and glycogen storage) (6). A large number of factors can activate or inhibit the AMPK signaling pathway, and one of these is the upstream signal, LKB1, a tumor suppressor. In experimental studies, inhibition of the LKB1-AMPK pathway results in tumor formation (7), whereas metformin activates the LKB1-AMPK pathway and inhibits cancer cell growth (8). In support of these findings, epidemiological studies have reported reduced cancer risk (9) and associated mortality (10) in type 2 diabetic patients treated with metformin compared with other antidiabetes drugs. This study argued that the anticancer effects of metformin, if any, would be most evident in patients with low HDL cholesterol.

RESEARCH DESIGN AND METHODS

—We selected a prospective cohort from the Hong Kong Diabetes Registry enrolled between 1 December 1996 and 8 January 2005 because drug dispensary data became fully computerized and available for analysis purposes in 1996. A detailed description of the Hong Kong Diabetes Registry is available elsewhere (11–13). Briefly, the registry was established at the Prince of Wales Hospital, the teaching hospital of the Chinese University of Hong Kong, which serves a population of >1.2 million. The referral sources of the cohort included general practitioners, community clinics, other specialty clinics, the Prince of Wales Hospital, and other hospitals. Enrolled patients with hospital admissions within 6–8 weeks prior to assessment accounted for <10% of all referrals. A 4-h assessment of complications and risk factors

From the [1]Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong Special Administrative Region (SAR), China; the [2]Hong Kong Institute of Diabetes and Obesity, The Chinese University of Hong Kong, Hong Kong SAR, China; and the [3]Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China.
Corresponding author: Xilin Yang, yang.xilin@cuhk.edu.hk or yxl@hotmail.com.
Received 4 August 2010 and accepted 16 October 2010.
DOI: 10.2337/dc10-1509
This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc10-1509/-/DC1.

was performed on an outpatient basis, modified from the European DiabCare protocol (14). Once a patient had undergone this comprehensive assessment, he/she was considered to have entered this study cohort and would be followed until the time of death. Ethical approval was obtained from the Chinese University of Hong Kong Clinical Research Ethics Committee. This study adhered to the Declaration of Helsinki, and written informed consent was obtained from all patients at the time of assessment, for research purposes.

By 2005, 7,387 diabetic patients were enrolled in the registry since December 1996. We sequentially excluded *1*) 328 patients with type 1 diabetes or missing data on types of diabetes; *2*) 45 patients with non-Chinese or unknown nationality; *3*) 175 patients with a known history of cancer or receiving cancer treatment at enrollment; *4*) 736 patients with missing values on any variables used in the analysis (see Table 1 for a list of variables); and *5*) 3,445 patients who used metformin during 2.5 years before enrollment. The remaining 2,658 patients were included in the analysis. The cutoff point of 2.5 years was chosen because any duration longer than that did not lead to any noticeable changes in the hazard ratios (HRs) and 95% CIs of metformin use for cancer (Supplementary Table 1).

Clinical measurements and data retrieval

Patients attended the center after an 8-h fast and underwent a 4-h structured clinical assessment that included laboratory investigations. A sterile, random-spot urinary sample was collected to measure albumin-to-creatinine ratio (ACR). In this study, albuminuria was defined as an ACR ≥2.5 mg/mmol in men and ≥3.5 mg/mmol in women. The abbreviated Modification of Diet in Renal Disease Study formula recalibrated for Chinese subjects (15) was used to estimate glomerular filtration rate (GFR) (expressed in mL/min per 1.73 m^2): estimated GFR = $186 \times (\text{SCR} \times 0.011)^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times 1.233$, where SCR is serum creatinine expressed as μmol/L (original mg/dL converted to μmol/L), and 1.233 is the adjusting coefficient for Chinese subjects. Total cholesterol, triglycerides, and HDL cholesterol were measured by enzymatic methods on a Hitachi 911 automated analyzer (Boehringer Mannheim, Mannheim, Germany) using reagent kits supplied by the manufacturer of the analyzer, whereas LDL cholesterol was calculated using the Friedewald equation (16). The precision performance of these assays was within the manufacturer's specifications.

Drug usage data were extracted from the Hong Kong Hospital Authority Central Computer System, which recorded all drug dispensary data in public hospitals, including the start dates and end dates for each of the drugs of interest. In Hong Kong, all medications are dispensed on

Table 1—*Clinical and biochemical characteristics of the study cohort stratified according to occurrence of cancer during follow-up period*

	Noncancer (*n* = 2,529)	Cancer (*n* = 129)	*P*
Baseline variables			
Age (years)	55 (44–66)	64 (55–72)	<0.0001*
Male sex	1,236 (48.9)	71 (55.0)	0.1718†
Employment status			<0.0001†
Full-time	926 (36.6)	29 (22.5)	
Housewife	699 (27.6)	36 (27.9)	
Retired	654 (25.9)	58 (50.0)	
Other	250 (9.9)	6 (4.7)	
Smoking status			0.0046†
Ex-smoker	365 (14.4)	26 (20.2)	
Current smoker	390 (15.4)	30 (23.3)	
Alcohol intake			0.0030†
Ex-drinker	298 (11.8)	28 (21.7)	
Current drinker	198 (7.8)	7 (5.4)	
BMI (kg/m^2)	24.3 (22.0–26.5)	23.9 (22.2–26.4)	0.6937*
Duration of diabetes (years)	4 (1–9)	5 (2–12)	0.0180*
SBP (mmHg)	132 (120–146)	134 (125–148)	0.0827*
Diastolic blood pressure (mmHg)	75 (68–82)	75 (67–82)	0.9706*
A1C (%)	6.9 (6.1–8.2)	7.2 (6.1–8.6)	0.2575*
LDL cholesterol (mmol/L)	3.20 (2.60–3.80)	2.30 (2.90–3.74)	0.0154*
HDL cholesterol (mmol/L)	1.26 (1.07–1.52)	1.25 (0.99–1.53)	0.3760*
HDL cholesterol <1.0 mmol/L	398 (15.7)	35 (27.1)	0.0006
Triglycerides (mmol/L)	1.26 (0.9–1.86)	1.16 (0.84–1.66)	0.0700*
ACR (mg/mmol)	1.54 (0.67–8.54)	3.07 (0.93–11.04)	0.0063*
Albuminuria	968 (38.3)	64 (49.6)	0.0100†
Estimate GFR (mL/min per 1.73 m^2)	105.5 (84.0–127.7)	99.8 (81.6–118.8)	0.1047*
Events and medications during follow-up§			
Death (all-cause)	188 (7.4)	68 (52.4)	<0.0001†
ACE inhibitors or ARBs	1,198 (47.4)	55 (42.6)	0.2933†
Statins	817 (32.3)	17 (13.2)	<0.0001†
Fibrates	206 (8.2)	7 (5.4)	0.2672†
Other lipid-lowering drugs	12 (0.5)	0 (0.0)	1.0‡
Acarbose	143 (9.5)	5 (2.5)	0.3902†
Glibenclamide	537 (21.2)	25 (19.4)	0.6150†
Gliclazide	1,001 (39.6)	41 (31.8)	0.0768†
Glimepiride	15 (0.6)	1 (0.8)	0.5499‡
Glipizide	217 (8.6)	21 (16.3)	0.0028†
Metformin	1,226 (48.5)	40 (31.0)	<0.0001†
Duration of metformin use (years)ξ	3.09 (1.21–5.65)	1.21 (0.57–3.57)	0.0008*
Mean daily metformin dosage (g)ξ	1.01 (0.82–1.56)	1.05 (0.64–1.42)	0.1981*
Pioglitazone	19 (0.8)	0 (0)	1.0‡
Rosiglitazone	72 (2.8)	1 (0.9)	0.2618‡
Tolbutamide	19 (0.8)	0 (0)	1.0‡
Follow-up time (years)	5.70 (3.23–7.44)	2.52 (1.09–4.51)	<0.0001†

Data are median (25th to 75th percentile) or *n* (%). *Derived from Wilcoxon two-sample test. †Derived from χ^2 test. ‡Derived from Fisher exact test. §From enrollment to the earliest date of cancer, death, or censoring. ξAmong metformin users only.

site in both inpatient and outpatient settings. These databases were matched by a unique identification number, the Hong Kong identity card number, which is compulsory for all residents in Hong Kong.

A trained team at the Hong Kong Hospital Authority coded all hospital admissions. All medical admissions of the cohort from enrollment to 30 July 2005 were retrieved from the Hong Kong Hospital Authority Central Computer System, which recorded admissions to all public hospitals in Hong Kong. Collectively, these hospitals provide 95% of the total hospital bed-days in Hong Kong (17). Additionally, mortality data from the Hong Kong Death Registry during the period were retrieved and cross-checked with hospital discharge status. Hospital discharge principle diagnoses, coded by the International Statistical Classification of Diseases and Related Health Problems 9th Revision (ICD-9), were used to identify cancer events. The outcome measure of this study was incident cancer (fatal or nonfatal: codes 140–208) during the follow-up period.

Statistical analyses

We used biological interactions to test whether metformin use was associated with a greater cancer risk reduction in patients with low HDL cholesterol than in those with normal or high HDL cholesterol. The Statistical Analysis System (release 9.10) was used to perform the statistical analysis (SAS Institute, Cary, NC), unless otherwise specified. Follow-up time was calculated as the period in years from the first enrollment since 1 December 1996 to the date of the first cancer event, death, or censoring, whichever came first. Cox proportional hazard regression was used to obtain the HRs and 95% CIs of the variables of interest.

We first plotted the full-range association of HDL cholesterol and cancer and further refined cutoff points of HDL cholesterol for cancer risk in the cohort without prevalent metformin users, using restricted cubic spline Cox models (11). Then, we examined the biological interaction for cancer risk between low HDL cholesterol and nonuse of metformin using three measures: *1*) relative excess risk caused by interaction (RERI); *2*) attributable proportion (AP) caused by interaction; and *3*) the synergy index (S) (18). A detailed calculation method of additive interaction, including the definition of three indicator variables, an SAS program, and a calculator in Microsoft Excel (www.epinet.se), was described by the authors. The RERI is the excess risk attributed to interaction relative to the risk without exposure. AP refers to the attributable proportion of disease, which is caused by the interaction in subjects with both exposures. S is the excess risk from both exposures when there is biological interaction relative to the risk from both exposures without interaction. A simulation study showed that RERI performed best and AP performed fairly well, but S was problematic in the measure of additivity in the proportional hazard model (19). The current study refined the criteria as either a statistically significant RERI >0 or AP >0 to indicate biological interactions.

The following two-step adjustment scheme was used in these analyses: *1*) only adjusting for LDL cholesterol–related cancer risk indicators (LDL cholesterol $\geq$3.80 mmol/L and LDL cholesterol $<$2.80 mmol/L plus albuminuria) (11,12), triglycerides (2), and high HDL cholesterol, where appropriate (2), and *2*) further adjusting for age, sex, employment status, smoking status, alcohol intake, duration of diabetes, BMI ($\geq$27.6 and $<$24.0 kg/m^2) (2), systolic blood pressure (SBP), and A1C (20) at enrollment and use of statins (13), fibrates, other lipid-lowering drugs, ACE inhibitors/angiotensin II receptor blockers (ARBs) (13), and insulin (20) during follow-up. Use of drugs during follow-up was defined as use of the drugs from enrollment to cancer, death, or censoring date, whichever came first. By definition, the use of any drugs after the first cancer event was coded as nonuse of these drugs, and any drug users had been given at least one prescription of the drug during follow-up. The total metformin dosage divided by the total number of days during which metformin was prescribed was used as daily metformin dosage. We also used propensity score to adjust for the likelihood of initiation of metformin during the follow-up period (21). The former was obtained using a logistic regression procedure that includes the following independent variables: age; sex; BMI; LDL cholesterol; HDL cholesterol; triglycerides; tobacco and alcohol intake; A1C; SBP; ln (ACR + 1); estimated GFR; peripheral arterial disease; retinopathy; sensory neuropathy; and history of cardiovascular disease (coronary heart disease, myocardial infarction, and stroke) at baseline (c statistics = 0.73). We then used stratified Cox models on deciles of the score to adjust for the likelihood of metformin use.

Sensitivity analyses were performed to address *1*) the impacts of undiagnosed cancer by limiting the analysis to patients who were followed for $\geq$2.5 years (n = 2170); *2*) the impact of incomplete exclusion of patients who used metformin during 2.5 years before enrollment by limiting the analysis to patients who were enrolled on or after 1 July 1998 (n = 1707); *3*) the impact of prevalent bias by reinclusion of 3,445 patients who used metformin during 2.5 years before enrollment; and *4*) inclusion of subjects with missing values in univariable analysis and without adjusting for the propensity score to maximize the valid sample size (n of the valid sample size = 2,996 and n of the sample size with missing values in HDL cholesterol = 53 [i.e., 1.74% missing-value rate]).

RESULTS

Characteristics of the patients

The median age of the cohort was 56 years (25th to 75th percentiles [interquartile range {IQR} 45–67]) at enrollment. During 13,808 person-years of follow-up (5.51 years [3.08–7.39]), 129 patients developed cancer. In the cohort, 16.3% (n = 433) of patients had low HDL cholesterol $<$1.0 mmol/L and 46.7% (n = 1,243) had HDL cholesterol $\geq$1.30 mmol/L. Patients with low HDL cholesterol were more likely to use insulin, develop cancer, and die prematurely. Patients who developed cancer were older, more likely to use tobacco and alcohol, and had longer disease duration. They had high LDL cholesterol, low HDL cholesterol, and albuminuria and were more likely to have premature death than those free of cancer. Patients who developed cancer were less likely to use statins and metformin during the follow-up period than patients without cancer (Table 1).

HDL cholesterol and cancer

Compared with patients with HDL cholesterol $\geq$1.0 but $<$1.3 mmol/L, patients with HDL cholesterol $<$1.0 mmol/L (HR 2.22 [95% CI 1.38–3.58]) and those with HDL cholesterol $\geq$1.3 mmol/L (1.61 [1.05–2.46]) had increased cancer risk in univariable analysis. After adjusting for other covariates (Supplementary Fig. 1), HDL cholesterol $<$1.0 mmol/L for cancer remained significant (2.41 [1.46–3.96]) but not HDL cholesterol $\geq$1.3 mmol/L (P = 0.1197). Additional subgroup univariable and multivariable analyses indicate that low HDL cholesterol

was associated with increased cancer risk only among those who did not use metformin but not among those who did (power = 0.37) (Table 2).

Use of metformin and cancer

Use of metformin was associated with a decreased risk of cancer in a dose-response manner. After adjusting for covariates, patients with HDL cholesterol <1.0 mmol/L and who were not treated with metformin had a 5.8-fold risk of cancer compared with the referent group, who had HDL cholesterol ≥1.0 and used metformin. Patients with HDL cholesterol ≥1.0 mmol/L but who were not treated with metformin also had higher cancer risk than the referent group. However, the cancer risk associated with HDL cholesterol <1.0 mmol/L was rendered nonsignificant among those who used metformin (Table 2 and Supplementary Fig. 2). There was a significant interaction between low HDL cholesterol and nonuse of metformin for cancer risk, after adjusting for covariates (AP 0.44 [95% CI 0.11–0.78]) (Table 3).

Consistently, the copresence of HDL cholesterol <1.0 mmol/L and nonuse of metformin was associated with an increased risk of cancer at sites other than the digestive organs and peritoneum and, to a lesser degree, cancers of the digestive organs and peritoneum. Copresence of both factors also was associated with an increased risk of fatal cancer and, to a lesser degree, nonfatal cancer (Table 4).

Table 2—*HRs of different combinations of low HDL cholesterol and metformin use for cancer risk in type 2 diabetes*

Exposures	*n* at risk	HR (95% CI)	*P*
HDL cholesterol <1.0 vs. ≥1.0 mmol/L			
Among metformin nonusers			
Model 1*	200	2.87 (1.57–5.25)	0.0006
Model 2*†	200	2.99 (1.60–5.61)	0.0006
Among metformin users			
Model 3*	233	1.66 (0.72–3.87)	0.2367
Model 4*†	233	1.61 (0.66–3.92)	0.2969
Average daily metformin dose in the whole cohort (per g)			
Model 5*‡	1,266	0.44 (0.32–0.62)	<0.0001
Model 6*†‡	1,266	0.50 (0.35–0.71)	<0.0001
Metformin users vs. nonusers			
Among patients with HDL cholesterol ≥1.0 mmol/L			
Model 7*‡	1,033	0.46 (0.28–0.74)	0.0013
Model 8*†‡	1,033	0.51 (0.31–0.82)	0.0059
Among patients with HDL cholesterol <1.0 mmol/L			
Model 9*‡	233	0.29 (0.13–0.61)	0.0013
Model 10*†‡	233	0.30 (0.13–0.70)	0.0052
Biological interaction models			
Model 11*‡			
HDL cholesterol <1.0 mmol/L plus nonuse of metformin	200	6.18 (3.35–11.40)	<0.0001
HDL cholesterol ≥1.0 mmol/L plus nonuse of metformin	1,192	2.35 (1.47–3.75)	0.0003
HDL cholesterol <1.0 mmol/L plus use of metformin	233	1.83 (0.87–3.88)	0.1140
HDL cholesterol ≥1.0 mmol/L plus use of metformin	1,033	Reference	
Model 12*†‡			
HDL cholesterol <1.0 mmol/L plus nonuse of metformin	200	5.75 (3.03–10.90)	<0.0001
HDL cholesterol ≥1.0 mmol/L plus nonuse of metformin	1,192	2.17 (1.35–3.49)	0.0013
HDL cholesterol <1.0 mmol/L plus use of metformin	233	2.02 (0.94–4.35)	0.0855
HDL cholesterol ≥1.0 mmol/L plus use of metformin	1,033	Reference	

*Adjusted for LDL cholesterol–related risk indicators (LDL cholesterol ≥3.8 mmol/L and LDL cholesterol <2.8 mmol/L plus albuminuria), HDL cholesterol ≥1.30 mmol/L (not for models 7 and 8), and the nonlinear association of triglycerides with cancer. †Further adjusted for age, sex, employment status, smoking status, alcohol intake, duration of diabetes, BMI (≥27.6 or <24.0 kg/m^2), A1C, and SBP at enrollment and use of statins, fibrates, other lipid-lowering drugs, ACE inhibitors/ARBs, and insulin during follow-up. ‡Stratified Cox model analyses on deciles of the propensity score of metformin use were included in models 5–12 to control for the likelihood of starting metformin therapy during follow-up.

Sensitivity analyses

The series of sensitivity analyses showed a consistent trend toward an interactive effect of nonuse of metformin and HDL cholesterol <1.0 mmol/L on the risk of cancer, although not all interactions in these sensitivity analyses reached statistical significance (Supplementary Tables 2 and 3).

CONCLUSIONS—In this study, we observed that HDL cholesterol <1.0 mmol/L and nonuse of metformin was associated with a 5.8-fold cancer risk compared with metformin users with HDL cholesterol ≥1.0 mmol/L. The significant additive interaction indicates that the increased cancer risk as a result of a combination of nonuse of metformin and HDL cholesterol <1.0 mmol/L was more than the addition of the risks attributed to the presence of either nonuse of metformin or low HDL cholesterol alone. In other words, the significant interaction suggests that the use of metformin may confer an extra cancer benefit in type 2 diabetic patients with low HDL cholesterol.

Although there are ongoing debates about the associations between insulin usage and cancer in diabetes, epidemiological studies have consistently found that the use of metformin is associated with reduced cancer risk. Among these studies, Libby et al. (9) reported that metformin use was associated with a 54% (95% CI 47–60) lower crude incidence and a 37% (25–47) lower adjusted incidence of cancer than metformin nonusers over a period of 10 years. In support of these findings, we also found a 50% lower adjusted cancer risk among metformin users with HDL cholesterol ≥1.0 mmol/L and a 72% lower adjusted risk among metformin users with HDL cholesterol <1.0 mmol/L.

Several lines of evidence support the pivotal role of AMPK, which can be

Table 3—*Measures for estimation of biological interaction between low HDL cholesterol and nonuse of metformin for the risk of cancer in type 2 diabetes*

Measures of biological interaction	Estimate (95% CI)	*P*
Between HDL cholesterol <1.0 mmol/L and nonuse of metformin		
Model 1*†		
RERI	3.00 (−0.14 to 6.14)	0.0611
AP	0.49 (0.18–0.79)‡	0.0017
S	2.38 (1.07–5.28)	0.1091
Model 2*†‡		
RERI	2.55 (−0.49 to 5.60)	0.0999
AP	0.44 (0.11–0.78)‡	0.0105
S	2.17 (0.94–4.99)	0.1331

*Adjusted for LDL cholesterol–related risk indicators (LDL cholesterol ≥3.8 mmol/L and LDL cholesterol <2.8 mmol/L plus albuminuria), HDL cholesterol ≥1.30 mmol/L, and the nonlinear association of triglyceride with cancer. †Further adjusted for age, sex, employment status, smoking status, alcohol intake, duration of diabetes, BMI (≥27.6 or <24.0 kg/m^2), A1C, and systolic blood pressure at enrollment and use of statins, fibrates, other lipid-lowering drugs, ACE inhibitors/ARBs, and insulin during follow-up. ‡Stratified Cox model analyses on deciles of the propensity score of use of metformin were used to control for likelihood of starting metformin therapy during follow-up.

triggered by a large number of upstream signals, in maintaining energy homeostasis by providing a balance between energy expenditure through lipolysis and energy storage through protein and glycogen synthesis. Activation of AMPK by the tumor suppressor, LKB1, promotes glucose uptake, increases fatty acid oxidation, and reduces protein and lipid synthesis. Metformin is known to activate the AMPK pathway, possibly through the activation of the *LKB1 suppressor* gene (22). On the other hand, hyperglycemia can downregulate *ApoA-I* gene transcription, which is the major lipoprotein component of HDL lipid particles (23). In this regard, ApoA-I has been shown to stimulate phosphorylation of AMPK and ACC (5). More recently, Kimura et al. (24) reported that HDL can activate AMPK through binding to both sphingosine 1-phosphate receptors/Gi proteins and scavenger receptor class B type I (SR-BI)/protein PDZK1, with LKB1 being involved in the SR-BI signaling. Given the close relationship between HDL cholesterol and the AMPK pathway, the interactive effects between metformin use and HDL cholesterol on cancer risk is thus plausible.

Several limitations in the study have been noticed. *1*) A single measurement of HDL cholesterol was used in the analysis. *2*) Hospital principle discharge diagnosis was used to retrieve cancer events in the cohort, and this approach may have missed a small number of cancer events. *3*) The use of drug dispensary data are an indirect method and may overestimate exposure because drug acquisition is only a surrogate marker for actual drug consumption. Although our definition of drug use should not introduce major bias (25), some unmeasured confounding factors may exist. *4*) The sample size of the study was not large enough to address whether there were sex-specific cutoff points of HDL cholesterol for the risk of cancer. *5*) There were insufficient numbers of patients/events to explore the relationships between HDL cholesterol status, metformin exposure, and risk of specific cancers. *6*) RERI did not reach statistical significance. However, RERIs were significant in sensitivity analyses 3 and 4 with larger sample sizes, suggesting that the marginally significant RERI in the analysis is possibly attributed to insufficient power. *7*) These findings were only derived from a Chinese cohort and need to be replicated in other ethnic populations.

In conclusion, the use of metformin might confer stronger benefits in reducing cancer risk in patients with HDL cholesterol <1.0 mmol/L. Although low HDL cholesterol is not an indication for metformin usage, if our findings can be independently replicated, patients with low HDL cholesterol with or without type 2 diabetes might be candidate subjects for clinical trials that formally test the anticancer effects of metformin or agents that modulate the ApoA-I–LKB1–AMPK pathway.

Table 4—*HRs of the copresence of HDL cholesterol <1.0 mmol/L and nonuse of metformin during follow-up versus all other groups for site-specific cancers and fatal and nonfatal cancers*

	Number of cancers	HR (95% CI)*	*P*
Cancer subtypes†			
1) Lip, oral cavity, and pharynx	6		
2) Respiratory and intrathoracic organs	16		
3) Genitourinary organs	16		
4) Lymphatic and hematopoietic tissue	19		
5) Bone, connective tissue, skin, and breast	7		
6) Other and unspecified sites	19		
Cancer at sites other than digestive organs and peritoneum (1–6 combined)	77	4.06 (2.39–6.89)	<0.0001
7) Digestive organs and peritoneum	65	2.31 (1.17–4.54)	0.0153
Fatal and nonfatal cancers‡			
Fatal cancer	56	3.42 (1.80–6.49)	0.0002
Nonfatal cancer	119	2.70 (1.67–4.37)	<0.0001

*Univariable Cox models with stratification on deciles of the propensity score of use of metformin during follow-up were used to obtain the HRs. †Classification was based on the ICD-9 (there are overlaps among site-specific cancers). ‡46 nonfatal cancer events developed before fatal cancer.

Acknowledgments—This study was supported by the Research Grants Council Direct Allocation (ref. no. 2009.2.010) and the Hong Kong Foundation for Research and Development in Diabetes, Lioa Wun Yuk Diabetes Memorial Fund, established under the auspices of the Chinese University of Hong Kong.

J.C.N.C. received a research grant and/or honorarium for consultancy or lecturing from Bayer, Daiichi-Sankyo, Eli Lilly, GlaxoSmithKline, Merck Sharp and Dohme, Merck Serono, Pfizer, AstraZeneca, sanofi-aventis, Novo Nordisk, and/or Bristol-Myers Squibb, all of which have been donated to the Chinese University of Hong Kong to support ongoing research and development. No other potential conflicts of interest relevant to this article were reported.

X.Y. contributed to the discussion, wrote the manuscript, and reviewed and edited the manuscript. W.Y.S., R.C.W.M., A.P.S.K., H.M.L.,

L.W.L.Y., C.-C.C., R.O., and G.T.C.K. researched data and contributed to the discussion. J.W.C. researched data, contributed to the discussion, and reviewed and edited the manuscript.

The authors thank all the medical and nursing staff of the Diabetes and Endocrine Centre of the Prince of Wales Hospital for recruiting and managing these patients.

References

1. Renehan A, Smith U, Kirkman MS. Linking diabetes and cancer: a consensus on complexity. Lancet 2010;375:2201–2202
2. Yang X, So WY, Ma RC, et al. Predicting values of lipids and white blood cell count for all-site cancer in type 2 diabetes. Endocr Relat Cancer 2008;15:597–607
3. Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. Nat Clin Pract Endocrinol Metab 2009;5:150–159
4. Vigneri P, Frasca F, Sciacca L, Pandini G, Vigneri R. Diabetes and cancer. Endocr Relat Cancer 2009;16:1103–1123
5. Han R, Lai R, Ding Q, et al. Apolipoprotein A-I stimulates AMP-activated protein kinase and improves glucose metabolism. Diabetologia 2007;50:1960–1968
6. Towler MC, Hardie DG. AMP-activated protein kinase in metabolic control and insulin signaling. Circ Res 2007;100:328–341
7. Huang X, Wullschleger S, Shpiro N, et al. Important role of the LKB1-AMPK pathway in suppressing tumorigenesis in PTEN-deficient mice. Biochem J 2008;412:211–221
8. Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer 2008;8:915–928
9. 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;32:1620–1625
10. Bowker SL, Majumdar SR, Veugelers P, Johnson JA. Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. Diabetes Care 2006;29:254–258
11. Yang X, So W, Ko GT, et al. Independent associations between low-density lipoprotein cholesterol and cancer among patients with type 2 diabetes mellitus. CMAJ 2008;179:427–437
12. Yang X, So WY, Ma RC, et al. Low LDL cholesterol, albuminuria, and statins for the risk of cancer in type 2 diabetes: the Hong Kong diabetes registry. Diabetes Care 2009;32:1826–1832
13. Yang X, Zhao H, Sui Y, et al. Additive interaction between the renin-angiotensin system and lipid metabolism for cancer in type 2 diabetes. Diabetes 2009;58:1518–1525
14. Piwernetz K, Home PD, Snorgaard O, Antsiferov M, Staehr-Johansen K, Krans M; the DIABCARE Monitoring Group of the St Vincent Declaration Steering Committee. Monitoring the targets of the St Vincent Declaration and the implementation of quality management in diabetes care: the DIABCARE initiative. Diabet Med 1993;10:371–377
15. Ma YC, Zuo L, Chen JH, et al. Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease. J Am Soc Nephrol 2006;17:2937–2944
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502
17. Yang X, So WY, Tong PC, et al.; the Hong Kong Diabetes Registry. Development and validation of an all-cause mortality risk score in type 2 diabetes. Arch Intern Med 2008;168:451–457
18. Andersson T, Alfredsson L, Källberg H, Zdravkovic S, Ahlbom A. Calculating measures of biological interaction. Eur J Epidemiol 2005;20:575–579
19. Li R, Chambless L. Test for additive interaction in proportional hazards models. Ann Epidemiol 2007;17:227–236
20. Yang X, Ko GT, So WY, et al. Associations of hyperglycemia and insulin usage with the risk of cancer in type 2 diabetes: the Hong Kong diabetes registry. Diabetes 2010;59:1254–1260
21. Joffe MM, Rosenbaum PR. Invited commentary: propensity scores. Am J Epidemiol 1999;150:327–333
22. Cazzaniga M, Bonanni B, Guerrieri-Gonzaga A, Decensi A. Is it time to test metformin in breast cancer clinical trials? Cancer Epidemiol Biomarkers Prev 2009;18:701–705
23. Murao K, Wada Y, Nakamura T, Taylor AH, Mooradian AD, Wong NC. Effects of glucose and insulin on rat apolipoprotein A-I gene expression. J Biol Chem 1998;273:18959–18965
24. Kimura T, Tomura H, Sato K, et al. Mechanism and role of high density lipoprotein-induced activation of AMP-activated protein kinase in endothelial cells. J Biol Chem 2010;285:4387–4397
25. Yang X, Ma RC, Chan JC. Response to comment on: Yang X, Ko GT, So WY, Ma RC, Yu LW, Kong AP, Zhao H, Chow CC, Tong PC, Chan JC. Associations of hyperglycemia and insulin usage with the risk of cancer in type 2 diabetes: the Hong Kong diabetes registry. Diabetes 59:1254–1260, 2010. Diabetes 2010;59:e19–e22