The Association of Metformin, Other Antidiabetic Medications, and Statins with the Incidence of Colon Cancer in Type 2 Diabetes Patients

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Conflict of interest

Mikko Marttila is employed by Orion Corporation. Orion Corporation had no role in the study design; collection, analysis, and interpretation of the data; writing this report; or decision to submit the article for publication.

Funding

This study was funded by grants from the Jane and Aatos Erkko Foundation, the Cancer Society of Finland, and Finnish Government Research Funds granted to the University Hospital of Oulu.

Abbreviations

ADM = Antidiabetic medication

CC = colon cancer

CRC = colorectal cancer

CI = confidence interval

DDD = defined daily dose

FinDM = Diabetes in Finland database

HMG-CoA = 3-hydroxy-3-methylglutaryl coenzyme A

HR = hazard ratio

ICD-10 = International Statistical Classification of Diseases and Related Health Problems, 10th revision

T2D = type 2 diabetes

Micro-abstract: Metformin and statins may have anticancer effects, with plausible cellular mechanisms. Our register study of 306,317 individuals found no evidence for a protective effect of antidiabetic medications, including metformin or statins, against colon cancer.

Abstract

Background: Metformin and statins may have anticancer effects, with plausible cellular mechanisms. However, the association of these agents with the risk of colorectal cancer (CRC) is unclear.

Materials and methods: This was a retrospective cohort study on a large population (N = 316,317) of persons with type 2 diabetes (T2D). The data were obtained from the Diabetes in Finland database and the Finnish Cancer Registry. In a full cohort analysis, hazard ratios (HRs), with their 95% confidence intervals (CIs) for ever use versus never use were estimated using a multiple Poisson regression model. A nested case–control design within the cohort was employed to examine the association of colon cancer (CC) with the defined daily dose (DDD) of medication, and the data

from this were analyzed by conditional logistic regression. The analyses were adjusted for the patient's age, sex, and duration of diabetes.

Results: In total, 1,351 cases of CC were diagnosed during 1996–2011. Insufficient evidence was found for an association of ever use of metformin (HR: 1.01, 95% CI: 0.90-1.14), other oral antidiabetic medications (ADMs) (HR: 1.05, 95% CI: 0.93-1.19), insulin (HR: 1.02, 95% CI: 0.86-1.22), and statins (HR: 0.94, 95% CI: 0.84-1.05) with the incidence of CC in the full cohort analysis. The results from the case–control analysis were similar, with no consistent trend in the incidence of CC by the cumulative dose.

Conclusions: This study found insufficient evidence for an association between metformin, insulin, other oral T2D medications, or statins and the incidence of CC.

Keywords: Cohort, Colorectal cancer, Epidemiology, Insulin, Nested case–control

Introduction

Type 2 diabetes (T2D) is associated with an elevated incidence of colorectal cancer (CRC), and the prognosis of T2D patients with CRC is worse than those without T2D[1,2]. Metformin is a widely used biguanide class drug for the treatment of T2D, with reported anticancer effects in preclinical studies[3]. In two recent meta-analyses, the use of metformin among T2D patients was associated with a decreased incidence of CRC[4,5]. However, some observational studies on metformin and cancer have suffered from time-related biases[6]. Two studies designed to avoid bias did not find any association between ever use of metformin and the risk of CRC among individuals with T2D[7,8], although long-term (≥ 5 y) use appeared to be associated with a reduced risk in a second study[8]. In meta-analyses, T2D insulin users were observed to have a greater risk of CRC than did non-insulin users[9,10].

Statins, 3 hydroxy-3 methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, are a class of lipid-lowering medications. Statins are widely used for T2D in Finland, with 46% of those diagnosed with T2D prescribed statins and up to 79% of those diagnosed with T2D and coronary heart disease using statins[11]. Previous research demonstrated antitumor effects of lipophilic statins *in vitro*[12]. A modest reduction in the risk of CRC was linked to statin usage in a meta-analysis of 40 studies[13].

There are fundamental differences in the pathogenesis of colon cancer (CC) and rectal cancer[14]. In this register-based, cohort, nested case–control study, we investigated the association of the use of metformin, other antidiabetic medications (ADMs), and statins with the incidence of CC in individuals with T2D. This study adhered to STROBE guidelines for observational studies[15].

Materials and Methods

Study population

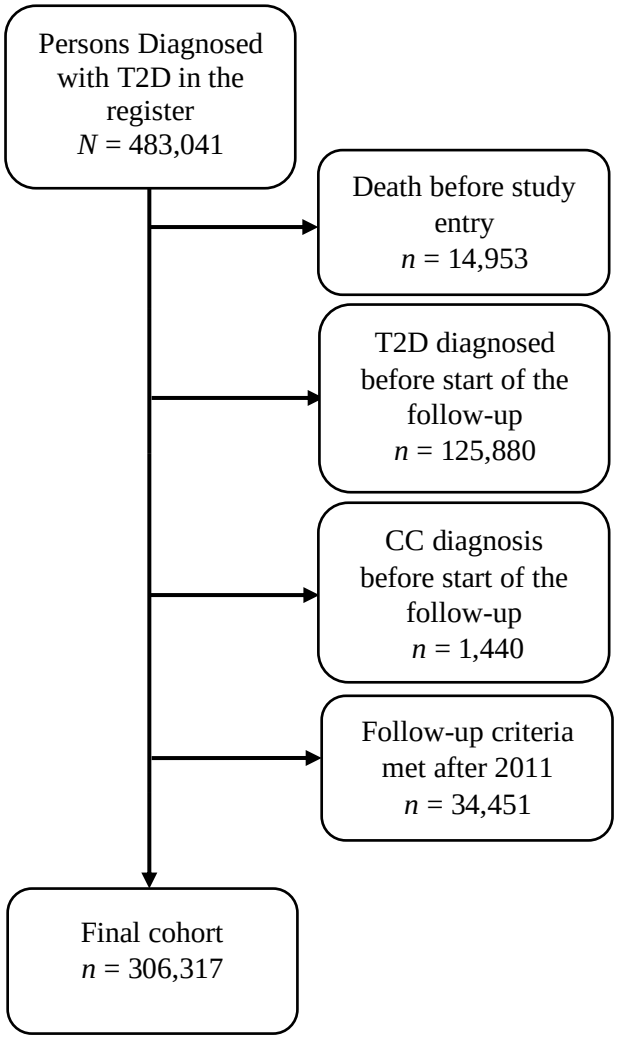
Data on individuals diagnosed with diabetes were obtained from the Diabetes in Finland database (FinDM), which was created for the purpose of epidemiological monitoring of diabetes in Finland[16]. FinDM is composed of register data from multiple databases: The Care Register for Health and the Hospital Discharge Register from the National Institute for Health and Welfare, the Special Refund Entitlement Register and the Prescription Register from the Social Insurance Institution of Finland, and the Cause of Death Register from Statistics Finland. The Special Refund Entitlement Register and Prescription Register contain information on all drug purchases prescribed by a physician and reimbursed by the Social Insurance Institution of Finland, beginning from 1994, which allows an accurate assessment of statin and ADM usage. Diabetes patients were identified from hospital records, starting from 1969 for inpatients and 1998 for outpatients, or from ADM reimbursements.

Diabetes was categorized as type 1 or type 2 diabetics, mainly according to first-line treatment. FinDM does not contain information about former treatment of diet-controlled diabetes. Thus, in some cases, the duration of diabetes may be longer than indicated in the register. FinDM has good national coverage in Southern Finland when compared to local registers[17]. The data from FinDM were linked with data from the Finnish Cancer Registry, which contains information on almost all cancer cases diagnosed in Finland since 1953[18]. The information includes the date of diagnosis, histology, morphology, and spread (local, advanced, or unknown). Completeness of the records has been estimated to be 96% for solid tumors[18]. Dates and causes of death for individuals were obtained from Statistics Finland. Linking was based on personal identification codes, which are unique to each resident of Finland.

Study cohort

The cohort selection process is presented in a flow chart (Fig. 1). Between 1 January 1996 and 31 December 2011, 483,041 individuals were diagnosed with T2D. The drug purchase history until the end of the study period was available for all those in the cohort. The follow-up started on the 40th birthday, or 1 year after a diagnosis of T2D, whichever occurred later. We excluded the first year after a T2D diagnosis from the follow-up to minimize the risk of reverse causality and detection biases. Patients diagnosed with CC (code C18 of the International Classification of Diseases 10th Revision (ICD-10) prior to the beginning of the follow-up were also excluded. The final cohort contained 306,317 individuals diagnosed with T2D.

Figure 1. Flow chart of the cohort selection process. T2D = type 2 diabetes, CC = colon cancer



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135 We defined CC as a diagnosis with ICD-10 code C18 and ICD-O-3 morphology code M-8140/3.
136 The code includes the following CCs: cancers of the cecum, appendix, ascending colon, right colic
137 flexure, transverse colon, left colic flexure, sigmoid, and unspecified.

138 We evaluated usage of ADM medication in three different categories: metformin, other oral ADMs,
139 and insulin. The use of statins was assessed as a separate variable. The exposure was defined as
140 beginning 365 days after the first purchase. This allowed a reasonable latency period for the
141 exposure and minimized reverse causality problems. The follow-up time was defined as ever or

never exposed after medication usage criteria were fulfilled. The follow-up ended on the date of CC diagnosis, death, emigration, or 31 December 2011, whichever occurred earliest.

A nested case–control study[19] embedded in the cohort was also performed. Up to 20 controls were randomly selected for each case subject with CC, individually matched on sex, age, and duration of diabetes (182 days) from those cohort members at risk on the date of the CC diagnosis of the case. In addition, we evaluated the cumulative effect of medication use, measured by the defined daily dose (DDD), on CC risk. The effects of cumulative usage were assessed in a nested case–control analysis using the total DDDs purchased during the follow-up

Statistical analysis

The statistical analyses were performed in R environment, version 3.5.2[20]. A person-period file was created using the Lexis tools[21] in the Epi package[22], which made it possible to split the individual follow-up time of each person simultaneously into appropriate periods of age, duration of T2D, and time-dependent medication use status.

In a full cohort analysis, hazard ratios (HRs), with their 95% confidence intervals (CI) for ever versus never use of each medication were estimated using a multiple Poisson regression model. A piecewise constant hazard pattern was assumed for the effects of current age and the duration of T2D. Age was split into 5-y intervals starting from age group 40–44 y. The duration of T2D was split into four categories: 1- < 3 y, 3- < 5 y, 5- < 8 y, and 8 - < 16 y. The Poisson regression model for the analysis of the full cohort data was fitted using the glm function of R.

In the nested case–control analysis of ever use of any ADMs and statins, HRs, with their 95% CIs were estimated using conditional logistic regression, equivalent to stratified proportional hazards model[19]. For the DDD data, cumulative doses were categorized according to tertiles. In the analysis

of the nested case–control data, the conditional logistic regression model was fitted using the clogit function of the survival package[23] of R.

Results

The final cohort included 306,317 individuals, covering 1,632,577 person-years and 1,349 incident cases of CC (Table 1). The overall incidence of CC in the cohort was 8.3 per 10,000 person-years, with the highest incidence found in the age group 80–89 y. Women accounted for 48.2% of the cohort population. The incidence of CC among women was lower than that among men, with an estimated HR of 0.75 (95% CI: 0.67-0.84). In the study population, 80.2% had ever used metformin, 52.5% had ever used other oral ADMs, and 16.4% had ever used insulin. In addition, 62.5% of the cohort members had used statins. Other oral ADMs included sulphonylureas (70.8% of other oral ADM users), dipeptidyl peptidase-4 inhibitors, glitazones, glinides, guar gum, and fixed combinations (Supplementary Table 1).

In the study cohort, the statins most commonly used were simvastatin (74.7% of statin users) and atorvastatin (27.0% of statin users) (Supplementary Table 1), both being classified as lipophilic statins.

Table 1. Distribution of person-years in the cohort, incidence rates of colon cancer (CC) (per 10,000), and number (%) of cases and controls matched for age, duration of diabetes, and medication use.

Variable	Value	*N*	Person-years in cohort	Incidence per 10,000	Cases (%)	Controls (%)
Total		306,317	1,632,577	8.3	1,349 (100.0)	24,493 (100.0)
Sex	Female	146,078	797,121	8.2	655 (48.6)	11,955 (48.8)
	Male	160,239	835,456	8.3	694 (51.5)	12,538 (51.2)
Age (y)	40-49	38,864	124,470	0.6	7 (0.5)	149 (0.59)
	50-59	99,696	356,633	2.9	102 (7.6)	2,038 (8.1)
	60-69	137,001	493,059	6.5	322 (28.9)	6,185 (24.5)
	70-79	115,171	419,157	12.5	523 (38.8)	9,645 (38.3)
	80-89	64,767	213,556	17.1	365 (27.1)	6,681 (26.5)
	90-107	11,093	25,699	11.7	30 (2.2)	510 (2.0)
Duration of diabetes (y)	1-<3	302,740	531,858	7.5	397 (29.4)	7,603 (29.3)
	3-<5	232,094	388,029	7.4	288 (21.4)	5,757 (22.2)
	5-<8	163,434	385,285	9.1	351 (26.0)	6,636 (25.6)
	8-<16	96,520	327,404	9.6	313 (23.2)	5,937 (22.9)
Metformin use	Ever	246,439	1,114,435	8.0	888 (65.8)	16,297 (65.7)
	Never	129,446	518,142	8.9	461 (34.2)	8,475 (34.2)
Other oral ADM use	Ever	147,676	845,588	9.0	761 (56.4)	13,713 (55.6)
	Never	239,976	786,989	7.5	588 (43.6)	10,961 (65.8)
Insulin use	Ever	50,566	216,062	8.0	173 (12.8)	3,100 (12.6)
	Never	303,508	1,416,515	8.3	1,176 (87.2)	21,607 (87.5)
Statin use	Ever	196,000	843,452	8.2	690 (51.2)	13,125 (52.9)
	Never	196,580	789,125	8.4	659 (48.9)	11,666 (47.1)

The associations of the incidence of CC with the medications studied are reported in Table 2. The estimated HRs, adjusted for age, sex, and duration of diabetes, with their 95% CIs were as follows: ever use of metformin (HR: 1.01, 95% CI: 0.90-1.14); insulin (HR: 1.02, 95% CI: 0.86-1.21); other oral ADMs (HR: 1.05, 95% CI 0.93-1.19); and statins (HR: 0.94, CI 95% 0.84-1.05), each compared to never use. . Similar results were obtained in the nested case-control analysis. No

192 evidence for an association between increasing cumulative doses and a reduced risk of CC was
193 found (Fig. 2).

194

195 **Table 2.** Adjusted estimated hazard ratios (HR), with their 95% confidence intervals (CIs) for ever
196 use of metformin, insulin, other oral ADMs, or statins and the incidence of CC compared to never
197 use. The full cohort data are based on Poisson regression and the nested case-control data are based
198 on conditional regression, both adjusted for patient age, sex and duration of diabetes.

Ever use	Full cohort	Case control
	HR (95% CI)[a]	HR (95% CI)[a]
Metformin	1.01 (0.90-1.14)	1.03 (0.90-1.16)
Other oral T2D medications	1.02 (0.93-1.19)	1.01 (0.89-1.14)
Insulin	1.00 (0.86-1.21)	1.04 (0.87-1.24)
Statins	0.94 (0.84-1.05)	0.93 (0.83-1.05)
Metformin versus other oral ADMs	0.98 (0.84-1.15)	1.02 (0.86-1.21)

[a]Adjusted for patient sex, age and duration of diabetes

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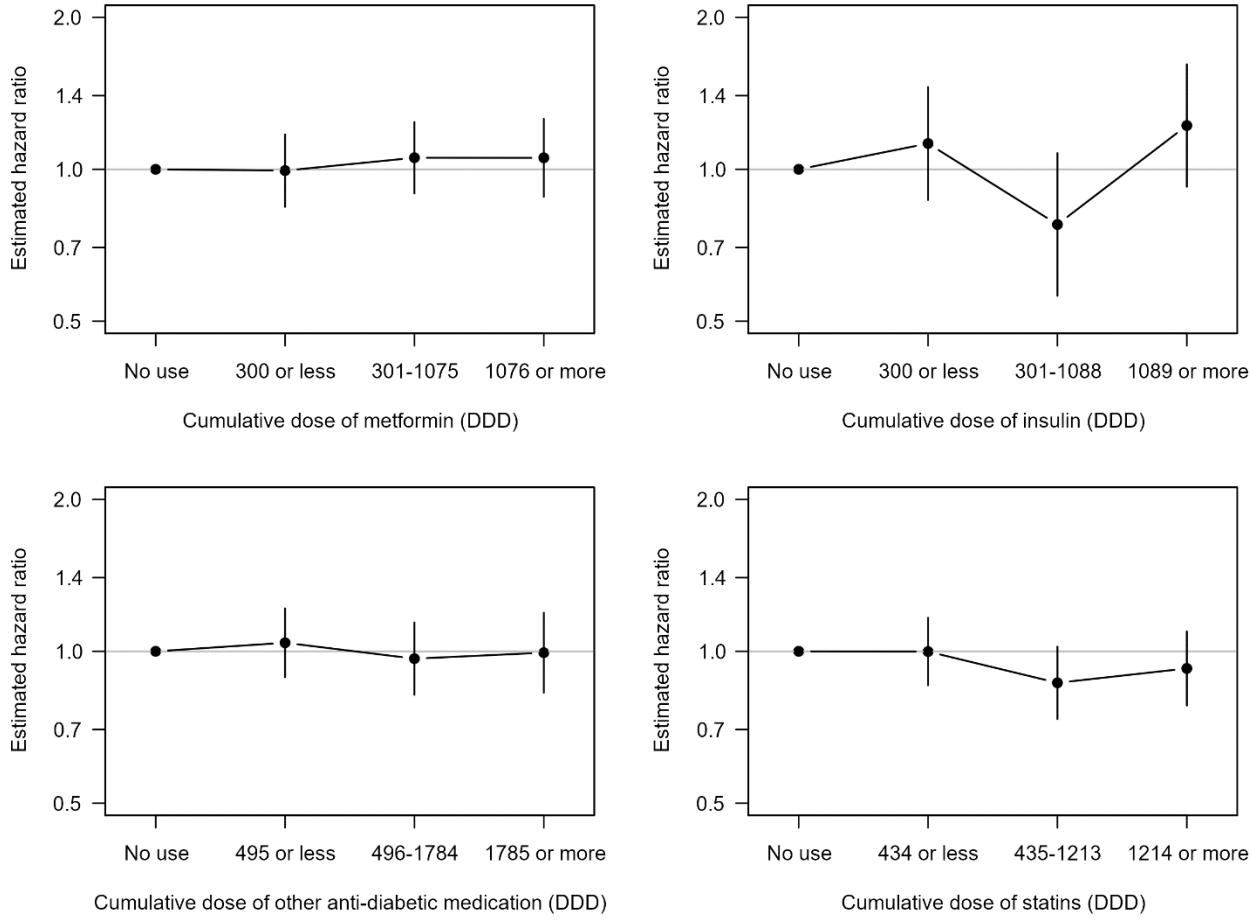
Figure 2. Estimated hazard ratios (HRs), with their 95% confidence intervals (CIs) for colon cancer (CC) by cumulative defined daily dose (DDD) in the different medication groups in the case–control analysis.



Discussion

In this large retrospective cohort study, also including a nested case–control analysis, we found no evidence for an association of the risk of colon cancer (CC) with the use of metformin, other oral ADMs, insulin, or statins in T2D patients.

There is strong evidence for an increased risk of colorectal cancers (CRC) associated with T2D[24]. CRC and T2D also share common risk factors, including obesity[25,26] and high meat intake[27]. A biochemical link exists, too, between the two diseases: T2D creates CRC promoting microenvironment through hyperinsulinemia and hyperglycemia[28]. Insulin acts as a growth factor, and higher levels of fasting insulin and C peptide have been associated with increased CRC risk in meta-analysis of 35 studies with 25,566 patients[29]. The same study found an association between a biomarker of hyperglycemia, Hba1C, and an increased risk for developing colorectal cancer. Hyperglycemia leads to formation of advanced glycation end products, which have been associated with increased proliferation and migration of CRC cells *in vitro*, in addition to hyperglycemia itself[28]. Hyperglycemia and AGES also lead to increased oxidative stress and inflammation, further promoting malignant progression. Other possible links between CRC and T2D include hyperlipidemia, increased inflammation, extracellular matrix alterations and altered microbiota.

Multiple anticancer effects of metformin have been reported in preclinical trials in many most solid cancer types, including extensive evidence from basic research on colorectal cancer [30-32]. The most commonly reported cancer-killing *in vitro* effects of metformin include the inhibition of mitochondrial complex 1, activation of AMPK, reduction of glucose levels by glucagon signaling suppression, and induction of cell cycle arrest and apoptosis[3]. Some of these mechanisms, however, might have been due to suprapharmacological doses used *in vitro*[33,34].

Previous meta-analyses have reported metformin use to be associated with a reduced incidence of both CRC and also colorectal adenomas in T2D patients[4,5,35]. On the other hand, an analysis of 46 observational studies on metformin and various cancers in T2D patients reported that only three of these studies had a low or no risk of bias[36]. Two of these three potentially unbiased studies analyzed the association between metformin and the risk of CRC and found no evidence for a reduced risk[37,38], which was in line with the results of the present study. Additionally, some observational studies have been criticized for overestimating the possible beneficial effect of metformin through

biases, including immortal time bias, time-window bias, and failure to adjust for baseline severity of the disease[6,36,39].

We compared ever-use of medication against never-use. This introduces a risk for potential bias due to possible differences in patient characteristics in these two categories[40]. Treatment might be withheld from persons with poor health, due to no perceived benefit in their state, introducing confounding by frailty, thus exaggerating the beneficial effect. Never-users of a medication can have a less severe diabetes and/or less risk factors for a severe disease. Since T2D and CC have common risk factors, including obesity, some never-users of medication might have a lower CC risk, thus resulting in a smaller apparent preventive effect of medication use. Ever-use versus never-use also introduces a potential outcome detection bias: persons who use medications are more likely to engage with the medical system, thus leading to increased cancer detection and seemingly increased number of cancer cases. The Finnish Cancer Registry contains no data on whether the cancer has been screen detected or not, and no organized screening program has been offered in Finland during our study time period. Colonoscopy screening for asymptomatic patients is not common in the Finnish healthcare system. Choosing an active comparator group, for example persons using a different oral antidiabetic medication than metformin would lead to a more comparable study groups and thus more reliable results with less risk of bias[40,41]. Most of our reference studies concerning metformin use and CRC have employed an user versus non-user design[2,4,5,8,35].

Insulin acts as a growth stimulating agent through the insulin-like growth factor system, and previous research has suggested that hyperinsulinemia increases the incidence of various cancer types in T2D patients[42]. Long-acting synthetic insulin analogs might have cancer-promoting effects due to prolonged receptor stimulation, elevated insulin levels, and different receptor interactions as compared to endogenic insulin and short-acting analogs. A number of studies have also reported that insulin use is associated with an elevated risk of CRC in individuals with T2D[9,10]. However, we

found no evidence for an association between insulin use and the incidence of CC in our study population.

Statins have been hypothesized to exert various effects leading to the inhibition of cancer, including CRC, suppression in preclinical studies[43]. Statins act by inhibiting HMG-CoA reductase, which leads to lower levels of mevalonate, a cholesterol precursor[44]. Tumor cells, especially those found in malignant tumors, have a greater demand for products synthetized from mevalonate. Statins also induce cell-cycle arrest by affecting regulatory proteins involved in the cell cycle, and they cause apoptosis in cancer cells. Previous research demonstrated that lipophilic statins, which were almost solely used in our study population, had a greater anticancer effect than did hydrophilic statins. In a meta-analysis of 42 studies, statin use was reported to be associated with a modest reduction in the incidence of CRC[44]. However, a subgroup analysis of 11 studies that analyzed CC separately found no evidence for an association of statin use with a reduced risk of CC. The results of this subgroup analysis were similar to ours.

The strengths of our study were the large cohort of individuals with T2D and the use of a database with good national coverage. In addition, our study design minimized the risk of detection and reverse causality biases, as we could adjust for the diabetes duration, amount of drug usage, age, and sex. However, some risk factors, such as dietary intake of fiber, red and processed meat, obesity, alcohol intake, and inflammatory bowel disease, could not be taken into account. As aspirin is available over the counter in Finland, data on aspirin usage were not reported in registers.

Conclusions

Preclinical and epidemiological studies have suggested that metformin and statins might have anticancer effects. In our study, we found no evidence for an association between the incidence of CC and the studied medications, with narrow CIs, which was in line with the findings of previous

observational studies designed to avoid common biases. In conclusion, we found no evidence for a protective effect of metformin, insulin, other oral ADMs, or statins against CC.

Clinical practice points

The association between the risk of CRC and metformin and statins were unclear in previous studies. We found no evidence for an association between metformin, statins, insulin, or other oral T2D medications with the risk of CC. Our study does not support the use of these medications for the prevention of CC. Furthermore, their usage does not seem to increase the risk of CC.

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