

# Impact of Glucose-Lowering Agents on the Risk of Cancer in Type 2 Diabetic Patients. The Barcelona Case-Control Study

Rafael Simó<sup>1,2\*</sup>, Oleguer Plana-Ripoll<sup>2,3,4</sup>, Diana Puente<sup>2,3</sup>, Rosa Morros<sup>2,3,5</sup>, Xavier Mundet<sup>2,3</sup>, Luz M. Vilca<sup>2,6</sup>, Cristina Hernández<sup>1</sup>, Inmaculada Fuentes<sup>7</sup>, Adriana Procupet<sup>5</sup>, Josep M. Taberner<sup>2,8</sup>, Concepción Violán<sup>2,3</sup>

**1** Grup de Recerca en Diabetis i Metabolisme i CIBER de Diabetis i Malalties Metabòliques Associades (CIBERDEM), Institut de Recerca Hospital Vall d'Hebron, Barcelona, Spain, **2** Universitat Autònoma de Barcelona, Bellaterra, Spain, **3** Institut Universitari d'Investigació en Atenció Primària Jordi Gol (IDIAP Jordi Gol), Barcelona, Spain, **4** Section of Epidemiology, Department of Public Health, Aarhus University, Aarhus, Denmark, **5** Institut Català de la Salut, Barcelona, Spain, **6** Servei de Farmacologia Clínica, Hospital Universitari Vall d'Hebron, Barcelona, Spain, **7** Servei Medicina Preventiva i Epidemiologia, Hospital Universitari Vall d'Hebron, Barcelona, Spain, **8** Servei d'Oncologia, Hospital Universitari Vall d'Hebron, Barcelona, Spain

## Abstract

**Background:** The aim of the present study is to evaluate the impact of glucose-lowering agents in the risk of cancer in a large type 2 diabetic population.

**Methods:** A nested case-control study was conducted within a defined cohort (275,164 type 2 diabetic patients attending 16 Primary Health Care Centers of Barcelona). Cases (n=1,040) comprised those subjects with any cancer diagnosed between 2008 and 2010, registered at the Cancer Registry of Hospital Vall d'Hebron (Barcelona). Three control subjects for each case (n=3,120) were matched by age, sex, diabetes duration, and geographical area. The treatments analyzed (within 3 years prior to cancer diagnosis) were: insulin glargine, insulin detemir, human insulin, fast-acting insulin and analogues, metformin, sulfonylureas, repaglinide, thiazolidinediones, dipeptidyl peptidase-4 inhibitors, and alpha glucosidase inhibitors. Conditional logistic regressions were used to calculate the risk of cancer associated with the use of each drug adjusted by age, BMI, dose and duration of treatment, alcohol use, smoking habit, and diabetes duration.

**Results:** No differences were observed between case and control subjects for the proportion, dose or duration of exposure to each treatment. None of the types of insulin and oral agents analyzed showed a significant increase in the risk of cancer. Moreover, no cancer risk was observed when glargine was used alone or in combination with metformin.

**Conclusions:** Our results suggest that diabetes treatment does not influence the risk of cancer associated with type 2 diabetes. Therefore, an eventual increase of cancer should not be a reason for biasing the selection of any glucose-lowering treatment in type 2 diabetic population.

**Citation:** Simó R, Plana-Ripoll O, Puente D, Morros R, Mundet X, et al. (2013) Impact of Glucose-Lowering Agents on the Risk of Cancer in Type 2 Diabetic Patients. The Barcelona Case-Control Study. PLoS ONE 8(11): e79968. doi:10.1371/journal.pone.0079968

**Editor:** Amar Abderrahmani, University of Lille Nord de France, France

**Received:** April 30, 2013; **Accepted:** October 8, 2013; **Published:** November 21, 2013

**Copyright:** © 2013 Simó et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** This work has received financial support by a grant for the development of independent clinical research from Ministry of Health and Social Policy EC10-338. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

\* E-mail: rafael.simo@vhir.org

## Introduction

Type 2 diabetes has been associated with an increased risk of cancer. This population indeed has a greater risk of three of the leading causes of cancer mortality such as pancreatic, colorectal and breast cancer [1]. In addition, type 2 diabetes is associated with substantial premature death rates from several types of cancer [2].

The etiology of this excess cancer risk is poorly understood. Type 2 diabetes and cancer have common risk factors including age, race/ethnicity, obesity, physical inactivity, and tobacco use [1]. Data from large randomized controlled trials of intensified glycemic control suggest that cancer risk is not reduced by

improving glycemic control in type 2 diabetes [3], and that both obesity and insulin resistance with or without hyperglycemia are also associated with an increased risk of cancer [4,5]. Therefore, factors other than glucose could be involved in the relationship between type 2 diabetes and cancer development. Among these factors it seems that hyperinsulinemia and/or insulin resistance could play an essential role. In fact, the presence of insulin resistance and hyperinsulinemia, may accelerate tumor growth [6].

The role of insulin in cancer promotion is suggested by studies associating circulating insulin levels and cancer of the colon, pancreas, and breast [1,6,7]. The association between exogenous insulin and cancer gained attention in 2009 when three

observational studies evaluating cancer risks with different types of insulin were published concurrently [8–10], fuelling speculation of an increased risk of cancer (in particular breast cancer) associated with the insulin analogue insulin glargine, due to its higher affinity for the IGF-1 receptor in comparison with human insulin. More recently, several studies have found a lack of relationship between insulin glargine and overall cancer incidence [11–14].

Apart from insulin, other glucose-lowering therapies have been involved in the relationship between type 2 diabetes and cancer. Several observational studies have suggested an increased risk of cancer or cancer mortality with sulfonylureas [15–17]. This finding could be explained by sulfonylureas capacity to increase circulating insulin levels. Glucagon-like peptide-1 Receptor (GLP-1R) agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors have also been associated with thyroid and pancreatic cancer [18]. By contrast, metformin [19–25] and thiazolidinediones (TZDs) [26–28] have been associated with a reduced risk of cancer. This association may be due to the ability of these drugs to reduce insulin resistance.

Given the clear relationship between type 2 diabetes and cancer incidence it seems important to dissect the potential role of any glucose-lowering therapy in the cancer risk. In this regard, the aim of the present study was to evaluate the impact of glucose-lowering agents in the risk of cancer in a large type 2 diabetic population.

## Methods

### Data Source

The information was obtained from the Catalan Institute of Health and electronically fielded by using the System for the Development of Research in Primary Care (SIDIAP) database. This comprises the clinical information coded in the corresponding medical records from 274 Primary Health Care Centers (PHCC) with a total of 3,414 general practitioners and with a global adult (over 15 years old) population of 7,434,632 subjects. The SIDIAP includes data from primary care electronic medical records (demographics, consultations with GPs, diagnoses, clinical variables, prescriptions and referrals), laboratory test results and medications (obtained from CatSalut prescription drug pharmacy invoice database) [29].

For the present study, the validity of the Electronic Health Records (EHR) data was assessed by checking whether the continuous variables were within biologically plausible ranges. The reliability of the EHR data was assessed by assuring the concordance between related variables such as frequency of diabetes diagnosis and frequency of antidiabetic drugs use. In addition, the prevalence of type 2 diabetes and the use of antidiabetic drugs obtained in the 16 PHCC included in the study was within the range reported in our country. Since the SIDIAP database was established, different studies have confirmed the validity of its information [29–33].

The diagnosis of type 2 diabetes and cancer was established according the *International Classification of Diseases*, 10<sup>th</sup> revision (ICD-10) [34]. A case was defined as a patient with cancer diagnosis retrieved from the Hospital Vall d'Hebron Cancer Registry. This is an anonymous specialized registry that includes all patients with cancer diagnosis attending this hospital and collects demographic and clinical data, cancer site, pathology description, cancer diagnosis date (the first ever mention of cancer irrespective of its stage), and death (if applicable).

This clinical investigation was conducted according to the principles expressed in the Declaration of Helsinki. The Study Protocol was approved by the Clinical Research Ethics Committee of Hospital Universitari Vall d'Hebron (Barcelona). This Com-

mittee waived the need for written informed consent. All data were anonymized and the confidentiality of medical records was respected at all times according to the law (Organic Law 15/1999 on the Protection of Personal Data. <http://www.boe.es/boe/dias/1999/12/14/pdfs/A43088-43099.pdf>). As this study was a retrospective analysis, there was no need for informed consent.

### Study Cohort

A total of 275,164 type 2 diabetic patients older than 40 years registered in the SIDIAP and attending 16 Primary Health Care Centers (PHCC) of Barcelona were included in the study.

### Case and control selection

A nested case-control study was conducted within the defined cohort following the Strobe rules [35].

Cases (n = 1040) comprised those type 2 diabetes subjects with any cancer [10th Revision (ICD-10) codes version: C00-C97 [34]] diagnosed between 2008 and 2010, registered at the Cancer Registry of Hospital Universitari Vall d'Hebron (Barcelona).

In order to perform the analysis of the present study both databases, SIDIAP and the Hospital Universitari Vall d'Hebron Cancer Registry were linked. Exclusion criteria were: 1) No sufficient elapsed time (<1 year) from diagnosis of diabetes and cancer detection. 2) Less than two visits to the PHCC in the last year. 3) Patients who had received treatment outside of the Catalan Institute of Health service system. 4) Patients in whom the diagnosis of cancer were non-coincident between the Cancer Registry of Hospital Universitari Vall d'Hebron and the SIDIAP database. Controls were obtained from the population with type 2 diabetes registered in SIDIAP (n = 275,164). After excluding those patients with a history of cancer and those with less than two visits at the PHCC, a total of 235,332 possible controls were obtained. Then, three controls for each case were matched by age (+/–5 years), sex, diabetes duration (+/–1.5 years), and geographical area (from the same PHCC).

### Exposure assessment

Data on medication use were obtained from the CatSalut prescription drug pharmacy invoice database. For each case, the treatment received during the 3 years before the diagnosis of cancer was taken into consideration. Treatment with antidiabetic drugs had to have started at least 6 months prior to the diagnosis of cancer. The variable treatment was gathered in 10 groups: insulin glargine, insulin detemir, human insulin, fast-acting insulin and analogues, metformin, sulfonylureas, repaglinide, thiazolidinediones (TZD), DPP-4 inhibitors, and alpha glucosidase inhibitors. For each treatment, exposure time and dose defined as DDD (Daily Dose Defined) divided by total number of days were considered in the analysis.

GLP-1R agonists were not included because they were not licensed in Spain.

### Variables

The information obtained from SIDIAP database included the following variables: gender, age (calculated from birth date), duration of diabetes (calculated from diabetes diagnosis date), BMI (body mass index) at the time of cancer diagnosis, smoking habit (one or more cigarettes per day) classified as former and current smokers versus nonsmokers, and alcohol intake (regular intake of >1 drink per day) classified as yes/no at the time of cancer diagnosis. HbA1c level measured within the 6 months previous to cancer diagnosis and the defined daily dose (DDD) and duration of

treatment (months) of glucose-lowering agents were taken into account. Finally, the use of statins and aspirin, two drugs commonly used by type 2 diabetic patients that have been associated with a reduced risk of cancer [36] were also considered.

Information about cancer diagnosis date, tumor location, pathological characteristics, date of death (if applicable) was also collected from the Cancer Registry of Hospital Universitari Vall d'Hebron.

### Statistical analysis

To estimate the sample size, the following proportions of treatments were used: insulin glargine (7.25%), insulin detemir (3.48%), insulin NPH (7.35%), and without pharmacological treatment (22.92%). To estimate the odds ratio (OR) of exposure to different insulins and oral hypoglycemic agents, we considered the prevalence of insulin detemir because it required a larger sample size. A total of 554 cases and 1662 controls were necessary in order to detect an OR at least of 2 in patients exposed to treatment compared to those not receiving, using a two-tailed significant alpha level of 0.05 and 80% of power. These calculations were performed with PASS 2008 and with EPIDAT, version 3.1.

An initial descriptive analysis was performed using mean (standard deviation) for quantitative variables and frequency (percentage) for qualitative variables. To assess differences between cases and controls Chi-squared or Fisher test for qualitative variables and T-test or Kruskal test for quantitative variables were performed.

We conducted a conditional logistic regression to estimate the crude and adjusted OR (95% CI) of risk of cancer for each treatment group. Since the design of the study is a nested case-control study (1 case and 3 matched controls), a total of 764 groups were considered for the analysis. In each group the case and the 3 controls could be either exposed or non-exposed. In order to calculate the adjusted OR the following confounders were taken into account: age, BMI, alcohol use, smoking and duration of diabetes. In order to take into account the dose and duration of each treatment, two more conditional logistic regression models were performed for each glucose-lowering drug. The main exposure was dose (DDD in tertiles vs. non-users) in the first one and duration (months in tertiles vs. non-users) in the second one. All these models were also adjusted for the same explanatory variables and for the use of other glucose-lowering drugs. The methodology chosen for the final models was the one developed by Hosmer and Lemeshow [37]. The level of statistical significance was 0.05 and all the analysis were performed using IBM SPSS Statistics 19 and R software version 2.10.1.

### Results

Out of 1040 diabetic patients with cancer according to the inclusion criteria, 276 cases were excluded. Among them, 164 were excluded because diabetes mellitus was diagnosed in the year previous to cancer diagnosis; 14 cases did not make at least 2 visits to PHCC during the year previous to cancer diagnosis and finally 98 cases were excluded because the diagnosis of cancer was not well defined. Therefore, a total of 764 patients (34.5% female and 65.5% male) diagnosed with cancer were considered for the study. The number of patients by cancer site is shown in Table 1.

From 235,332 possible controls, 2,292 (34.5% women and 65.5% men) were selected according to similarity with the corresponding assigned case (3 controls for each case). Figure 1 show the flow chart of the selection patient process.

**Table 1.** Number of cases by cancer site included in the study.

Cancer Site	n	%
Colon	108	14.14
Lung	96	12.57
Prostate	96	12.57
Breast	81	10.60
Urinary bladder	80	10.47
Stomach	43	5.63
Uterus	40	5.24
Liver	40	5.24
Oral cavity	36	4.71
Pancreas	24	3.14
Other	120	15.69

doi:10.1371/journal.pone.0079968.t001

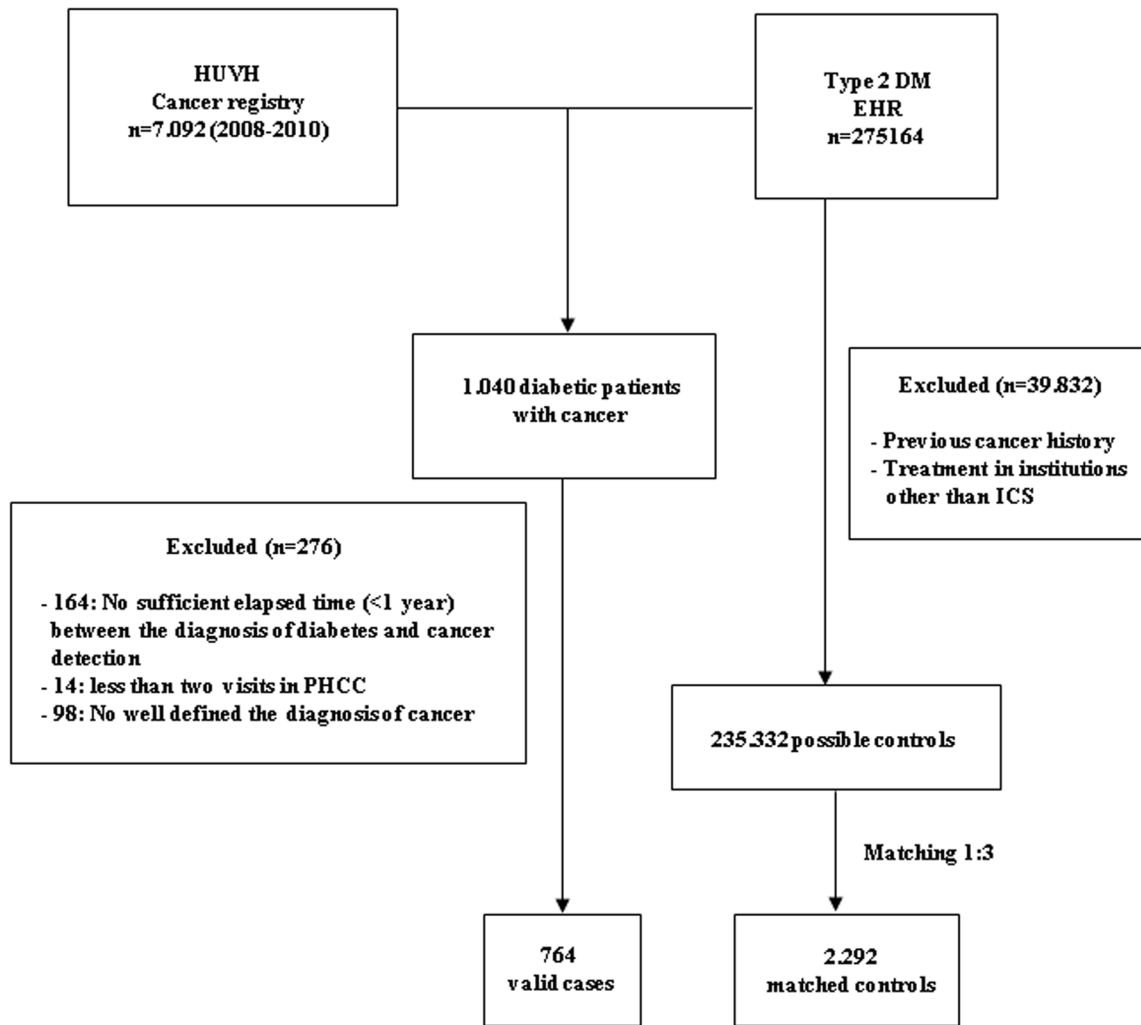
Information regarding cases and controls related to demographic characteristics and treatment received are summarized in Tables 2 and 3. The mean age of cases and controls were 72 (SD 9.1). As reported in Table 2, case subjects and matched controls were similar in age, gender, diabetes duration and HbA1c levels. BMI and smoking habit were higher in cases than in control group but did not reach statistical significance.

Regarding antidiabetic treatment, no statistically significant differences were observed between case and control subjects for the proportion, dose or duration of exposure to each treatment (Table 3). Since a dose higher than 0.3 UI/Kg/day has been associated with an increase of cancer risk [38] we have also considered this cut-off point in the analysis of the results. Figure 2 shows the risk of cancer associated with several types of insulin and oral agents analyzed as “use” versus “non-use”, and adjusted for age at cancer diagnosis, BMI, alcohol consumption, tobacco and duration of diabetes. None of them showed a significant increase in the risk of cancer. Given that no differences between cases and controls in the use of statins and aspirin were observed (data not shown), these variables were not included in the multivariate analysis.

When considering the dose and duration of each glucose-lowering drug, no statistically significant differences were observed. Among those patients who received glargine treatment, the adjusted OR of DDD divided by duration of treatment in days was 0.55 (95% CI = 0.24–1.25) in the first tertile, 1.37 (95% CI 0.70–2.66) in the second tertile and 1.11 (95% CI 0.55–2.25) in the third tertile. The OR of duration of treatment in months among those who received glargine treatment was 1.10 (95% CI = 0.55–2.22), 0.95 (95% CI = 0.55–1.64) and 0.68 (95%CI = 0.15–3.17) in the first, second and third tertiles, respectively. For both analyses ‘no glargine treatment’ was the reference category. Finally, no increased cancer risk was observed when glargine was used alone or in combination with metformin (OR 0.94 [95%CI 0.48–1.86] vs. 0.99 [95%CI 0.58–1.69]).

### Discussion

This study was aimed to evaluate the impact of glucose-lowering agents in the risk of cancer in a large type 2 diabetic population. We have found a lack of relationship between glucose-lowering therapies and cancer incidence.



**Figure 1. Flow chart of selection of cases and controls.** EHR: Electronical Health Record (Barcelona region); HUVH: Hospital Universitari Vall d'Hebron. ICS: Catalan Institute of Health; PHCC: Primary Health Care Centers.  
doi:10.1371/journal.pone.0079968.g001

In recent years, insulin glargine has been suggested to increase the risk of breast cancer in type 2 diabetic patients [8–10,39]. By contrast, metformin seemed to reduce the cancer in the same population of patients [19–25,40]. However, several potential caveats and methodological concerns have been raised in some of these studies including but not limited to cohort definitions, data elements and analytical approach.

The possible relationship between insulin glargine and the increase of cancer risk has been a topic which has generated intense debate in recent years. A significant increased risk for all types of cancer [8], breast cancer in women [9,10,12], and prostate and pancreatic cancer in men [11] has been reported in some studies. Conversely, some others have found a neutral effect [11–14] or even a significant protection for overall cancer incidence [38]. In our study, we did not find any increase of risk of cancer associated with insulin glargine with a mean exposition time of 2 years. Our results agree with the recent French nationwide cohort study based on administrative databases with a median follow-up of 2.67 years [41], and the ORIGIN trial with a prospective follow up of up to 7 years [42]. Moreover, we did not find any relationship between doses of insulin glargine and risk of cancer.

Insulin glargine is a recombinant DNA analog of human insulin with three amino acid substitutions: two arginine residues are added to the chain B at positions 31 and 32 and glycine is substituted for histidine at position 21 in the A chain. These amino acid substitutions result in an increased binding affinity for the insulin and insulin-like growth factor-1 (IGF-1) receptors, six to eight times that of human insulin [42]. *In vitro* studies have shown that glargine is more mitogenic than human insulin and promotes proliferation of certain tumor cells [43]. However, this mitogenic activity has been shown exclusively at supraphysiological concentrations (nanomolar/micromolar) and it is related to the expression of the IGF-1 receptor; the effect being present in cells with high levels of the receptor and absent in cells with limited or no IGF-1 receptor expression. In animal studies, glargine did not promote tumor growth, despite being administered at these supraphysiological concentrations, which are unlikely to be achieved in the clinical practice as the doses needed to produce these concentrations are liable to lead to hypoglycemia. Furthermore, glargine in *in vivo* experiments is rapidly transformed into its metabolites, the metabolic and mitogenic characteristics of which have been shown to be broadly equal to or even lower than human insulin [44]. Finally, the concentration of insulin glargine reaching the tumoral

**Table 2.** Clinical characteristics of the diabetic population included in the study.

		TOTAL	Cases	Controls	p-value
N of patients		3056	764	2292	
Gender	n(%)	3056 (100)	764 (100)	2292 (100)	
	Male	2000 (65.5)	500 (65.5)	1500 (65.5)	
	Female	1056 (34.5)	264 (34.5)	792 (34.5)	1.000
Age at cancer in years	n (%)	3056 (100)	764 (100)	2292 (100)	
	mean (SD)	72.0 (9.1)	72.0 (9.1)	72.0 (9.1)*	0.975
Age at DM in years	n (%)	3056 (100)	764 (100)	2292 (100)	
	mean (SD)	65.6 (9.5)	65.6 (9.5)	65.6 (9.5)	0.961
Diabetes' duration in years	n (%)	3056 (100)	764 (100)	2292 (100)	
	mean (SD)	6.4 (4.4)	6.4 (4.4)	6.4 (4.4)	0.864
BMI in kg/m <sup>2</sup>	n (%)	2602 (85.1)	669 (87.6)	1933 (84.3)	
	mean (SD)	29.6 (4.6)	29.3 (4.6)	29.2 (4.6)	0.057
Alcohol	n (%)	3056 (100)	764 (100)	2292 (100)	
	yes	110 (3.6)	31 (4.1)	79 (3.4)	
	no	2946 (96.4)	733 (95.9)	2213 (96.6)	0.433
Tobacco	n (%)	2932 (95.9)	732 (95.8)	2200 (96.0)	
	yes/former	1073 (36.6)	290 (39.6)	783 (35.6)	
	never	1859 (63.4)	442 (60.4)	1417 (64.4)	0.051
HbA1C <sup>†</sup>	n (%)	2000 (65.4)	522 (68.3)	1478 (64.5)	
	mean (SD)	6.9 (1.4)	6.9 (1.4)	6.9 (1.4)	0.812

\*Age at day of cancer of the corresponding case.

<sup>†</sup>At least into the 6 months previous to cancer diagnosis.

m (SD) = mean and standard deviation.

DM = Diabetes Mellitus type 2.

doi:10.1371/journal.pone.0079968.t002

cells is unknown but, as can be deduced, much lower than that used *in vitro*. Thus, the suggestion of increased relative mitogenic potency of insulin glargine seen in some cell lines does not appear to carry over to the *in vivo* behavior in animals and humans.

A small number of studies found a higher risk of cancer or cancer death among individuals with diabetes who were treated with sulfonylureas compared with those treated with metformin or other anti-diabetic drugs [15–17]. However, a recent meta-analysis did not find that sulfonylurea affected the risk of any type of cancer [45].

Several studies have suggested that both metformin [19–25] and TZD [26–28] are associated with a reduced risk of cancer in diabetic subjects. Given the biologically plausible link between diabetes and cancer, mediated through insulin resistance and hyperinsulinemia, this effect may be due to the ability of these drugs to reduce insulin resistance, although there may also be specific cellular mechanisms, mediated in part through AMP-activated protein kinase (AMPK) signaling pathways [46]. However, a recent meta-analysis failed to confirm this protective action of metformin [47] and even an increased risk of bladder cancer has been associated with TZDs [48]. We did not find that either metformin or TZDs had any effect on the risk of cancer in our type 2 diabetic population.

Incretin mimetics have been associated with an increased risk of pancreatic cancer [18]. However, this association was based on the Food Drug Administration Adverse Event Reporting System (FAERS) database which cannot be considered an acceptable registry to compare adverse event rates between drugs because of reporting biases and incomplete data. We found a lack of relationship between DDP-4 inhibitors and the risk of cancer.

Most of the published results on the potential influence of glucose-lowering treatments and cancer incidence have been drawn from observational studies with different approaches. This is considered to be the main reason for the controversial results between the studies. In addition, there are several limitations in the clinical assessment in these observational studies including the failure to correct for BMI, the duration of diabetes, the dosage of glucose-lowering treatments, the impossibility of breaking down the risk of cancer from a general to a tumor-specific risk, and the lack of information on tobacco use. In our study, we have considered all these confounding factors and we have not found any effect of glucose-lowering agents on the risk of cancer. In addition, all the patients included in the study were periodically followed by physicians and nurses working for the same health-care provider (*Catalan Health Institute*) using similar protocols and the same electronic clinical record registry. Furthermore, a central Cancer Registry was used. This is worth mentioning because 98 out of 1,040 diabetic patients were excluded because of discrepancies between the clinical record and the cancer registry. Another point to be commented on is the so called “protopathic bias”. This is the bias towards the use of more potent antidiabetic agents due to the poor glycemic control secondary to the presence of a hidden cancer. In this regard, the protopathic bias has been reported as the main factor accounting for the increased risk of cancer in the first 6 months after starting treatment with insulin and sulfonylureas [49]. In the present study the 6 month window for antidiabetic treatment prior to cancer diagnosis seems a sufficient period to significantly reduce any eventual protopathic bias. Finally, a potential bias due to the use of statins and aspirin, two drugs commonly used by type 2 diabetic patients that exhibit



**Table 3.** Glucose-lowering agents received by the diabetic population included in the study.

		TOTAL	Cases	Controls	p-value
N of any treatment		2438	609	1829	
Insulin Glargine	n(%)	128 (4.2)	31 (4.1)	97 (4.2)	0.917
	Duration months, m(SD)	20 (10.9)	20.1 (12.2)	19.9 (10.5)	0.920
	n° DDD, m(SD)	455.5 (305.9)	475 (309.8)	449.2 (305.9)	0.709
	n° DDD/days, m (SD)	0.84 (0.44)	0.88(0.36)	0.83 (0.46)	0.336
	Units/Kg/day, n°	108	24	84	
	Units/Kg/day, m (SD)	0.44 (0.24)	0.49 (0.21)	0.43 (0.25)	0.128
	Units/Kg/day≥0.3, n(%)	81 (75.0)	21 (87.5)	60 (71.4)	0.180
Human Insulin NPH	n(%)	392 (12.8)	90 (11.8)	302 (13.2)	0.349
	Duration months, m(SD)	27 (10.9)	27.1 (11.5)	26.9 (10.7)	0.668
	n° DDD,m(SD)	781.2 (616.8)	743.9 (497.6)	792.4 (648.4)	0.805
Insulin Detemir	n(%)	98 (3.2)	27 (3.5)	71 (3.1)	0.554
	Duration months, m(SD)	20.2 (10.7)	21.2 (11.3)	19.8 (10.6)	0.588
	n° DDD, m(SD)	567.5 (501.0)	476.4 (318.0)	602.1 (553.1)	0.408
Fast-acting insulin and analogues	n(%)	184 (6.0)	53 (6.94)	131 (5.7)	0.220
	Duration months, m(SD)	27.8 (9.7)	29.5 (8.5)	27.2 (10.1)	0.277
	n° DDD,m(SD)	892.9 (625.6)	999.5 (711.3)	849.8 (584.9)	0.255
Metformin	n(%)	1762 (57.7)	430 (56.3)	1332 (58.1)	0.375
	Duration months, m(SD)	27.9 (10.1)	27.6 (10.4)	28 (10.0)	0.433
	n° DDD, m(SD)	619.7 (386.1)	613.8 (389.2)	621.6 (385.2)	0.648
Sulfonylureas	n(%)	1233 (40.4)	294 (38.5)	939 (41.0)	0.233
	Duration months, m(SD)	28 (9.9)	28.6 (9.5)	27.8 (10.0)	0.221
	n° DDD, m(SD)	1114.2 (909.5)	1086.5 (834.6)	1122.9 (932.0)	0.971
Repaglinide	n(%)	164 (5.4)	40 (5.2)	124 (5.4)	0.926
	Duration months, m(SD)	23.8 (11.5)	22.9 (10.7)	24.1 (11.8)	0.474
	n° DDD, m(SD)	527.7 (466.1)	564.7 (477.9)	515.8 (463.6)	0.799
Thiazolidinediones	n(%)	146 (4.8)	33 (4.3)	113 (4.9)	0.557
	Duration months, m(SD)	22.5 (10.6)	22.1 (11.2)	22.6 (10.5)	0.874
	n° DDD,m(SD)	661.1 (440.5)	619.3 (439.2)	673.3 (442.1)	0.398
DPP-4 Inhibitors	n(%)	75 (2.5)	19 (2.5)	56 (2.4)	1.000
	Duration months,m(SD)	11.8 (6.6)	10.7 (5.3)	12.1 (6.9)	0.591
	n° DDD, m(SD)	367.9 (213.5)	308.7 (164.9)	388.1 (225.4)	0.209
Alpha Glucosidase Inhibitors	n(%)	115 (3.8)	24 (3.1)	91 (4.0)	0.325
	Duration months, m(SD)	23 (12.0)	19.3 (10.6)	24 (12.2)	0.038
	n° DDD, m(SD)	382 (350.3)	263.2 (204.4)	413.4 (374.2)	0.132

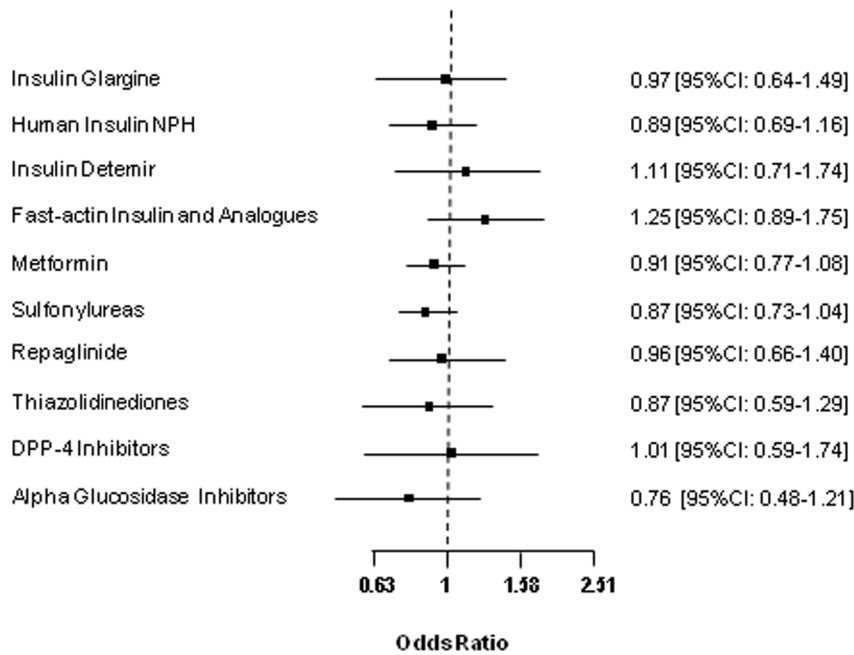
m (SD) = mean and standard deviation.

doi:10.1371/journal.pone.0079968.t003

an antineoplastic effect [36] can be ruled out because we did not find any differences between cases and controls in the use of these drugs.

This study has several limiting factors: 1) The information regarding glucose lowering treatments in HER was only available since 2005, and, therefore, we have no information on the occurrence of cancers after long-term exposure. 2) Our analysis only attributed a case of cancer when this event occurred at least 6 months after the start of exposure, a period that could be considered acceptable by exploring the antidiabetic drugs with an eventual oncogenic effect but probably short for drugs with potential protective effects like metformin. 3) Specific information on the effect of glucose-lowering agents by cancer site is not

provided because the low number of cancers per site prevented us to performing a valid statistical analysis. 4) Another problem is that a high number of patients were taking more than one antidiabetic drug, thus making it very difficult to dissect the cancer risk attributed to an individual treatment [50]. In addition, the comparator group for each antidiabetic drug was composed of the patients taking the remaining antidiabetic drugs. Ideally, the clinical effect of each antidiabetic drug should be evaluated by analyzing only diabetic patients with monotherapy and comparing them with those with no pharmacological therapy (only diet). However, this ideal scenario can hardly be achieved because a high percentage of type 2 diabetic patients are not in monotherapy, and treatments based only on



**Figure 2. Risk of cancer associated with drugs (use vs. non-use).** OR [CI95%] adjusted for age, BMI, duration of diabetes, alcohol and tobacco consumption.  
doi:10.1371/journal.pone.0079968.g002

diet are not now currently recommended. In fact, few studies comparing the incidence of cancer with antidiabetic drugs in monotherapy have been reported [35]. 5) Another limiting factor is the absence of available data on GLP-1 R agonists. These drugs were not included in data analyses because they were recently introduced into our country at study entry, thus resulting in a very low number of cases. 6) Finally, we rely on doses dispensed rather than dose administered, a reasonable but imperfect measure of real use.

In conclusion, our results suggest that anti-diabetic treatment does not influence the risk of cancer associated with type 2 diabetes. Therefore, an eventual increase of cancer should not be a

reason for biasing the selection of any glucose-lowering treatment in type 2 diabetic population.

**Acknowledgments**

The authors acknowledge the English language review by Mick Willy.

**Author Contributions**

Conceived and designed the experiments: RS OPR JT CV. Performed the experiments: DP RM XM LV CH IF AP. Analyzed the data: RS OPR DP LV CH AP CV. Contributed reagents/materials/analysis tools: AP JT CV. Wrote the paper: RS OPR DP RM XM LV CH IF AP JT CV.

**References**

- Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, et al (2010) Diabetes and cancer: a consensus report. *Diabetes Care* 33: 1674–1685.
- The Emerging Risk Factors collaboration, Seshasai SR, Kaptoge S, Thompson A, Di Angelantonio E, et al. (2011) Diabetes mellitus, fasting glucose, and risk of cause-specific death. *N Engl J Med* 364: 829–841.
- Johnson JA, Bowker SL (2011) Intensive glycaemic control and cancer risk in type 2 diabetes: a meta-analysis of major trials. *Diabetologia* 54: 25–31.
- Smith U, Gale EA (2009) Does diabetes therapy influence the risk of cancer? *Diabetologia* 52: 1699–1708.
- Renchan AG, Roberts DL, Dive C (2008) Obesity and cancer: pathophysiological and biological mechanisms. *Arch Physiol Biochem* 114: 71–83.
- Hsu IR, Kim SP, Kabir M, Bergman RN (2007) Metabolic syndrome, hyperinsulinemia, and cancer. *Am J Clin Nutr* 86: s867–s871.
- Giovannucci E, Michaud D (2007) The role of obesity and related metabolic disturbances in cancers of the colon, prostate, and pancreas. *Gastroenterology* 132: 2208–2225.
- Hemkens LG, Grouven U, Bender R, Günster C, Gutschmidt S, et al. (2009) Risk of malignancies in patients with diabetes treated with human insulin or insulin analogues: a cohort study. *Diabetologia* 52: 1732–1744.
- Jonasson JM, Ljung R, Talbäck M, Haglund B, Gudbjörnsdóttir S, et al. (2009) Insulin glargine use and short-term incidence of malignancies—a population-based follow-up study in Sweden. *Diabetologia* 52: 1745–1754.
- Colhoun HM, SDRN Epidemiology Group (2009) Use of insulin glargine and cancer incidence in Scotland: a study from the Scottish Diabetes Research Network Epidemiology Group. *Diabetologia* 52: 1755–1765.
- Chang CH, Toh S, Lin JW, Chen ST, Kuo CW, et al. (2011) Cancer risk associated with insulin glargine among adult type 2 diabetes patients- a nationwide cohort study. *PLoS ONE* 6: e21368.
- Suissa S, Azoulay L, Dell’Aniello S, Evans M, Vora J, et al (2011) Long-term effects of insulin glargine on the risk of breast cancer. *Diabetologia* 54: 2254–2262.
- Morden NE, Liu SK, Smith J, Mackenzie TA, Skinner J, et al (2011) Further exploration of the relationship between insulin glargine and incident cancer: a retrospective cohort study of older Medicare patients. *Diabetes Care* 34: 1965–1971.
- Lind M, Fahlén M, Eliasson B, Odén A (2010) The relationship between the exposure time of insulin glargine and risk of breast and prostate cancer: an observational study of the time-dependent effects of antidiabetic treatments in patients with diabetes. *Prim Care Diabetes* 6:53–59.
- Bowker SL, Majumdar SR, Veugelers P, Johnson JA (2006) Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. *Diabetes Care* 29: 254–258.
- Monami M, Balzi D, Lamanna C, Barchielli A, Masotti G, et al (2007) Are sulphonylureas all the same? A cohort study on cardiovascular and cancer-related mortality. *Diabetes Metab Res Rev* 23: 479–484.
- Monami M, Lamanna C, Pala L, Bardini G, Cresci B, et al. (2008) Treatment with insulin secretagogues and cancer-related mortality in type 2 diabetic patients: a retrospective cohort study. *Exp Clin Endocrinol Diabetes* 116:184–189.
- Elashoff M, Matveyenko AV, Gier B, Eashoff R, Butler PC (2011) Pancreatitis, pancreatic and thyroid cancer with glucagon-like peptide-1–based therapies. *Gastroenterology* 141:150–156.
- Evans JMM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD (2005) Metformin and reduced risk of cancer in diabetic patients. *BMJ* 330: 1304–1305.
- Currie CJ, Poole CD, Gale EA (2009) The influence of glucose lowering therapies on cancer risk in type 2 diabetes. *Diabetologia* 52: 1766–1777.

21. Libby G, Donnelly LA, Donnan PT, Alessi DR, Morris AD, et al (2009) New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. *Diabetes Care* 32: 1620–1625.
22. Decensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, et al. (2010) Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. *Cancer Prev Res (Phila)* 3: 1451–1461.
23. Zhang ZJ, Zheng ZJ, Kan H, Song Y, Cui W, et al. (2011) Reduced Risk of Colorectal Cancer With Metformin Therapy in Patients With Type 2 Diabetes: A meta-analysis. *Diabetes Care* 34: 2323–2328.
24. Noto H, Goto A, Tsujimoto T, Noda M (2012) Cancer risk in diabetic patients treated with metformin: a systematic review and meta-analysis. *PLoS One* 7(3):e33411
25. Col NF, Ochs L, Springmann V, Aragaki AK, Chlebowski RT (2012) Metformin and breast cancer risk: a meta-analysis and critical literature review. *Breast Cancer Res Treat* 135: 639–646.
26. Lewis JD, Capra AM, Achacoso NS, Ferrara A, Levin TR, et al. (2008) Thiazolidinedione therapy is not associated with increased colonic neoplasia risk in patients with diabetes mellitus. *Gastroenterology* 135: 1914–1923.
27. Govindarajan R, Ratnasingham L, Simmons DL, Siegel ER, Midathada MV, et al. (2007) Thiazolidinediones and risk of lung, prostate, and colon cancer in patients with diabetes. *J Clin Oncol* 25: 1476–1481.
28. Koro C, Barrett S, Qizilbash N (2007) Cancer risks in thiazolidinedione users compared to other anti-diabetic agents. *Pharmacoepidemiol Drug Saf* 16: 485–492.
29. Bolibar B, Fina Avilés F, Morros R, Del Mar Garcia-Gil M, Hermosilla E, et al. (2012) Grupo SIDIAP: [SIDIAP database: electronic clinical records in primary care as a source of information for epidemiologic research]. *Med Clin (Barc)* 138: 617–621.
30. García-Gil MM, Hermosilla E, Prieto-Alhambra D, Fina F, Rosell M, et al. (2011) Construction and validation of a scoring system for the selection of high-quality data in a Spanish population primary care database (SIDIAP). *Inform Prim Care* 19:135–145.
31. Ramos R, Balló E, Marrugat J, Elosua R, Sala J, et al. (2012) Validity for use in research on vascular diseases of the SIDIAP (Information System for the Development of Research in Primary Care): the EMMA study. *Rev Esp Cardiol (Engl Ed)* 65:29–37.
32. Vinagre I, Mata-Cases M, Hermosilla E, Morros R, Fina F, et al. (2012) Control of glycemia and cardiovascular risk factors in patients with type 2 diabetes in primary care in Catalonia (Spain). *Diabetes Care* 35: 774–779.
33. Violán C, Foguet-Boreu Q, Hermosilla-Pérez E, Valderas JM, Bolibar B, et al. (2013) Comparison of the information provided by electronic health records data and a population health survey to estimate prevalence of selected health conditions and multimorbidity. *BMC Public Health* 13:251e.
34. ICD-10 International Statistical Classification of Diseases and Related Health Problems 10th Revision Version for 2007. World Health Organization. WHO international website. Available: <http://www.who.int/classifications/apps/icd/icd10online>. Accessed 2012 Sep 10.
35. STROBE statement Website. Available: [http://www.strobstatement.org/fileadmin/Strobe/uploads/checklists/STROBE\\_checklist\\_v4\\_case-control.pdf](http://www.strobstatement.org/fileadmin/Strobe/uploads/checklists/STROBE_checklist_v4_case-control.pdf). Accessed 2012 Sep 20.
36. Singh S, Singh PP (2013) Statin a day keeps cancer at bay. *World J Clin Oncol* 4:43–46.
37. Hosmer DW, Lemeshow S (2000) *Applied Logistic Regression*. New York: John Wiley & Sons, 2000.
38. Mannucci E, Monami M, Balzi D, Cresci B, Pala L, et al. (2006) Doses of insulin and its analogues and cancer occurrence in insulin-treated type 2 diabetic patients. *Diabetes Care* 33: 1997–2003.
39. Ruitter R, Visser LE, van Herk-Sukel MP, Coebergh JW, Haak HR, et al. (2012) Risk of cancer in patients on insulin glargine and other insulin analogues in comparison with those on human insulin: results from a large population-based follow-up study. *Diabetologia* 55:51–62.
40. Ruitter R, Visser LE, van Herk-Sukel MP, Coebergh JW, Haak HR, et al. (2012) 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* 35: 119–124.
41. Fagot JP, Blotière PO, Ricordeau P, Weill A, Alla F, et al. (2013) Does Insulin Glargine Increase the Risk of Cancer Compared With Other Basal Insulins?: A French nationwide cohort study based on national administrative databases. *Diabetes Care* 36: 294–301.
42. ORIGIN Trial Investigators, Gerstein HC, Bosch J, Dagenais GR, Díaz R, et al. (2012) Basal insulin and cardiovascular and other outcomes in dysglycemia. *N Engl J Med* 367: 319–328.
43. Ciaraldi TP, Sasaoka T (2011) Review on the in vitro interaction of insulin glargine with the insulin/insulin-like growth factor system: potential implications for metabolic and mitogenic activities. *Horm Metab Res* 43: 1–10.
44. Pierre-Eugene C, Pagesy P, Nguyen TT, Neuillé M, Tschank G, et al. (2012) Effect of insulin analogues on insulin/IGF1 hybrid receptors: increase activation by glargine but not by its metabolites M1 and M2. *PLoS One* 2102, 7:e-41992.
45. Soranna D, Scotti L, Zambon A, Bosetti C, Grassi G, et al. (2012) Cancer risk associated with use of metformin and sulfonylurea in type 2 diabetes: a meta-analysis. *Oncologist* 17: 813–822.
46. Rattan R, Ali Fehmi R, Munkarah A (2012) Metformin: an emerging new therapeutic option for targeting cancer stem cells and metastasis. *J Oncol* 2012: 928127.
47. Stevens RJ, Ali R, Bankhead CR, Bethel MA, Cairns BJ, et al. (2012) Cancer outcomes and all-cause mortality in adults allocated to metformin: systematic review and collaborative meta-analysis of randomised clinical trials. *Diabetologia* 55: 2593–2603.
48. Mamtani R, Haynes K, Bilker WB, Vaughn DJ, Strom BL, et al. (2012) Association between longer therapy with thiazolidinediones and risk of bladder cancer: a cohort study. *J Natl Cancer Inst* 104: 1411–1421.
49. van Staa TP, Patel D, Gallagher AM, de Bruin ML (2012) Glucose-lowering agents and the patterns of risk for cancer: a study with the General Practice Research Database and secondary care data. *Diabetologia* 55: 654–665.
50. Singh S, Singh PP, Singh AG, Murad MH, Sanchez W (2013) Anti-diabetic medications and the risk of hepatocellular cancer: a systematic review and meta-analysis. *Am J Gastroenterol* 108: 881–891.