Research Article

Cancer Epidemiology, Biomarkers & Prevention

Antidiabetic Medications and the Risk of Colorectal Cancer in Patients with Diabetes Mellitus: A Systematic Review and Meta-analysis

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Abstract

Background: Antidiabetic medications (ADM) may modify colorectal cancer risk in patients with diabetes mellitus. We performed a systematic review and meta-analysis, evaluating the effect of metformin, thiazolidinediones (TZD), sulfonylureas, and insulin on colorectal cancer risk in diabetic patients.

Methods: We conducted a systematic search of multiple bibliographic databases, up to September 2012, for articles that evaluated exposure to metformin, TZD, sulfonylureas, and insulin, reported colorectal cancer risk in patients with diabetes mellitus, and reported OR or provided data for their estimation. Summary OR estimates with 95% confidence intervals (CI) were estimated using the random-effects model.

Results: Fifteen studies reporting 13,871 cases of colorectal cancer in 840,787 patients with diabetes mellitus were included. Meta-analysis of observational studies showed an 11% reduction in colorectal cancer risk associated with metformin use ($n = 9$ studies; OR, 0.89; 95% CI, 0.81–0.99), whereas TZD use was not associated with colorectal cancer risk ($n = 5$ studies; OR, 0.96; 95% CI, 0.87–1.05). Conversely, a trend toward higher colorectal cancer risk was observed with sulfonylurea ($n = 7$ studies; OR, 1.11; 95% CI, 0.97–1.26) and insulin ($n = 9$ studies; OR, 1.33; 95% CI, 0.91–1.94) use, although these associations were not statistically significant. There was considerable heterogeneity across studies, partly explained by study location and adjustment for concomitant use of other ADMs. *Post-hoc* analysis of randomized controlled trials did not reveal any significant association between ADM and colorectal cancer risk.

Conclusions: Meta-analysis of published studies supports a protective association between metformin use and colorectal cancer risk in patients with diabetes mellitus.

Impact: Clinical trials on the chemopreventive effect of metformin against colorectal cancer are warranted.
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Introduction

Colorectal cancer is the third most common malignancy worldwide, with a 6% lifetime risk of developing this cancer; half the patients diagnosed with this cancer die from it (1). Most cases of sporadic colorectal cancer are thought to arise from dysplastic adenomas (2). Regular screening with colonoscopic resection of premalignant polyps is the preferred approach to preventing sporadic colorectal cancers, and has been associated with decreased mortality (3). Unfortunately, suboptimal adherence, access, and expense limit population-wide adoption of screening colonoscopy. Given limitations of screening tests and poor prognosis associated with colorectal cancer, there is great emphasis on identifying high-risk patients and exploring chemopreventive strategies to reduce the burden of colorectal cancer.

Diabetes mellitus is an established, independent risk factor for colorectal cancer, with a reported 30% to 40% higher risk as compared with nondiabetic patients (4–8). The putative carcinogenic effects of diabetes mellitus may be attributable to insulin- and insulin-like growth factors and/or an obesity-associated chronic inflammatory state (9). Encouragingly, several preclinical studies have shown that conventional antidiabetic medications (ADM) may modify the risk of some cancers, including colorectal cancer. For example, metformin has been shown to have antineoplastic effects through both insulin-dependent and insulin-independent mechanisms (10). Thiazolidinediones (TZD) have been postulated to trigger cell-growth arrest, induce apoptosis, and prevent cancer-cell invasion (11). In contrast, sulfonylureas, which stimulate insulin secretion, and insulin itself may increase cell proliferation and inhibit apoptosis, thereby promoting oncogenesis (12). To date, epidemiologic studies have shown that

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metformin use in diabetic patients may be associated with lower risk of colorectal cancer (13, 14), whereas insulin and insulin secretagogues may be associated with higher colorectal cancer risk (15, 16). However, existing data remain inconsistent (17–19). Previous meta-analyses are limited in evaluating the risk modification with metformin alone (failing to account for the concomitant cancer-modifying effects of other ADMs; refs. 20, 21) or have included a very small number of studies (22).

To better understand the association between commonly prescribed ADMs and colorectal cancer risk, we performed a systematic review and meta-analyses of observational studies and randomized controlled trials (RCT) that investigated the effect of metformin, TZD, sulfonylureas, and insulin on the risk of developing colorectal cancer in patients with diabetes mellitus.

Materials and Methods

This systematic review was conducted following guidance provided by the Cochrane Handbook (23) and is reported according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (24). The process followed *a priori* established protocol.

Search strategy

First, a systematic literature search of MEDLINE (1966 through September 30 2012), Embase (1988 through September 30 2012), and Web of Science (1993 through September 30 2012) databases was conducted by two study investigators (S. Singh and H. Singh) for all relevant articles on the association between ADM use and risk of colorectal cancer in patients with diabetes mellitus. Medical subject heading terms used in the search included "hypoglycemic agents," "metformin," "sulfonylurea compounds," "thiazolidinediones," and "insulin" combined with "neoplasms." The title and abstract of studies identified in the search were reviewed by two authors independently (S. Singh and P. Singh) to exclude studies that did not answer the research question of interest. The full text of the remaining articles was examined to determine whether it contained relevant information. Next, bibliographies of the selected articles, as well as review articles on the topic were manually searched for additional articles. Third, manual search of abstracts from major gastroenterology and oncology conferences (2005–2012) was performed for additional abstracts on the topic.

Selection criteria

Studies considered in this meta-analysis were either observational studies or RCTs that met the following inclusion criteria: (i) evaluated and clearly defined exposure to ADMs, (ii) reported colorectal cancer risk in patients with diabetes mellitus, and (iii) reported relative risk or OR or provided data for their calculation. Inclusion was not otherwise restricted by study size, language, or publication type. When there were multiple publications from the same population, only data from the most comprehensive report were included. We excluded studies that compared one form of insulin (like long-acting insulin glargine) with other forms of insulin and studies that reported the risk of colorectal adenomas with ADMs. The flow diagram summarizing study identification and selection is shown in Fig. 1.

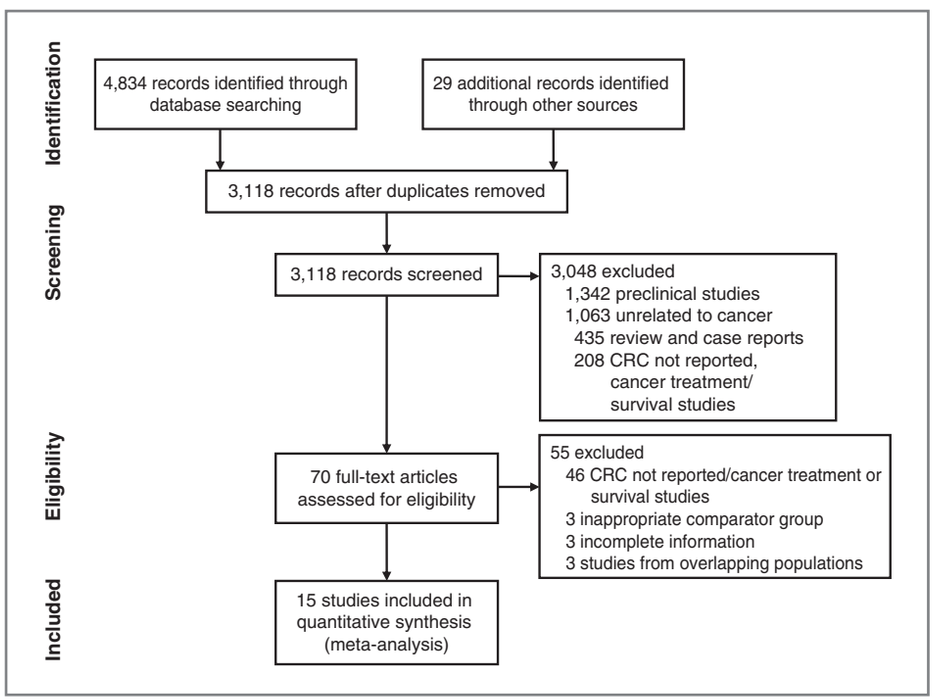

Figure 1. Flowsheet summarizing study identification and selection. CRC, colorectal cancer.

The methodologic quality of observational studies was assessed by two authors independently (S. Singh and H. Singh) using the Newcastle–Ottawa scale (25). In this scale, studies were scored across three categories: selection (4 questions) and comparability (2 questions) of study groups, and ascertainment of the outcome of interest (3 questions), with all questions with a score of one except for comparability of study groups, in which separate points were awarded for controlling age and/or sex (maximum two points). The methodologic quality of RCTs was assessed using the Cochrane Collaboration's tool for assessing risk of bias in randomized trials (26). This tool focuses on the adequacy of randomization and allocation concealment procedures, blinding, and loss to follow-up. Any discrepancies were addressed by a joint reevaluation of the original article.

Data abstraction

Data were independently abstracted onto a standardized form by two reviewers (S. Singh and P. Singh). The following data were collected from each study: study design, time period of study/year of publication, country of the population studied, primary outcome reported, type of ADM, dose and duration of use (if reported), information source of exposure ascertainment and outcome assessment, total number of persons in each group (exposed vs. not exposed), OR, and 95% confidence intervals (CI) with and without adjustment for confounding factors. When data on males and females were reported separately, we pooled these to derive a summary estimate for the study. For all analysis, referent group was composed of patients with diabetes mellitus not exposed to medication of interest. Data on the following risk factors for colorectal cancer were extracted from each study, where available: age, sex, ethnicity, body mass index (BMI), family history of colorectal cancer, smoking, alcohol, physical activity, dietary factors (red meat, fat intake, fruits, and vegetables), diabetes mellitus severity and duration, use of aspirin and/or nonsteroidal anti-inflammatory drugs, use of statins or hormone replacement therapy, as well as frequency of screening colonoscopy in study participants. Conflicts in data abstraction were resolved by consensus, referring back to the original article.

Outcomes assessed

The primary analysis focused on assessing the risk of colorectal cancer in patients with diabetes mellitus based on the type of ADM used. *A priori* hypotheses to explain potential heterogeneity in the direction and magnitude of effect among different observational studies included location of study (Western population vs. Asian population), study design (case–control vs. cohort), and whether the study adjusted for the concomitant use of other ADMs besides the index medication. Because of significant differences in the design of observational studies and *post-hoc* analysis of RCTs, data from these RCTs were analyzed and presented separately.

Statistical analysis

We used the random-effects model described by DerSimonian and Laird to calculate meta-analytic OR and 95% CI (27). Because outcomes were relatively rare, ORs were considered approximations of relative risk. Adjusted ORs reported in studies were used for analysis to account for confounding variables. We assessed heterogeneity between study-specific estimates using two methods (28, 29). First, the Cochran Q statistical test for heterogeneity, which tests the null hypothesis that all studies in a meta-analysis have the same underlying magnitude of effect, was carried out. Because this test is underpowered to detect moderate degrees of heterogeneity, a P value of less than 0.10 was considered suggestive of significant heterogeneity. Second, to estimate what proportion of total variation across studies was due to heterogeneity rather than chance, I^2 statistic was calculated. In this, a value of less than 30%, 30%–60%, 61%–75%, and more than 75% was suggestive of low, moderate, substantial, and considerable heterogeneity, respectively (29). Once heterogeneity was noted, between-study sources of heterogeneity were investigated using subgroup analyses by grouping original estimates according to study characteristics (as described above). In this analysis, a test of interaction comparing the two subgroups was performed (30); if the P value for difference between subgroups was less than 0.10, it was considered statistically significant (i.e., $P < 0.10$ suggested that stratifying on the basis of that particular study characteristic partly explained the heterogeneity observed in the analysis). We assessed for publication bias quantitatively using the Egger regression test (publication bias present if $P \leq 0.10$; ref. 31), and qualitatively, by visual inspection of funnel plots of the logarithmic OR versus their standard errors (32). All P values were two tailed. For all tests (except for heterogeneity and publication bias), a P value of less than 0.05 was considered statistically significant. All calculations and graphs were performed using Comprehensive Meta-Analysis (CMA) version 2 (Biostat; ref. 33).

Results

From a total of 3,118 unique studies identified using the search strategy, 15 studies fulfilled the inclusion criteria and were included in meta-analysis (5 case–control, 8 cohort, and 2 RCTs; refs. 13, 14, 16–19, 34–42). These studies cumulatively reported 13,871 colorectal cancer cases in 840,787 patients with diabetes mellitus. There were four Taiwanese studies from the same cohort (15, 34, 43, 44), and hence, only one report was included in the main analysis (34). There were three studies that compared the association between insulin glargine (as compared with nonglargine insulin) and the risk of cancer, and these were excluded from analysis (45–47).

Characteristics of included studies

The characteristics of the included studies are shown in Table 1. All studies were population based. Thirteen studies represented Western populations (7 based in the

Table 1. Characteristics of included studies assessing the risk of colorectal cancer in patients with diabetes mellitus treated with ADMs

Study	Design	Location/setting	Time period; mean F/U (y)	Exposure ascertainment	Outcome assessment	Total subjects[a]	CRC cases[a]	Metformin (%)	TZD (%)	SU (%)	Insulin (%)	Confounding variables adjusted for
Observational studies: cohort studies												
Campbell and colleagues (18) 2010	Cohort	USA; population-based	1992–2007; NR	Self-report	Self-reported with independent validation	154,975 (11,335 with DM)	2,809 (335)	—	—	—	25.4	1–11, 14, 17, 18
Currie and colleagues (13) 2009	Cohort	UK; population-based	NR; 2.4	Pharmacy prescription database	Read diagnostic codes	59,609	292	72.1	—	33.9	16.0	1, 2, 6, 10, 15
Ferrara and colleagues (19) 2011	Cohort	USA; population-based	1997–2005; 2.5–3.7	Pharmacy prescription database	Cancer Registry	252,467	1,650	43.5	12.6	52.8	20.4	1, 2, 4, 10, 15, 16
Govindarajan and colleagues (35) 2007	Cohort	USA; population-based	1997–2004; NR	Medical record review	ICD-9 diagnostic codes	87,678	1,137	—	12.9	—	—	1–4, 10, 15
Libby and colleagues (14) 2009	Cohort	Scotland; population-based	1993–2004; NR	Pharmacy prescription database	ICD-9 diagnostic codes, with record linkage	8,170	116	50.0	—	36.4	10.1	1–3, 6, 10, 15, 16
Morden and colleagues (39) 2011	Cohort	USA; population-based	2003–2008; ∼2	Pharmacy prescription database	ICD-9 diagnostic codes	81,681	428	18.7	—	—	100	1–4, 6, 10, 14, 16, 17
Oliveria and colleagues (40) 2007	Cohort	USA; population-based	2000–2004; 3.9	Pharmacy prescription database	ICD-9 codes and medical record review	191,223	383	—	—	—	—	1, 2, 16
Ruiter and colleagues (41) 2012	Cohort	Netherlands; population-based	1998–2008; 2.8–4.6	Pharmacy prescription database	ICD-9 diagnostic codes on hospital discharge	85,289	527	61.8	0	38.2	0	1, 2
Observational studies: case–control studies												
Bodmer and colleagues (17) 2012	C-C	UK; population-based	1995–2009; NR	Pharmacy prescription database	Read diagnostic codes	6,439	920	43.1	—	49.6	16.8	1–3, 6, 10–12, 14–16
Chang and colleagues (34) 2012	C-C	Taiwan; population-based	2000–2008; 7.9	Pharmacy prescription database	ICD-9 diagnostic codes with record linkage with the National Cancer Register	35,912	7,200	79.7	17.8	90.5	31.8	1, 2, 11, 15, 16
Koro and colleagues (38) 2007	C-C	USA; population-based	1997–2004; 1.5	Pharmacy prescription database	ICD-9 diagnostic codes	2,435	408	—	20.2	—	5.6	1, 2, 15
Vinikoor and colleagues (42) 2009	C-C	USA; population-based	2001–2006	Self-report	Histologically confirmed CRC	1,995 (341)	1,007 (183)	—	—	—	29.3	1, 2, 5, 11, 13, 17
Yang and colleagues (16) 2004	C-C	UK; population-based	1987–2004	Pharmacy prescription database	Read diagnostic codes, with independent validation	1,320	125	16.8	—	41.4	9.8	1–3, 10, 11, 15
Randomized controlled trials												
ADOPT (36) 2006	RCT	USA, Europe; hospital-based	2000–2006; 4.0	RCT	Adverse event review	4,351	21	33.4	33.1	33.5	—	1–4, 6, 10, 11, 16, 17
ORIGIN (37) 2012	RCT	Americas, Europe, Asia; hospital-based	2003–2011; 6.2	RCT	Adverse event review	12,537	146	27.4	—	29.6	50.0	1–3, 6, 10–12, 15, 16

Abbreviations: C-C, case–control; CRC, colorectal cancer; DM, diabetes mellitus; NR, not reported; SU, sulfonylureas.

[a]Where required, the number in brackets represents the number of patients with colorectal cancer with preceding diagnosis of diabetes mellitus; variables adjusted for: 1, age; 2, sex; 3, BMI; 4, ethnicity; 5, family H/O colorectal cancer; 6, smoking; 7, alcohol; 8, physical activity; 9, dietary factors (red meat, fat intake, fruits, and vegetables); 10, duration of diabetes mellitus or severity; 11, aspirin/NSAID; 12, statin use; 13, vitamin D/calcium intake; 14, hormone replacement therapy; 15, other ADMs; 16, other comorbidities (coronary artery disease, chronic kidney disease, etc.); 17, socioeconomic status; 18, GI procedures (lower gastrointestinal endoscopy, colonic surgery).

United States, 5 based in Europe, and 1 multicenter RCT; refs. 13, 14, 16–19, 35, 36, 38–42), one study was performed in an Asian population (Taiwan; ref. 34), and one study was a multicenter RCT across the United States, Europe, and Asia (37). The earliest study period began in 1987, and the latest ended in 2011. There was a small overlap of time period in two studies performed using the UK General Practice Research Database (16, 17).

Most patients in the included studies were on multiple ADMs for management of diabetes mellitus, and the comparator group for estimation of OR was based on exposure to medication of interest and nonexposure to the same medication. Three studies included only patients on monotherapy for diabetes mellitus, in which follow-up was censored when a second ADM was added (13, 40, 41). In six studies, the adjusted OR for the drug of interest accounted for the simultaneous use of other ADMs (14, 17, 19, 34, 35, 37).

Quality of included studies

The overall methodologic quality of this body of evidence was moderate to high. Supplementary Tables 1 and 2 show the performance of studies on the Newcastle–Ottawa scale. For most studies, exposure was ascertained from prescription pharmacy database; outcome assessment was based on standard medical diagnostic codes with independent validation of a random sample. The duration and adequacy of follow-up in cohort and nonresponse rate in case–control studies were inconsistently reported. Most studies adjusted for the following confounders: age (15 of 15), sex (15/15), BMI (seven of 15), diabetes mellitus duration and/or severity (10 of 15), smoking (seven of 15), family history of colorectal cancer (two of 15), use of aspirin and/or nonsteroidal anti-inflammatory drugs (seven of 15), or use of statins and/or hormone replacement therapy (three of 15). Colonoscopy rates were reported and adjusted for in only one study (Table 1) (18). The quality of the randomized trials was moderate.

Metformin and risk of colorectal cancer

Of the ten studies (nine observational and one RCT) that reported on the association between metformin use and colorectal cancer risk, four demonstrated an apparent chemoprotective association (13, 14, 40, 41) and 6 reported no significant relationship (16, 17, 19, 34, 36, 39). Meta-analysis of observational studies demonstrated that metformin use (as compared with nonuse) was associated with a statistically significant 22% reduction in colorectal cancer incidence ($n = 9$ studies; unadjusted OR, 0.78; 95% CI, 0.63–0.96). When only the adjusted OR estimates from each study were analyzed, the summary estimate decreased to 11% reduction in colorectal cancer risk (adjusted OR, 0.89; 95% CI, 0.80–0.99; Supplementary Fig. S1). There was substantial heterogeneity between studies (Cochran Q test $P < 0.01$; $I^2 = 62\%$), which could be explained partly by study location (Western vs. Asian) and study design (case–control vs. cohort; Table 2).

The chemopreventive association was stable and significant on restricting analysis to studies that adjusted for concomitant ADM use ($n = 7$ studies; adjusted OR, 0.88; 95% CI, 0.78–0.99) and studies that compared metformin monotherapy with other ADM use ($n = 3$ studies; adjusted OR, 0.72; 95% CI, 0.53–0.98). This association between metformin use and colorectal cancer risk was most prominent in the Western populations ($n = 8$ studies; adjusted OR, 0.86; 95% CI, 0.76–0.97). *Post-hoc* analysis of RCT (7 cases of colorectal cancer, 1,454 patients on metformin) revealed no significant chemopreventive or oncogenic effect of metformin ($n = 1$ study; adjusted OR, 1.00; 95% CI, 0.40–2.47; ref. 36).

TZD and risk of colorectal cancer

Six studies (five observational and one RCT) reported on the association between TZD use and colorectal cancer risk, and none demonstrated a significant protective or harmful relationship (19, 34–36, 38, 40). On meta-analysis of five observational studies, TZD seemed to have a neutral effect on colorectal cancer risk in patients with

Table 2. Subgroup analysis of studies comparing the association between metformin and colorectal cancer risk

Subgroup analysis		Metformin			*P* value for the difference between subgroups
		N	OR	95% CI	
Study design	Case–control	3	1.04	0.94–1.15	<0.01
	Cohort	6	0.82	0.71–0.94	
Study location	Western	8	0.86	0.76–0.97	0.02
	Asian	1	1.04	0.93–1.16	
Adjusted for other ADMs	Yes	7	0.88	0.78–0.99	0.54
	No	2	0.95	0.75–1.20	

NOTE: For each ADM, subgroup analyses were performed by grouping studies on the basis of study design, geographic location of study sample, and whether the studies adjusted for concomitant use of other ADMs. $P_{interaction}$ refers to *P* value for a test of interaction to assess for (statistically significant) differences in summary estimates based on subgroups; a value of <0.10 was considered statistically significant.

Table 3. Subgroup analysis of studies comparing the association between TZD and colorectal cancer risk

		TZD			
Subgroup analysis		*N*	OR	95% CI	*P* value for difference between subgroups
Study design	Case–control	2	0.98	0.84–1.14	0.74
	Cohort	3	0.95	0.83–1.07	
Study location	Western	4	0.97	0.86–1.08	0.81
	Asian	1	0.94	0.78–1.14	
Adjusted for other ADMs	Yes	3	0.92	0.82–1.04	0.28
	No	2	1.04	0.88–1.23	

NOTE: For each ADM, subgroup analyses were performed by grouping studies on the basis of the study design, geographic location of the study sample, and whether the studies adjusted for concomitant use of other ADMs. $P_{interaction}$ refers to a *P* value for a test of interaction to assess for (statistically significant) differences in summary estimates based on subgroups; a value of <0.10 was considered statistically significant.

diabetes mellitus (unadjusted OR, 0.97; 95% CI, 0.92–1.03; adjusted OR, 0.96; 95% CI, 0.87–1.05; Supplementary Fig. S2). The results were consistent across all studies with low heterogeneity (Cochran *Q* test $P = 0.75$; $I^2 = 0\%$). No significant difference was noted in subgroup analysis based on study design, location, or adjustment for concomitant use of other ADMs (Table 3). *Post-hoc* analysis of the ADOPT trial (4 cases of colorectal cancer; 1,456 patients on rosiglitazone) revealed no significant chemopreventive or oncogenic association between TZD and colorectal cancer risk ($n = 1$ study; adjusted OR, 0.47; 95% CI, 0.16–1.39; 36).

Sulfonylureas and risk of colorectal cancer

Of eight studies (seven observational and one RCT) that reported on the association between sulfonylurea use and colorectal cancer risk, three studies found an increased risk of colorectal cancer with sulfonylurea use (as compared with nonuse; refs. 13, 34, 41) and five showed no significant association (16, 17, 19, 36, 40). Meta-analysis of seven observational studies demonstrated that there was a statistically insignificant increased colorectal cancer risk with sulfonylurea use among diabetic patients (unadjusted OR, 1.20; 95% CI, 0.98–1.46). This association was persistent, albeit less prominent after adjusting for potential confounders (adjusted OR, 1.11; 95% CI, 0.97–1.26; Supplementary Fig. S3). This summary estimate was also stable on meta-analysis of sulfonylurea monotherapy studies ($n = 3$ studies; adjusted OR, 1.18; 95% CI, 0.92–1.51), but on analysis restricted to studies that adjusted for concomitant effects of other ADMs, there was 13% increased risk of colorectal cancer ($n = 7$ studies; adjusted OR, 1.13; 95% CI, 1.00–1.29). There was considerable heterogeneity across studies (Cochran *Q* test $P < 0.01$; $I^2 = 79\%$), which could be explained on the basis of study location (Western vs. Asian) and concomitant effect of other ADMs (Table 4). Unlike metformin, the association between sulfonylurea use and colorectal cancer risk was

Table 4. Subgroup analysis of studies comparing the association between sulfonylureas and colorectal cancer risk

		Sulfonylureas			
Subgroup analysis		*N*	OR	95% CI	*P* value for the difference between subgroups
Study design	Case–control	3	1.04	0.73–1.48	0.72
	Cohort	4	1.11	0.97–1.28	
Study location	Western	6	1.05	0.93–1.19	<0.01
	Asian	1	1.39	1.22–1.58	
Adjusted for other ADMs	Yes	6	1.13	1.00–1.29	0.09
	No	1	0.80	0.55–1.17	

NOTE: For each ADM, subgroup analyses were performed by grouping studies on the basis of the study design, geographic location of the study sample, and whether the studies adjusted for concomitant use of other ADMs. $P_{interaction}$ refers to a *P* value for a test of interaction to assess for (statistically significant) differences in summary estimates based on subgroups; a value of <0.10 was considered statistically significant.

Table 5. Subgroup analysis of studies comparing the association between insulin and colorectal cancer risk

		Insulin			
Subgroup analysis		*N*	OR	95% CI	*P* value for the difference between subgroups
Study design	Case–control	5	1.48	0.87–2.53	0.39
	Cohort	4	1.15	0.94–1.40	
Study location	Western	8	1.18	0.99–1.40	<0.01
	Asian	1	2.56	2.40–2.72	
Adjusted for other ADMs	Yes	5	1.34	0.80–2.25	0.88
	No	4	1.28	0.92–1.78	

NOTE: For each ADM, subgroup analyses were performed by grouping studies on the basis of the study design, geographic location of the study sample, and whether the studies adjusted for concomitant use of other ADMs. $P_{interaction}$ refers to a *P* value for a test of interaction to assess for (statistically significant) differences in summary estimates based on subgroups; a value of <0.10 was considered statistically significant.

not statistically significant in the Western population studies ($n = 6$ studies; adjusted OR, 1.06; 95% CI, 0.94–1.19) but more prominent in Asian subjects. *Post-hoc* analysis of the ADOPT trial (10 cases of colorectal cancer; 1,441 patients on glibenclamide) on oral antidiabetic monotherapy revealed no significant chemopreventive or oncogenic effect of sulfonylureas ($n = 1$ study; adjusted OR, 1.84; 95% CI, 0.78–4.35; ref. 36).

Insulin and risk of colorectal cancer

Ten studies (nine observational and one RCT) reported on the association between insulin use (long and/or shorting-acting) and colorectal cancer risk in patients with diabetes mellitus. Three studies demonstrated an increased risk of colorectal cancer (13, 16, 34), whereas seven studies reported null association (17–19, 37, 38, 40, 42). On meta-analysis of nine observational studies, there was a trend toward increased colorectal cancer risk associated with insulin use (as compared with nonuse), both on unadjusted (unadjusted OR, 1.28; 95% CI, 0.88–1.86) and adjusted analysis (adjusted OR, 1.33; 95% CI, 0.91–1.94), although this was not statistically significant (Supplementary Fig. S4). The results were considerably heterogeneous (Cochran *Q* test $P < 0.01$; $I^2 = 96\%$), which was explained by location where the study was performed (Table 5). On exclusion of the one outlier Asian study (34), the heterogeneity decreased (Cochran *Q* test $P = 0.02$; $I^2 = 57\%$). *Post-hoc* analysis of the ORIGIN trial (76 cases of colorectal cancer; 6,264 patients on insulin glargine) did not show a significant difference in colorectal cancer risk with insulin glargine use (as compared with "standard of care"; $n = 1$ study; adjusted OR, 1.09; 95% CI, 0.79–1.51; ref. 37).

On further subgroup analysis, this apparent oncogenic association was less prominent in the Western population studies ($n = 8$ studies; adjusted OR, 1.18; 95% CI, 0.99–1.40) and most prominent in the Taiwanese study (adjusted OR, 2.56; 95% CI, 2.40–2.72). The results were stable across study design irrespective of whether analysis was adjusted for concomitant use of other ADMs.

Stratified analysis

On the basis of data from four studies, duration–response relation between insulin use and colorectal cancer risk was not observed [short duration insulin use (0–2 years): adjusted OR, 1.27; 95% CI, 0.98–1.63; long duration insulin use (2–5 years): adjusted OR, 1.37; 95% CI, 0.93–2.01; refs. 16–18, 42]. On subgroup analysis, there was no appreciable difference in the effects of insulin on colorectal cancer risk, based on sex [$n = 2$ studies; males vs. females; adjusted OR (95% CI): 0.93 (0.62–1.41) vs. 0.97 (0.70–1.34); refs. 17, 18] or tumor location (colon cancer: $n = 2$ studies; adjusted OR, 1.10; 95% CI, 0.93–1.29; rectal cancer: $n = 3$ studies; adjusted OR, 1.07; 95% CI, 0.83–1.37; refs. 18, 19, 42). Sufficient information was not available to perform a similar stratified analysis based on sex, tumor location, or duration–response relationship for other ADMs (metformin, TZD, or sulfonylureas) and risk of colorectal cancer.

Sensitivity analysis and publication bias

To assess whether any one study had a dominant effect on the meta-analytic OR, each study was excluded and its effect on the main summary estimate and Cochran *Q* test *P* value for heterogeneity was evaluated; the results were unchanged. When we excluded the studies with the most weight for each individual analysis (Ruiter and colleagues for metformin and sulfonylureas, Chang and colleagues for TZD and insulin; refs. 34, 41), the conclusions of the main analysis did not change significantly for metformin (adjusted OR, 0.87; 95% CI, 0.76–1.00), TZD (adjusted OR, 0.99; 95% CI, 0.91–1.08), sulfonylureas (adjusted OR, 1.11; 95% CI, 0.92–1.33), or insulin (adjusted OR, 1.15; 95% CI, 0.99–1.34). On replacing one Taiwanese study (34) with other similar population-based cohort studies from the same Taiwanese population (15, 43), there was no

significant change in overall association of colorectal cancer with metformin (adjusted OR, 0.85; 95% CI, 0.76–0.96), sulfonylureas (adjusted OR, 1.06; 95% CI, 0.96–1.17) or insulin (adjusted OR, 1.27; 95% CI, 0.94–1.69). Because of significant heterogeneity between studies, a single summary estimate for number needed to treat with metformin to prevent one case of colorectal cancer (or number needed to treat with sulfonylureas or insulin to cause one case of colorectal cancer) could not inferred.

There was no evidence of publication bias, both quantitatively ($P = 0.56$ for metformin, $P = 0.23$ for TZD, $P = 0.94$ for sulfonylureas, $P = 0.15$ for insulin), and qualitatively, on visual inspection of the funnel plot for studies (figures not shown).

Discussion

In this comprehensive meta-analysis of 15 studies analyzing the association between conventional ADMs and colorectal cancer risk in more than 840,000 patients with primarily type II diabetes mellitus, we found that metformin use was associated with a modest, yet statistically significant, protective association (11% risk reduction), whereas TZD, sulfonylurea, and insulin use were associated with neutral or possibly slightly increased risks (compared with nonuse of the ADM of interest). The observed benefits associated with metformin use were evident even after accounting for the simultaneous cancer-modifying effects of multiple ADMs, and was stable across observational study designs and on replacing studies with similar studies from the same cohorts. However, the reported meta-analysis was limited by substantial heterogeneity across studies that could be explained by differences in study design and/or location. On the basis of the data from our study, we speculate that the variable effects of different ADMs on cancer risk modification may at least partially explain why intensive glucose lowering through combination therapy is not always associated with lower cancer risk (48, 49).

The antineoplastic effect of metformin is postulated to be mediated by activation of adenosine monophosphate-activated protein kinase (AMPK) and consequent inhibition of the mammalian target of rapamycin (mTOR) pathway, a downstream effector of growth factor signaling that is frequently activated in malignant cells (50). In addition, metformin may also inhibit cell growth and promote cell senescence by inhibiting cyclin D1 expression and pRb phosphorylation (51). The direct chemopreventive effect of metformin has been demonstrated in nondiabetic animal models of colorectal cancer. In the adenomatous polyposis coli ($APC^{Min/+}$) mice, a murine model of familial adenomatous polyposis, metformin has been shown to suppress intestinal polyposis (52). Metformin also suppresses azoxymethane-induced formation of colorectal aberrant crypt foci (ACF), a reliable surrogate biomarker of colorectal cancer, by activating AMPK (53, 54). This suppression of ACF was also demonstrated in a pilot study on chemopreventive effects of low dose oral metformin in nondiabetic human subjects (55). Besides a tumor-cell specific effect, metformin has a systemic effect, improving insulin sensitivity and promoting weight loss (10). On the other hand, sulfonylureas, by increasing insulin secretion, and exogenous insulin itself, can promote oncogenesis either directly or indirectly by increasing insulin-like growth factor 1 activity, resulting in abnormal stimulation of multiple cellular signaling cascades, enhancing growth factor-dependent cell proliferation and affecting cell metabolism (12). Previous observations support a functional, albeit limited, role of hyperinsulinemia and aberrant glucose homeostasis due to insulin resistance in the pathogenesis of colorectal neoplasia (56–58).

Differences observed in the Western and Asian population in our analysis should be interpreted with caution as only one of the included studies represented an Asian population. The differential association may be due to differences in dietary habits and/or other cultural behaviors. *In vivo* studies have shown that metformin has greatest antineoplastic activity in mice receiving a high-energy diet associated with hyperinsulinemia as compared with mice receiving a control diet (59). The oncogenic association between insulin use and colorectal cancer risk was more pronounced in the Asian population for unclear reasons. This increased susceptibility of Asians to the oncogenic effect of insulin has also been observed in patients with hepatocellular cancer (60).

The strengths of our study include the comprehensive and simultaneous assessment of the effects of all conventional ADMs on risk modification of colorectal cancer; performance of unadjusted analysis and meta-analysis of adjusted risk estimates reported in the studies to account for the impact of potential confounders as well as multiple subgroup analysis to ensure stability of the association and identify factors responsible for heterogeneity. Although Zhang and colleagues recently performed a meta-analysis of metformin and colorectal cancer risk (21), they did not account for the potential cancer-modifying effects of other ADMs. In their analysis of four studies, they observed a 37% lower colorectal cancer risk with metformin use; furthermore, subgroup analyses were not reported. In our study, the inclusion of more studies and simultaneous assessment of cancer-modifying effect of other ADMs resulted in a lower risk estimate associated with metformin than has been reported in other meta-analyses (20–22). Soranna and colleagues estimated the association between metformin and sulfonylureas and risk of all-cause cancer; however, they were unable to estimate the impact of sulfonylureas, TZD, and insulin on colorectal cancer risk separately (22).

Limitations

There were several limitations to our meta-analysis that merit consideration. First, the cancer-modifying association between ADM and colorectal cancer risk were based on data from observational studies, which accounted for nearly all of the included colorectal cancer cases ($n =$ 13,704 cases; 98.9%); no statistically significant association

was apparent based on the RCT cases (167 cases; 1.1%). These RCTs were not adequately powered to detect a significant difference between classes of ADMs with respect to colorectal cancer risk reduction and subjects included in these studies were not systematically screened for colorectal cancer, which might conceivably have introduced some degree of detection bias. Observational studies of ADMs on cancer risk are prone to immortal time bias and inherent time-lagging issues, when comparing first line treatment with metformin with second- and third-line treatments with other agents (61). This may potentially overestimate the apparent chemopreventive effect of metformin, as well as the oncogenic effect of insulin. Second, despite adjusting for potential covariates when such data were available, the possibility of residual confounding from obesity or other factors such as confounding by indication and severity cannot be excluded (62, 63). Moreover, the included studies could not account for "healthy user effect", wherein the diagnosis of diabetes mellitus may potentially modify dietary consumption and physical activity of patients as well as influence uptake of preventive health measures like screening colonoscopy. Third, the nature of the comparator group for each individual ADM was composed of other ADMs, which may have inherent cancer-modifying effects. We tried to account for this by performing a subgroup analysis restricted to those studies that reported OR after adjusting for the effect of other ADMs and observed stable results. Finally, the individual studies were limited in reporting an association between classes of ADMs and site-specific colorectal cancer risk modification. Hence, we could not establish whether different ADMs are differentially associated with risk of proximal or distal colorectal cancer.

Conclusion

On the basis of the results of this meta-analysis, it seems that metformin use may be associated with a lower risk of colorectal cancer in diabetic patients, whereas sulfonylureas and insulin do not significantly increase colorectal cancer risk. These data support the hypothesis that in diabetic patients at high risk for colorectal cancer (due to their metabolic condition with or without other risk factors) who are candidates for pharmacologic therapy may be best served by metformin. Our data also suggest that the treatment of diabetes mellitus with metformin, in combination with other ADMs, is not associated with an increased risk for colorectal cancer, however, this observation needs further investigation. Because the observed magnitude of colorectal cancer risk reduction associated with metformin was relatively modest, the number needed to treat to prevent one case of colorectal cancer would be large. A definitive and randomized chemoprevention trial is needed to rigorously assess the effects of metformin on incident colorectal cancer in diabetic patients, but would be lengthy, logistically challenging, and resource intensive. To facilitate further clarification of metformin's colorectal cancer chemopreventive potential, clinical evaluation in an enriched patient population (i.e., history of sporadic colorectal neoplasia) is ongoing (64).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. Singh, M.H. Murad, P.J. Limburg
Development of methodology: S. Singh, H. Singh, M.H. Murad
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Singh, H. Singh, P.P. Singh, M.H. Murad
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Singh, P.P. Singh
Writing, review, and/or revision of the manuscript: S. Singh, H. Singh, P.P. Singh, M.H. Murad, P.J. Limburg
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Singh
Study supervision: M.H. Murad, P.J. Limburg

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References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69–90.
2. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–67.
3. Zauber AG, Winawer SJ, O'Brien MJ, Lansdorp-Vogelaar I, van Ballegooijen M, Hankey BF, et al. Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. N Engl J Med 2012; 366:687–96.
4. Hu FB, Manson JE, Liu S, Hunter D, Colditz GA, Michels KB, et al. Prospective study of adult onset diabetes mellitus (type 2) and risk of colorectal cancer in women. J Natl Cancer Inst 1999;91: 542–7.
5. Limburg PJ, Anderson KE, Johnson TW, Jacobs DR Jr, Lazovich D, Hong CP, et al. Diabetes mellitus and subsite-specific colorectal cancer risks in the Iowa Women's Health Study. Cancer Epidemiol Biomarkers Prev 2005;14:133–7.
6. Yang YX, Hennessy S, Lewis JD. Type 2 diabetes mellitus and the risk of colorectal cancer. Clin Gastroenterol Hepatol 2005;3:587–94.
7. Yuhara H, Steinmaus C, Cohen SE, Corley DA, Tei Y, Buffler PA. Is diabetes mellitus an independent risk factor for colon cancer and rectal cancer? Am J Gastroenterol 2011;106:1911–21.
8. Larsson SC, Orsini N, Wolk A. Diabetes mellitus and risk of colorectal cancer: a meta-analysis. J Natl Cancer Inst 2005;97: 1679–87.
9. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, et al. Diabetes and cancer: a consensus report. CA Cancer J Clin 2010;60:207–21.
10. Gallagher EJ, LeRoith D. Diabetes, cancer, and metformin: connections of metabolism and cell proliferation. Ann N Y Acad Sci 2011; 1243:54–68.
11. Okumura T. Mechanisms by which thiazolidinediones induce anticancer effects in cancers in digestive organs. J Gastroenterol 2010;45: 1097–102.
12. 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;29:254–8.

13. Currie CJ, Poole CD, Gale EA. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. Diabetologia 2009;52: 1766–77.
14. 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.
15. Chang CH, Lin JW, Wu LC, Lai MS, Chuang LM. Oral insulin secretagogues, insulin, and cancer risk in type 2 diabetes mellitus. J Clin Endocrinol Metab 2012;97:1170–5
16. Yang YX, Hennessy S, Lewis JD. Insulin therapy and colorectal cancer risk among type 2 diabetes mellitus patients. Gastroenterology 2004;127:1044–50.
17. Bodmer M, Becker C, Meier C, Jick SS, Meier CR. Use of metformin is not associated with a decreased risk of colorectal cancer: a case-control analysis. Cancer Epidemiol Biomarkers Prev 2012;21:280–6.
18. Campbell PT, Deka A, Jacobs EJ, Newton CC, Hildebrand JS, McCullough ML, et al. Prospective study reveals associations between colorectal cancer and type 2 diabetes mellitus or insulin use in men. Gastroenterology 2010;139:1138–46.
19. Ferrara A, Lewis JD, Quesenberry CP Jr, Peng T, Strom BL, Van Den Eeden SK, et al. Cohort study of pioglitazone and cancer incidence in patients with diabetes. Diabetes Care 2011;34:923–9.
20. Noto H, Goto A, Tsujimoto T, Noda M. Cancer risk in diabetic patients treated with metformin: a systematic review and meta-analysis. PLoS ONE 2012;7:e33411.
21. Zhang ZJ, Zheng ZJ, Kan H, Song Y, Cui W, Zhao G, et al. Reduced risk of colorectal cancer with metformin therapy in patients with type 2 diabetes: a meta-analysis. Diabetes Care 2011;34:2323–8.
22. Soranna D, Scotti L, Zambon A, Bosetti C, Grassi G, Catapano A, et al. Cancer risk associated with use of metformin and sulfonylurea in type 2 diabetes: a meta-analysis. Oncologist 2012;17:813–22.
23. Higgins JP, Green S, editors. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from: www.cochrane-handbook.org.
24. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med 2009;151:264–9, W264.
25. Wells GA, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses [cited December 15, 2012]. Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp
26. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ 2011;343:d5928.
27. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–88.
28. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557–60.
29. Kanwal F, White D. "Systematic Reviews and Meta-analyses" in Clinical Gastroenterology and Hepatology. Clin Gastroenterol Hepatol 2012;10:1184–6.
30. Altman DG, Bland JM. Interaction revisited: the difference between two estimates. BMJ 2003;326:219.
31. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629–34.
32. Easterbrook PJ, Berlin JA, Gopalan R, Matthews DR. Publication bias in clinical research. Lancet 1991;337:867–72.
33. Borenstein M, Higgins J, Rothstein H. Comprehensive Meta-analysis In. Version 2 ed. Englewood NJ: Biostat; 2005.
34. Chang CH, Lin JW, Wu LC, Lai MS, Chuang LM, Chan KA. Association of thiazolidinediones with liver cancer and colorectal cancer in type 2 diabetes mellitus. Hepatology 2012;55:1462–72.
35. Govindarajan R, Ratnasinghe L, Simmons DL, Siegel ER, Midathada MV, Kim L, et al. Thiazolidinediones and the risk of lung, prostate, and colon cancer in patients with diabetes. J Clin Oncol 2007;25:1476–81.
36. Home PD, Kahn SE, Jones NP, Noronha D, Beck-Nielsen H, Viberti G. Experience of malignancies with oral glucose-lowering drugs in the randomised controlled ADOPT (A Diabetes Outcome Progression Trial) and RECORD (Rosiglitazone Evaluated for Cardiovascular Outcomes and Regulation of Glycaemia in Diabetes) clinical trials. Diabetologia 2010;53:1838–45.
37. ORIGIN Trial Investigators, Gerstein HC, Bosch J, Dagenais GR, Díaz R, Jung H, et al. Basal insulin and cardiovascular and other outcomes in dysglycemia. N Engl J Med 2012;367:319–28
38. Koro C, Barrett S, Qizilbash N. Cancer risks in thiazolidinedione users compared to other anti-diabetic agents. Pharmacoepidemiol Drug Saf 2007;16:485–92.
39. Morden NE, Liu SK, Smith J, Mackenzie TA, Skinner J, Korc M. Further exploration of the relationship between insulin glargine and incident cancer: a retrospective cohort study of older Medicare patients. Diabetes Care 2011;34:1965–71.
40. Oliveria S, Koro C, Ulcickas Yood M, Sowell M. Cancer incidence among patients treated with antidiabetic pharmacotherapy. Diabetes Metab Syndr: Clin Res Rev 2008;2:47–57.
41. Ruiter R, Visser LE, van Herk-Sukel MP, Coebergh JW, Haak HR, Geelhoed-Duijvestijn PH, et al. Lower risk of cancer in patients on metformin in comparison with those on sulfonylurea derivatives: results from a large population-based follow-up study. Diabetes Care 2012;35:119–24.
42. Vinikoor LC, Long MD, Keku TO, Martin CF, Galanko JA, Sandler RS. The association between diabetes, insulin use, and colorectal cancer among Whites and African Americans. Cancer Epidemiol Biomarkers Prev 2009;18:1239–42.
43. Lee MS, Hsu CC, Wahlqvist ML, 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.
44. Tseng CH. Diabetes, metformin use, and colon cancer: a population-based cohort study in Taiwan. Eur J Endocrinol 2012;167:409–16.
45. Blin P, Lassalle R, Dureau-Pournin C, Ambrosino B, Bernard MA, Abouelfath A, et al. Insulin glargine and risk of cancer: a cohort study in the French National Healthcare Insurance Database. Diabetologia 2012;55:644–53.
46. Colhoun HM. Use of insulin glargine and cancer incidence in Scotland: a study from the Scottish Diabetes Research Network Epidemiology Group. Diabetologia 2009;52:1755–65.
47. Ljung R, Talback M, Haglund B, Jonasson JM, Gudbjornsdottir S, Steineck G. Insulin glargine use and short-term incidence of malignancies - a three-year population-based observation. Acta Oncol 2011;50:685–93.
48. Johnson JA, Bowker SL. Intensive glycaemic control and cancer risk in type 2 diabetes: a meta-analysis of major trials. Diabetologia 2011;54: 25–31.
49. Stefansdottir G, Zoungas S, Chalmers J, Kengne AP, Knol MJ, Leufkens HG, et al. Intensive glucose control and risk of cancer in patients with type 2 diabetes. Diabetologia 2011;54:1608–14.
50. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 2001;108:1167–74.
51. Ben Sahra I, Laurent K, Loubat A, Giorgetti-Peraldi S, Colosetti P, Auberger P, et al. The antidiabetic drug metformin exerts an antitumoral effect *in vitro* and *in vivo* through a decrease of cyclin D1 level. Oncogene 2008;27:3576–86.
52. Tomimoto A, Endo H, Sugiyama M, Fujisawa T, Hosono K, Takahashi H, et al. Metformin suppresses intestinal polyp growth in APCMin/$^{+}$ mice. Cancer Sci 2008;99:2136–41.
53. Hosono K, Endo H, Takahashi H, Sugiyama M, Uchiyama T, Suzuki K, et al. Metformin suppresses azoxymethane-induced colorectal aberrant crypt foci by activating AMP-activated protein kinase. Mol Carcinog 2010;49:662–71.
54. Takayama T, Katsuki S, Takahashi Y, Ohi M, Nojiri S, Sakamaki S, et al. Aberrant crypt foci of the colon as precursors of adenoma and cancer. N Engl J Med 1998;339:1277–84.
55. Hosono K, Endo H, Takahashi H, Sugiyama M, Sakai E, Uchiyama T, et al. Metformin suppresses colorectal aberrant crypt foci in a short-term clinical trial. Cancer Prev Res 2010;3:1077–83.

56. Limburg PJ, Stolzenberg-Solomon RZ, Vierkant RA, Roberts K, Sellers TA, Taylor PR, et al. Insulin, glucose, insulin resistance, and incident colorectal cancer in male smokers. Clin Gastroenterol Hepatol 2006;4:1514–21.
57. Schoen RE, Weissfeld JL, Kuller LH, Thaete FL, Evans RW, Hayes RB, et al. Insulin-like growth factor-I and insulin are associated with the presence and advancement of adenomatous polyps. Gastroenterology 2005;129:464–75.
58. Ortiz AP, Thompson CL, Chak A, Berger NA, Li L. Insulin resistance, central obesity, and risk of colorectal adenomas. Cancer 2012;118:1774–81.
59. Algire C, Zakikhani M, Blouin MJ, Shuai JH, Pollak M. Metformin attenuates the stimulatory effect of a high-energy diet on *in vivo* LLC1 carcinoma growth. Endocr Relat Cancer 2008;15:833–9.
60. Singh S, Singh PP, Singh AG, Murad MH, Sanchez W. Anti-diabetic medications and the risk of hepatocellular cancer: a systematic review and meta-analysis. Am J Gastroenterol 2013;108:881–91
61. Suissa S, Azoulay L. Metformin and the risk of cancer: time-related biases in observational studies. Diabetes Care 2012;35:2665–73.
62. Pocock SJ, Elbourne DR. Randomized trials or observational tribulations? N Engl J Med 2000;342:1907–9.
63. Singh S, Singh PP, Singh AG, Murad MH, McWilliams RR, Chari ST. Anti-diabetic medications and risk of pancreatic cancer in patients with diabetes mellitus: a systematic review and meta-analysis. Am J Gastroenterol 2013;108:510–9
64. Higurashi T, Takahashi H, Endo H, Hosono K, Yamada E, Ohkubo H, et al. Metformin efficacy and safety for colorectal polyps: a double-blind randomized controlled trial. BMC Cancer 2012;12:118.