ASSOCIATION OF METFORMIN WITH BREAST CANCER INCIDENCE AND MORTALITY: A SYSTEMATIC REVIEW AND META-ANALYSIS

ASSOCIATION OF METFORMIN WITH BREAST CANCER INCIDENCE AND MORTALITY: A SYSTEMATIC REVIEW AND META-ANALYSIS

By

GRACE HOI LUM TANG, B.Sc.

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Author: Grace Hoi Lum Tang, BSc.

Supervisor: Dr. Paola Muti

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LAY ABSTRACT

Metformin is a common drug for people with diabetes. Animal studies have shown that metformin may also prevent breast cancer and improve overall survival. However in clinical research, the evidence is inconclusive. A literature review and analysis was conducted on all studies that compared metformin with other antidiabetic drugs to determine the effect on breast cancer diagnosis and prognosis.

It was found that metformin may improve overall survival of diabetic breast cancer patients. However, metformin was not shown to prevent the diagnosis of breast cancer. These results should be interpreted with caution due to the many limitations in observational research. Clinical trials are needed to truly determine the role of metformin in breast cancer risk and mortality.

ABSTRACT

Background Preclinical data suggests that metformin may have anti-cancer effects to reduce breast cancer incidence and improve cancer prognosis. However, the current evidence in observational studies is inconclusive. A systematic review and meta-analysis was conducted to assess the effect of metformin on the incidence and mortality of breast cancer in diabetic patients.

Methods A comprehensive literature search was performed on Medline (Pubmed), EMBASE, and the Cochrane library from inception to November 2016 with no language restrictions. Outcomes were incidence of breast cancer and all-cause mortality. Risk of bias and overall quality of evidence was assessed using the Newcastle Ottawa Scale and GRADE respectively. A meta-analysis was performed using the most adjusted odds ratios (ORs) or hazard ratios (HRs) and 95% confidence intervals (95% CI) as effect measures.

Results A total of 12 observational studies were included for breast cancer incidence and 11 studies for all-cause mortality. No significant association was found between metformin exposure and incidence of breast cancer (OR: 0.93, 95% CI: 0.85-1.03, $I^2 = 35\%$). A 45% risk reduction was observed for all-cause mortality (HR: 0.55, 95%CI: 0.44-0.70, $I^2=81\%$). Presence of publication bias is strongly suspected for both outcomes.

Conclusion The use of metformin in standard cancer therapy may improve overall survival of diabetic patients with breast cancer. No effect of metformin on the incidence of breast cancer was observed. Interpretation of results is limited by the observational nature of the studies and methodological biases. Clinical trials are warranted to determine the role of metformin in breast cancer risk reduction and prognosis.

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LIST OF ABBREVIATIONS

ADM	Antidiabetic medication
AMPK	Adenosine Monophosphate-activated Protein Kinase
BMI	Body Mass Index
CI	Confidence Interval
CTG	Clinical Trials Group
CVD	Cardiovascular Disease
DCF	Data Collection Form
DDFS	Distant Disease-free Survival
DFS	Disease-free Survival
EMR	Electronic Medical Record
ER	Estrogen Receptor
GRADE	Grading of Recommendations Assessment, Development, and Evaluation
Hb1Ac	Glycated Hemoglobin
HER2	Human Epidermal Growth Factor Receptor 2
HR	Hazard Ratio
ICES	Institute of Clinical Evaluative Sciences
IGF	Insulin Growth Factor-1
IHC	immunohistochemistry
ITT	Intention-to-treat
LKB1	Liver Kinase B1
mTOR	Mammalian Target of Rapamycin
NOS	Newcastle-Ottawa Scale
OR	Odds Ratio
pCR	Pathological Complete Response
PR	Progesterone Receptor
PRISMA	Preferred Reporting for Systematic Reviews and Meta-Analyses
RCT	Randomized Controlled Trials
RoB	Risk of Bias
ROS	Reactive Oxygen Species
RR	Relative Risk
SoF	Summary of Findings
SRR	Summary Relative Risk
T2DM	Type 2 Diabetes Mellitus
TSC2	Tuberous Sclerosis Complex Protein 2
TZD	Thiazolidinediones

DECLARATION OF ACADEMIC ACHIEVEMENT

As the primary author of this Master's thesis, I was responsible for most of the work presented. I designed the study, refined the research question and methods, and drafted the protocol. I was responsible for organizing the administrative aspects of this study which included: timeline of project, coordination with the second and third reviewer, designing the search strategy, retrieving all titles/abstracts, organizing the broad and secondary screen, full text review, designing the broad/secondary screen questions, and designing of the data extraction forms. As the primary author, I screened every article that was included in the broad and secondary screen, and extracted all the necessary data. I managed the data extraction forms and database, and prepared all the data for meta-analysis. I calculated the reviewer agreement, performed the meta-analyses and all related secondary analyses. Finally, I drafted every section of this manuscript and created all tables and diagrams.

1.0 INTRODUCTION

1.1 Breast Cancer

Breast cancer develops from the breast tissue and cells [1]. It is the most common cancer diagnosis in Canada [1]. In 2016, approximately 25,700 Canadian women were diagnosed with breast cancer, representing 26% of all newly diagnosed cancer cases in women [1]. Breast cancer is the second leading cause cancer of death in women, accounting for 13% of female cancer deaths. In Canada, the estimated 5-year survival of breast cancer is approximately 87%, ranging from 100% (stage I) to 22% (stage IV) [1]. Most recent population data suggests that there are approximately 90,685 women who were diagnosed since 2004 and remain living today [1].

At the global level, breast cancer is the most common cancer in the world [2]. In 2013, it was reported that there were 1.8 million incident cases of breast cancer and 464,000 deaths [2]. Incidence and survival rates vary greatly worldwide between developed and less developed countries [2]. Breast cancer 5-year survival rates in North America range from 80% or over, in Sweden and Japan range around 60%, and in low income countries range below 40% [3]. The incidence of breast cancer in developing countries is also rapidly increasing compared to the stable decline in developed countries [2]. The difference in diagnosis and prognosis in breast cancer in high versus low income countries can be explained by the lack of early detection programs and lack of access to diagnostic and treatment facilities, resulting in women presenting with late-stage disease [3].

In addition to access to healthcare, several risk factors play a role in the diagnosis and prognosis of breast cancer. Known risk factors include but are not limited to: family history, BRCA gene mutation, hormone replacement therapy, obesity, and alcohol use [1]. Factors that determine the prognosis of breast cancer include: stage at diagnosis, lymph node invasion, age,

hormone receptor status (estrogen, progesterone), and human epidermal growth factor receptor 2 (HER2) status [4].

1.1.1 Breast Cancer Classification

Breast cancer is a heterogeneous disease and can be classified into several subtypes based on distinct molecular profiles [5]. Classical immunohistochemistry (IHC) markers include estrogen receptors (ER), progesterone receptor (PR), HER2, ki67, and p53 [6,7]. Based on IHC classifications and differential expression of various genes, breast cancer can be further categorized into five major molecular subtypes: luminal A, luminal B, HER2 over-expression, basal (triple negative), and normal-like [5,6]. Prognosis and response to treatment is dependent on the combination of IHC classifications and traditional clinicopathological variables (i.e. tumour size, nodal invasion) [6].

1.2 Type 2 Diabetes Mellitus

Diabetes mellitus is a group of metabolic diseases where the body cannot maintain appropriate glycemic levels [8]. Type 2 diabetes mellitus (T2DM), also known as noninsulin-dependent diabetes, occurs when the body resists the effect of insulin or insulin production is inadequate to maintain glucose levels. Symptoms of T2DM include but are not limited to: frequent urination, weight change, blurred vision, unusual thirst, and extreme fatigue [8].

T2DM is one of the largest health burdens worldwide and in Canada [8,9]. In Canada, the prevalence of T2DM has significantly increased by 70% between 1999 to 2009 [8]. Notably, this increase was seen in the 35 to 39 and 40 to 44 age groups; likely due to the increasing rates of obesity, a significant risk factor for T2DM [8]. Moreover, the estimated prevalence of T2DM

in 2016 is approximately 3.5 million Canadians [10]. According to the Canadian Diabetes Cost Model, the projected prevalence of T2DM in 2026 is 4.9 million [10].

T2DM itself typically does not directly lead to mortality; however, its associated complications often lead to premature death. Complications of T2DM include but are not limited to: cardiovascular disease (CVD), renal failure, blindness, and neurological abnormalities [8,10]. Diabetics are three times more likely to be hospitalized with CVD compared to those without [8]. A report by the Institute of Clinical Evaluative Sciences (ICES) reported that diabetes contributes to 30% of strokes, 50% of kidney failure requiring dialysis, and 70% of non-traumatic lower limb amputations [11].

Risk factors for T2DM can be divided into modifiable and non-modifiable factors. Key modifiable risk factors include: obesity, physical inactivity, unhealthy eating, and tobacco smoking [8]. Non-modifiable risk factors for T2DM include family history and ethnicity[8].

The goal of T2DM management is to improve glucose control to reduce symptoms and delay related complications. The most common antidiabetic medication (ADM) in T2DM patients are oral hypoglycemic agents (metformin, sulfonylureas, thiazolidinediones (TZD)), and exogenous insulin [12]. In brief, oral hypoglycemic agents have three mechanisms of action: 1) stimulating insulin release from secreting beta cells in the pancreas, 2) enhancing insulin sensitivity in hepatic and peripheral tissues, and 3) reducing hepatic glucose output [13].

1.3 Type 2 Diabetes Mellitus and Breast Cancer

T2DM and cancer are two common multifactorial chronic diseases that can significantly decrease quality of life and overall survival [12,14]. The association between diabetes and cancer was identified as early as 1932 [12,15]. T2DM and cancer share many risk factors such as age,

sex, smoking, obesity, physical inactivity, and ethnicity [12,14,15]. Several meta-analyses and epidemiological studies have identified an increased risk for several types of cancers among diabetics [14].

T2DM is an independent risk factor for breast cancer, with increased risk ranging from 20% to 27% [16–19]. A meta-analysis by Boyle *et al.*, reported a relative risk (RR) of 1.27 (95% Confidence Interval (CI), 1.16-1.39), for breast cancer in women with diabetes. After adjustment for body mass index (BMI), the RR lowered to 1.16 (95% CI, 1.08-1.24), but remained statistically significant with a low heterogeneity [16].

Furthermore, large population based studies have also identified T2DM as an independent risk factor for breast cancer [20,21]. An Ontario study using ICES data confirmed this fact for advanced stage breast cancer, after adjusting for mammogram, age, income quintile, and comorbidities [20]. Furthermore, Michels *et al.* showed that women with T2DM had an elevated incidence of breast cancer compared to those without (hazard ratio (HR) =1.17, 95% CI=1.01-1.35) in the Nurses' Health Study [21].

The biological mechanism of breast cancer and T2DM has yet to be elucidated. Many studies have suggested that diabetes may play a role in malignant transformation and growth through hyperglycemia-related oxidative stress, insulin resistance, advanced glycation end products, and chronic low-grade inflammation [12,15,22,23]. Insulin can act as a growth hormone and is frequently over-expressed in malignant cells [20,24,25]. Altered glucose metabolism and fasting hyperglycemia have also been associated with breast cancer development and mortality in post-menopausal women [26–28]. A prospective multi-center case-cohort study found that the presence of metabolic syndrome (i.e. T2DM) was associated with increased breast cancer risk in all women, in particular in post-menopausal women [27]. Furthermore, it has been

reported that the Western sedentary lifestyle characterized by lack of physical activity and high fat diets may attribute to T2DM and breast cancer [24,29].

1.4 Literature Review of Metformin and Breast Cancer

There is emerging evidence suggestive of potential anti-cancer activity from metformin. Metformin, an inexpensive oral biguanide, is the most commonly prescribed first line therapy for T2DM patients to reduce blood glucose concentrations [30,5,31]. In brief, metformin has an anti-hyperglycemic effect mediated by inhibiting gluconeogenesis, decreasing glucose absorption from the small intestine, increasing glucose uptake in cells, and decreasing plasma free fatty acid concentration [30].

Metformin has an excellent safety profile with minimal side effects; mild gastrointestinal side effects (e.g. diarrhea, nausea, irritation of abdomen) are most commonly reported [30,32]. The major toxicity reported for metformin use is lactic acidosis, although this is very rare (9 per 100,000 cases) [5]. Furthermore, metformin has been administered alongside most cancer therapies without any known significant interactions [33]. Therefore, metformin is an excellent candidate as a potential anti-cancer drug to prevent cancer or delay cancer progression with minimal risks.

1.4.1 Preclinical Studies

Metformin as an anti-cancer therapy has been extensively studied in the preclinical setting, with several proposed direct, through the induction of cell metabolic modifications and indirect mechanisms through its blood glucose lowering properties and anti-inflammatory effects [15,30,5,34–37]. While the exact mechanisms have yet to be elucidated, the most widely accepted mechanism of action is the indirect activation of adenosine monophosphate-activated

protein kinase (AMPK) [5,15,38]. AMPK is an established molecular regulator of cell metabolism to suppress tumour growth [5,15,38]. The activation of AMPK has been shown to inhibit the mammalian target of rapamycin (mTOR) signaling pathway, known to promote cell growth and tumourgenesis [5,15,30,39,40]. AMPK increases tuberous sclerosis complex protein 2 (h2) activity, leading to the inactivation of mTOR and thereby decreasing protein synthesis and cell growth of cancer cells [15,30,41].

It has also been proposed that metformin can indirectly reduce the level of circulating insulin and insulin like growth factor 1 (IGF-1) [15]. Insulin/IGF-1 is involved in carcinogenesis through upregulation of the insulin/IGF receptor signalling pathway [15]. Through the insulin receptor substrate, a signal is transmitted to phosphoinositide 3-kinase and Akt/protein kinase B which indirectly activates the mTOR pathway [15]. As described by LeRoith *et al.,* many IGFs are expressed by various cancers including breast, and it may play an important role in changes of cellular metabolism that are typical of tumour cells [15,42].

Other proposed mechanisms of metformin as potential anticancer therapy include liver kinase B1 (LKB1) as a major upstream kinase of AMPK [5,15,43,44]. Phosphorylation of AMPK by LKB1 may potentially lead to the inhibition of mTOR [5,15]. A study by Shen *et al.,* reported that LKB1 plays a role in tumour suppression in human breast cancer [44]. When LKB1 protein is overexpressed in breast cancer cells, it can result in growth inhibition mediated by G1 arrest of the cell cycle [44]. Furthermore, low expression of LKB1 protein in human breast cancer is significantly associated with a shorter survival [44].

In specific breast cancer subtypes, Hadad *et al.* found that metformin can act as a growth inhibitor in both ER-positive and ER-negative breast cancer cells *in vitro* and arrest cells in the G1 cell cycle phase [45]. The authors hypothesize that the effect is likely mediated by AMPK

activation, and partly by the inhibition of fatty acid synthesis via acetyl-CoA carboxylase phosphorylation [45]. Another study found that metformin can decrease HER2-postiive expression by inhibiting p70S6K1 in human breast cancer cells, which is a downstream effector for mTOR [30,46].

Other proposed other mechanisms of action for metformin as anti-cancer therapy includes: inhibition of mitochondrial complex in the electron transport chain, and inhibition of *Ras*-induced reactive oxygen species (ROS) and DNA damage [47,48]. Nonetheless, the mode of action of metformin as anti-cancer therapy for diagnosis or prognosis remains unclear and under further investigation [5].

1.4.2 Systematic Reviews and Meta-Analyses

There are numerous epidemiological studies regarding the use of metformin as an agent for breast cancer prevention or as additional treatment for breast cancer. Observational studies range from single-center studies to large population based studies using administrative insurance claims data or electronic health records.

To date, there are several systematic reviews and meta-analyses published investigating the use of metformin in all types cancers in the diagnostic and prognostic setting [33,48–52]. The general consensus of these studies suggest that metformin may be associated with a reduction in risk of cancer and cancer related mortality [33,48–52]. However, few have focused on the association between metformin and breast cancer exclusively.

To our knowledge, there are four published systematic reviews and meta-analyses that investigated the incidence and mortality of metformin therapy in breast cancer patients only [53–56]. A meta-analysis by Col *et al.* pooled 7 studies investigating the use of metformin and breast

cancer found a significant risk reduction (Odds Ratio (OR)=0.83, 95%CI=0.71-0.97, $I^{2=}$=51%) [53]. In a subgroup analysis comparing longer duration of metformin, a stronger association was found (OR=0.75, 95%CI=0.62-0.91) [53]. Xu *et al.* investigated the effect of metformin to standard therapy on the prognosis of breast cancer survival and all-cause mortality [54]. The authors reported that metformin was associated with a 47% decreased risk of death in breast cancer patients (HR=0.53, 95%CI=0.39-0.71, I^2=78.9%) [54]. Yang *et al.* investigated metformin use for both breast cancer incidence and mortality in a total of 11 and 7 studies, respectively [55]. They found metformin did not reduce the incidence of breast cancer (RR=0.96, 95%CI 0.761-1.221, I^2=90.2%), but was associated with a reduction in all-cause mortality (RR=0.65, 95%CI 0.48-0.87, I^2=78.9%) [55]. Lastly, the most recent meta-analysis was conducted in 2016 by Moradi-Joo *et al.*, comparing metformin and sulfonylureas in T2DM patients for the risk of breast cancer [56]. The authors did not find a significant association in the pooled analysis (RR: 0.92, 95% CI=0.63-1.34, I^2=94%) [56].

While all studies used systematic review methodology and statistical methods, the quality of the studies varies. Yang *et al.* only analyzed articles available from PubMed, which limited the number of available articles for review [55]. Two systematic reviews did not conduct a manual search of grey literature through conference proceedings or bibliographies of published articles [55,56]. Another study by Xu *et al.* did not extract data in duplicate, which may reduce the accuracy of results [54]. Notably, all published studies did not use the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system to evaluate overall study quality and evidence [54–58]. Furthermore, all previously published meta-analyses did not consider the effect of time-related biases as described by Suissa & Azoulay [59]. The evidence currently supporting the role of metformin in breast cancer is inconsistent in the

literature. Therefore, we performed a meta-analysis which includes all recently published studies (including search of grey literature), and incorporates GRADE to evaluate the quality of studies.

2.0 OBJECTIVE

A systematic review and meta-analysis was performed with the objective to assess the current literature regarding the use of metformin and the incidence and mortality of breast cancer in T2DM patients compared to other commonly prescribed ADM (sulfonylureas, TZD and insulin therapy).

3.0 METHODS

The protocol was published on PROSPERO international prospective register of systematic reviews in January 2017 (CRD42017054888) [60]. This systematic review and meta-analysis was conducted following the Cochrane Handbook, and was reported as per Preferred Reporting for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [61,62].

3.1 Study Design Overview

This systematic review and meta-analysis examined the incidence and all-cause mortality of breast cancer in T2DM patients who have taken metformin compared to other common ADM. The research question is "Among type 2 diabetes mellitus patients, what is the incidence or mortality of breast cancer who received metformin therapy compared to those who received other therapy?"

3.2 Criteria for Selection Studies for Review

Inclusion Criteria:

Type of Studies: randomized controlled trials (RCTs), quasi-RCTs, cohort studies (prospective, retrospective), case control studies, quasi-experimental studies (interrupted time series studies, controlled before-and-after studies). No language restrictions as studies can be translated by outside resources.

Type of Participants:

For incidence studies: Females aged 18 years or older who were clinically diagnosed with T2DM

For mortality studies: Females aged 18 years or older who were clinically diagnosed with T2DM and breast cancer

Type of Intervention: Use of metformin oral therapy.

Type of Comparator: Other antidiabetic medications (e.g. sulfonylureas, TZD, insulin).

Type of Outcome:

- Incidence of breast cancer clinically diagnosed and pathologically confirmed
- All-Cause Mortality

Exclusion Criteria: Abstracts or conferences proceedings of studies without sufficient data to generate estimates of effect

3.3 Search Strategy

An electronic literature search on Medline (Pubmed) from 1946 to November 2016, EMBASE from OVID platform from 1966 to November 2016, and the Cochrane library from 1992 to November 2016 were conducted. The electronic search strategy was developed in consultation with individuals with a library sciences background. Search terms were used such as "metformin", "diabetes type 2", and "breast neoplasms". Search strategies were modified

appropriately for each respective database interface. See Appendix 1 for the Medline, EMBASE, and Cochrane Library full search strategies.

Additionally, studies were identified through bibliographies of published systematic reviews [53–56]. Grey literature was identified through a manual search from clinical trials registries and conference proceedings from major oncology and diabetes meetings (see Appendix 2 for list of sources). There were no language restrictions, as studies and abstracts could be translated from outside resources.

3.4 Data Collection

3.4.1 Selection of Studies

All citations identified from literature searches were merged, de-duplicated, and stored in EndNote X8 (Thomas Reuters, New York). Two reviewers (GT, MS) independently reviewed a list of citations by title and abstract (broad screen) using a standardized pilot-tested data collection form (DCF). Articles that did not satisfy the inclusion criteria (*see Section 3.2*) were excluded.

From the list of included studies from the broad screen, full published articles were retrieved. The two reviewers (GT, MS) used a standardized pilot-tested DCF for the secondary screen to determine the final selection of relevant studies. Conference proceedings found in grey literature that were not published as full text articles were also excluded. To avoid overlapping patient populations, articles with duplicate datasets were assessed by the most recent publication, and/or larger study duration, and population. Studies must also report a risk estimate (e.g. OR, RR, HR) with an estimate of precision, such as standard error or 95% CI, relating to the use of metformin by using regression models. Any disagreement or uncertainty in the broad and

secondary screen were resolved by a consensus or a consultation of a third party (AL). An unweighted kappa score was calculated to ensure agreement between the two independent reviewers (GT, MS) for inter-rater concordance for both broad and secondary screen.

3.4.2 Data Extraction and Management

After final selection of full text articles for quantitative analysis, the two reviewers (GT, MS) independently extracted information from each study. A standardized pilot-tested DCF was used to collect: study characteristics (date of publication, type of study), population characteristics (sample size, dose, duration), exposure ascertainment, adjustment for confounders, and outcome assessment (crude and adjusted OR, RR, or HR). The most fully adjusted estimate was recorded if several estimates were reported in the same article. Please see Appendix 3 for template of the data extraction forms. When appropriate, effect estimates and 95% CI were inverted to ensure comparator(s) (i.e. non-metformin) was the reference value. If studies included both non-diabetic patients and diabetics not on metformin treatment as comparators, we extracted only diabetics not on metformin treatment to minimize confounding by diabetes status.

3.4.3 Assessment of Risk of Bias in Included Studies

The Newcastle-Ottawa Scale (NOS) for non-randomized studies was used to assess risk of bias (RoB) for the included studies. The NOS uses a nine point ‘star system’ in which individual studies are judged based on: 1) selection of study groups (four stars), 2) comparability of groups (two stars), and 3) ascertainment of exposure and outcomes (three stars) [63,64]. The

acceptability criteria for the NOS was reviewed with an expert from the field of diabetes and breast cancer (PM).

The two reviewers (GT, MS) independently assessed each study using the NOS using a standardized pilot-tested DCF, and provided an overall score based on the assessment criteria (from 0 to 9 stars). Disagreements were resolved by consensus or a consultation of a third party (AL). To our knowledge, there is no established cut off for a 'low', 'moderate', or 'high' RoB for the NOS. As such, the authors relied on previous literature to determine a high RoB as a score of ≤ 5, moderate RoB as a score between 6 and 7, and low RoB as a score between 8 and 9 [65]. A weighted kappa score was calculated to ensure agreement between the two independent reviewers (GT, MS) for inter-rater concordance.

3.4.5 Grading of Recommendations Assessment, Development, and Evaluation (GRADE)

The overall quality of evidence across studies was assessed using the GRADE system as outlined by the GRADE Working Group [58]. The quality of evidence was summarized in four categories: high (4), moderate (3), low (2), and very low quality (1) [57]. The rating of the quality of evidence reflects the confidence that the estimates of effect are correct [57]. A body of evidence from observational studies starts as 'low quality (2)', whereas a body of evidence from RCTs start as 'high quality (4)' [57]. As per the GRADE criteria, reasons for quality to be rated downwards include: RoB, inconsistency, indirectness, imprecision, and publication bias [57,58]. Reasons to raise quality include: large magnitude of effect, dose-response relation, and all plausible residual confounding [57,58]. The primary author (GT) assessed the body of evidence for each outcome using the GRADE criteria for reasons to rate down or up, and a second author (HS) reviewed for accuracy.

Reasons to downgrade in RoB include: failure to develop and apply appropriate eligibility criteria, flawed measurement of both exposure/outcome, failure to control confounding, and short follow-up [66]. For inconsistency, differences in population and intervention, high heterogeneity identified by I^2, and overlapping CIs may downgrade the rating [67]. In regards to the directness of evidence, reasons to downgrade include: poor generalizability, transferability, and applicability [68]. Reasons to rate down for imprecision include small sample size and wide CI (i.e. uncertainty about magnitude of effect) [69]. Publication bias should always be suspected in rating of evidence, and marked down if funnel plot appears asymmetrical [70].

The three primary reasons for rating up the quality of evidence include: 1) when a large magnitude of effects exist, 2) when there is a dose-response gradient, and 3) when all plausible confounders or other biases were accounted for to increase the confidence in the estimated effect [71].

The effect estimates and quality of evidence were summarized in a GRADE evidence profile and summary of findings (SoF) table using GRADEPro (GRADE Working Group, McMaster University) [72]. The GRADE evidence profile presents a detailed quality assessment and judgement of each determining factor [58]. In contrast, the SoF table includes an assessment for each outcome, without detailed explanations [58].

3.4.6 Agreement Statistics

Agreement statistics were calculated using an online kappa calculator tool [73] between the two independent reviewers (GT, MS) for inter-rater concordance. An unweighted kappa

score was calculated after the initial comparison between reviewers for the broad and secondary screen. A weighted kappa was used for the RoB because the NOS uses an ordinal scale [74].

The value of the unweighted and weighted kappa was interpreted as by Altman *et al.* [75]. A kappa of <0.20 indicates poor strength of agreement, 0.21-0.4 indicates fair strength of agreement, 0.41-0.60 indicates moderate strength of agreement, 0.61-0.80 indicates good strength of agreement, and 0.81-1.00 indicates very good strength of agreement.

3.5 Data Analysis

3.5.1 Data Synthesis and Measure of Treatment Effect

Meta-analyses were performed using Review Manager Software 5.3 (Revman Computer Program, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012). Meta-analyses of observational studies were conducted using included studies that were sufficiently homogenous in population, and have appropriate intervention and outcomes to provide a meaningful summary of effect. All studies were pooled and weighted according to the inverse variance method using the calculator function. It is important to note that by using the calculator function in Revman 5.3, there may be minor differences in the upper 95%CI than what studies have reported. The random effects model was chosen as the main model of interest *a priori*; as the true effect sizes varies from study to study and the model assumes that there is inherent heterogeneity in the included studies [61,76].

Two separate forest plots were conducted for this study, one for each outcome (incideince and all-cause mortality). Only the most fully adjusted ORs and HRs were pooled in the final meta-analysis as unadjusted OR/HRs were not provided in most included studies. The longest duration of metformin use was pooled in the main analysis.

Dichotomous variables (i.e. diagnosis of breast cancer) were summarized in ORs for case-control studies. Time to event variables (i.e. time to breasts cancer diagnosis) were summarized in HRs for cohort studies. In the interest of capturing ORs from cohort studies in meta-analyses, HRs were converted to RR and then to OR using conversion equations. The conversion equations were obtained in private discussion with Prof. Diane Heels-Andsell, a statistician that specializes in meta-analysis from the Department of Health Research Methods, Evidence, and Impact, McMaster University. HR to OR conversions were completed in duplicate with another reviewer (MS) to ensure accuracy. Please see Appendix 4 for a summary and sample of HR to RR to OR calculations. For forest plots involving all-cause mortality, time to event variables (i.e. time to death) were summarized in HRs in cohort studies.

3.5.2 Missing Data

When appropriate, study authors were contacted for additional or missing data. Complete case analysis (i.e. include only available data) was conducted for any studies with missing data at random or with patients who were lost to follow-up. A sensitivity analysis was initially planned to assess the impact of missing data as per recommendations from the Cochrane Handbook [61]. However, due to unknown degree of missingness in several articles, the analysis was not performed.

3.5.3 Assessment of Heterogeneity

Heterogeneity was assessed using Cochran's Q statistic based on a χ^2 distribution (significant at $p < 0.10$) [61]. Degree of heterogeneity was interpreted using the chi-square I^2 test. An I^2 value $\leq 25\%$ was deemed low heterogeneity, 26 to 50% as moderate heterogeneity, and

>50% as high heterogeneity [61]. Visual inspection of forest plots also assessed heterogeneity by identifying the extent of overlap in the confidence intervals.

3.5.4 Subgroup Analyses

We conducted subgroup analyses to account for any significant levels of heterogeneity using the Sun *et al.* criteria [77]. A few sources of heterogeneity were hypothesized *a priori* including: age and BMI for both incidence and mortality analyses. However, subgroup analyses were not carried out due to similar age distribution among all studies and limited information regarding BMI in the metformin and non-metformin groups. Alternatively, a sensitivity analysis for BMI was conducted to compare studies that adjusted BMI compared to those who did not (see *Section 3.5.5*). All subgroup analyses were conducted using the random effects model. If a study contributed >50% of the total weight of all studies evaluated, an exploratory analysis was carried out such that the most weighted study will be removed.

A subgroup analysis was carried out for the duration of metformin treatment (>3 years vs. ≤3 years) for incidence of breast cancer. The decision to have a 3-year cutoff was based on Col *et al.*'s previous subgroup analysis [53]. Studies that reported ≥1 year were pooled as <3 years in the analysis. It was hypothesized that those with longer exposure to metformin will have better outcomes (lower frequency of breast cancer incidence) compared to those who did not because of the beneficial biological effects of metformin. There was an insufficient amount of studies for subgroup analysis for all-cause mortality.

For studies investigating the incidence of breast cancer, a subgroup analysis was conducted for each type of pooled ADM (i.e. sulfonylurea, insulin or 'non-metformin'). The main analysis included all types of ADMs, which introduces variability in the comparators and heterogeneity. It was hypothesized that metformin will have a stronger protective effect when

compared to insulin. A recent meta-analysis suggests that insulin may induce breast tumour progression by upregulating mitogenic signaling pathways [78]. It was hypothesized that sulfonylurea will have a null effect on breast cancer risk, due to the limited amount of evidence in the literature [79]. For studies that reported 'other ADM', they were pooled in an unspecified 'non-metformin' subgroup. There was an insufficient amount of studies for subgroup analysis for all-cause mortality, as all studies used 'non-metformin' as the comparator.

Lastly, a subgroup analysis was carried out to compare studies with time related-biases such as immortal time bias, time window bias, and time lag/latency bias. As described by Suissa & Azoulay, the effect of time-related biases are consistent and frequent in pharmacoepidemiologic studies [59] It was hypothesized that the effect size for studies without time-related biases will be closer to the null compared to studies with time-related biases.

3.5.5 Sensitivity Analyses

Sensitivity analyses were applied to test the robustness of estimates and any outlying study results. All sensitivity analyses were conducted using a random effects model. If a study contributed >50% of the total weight of all studies evaluated, an exploratory analysis was carried out such that the most weighted study will be removed. Sensitivity analyses for this study are as follows:

1. **Quality of Studies:** As stated *a priori*, an analysis was carried out for high/moderate RoB versus low RoB studies as measured using the NOS (i.e. studies with a score ≤ 7 vs. studies with a score >7).

2. **Obesity:** To compare studies that adjusted for obesity and/or body mass index (BMI), a known time-dependent confounder [80]. Obesity is an important confounder for patients with T2DM, as it increases risk for breast cancer and mortality.
3. **Type of Study:** To compare studies that investigated all types of cancer versus breast cancer specific studies. This sensitivity analysis was only conducted for breast cancer incidence as most studies investigating mortality were mostly breast-cancer specific.
4. **Fixed Effect Model:** A fixed effect model was used as a sensitivity analysis to compare differences between the random effects model. A fixed effect model assumes that all studies pooled share a common true effect size, and the source of variation is within-study estimation [76].

3.5.6. Publication Bias

Publication bias was assessed by using the Egger's funnel plot for diagnosis and prognosis articles [81]. A funnel plot shows the relationship between study effect size and its precision [82]. The premise of publication bias is that small studies with unfavorable results will not be published compared to larger studies [82]. Presence of publication bias was determined by the asymmetrical shape of the funnel plot [81].

4.0 RESULTS

4.1 Literature Search Results

A total of 1171 records were identified through Medline (Pubmed) (n=264), EMBASE (n=773), and the Cochrane Library (n=134). An additional 40 records were identified from other sources such as conference proceedings and grey literature. After de-duplication (n=306

removed), a total of 905 abstracts/titles were eligible for the broad screen. An additional 4 articles were identified through previously published systematic reviews and meta-analyses. Of these, 69 full text articles were retrieved for eligibility screening using the inclusion and exclusion criteria stated *a priori* (see *Section 3.2*). A total of 46 studies were excluded (*see Section 4.3*). The remaining 23 studies were eligible for quantitative analysis. Please see Appendix 5 for the PRISMA diagram of the search strategy.

4.2 Study Characteristics of Included Studies

A total of 23 articles met the inclusion criteria for quantitative analysis. Of the 23 studies, 12 articles assessed the incidence of breast cancer and 11 assessed all-cause mortality. Data extraction of all articles was completed in duplicate with a second reviewer (MS).

4.2.1. Breast Cancer Incidence

For the incidence of breast cancer, all 12 studies were published in peer-reviewed journals in the English language [83–94]. A total of 11 studies used a cohort design, and 1 study used a nested-case control design. Of the 11 cohort studies, 10 studies were retrospective and 1 study was prospective. A total of 4 studies were conducted in the United States, 2 studies were conducted in Taiwan, and 6 studies were conducted in Europe. All studies used a population-based database for exposure ascertainment, except for Soffer *et al.*, who used the hospital electronic medical record (EMR). Outcome ascertainment was assessed through cancer registries or diagnostic codes.

A range of comparators was used against metformin, including: sulfonylureas, insulin, ‘non-metformin’, and ‘other ADM’. A total of 7506 users of metformin and 8724 non-metformin

users were reported from the breast cancer specific studies. Of the metformin users, an estimated total of 1238 were diagnosed with breast cancer compared to the estimated 1673 from the comparator group. An accurate sample of all users and breast cancer events could not be calculated, as some studies analyzed all cancers and did not report breast-cancer specific sample sizes. All studies reported adjusted ORs or HRs with 95%CI. Please refer to Tables A6.1 and A6.2 for summary of the included studies and main outcomes.

4.2.2. All-Cause Mortality

A total of 11 studies were included and published in peer-review journals in English for all-cause mortality in breast cancer patients [51,95–104]. All studies used a retrospective cohort design. A total of 6 studies were conducted in North America, 2 in Europe, 2 in Asia, and 1 in Egypt. Population-based databases were used for 6 studies, and hospital EMRs were used in 5 studies. All studies used a death registry or EMR records for outcome ascertainment.

For all studies, 'non-metformin' was the comparator used against metformin. A reported total of 3400 metformin users and 2987 non-metformin users were pooled in the analysis. An estimated total of 491 deaths were reported for metformin users and estimated 1220 deaths for non-metformin users. An accurate number of users and deaths could not be reported as some studies did not disclose all information. Please refer to Tables A7.3 and A7.4 for summary of included studies and main outcomes.

4.3 Study Characteristics of Excluded Studies

There was a total of 46 studies that were excluded during the full-text (secondary) screen. Most studies (n=13) were excluded due to the limited available data (i.e. conference

proceedings). Other reasons for study exclusion were: inappropriate outcomes (n=10), duplicate articles (n=8), inappropriate ADM comparator(s) (n=7), overlapping databases (n=5), and inappropriate study design (n=2); 1 article was inaccessible via the McMaster University Health Sciences Library.

4.4 Risk of Bias in Included Studies

The NOS for case-control and cohort studies was used to assess the RoB for individual studies. RoB assessment of all articles was completed in duplicate with a second reviewer (MS). For studies investigating the incidence of breast cancer, most studies (n=7) had a score between 6 to 7, indicating a moderate risk of bias. The remaining studies (n=5) had a score between 8 to 9, implying a low risk of bias. All studies had excellent selection criteria, satisfying a total of four stars. Most studies controlled for at least 3 additional risk factors, and all studies controlled for age (comparability criteria). Sources of bias for breast cancer incidence studies were inadequate follow-up period and unadjusted confounders. Please see Tables A8.5 and A8.6 for the NOS assessment for incidence of breast cancer studies.

For studies investigating all-cause mortality, 5 studies reported a low risk of bias assessment, 5 studies had a moderate risk of bias, and 1 study had a high risk of bias. All studies had excellent selection criteria; except El-Benhawy *et al*., who had a small sample size of metformin and non-metformin users. Most studies controlled for at least 3 additional risk factors, and all studies controlled for age (comparability criteria). The main sources of bias were inappropriate outcome ascertainment and inadequate follow-up. Please see Tables A9.7 for the NOS assessment for all-cause mortality studies.

4.5 Agreement Statistics

Agreement statistics were calculated using an online kappa calculator tool [73] to determine inter-rater concordance between the two reviewers (GT, MS). An unweighted kappa of 0.46 was calculated for the broad screen, indicating moderate agreement between the two reviewers [75]. An unweighted kappa of 0.78 was calculated for the secondary screen, indicating a good agreement [75]. A weighted kappa was used for the RoB because the NOS scoring system uses an ordinal scale [74]. A weighted kappa of 0.29 was calculated for the NOS assessments for all included studies, indicating a fair agreement between reviewers. No consistent themes were identified as sources of disagreement.

4.6 Effects of Intervention: Metformin and Incidence of Breast Cancer

4.6.1 Metformin and Incidence of Breast Cancer – Main Analysis

A total of 12 studies with 15 risk estimates were pooled in the meta-analysis, the results are shown in Figure A10.2. Metformin was reported as a risk for breast cancer for one study (OR: 1.28, 95% CI: 1.05-1.56) [87]. Three studies reported a weak (ORs range from 0.56 to 0.95) protective effect of metformin for the incidence of breast cancer [84,88,89]. The remaining estimates did not report any significant associations.

The overall effect size using the random effects model did not demonstrate a significant protective effect of metformin against breast cancer (OR: 0.93, 95% CI: 0.85-1.03). A moderate amount of heterogeneity was detected between studies (I^2=35%). We also performed Egger's funnel plot, which suggested the presence of publication bias due to the asymmetrical shape (Figure A10.3).

4.6.2 Metformin and the Incidence of breast cancer – Subgroup Analyses

Three subgroup analyses were conducted for duration of metformin treatment, types of ADM, and presence of time-related biases. Please see Table A10.8 for a summary of results.

For those who received metformin treatment ≥3 years, there was a marginally significant protective effect (OR: 0.95, 95%CI: 0.91-0.99, I^2=0%) in the 4 pooled studies. However, Ruiter *et al.* contributed to 98% of the weight towards the overall effect. After removal, the effect was no longer significant (OR: 0.88, 95%CI: 0.62-1.23, I^2=15%). A total of 8 effect sizes were pooled for treatment duration <3 years. There was no significant effect detected in the pooled analysis (OR: 0.85, 95%CI: 0.72-1.00, I^2=35%).

Three types of ADMs were identified as comparators against metformin: sulfonylureas, insulin, and "non-metformin". No protective effect of metformin was reported when compared to sulfonylureas (OR: 0.94, 95%CI: 0.85-1.04, I^2=14%). An exploratory analysis was carried out as Ruiter *et al.* contributed to 71.2% of weight to the overall effect. After removal, the effect was further weakened (OR: 0.88, 95%CI: 0.68-1.13, I^2=31%). For insulin, no significant effect was identified (OR: 1.06, 95%CI: 0.83-1.36, I^2=39%). The "non-metformin" group included studies that did not specify the comparator. A total of 5 studies were pooled as non-metformin and a marginally protective effect of metformin was identified (OR: 0.85, 95%CI: 0.74-0.97, I^2=0%). An exploratory analysis was carried out to remove Soffer *et al*., as it contributed to 53% of the weight. After removal, the effect was slightly stronger (OR: 0.80, 95%CI: 0.66-0.97, I^2=0%).

All studies were reviewed to determine the presence of time-related bias. Please see Appendix 11 for description of time-related biases for included studies. Studies with the presence of time-related biases (i.e. immortal time bias, time window bias, time lag/latency bias, time dependent confounders) did not show any significant protective effect (OR: 0.89, 95% CI: 0.70-

1.13, I^2=59%). Of the 7 studies that accounted for time-related biases; there was a weak protective effect of metformin (OR: 0.94, 95% CI: 0.91-0.98, I^2=0%). Of note, Ruiter *et al.* contributed 88.5% of the weight in the analysis. After removal, the association was stronger but no longer significant (OR: 0.90, 95%CI: 0.80-1.02, I^2=0%).

4.6.3 Metformin and the Incidence of Breast Cancer – Sensitivity Analyses

A total of 4 sensitivity analyses were carried out as described in *Section 3.5.5.* Please see Table A10.9 for a summary of results. Sensitivity analyses were conducted to compare high quality studies versus low quality studies as determined by the NOS for risk of bias. For studies with high risk of bias (i.e. NOS score ≤ 7), no effect was observed (OR: 0.93, 95%CI: 0.80-1.09) with moderate heterogeneity (I^2=46%). Moreover, no effect was observed for low RoB (i.e. NOS score >7) (OR: 0.80, 95%CI: 0.80-1.02, I^2=1%).

We conducted sensitivity analyses to compare studies that adjusted for obesity and/or BMI. Studies that adjusted for BMI/obesity did not show any meaningful effect (OR: 0.90, 95%CI: 0.73-1.11, p-0.34, I^2=56%). Studies that did not consider the effect of BMI/obesity for breast cancer risk detected a marginal benefit of metformin (OR: 0.95, 95%CI: 0.91-0.98, I^2=0%). However, Ruiter *et al.* contributed to 90.6% of the weight, and was removed in the exploratory analysis. After removal, no significant effect was observed (OR: 0.90, 95%CI: 0.79-1.03, I^2=0%).

Sensitivity analyses to compare studies that investigated all types of cancer and breast cancer specific studies were carried out. No effect was observed for breast-cancer specific studies (OR: 0.82, 95%CI: 0.67-1.01, I^2=0%). A total of 12 effect sizes were pooled for studies

that investigated all types of cancer. No significant effect was observed for metformin use and incidence of breast cancer (OR: 0.95, 95%CI: 0.86-1.06, I^2=38%).

Lastly, a sensitivity analysis for the fixed effects model was conducted to evaluate the differences from the random effects model. A marginal protective effect of metformin and breast cancer incidence was detected (OR: 0.95, 95%CI: 0.91-0.99, I^2=35%). However, Ruiter *et al.* contributed to 80.5% of the weight. After removal, the association was weakened and no longer significant (OR: 0.95, 95%CI: 0.87-1.04, I^2=40%).

4.7 Effects of Intervention: Metformin and All-Cause Mortality

4.7.1 Metformin and All-Cause Mortality – Main Analysis

A total of 11 studies with 16 estimates were pooled in the final meta-analysis. The results are shown in Figure A12.4. All studies reported a significant protective effect of metformin and all-cause mortality, except for 5 studies that crossed the line of no effect [51,96,99,103,104]. Notably, the 5 studies had an estimated effect <1, indicating a non-significant protective effect.

The overall effect of the studies using the random effects model demonstrated a statistically significant protective effect of metformin against all-cause mortality (HR: 0.55, 95%CI: 0.44-0.70). However, there was a significant degree of heterogeneity (I^2=81%) identified. We also performed Egger's funnel plot which suggested presence of publication bias (Figure A12.5).

4.7.2. Metformin and All-Cause Mortality – Subgroup Analysis

A subgroup analysis was carried out to compare studies with time-related biases (i.e. immortal time bias, time window bias, time lag/latency bias, time dependent confounders) versus

studies without. Please see Table A12.12 for a summary of results. All studies were reviewed to determine the presence of time-related biases and are described in Table A11.11.

A total of 7 studies (12 effect sizes) with time-related biases showed a significant reduction in all-cause mortality from metformin use (HR: 0.48, 95%CI: 0.49-0.59, $p < 0.00001$, I^2=17%). For the 4 studies that accounted for time-related biases, a marginally significant protective effect of metformin was demonstrated (HR: 0.75, 95%CI: 0.58-0.95). However, there was high degree of heterogeneity between the studies (I^2=81%).

4.7.3. Metformin and All-Cause Mortality – Sensitivity Analyses

A total of 3 sensitivity analyses were carried out as described in *Section 3.5.5*. Please see Table A12.13 for a summary of results. Sensitivity analyses were conducted to compare high quality studies versus low quality studies as determined by the NOS. For studies with high RoB (i.e. NOS score ≤ 7), a significant protective effect was observed (HR: 0.58, 95% CI: 0.47-0.71, $p < 0.00001$, I^2=35%). For studies with low RoB (i.e. NOS score >7), a significant protective effect of metformin and all-cause mortality was reported (HR: 0.55, 95%CI: 0.36-0.83, I^2=85%).

We also conducted sensitivity analyses to compare studies that adjusted for obesity and/or BMI. Studies that adjusted for BMI/obesity showed a significant protective effect with no heterogeneity (HR: 0.50, 95% Ci: 0.43-0.59, I^2=0%). Studies that did not consider the effect of BMI/obesity for all-cause mortality detected a marginal significant effect and high degree of heterogeneity (HR: 0.74, 95%CI: 0.55-1.01, I^2=77%).

Lastly, a sensitivity analysis for the fixed effects model was conducted to evaluate the differences from the random effects model. A significant protective effect of metformin and all-cause mortality was detected with high heterogeneity (HR: 0.90, 95%CI: 0.85-0.94, I^2=81%).

However, Lega *et al.* contributed to 84.2% of weight in the model. After removal, the association strengthened (HR: 0.58, 95%CI: 0.51-0.65) with a moderate degree (I^2=32%) of heterogeneity.

4.8 GRADE

Evidence profile and summary of finding tables are presented in Tables A13.14 and A13.15. For incidence of breast cancer, the overall certainty of estimates was 'very low', mainly due to risk of bias and the presence of publication bias. For all-cause mortality, the overall quality was 'very low' because of the risk of bias, unexplained heterogeneity, and presence of publication bias. These results suggest that the study authors have very little confidence in the effect estimate, and that the true effect is likely to be different from the estimate of effect [57].

5.0 DISCUSSION

5.1 Summary of Principal Findings

In the present study, we conducted a systematic review and two separate meta-analyses regarding the association of metformin on the risk of breast cancer incidence and all-cause mortality in diabetic patients. We did not find a significant relationship between metformin exposure and incidence of breast cancer in our main analysis (OR: 0.93, 95% CI: 0.85-1.03, I^2 = 35%). In our meta-analysis regarding metformin exposure and all-cause mortality, we identified a 45% risk reduction in 11 pooled studies (HR: 0.55, 95%CI: 0.44-0.70, I^2=81%). In both meta-analyses, presence of publication bias was identified using Egger's funnel plot.

5.2 Comparison with Existing Systematic Reviews and Meta-Analyses

5.2.1 All Cancers Meta-Analyses

Our results regarding breast cancer incidence do not differ from previous meta-analyses that included a breast cancer subgroup from all types of malignancies [48,50,52]. Previous studies did not report a significant effect, with the exception of Zhang *et al.*, who reported a marginal protective effect of 6% (summary relative risk (SRR): 0.94, 95%CI: 0.91-0.97, I^2=38.8%) [49]. However, this study only pooled 7 effect sizes, and did not assess RoB in the individual studies nor consider presence of other methodological biases (i.e. time-related biases).

For mortality, our results demonstrated the strongest magnitude of effect and the highest degree of heterogeneity compared to previous studies [33,49,51]. Zhang *et al.* reported a marginal protective effect (SRR: 0.63, 95%CI: 0.40-0.99, I^2=31%) [49]. Previous studies by Lega *et al.* and Coyle *et al.* did not report a significant reduction in mortality (HR: 0.81, 95% CI: 0.64-1.04, I^2=63% and HR: 0.99, 95%CI: 0.92-1.05, I^2=0%, respectively) [33,51]. Notably, these results are from small subgroup analyses of breast tumours that only pooled 3 to 4 studies. Therefore, larger meta-analyses of all cancers may not reflect accurate results regarding breast cancer mortality

5.2.2 Breast-Cancer Specific Meta-Analyses (Incidence)

In the literature, there are few systematic reviews and meta-analyses that exclusively investigated the effect of metformin on breast cancer. The first meta-analysis of the literature was published in 2012 by Col *et al.* who pooled a total of 7 studies regarding the incidence of breast cancer. The authors' analyses supported the protective effect of metformin (OR: 0.83, 95% CI: 0.71-0.97, I^2=51%) [53]. Furthermore, the authors reported a stronger association for

studies that had a longer duration (i.e. > 3 years) of metformin use (OR: 0.75, 95%CI: 0.62-0.91) [53]. In the present study, a similar trend of a stronger effect when metformin treatment was ≥ 3 years (OR: 0.95, 95%CI: 0.91-0.99, I^2=0%) was found. However, the exploratory analysis concluded that this effect was mostly driven by the weight of Ruiter *et al*., and was insignificant after removal (OR: 0.88, 95%CI: 0.62-1.23, I^2=15%). Potential explanations why Col *et al.* reported significant results include earlier published studies with time-related biases (see *Section 5.3*) and fewer studies pooled.

When comparing high versus low quality studies, our findings did not report any differences using the NOS assessment. However, Col *et al*. reported a stronger protective effect when stratified for high quality studies (OR: 0.80, 95%CI: 0.69-0.92) [53]. Since Col *et al.*'s published study in 2012, there has been a growing amount of research using large administrative insurance claims databases and electronic health records. A total of 6 studies have been published since Col *et al*.'s meta-analysis and have been included into the present study [84,90–92,94,105].

In 2015, Yang *et al.* reported that metformin did not reduce the incidence of breast cancer after pooling 11 studies and the potential presence of publication bias (RR: 0.94, 95%CI: 0.76-1.22) [55]. These results are similar to our findings, most likely due to overlapping included studies. However, Yang *et al.* reported a considerable amount of heterogeneity of I^2=90.7% compared to ours of 35% [55]. The authors conducted further secondary analyses to explore the effect of ethnicity and study design (cohort versus case-control) and did not any find reductions in incidence related to metformin exposure [55].

The most recent meta-analysis was published in 2016 by Moradi-Joo *et al.*, who compared metformin to sulfonylureas therapy in diabetic patients [56]. The study reported a RR

of 0.92, 95%CI: 0.63-1.34, with I^2=94%. In our secondary analysis comparing metformin and sulfonylureas therapy, we found similar results (OR: 0.94, 95%CI: 0.85-1.04, I^2=14%). Interestingly, the authors reported metformin was protective of breast cancer in their conclusion (RR: 0.63, 95%CI: 0.56-0.70, I^2=0%) [56]. However, the authors inappropriately removed studies (step-by-step method) until there was no heterogeneity and did not investigate publication bias.

5.2.3 Breast-Cancer Specific Meta-Analyses (All-Cause Mortality)

There are two recently published systematic reviews and meta-analyses that investigated the effect of metformin and all-cause mortality [54,55]. Yang *et al.* reported that metformin therapy was associated with decreased all-cause mortality (RR: 0.65, 95%CI: 0.48-0.87, I^2=78.9%), with no obvious publication bias after pooling 7 studies [55]. Xu *et al.* found a stronger protective effect against all-cause mortality in diabetic breast cancer patients (HR: 0.53, 95%CI: 0.39-0.71, I^2=78.9%) after pooling 14 effect sizes [54]. However, presence of publication bias was observed using Egger's test [54].

Results from our meta-analysis are parallel with previous studies (HR: 0.55, 95%CI: 0.44-0.70). We also identified a high degree of heterogeneity (I^2=81%) and presence of publication using Egger's funnel plot. To account for heterogeneity, we conducted a sensitivity analysis to compare studies with high and low RoB based on the NOS assessment score. Studies with a low RoB showed a weaker protective effect and a wider confidence interval (HR: 0.55, 95%CI: 0.36-0.83), but remained statistically significant. Interestingly, the degree of heterogeneity increased to 85% for low RoB studies. Previous meta-analyses did not conduct secondary analyses on the impact of individual study quality to explain the high heterogeneity.

Yang *et al.* investigated the effect of ethnicity and study design on metformin exposure and all-cause mortality in diabetic breast cancer patients. The authors found that in case-control studies and Caucasian participants, metformin significantly decreases all-cause mortality [55]. Xu *et al.* also conducted several sensitivity analyses in diabetic breast cancer patients with and without metformin exposure [54]. The authors found that studies that adjusted for age, types of ADM, and types of chemotherapy were protective against all-cause mortality [54]. However, these results must be interpreted cautiously as there was a high degree of heterogeneity in the pooled effect sizes and small number of studies.

5.3 Challenges in Observational Studies

In the field of pharmacoepidemiology, there are several challenges conducting methodologically robust observational studies. While we have critically evaluated individual studies using the NOS for selection, comparability, and outcome, we also must consider the presence of time-related biases and time-dependent confounders.

5.3.1 Time-Related Biases

In observational studies, especially those that investigate drug therapy, time-related biases are common [48,59]. A review by Suissa & Azoulay suggested that time-related biases can greatly exaggerate the protective effect of metformin found in several published observational studies [59]. The three common time-related biases are: immortal time bias, time-window bias, and time-lag bias [59].

Immortal time bias is introduced when time-fixed cohort analyses misclassifies unexposed time as exposed time [48,59,106]. This is most frequently found in studies that

compare ‘users’ versus ‘non-users’ [48,59,106]. Immortal time bias can arise between cohort entry and date of first exposure to metformin, where ‘users’ can be misclassified, excluded or unaccounted for in the study design or analysis [48]. To address this bias, a Cox PH analysis with time dependent factors can be used. This allows for variables to change value over time, and the corrected value is used to calculate the HR when the event occurs [59,106]. Alternatively, an intention-to-treat (ITT) analysis can be used such that the first ADM used is classified as exposed time, irrespective of switching or additional medications [59].

Time window bias is present when there are differential exposure opportunities between subjects [59]. For example, when there are differences in the duration of exposure (or treatment) between the cases and control [59]. To address time-window bias, case-control studies can match for the duration of diabetes to indirectly minimize differences in disease severity [59]. However, this matching does not correct the differential lengths of treatment time between cases and control. Alternatively, study authors can avoid this bias by accounting for the duration of treatment in the cases and controls in the study design stage [59,107]. This can be presented in the inclusion or exclusion criteria (e.g. including participants with 1 year of treatment).

Lastly, time lag bias is introduced by comparing treatments given at different stages of the disease [59]. This bias is particularly prevalent in studies comparing first line therapy to second or third line therapies [59]. In the case of diabetes, patients are unlikely to be at the same stage, which can introduce confounding by disease duration (i.e. long duration of diabetes increases risk of cancer incidence) [59]. Time lag bias is also present when first line therapy is associated with an increased risk of cancer. After a long exposure period, it is more likely that the cancer will occur during the second-line therapy [59]. Therefore, this creates an incorrect case attribution to the second line treatment [59]. The presence of time-lag bias ignores the fact

that a previous exposure may have affected future cancer risk. To minimize this bias, authors can apply time-lag periods to exclude cancer diagnoses within a specified period after entry into the cohort (e.g. patients that were diagnosed with cancer in the first year of follow-up were excluded) [59].

A review by van Walraven & Austin suggested that time-related biases are more likely to be present in administrative database research compared to primary data collection [106]. This is likely due to post-baseline covariates that are easily extracted in databases compared to primary data collection [106]. In our meta-analyses of breast cancer incidence and all-cause mortality among diabetic patients, 87% (20/23) of our included studies used administrative databases.

To account for the presence of time-related biases in our meta-analyses, we conducted subgroup analyses to compare studies that were designed or analyzed to avoid immortal time bias, time window bias, and time lag bias. Please see Tables A11.10 and A11.11 for summary of time-related biases in breast cancer incidence and all-cause mortality, respectively.

In our subgroup analyses, we found a significant association between metformin and incidence of breast cancer in studies that accounted for time-related biases (OR: 0.94, 95%CI: 0.91-0.98, I^2=0%). However, most of this effect was driven by Ruiter *et al.*, who contributed 88.1% of the weight. After removal, the effect was no longer significant (OR: 0.90, 95%CI: 0.80-1.02, I^2=0%). Our findings are similar to Gandini *et al.*'s meta-analysis that demonstrated a marginal effect after pooling 6 studies with no time-related biases for breast cancer incidence (SRR: 0.94, 95%CI: 0.90-0.99, I^2=32%) [48]. Our findings support Suissa & Azoulay's claim that studies that used time-dependent techniques to avoid time-related biases tend to find no association between metformin and cancer incidence because presence of these biases can greatly exaggerate the protective effect of metformin [59].

For all-cause mortality, we identified a marginal protective effect for studies that accounted for time-related biases (HR: 0.75, 95%CI: 0.58-0.98). However, there was a high degree of heterogeneity (I^2=81%) and a small number of studies pooled (n=4). Although these studies were designed and analyzed with no time-related biases, the presence of other methodological biases (e.g. confounding bias, selection bias) may be present. To our knowledge, there are no existing meta-analyses for all-cause mortality that investigated the presence of time-related biases in breast cancer.

5.3.2 Time-Dependent Confounders

In addition to time-related biases, the presence of time-dependent confounders may also affect results. A critical review of existing literature by Farmer *et al.* defined a time-dependent confounder as a variable that satisfies 3 conditions: 1) changes throughout time, 2) predictive of treatment initiation, and 3) associated with the outcome of interest [80]. When time-dependent confounders are detected in the study, standard statistical models cannot estimate the true causal effect of time-varying treatment [80,108].

In the case of diabetes and cancer risk, BMI and glycated hemoglobin (Hb1Ac) are known time-dependent confounders [80]. Both BMI and Hb1Ac are predictive of metformin use, based on treatment guidelines for diabetes [80,109]. However, the effect of metformin will also have influence in future HbA1c levels and BMI [80]. There is also ongoing evidence that BMI and HbA1c can increase cancer risk and tumour proliferation [80,110,111].

The relationship between breast cancer and BMI or it's surrogate, obesity, is complex [48,80,110,112,113]. A meta-analysis of 12 prospective studies found that obesity was associated with an increased risk of breast cancer in postmenopausal women [112]. Preclinical research

suggests the role of inflammation, adipocyte size, and aromatase expression may play a role in the relationship between BMI and breast cancer outcomes [113]. Furthermore, potential mechanisms such as insulin and IGF-1 are directly impacted by metformin [48].

To account for the presence of BMI as a time-dependent confounder, we conducted sensitivity analyses for studies that adjusted BMI or obesity. Our findings did not suggest any statistically significant reduction in breast cancer incidence (OR: 0.90, 95%CI: 0.73-1.11, I^2=56%). Similar findings were reported by Gandini *et al.*, who pooled breast cancer subgroups for BMI adjustment (SRR: 0.82, 95%CI: 0.67-1.00, I^2=48%) [48]. These results support Farmer *et al*.'s hypothesis that studies least likely to be affected by bias does not support a causal effect between metformin and cancer risk [80].

For all-cause mortality after BMI adjustment, our findings suggest that there is a protective effect of metformin (HR: 0.50, 95%CI: 0.43-0.59, I^2=0%). Similar protective effect was reported by Xu *et al*., with a 57% reduction in all-cause mortality (HR: 0.43, 95%CI: 0.34-0.55, I^2=0%) [54]. However, these results must be interpreted cautiously as the relationship between BMI and cancer by metformin in a time-dependent context is complex.

A sensitivity analysis of HbA1c was not conduced for both incidence and all-cause mortality studies, as only 2 studies adjusted for its effect [83,85].

5.4 Possible Mechanisms and Explanation for Findings

After conducting several secondary analyses, the protective effect of metformin against all-cause mortality remained statistically significant. There are a few potential explanations for this observed effect. First, the mortality rate in the 'non-metformin' patient group may be explained by non-cancerous death such as diabetes complications (e.g. CVD) [54,114]. In our

study, we did not solely pool breast cancer-specific mortality. However, a previous meta-analysis by Xu *et al.* reported that there was an observed benefit of metformin for breast cancer-specific mortality [54]. Secondly, a study by Jiralerspong *et al.* reported that diabetic patients with breast cancer receiving metformin and neoadjuvant chemotherapy have a higher pathological complete response (pCR) compared to diabetics that did not take metformin [115]. This further suggests that metformin may have anti-tumour effects in patients with breast cancer. In contrast, we did not identify a protective effect of metformin in regards to the incidence of breast cancer. Reasons are further explored in the study limitations (*Section 5.5*).

From a physiological perspective, possible mechanisms of action for metformin as anti-cancer therapy in breast cancer are summarized in *Section 1.4.1.* In brief, the activation of AMPK to inhibit mTOR signaling pathway is the most widely accepted mechanism of action [5,15,30,39,40]. AMPK increases tuberous sclerosis complex protein 2 (TSC2) activity, leading to the inactivation of mTOR, and thereby decreasing protein synthesis and cell growth of cancer cells [15,30,41]. Other mechanisms include metformin's ability to reduce circulating levels of insulin and ICF-1, which is known to promote carcinogenesis [15].

Lastly, the presence of publication bias may also contribute to the observed effects. In the present study, publication bias is strongly suspected due to the asymmetrical shape of the funnel plot (see Figures A10.3 and A12.5). Both funnel plots for breast cancer incidence and all-cause mortality suggest that the missing studies are studies that show that metformin is harmful (OR > 1). Therefore, if it is true that these studies are not published, this could greatly affect the overall interpretation of these results.

5.5 Limitations

While the current analyses are based on the best available evidence, there are several limitations in the literature. Therefore, the results should be interpreted with caution. The included studies were mostly retrospective, and varied in: study population, diabetes duration, length of metformin exposure, and adjustment of confounding factors. This can lead to over or underestimation of results and introduction of many types of methodological biases. Furthermore, as Farmer *et al.* discussed in their critical review, there is a potential impact of less easily detectable biases (i.e. time-related biases and time-dependent confounders) which can contribute to the largest protective effects of metformin [80].

In the present study, metformin dose and adherence were not closely evaluated. As previously described by Col *et al.,* metformin dose at baseline may not represent dose during follow-up, as treatment and disease severity can change [53]. While a subgroup analysis was conducted comparing length of metformin treatment (≥3 years versus < 3 years), studies that reported "≥1 years" as "<3 years" were grouped. This may introduce inaccuracy in the subgroup results.

To assess RoB in individual studies, we used the NOS which may introduce some limitations. First, there are no validated cutoffs for high and low RoB using the nine-point star system. We therefore relied on previous literature to determine thresholds for high, moderate, and low RoB. This may introduce inaccuracy in our RoB final assessments. Secondly, the specific criteria for the NOS was developed by the authors and may not capture all acceptability criteria for selection, comparability and outcome. Results from the NOS can influence the results GRADE's rating for RoB. Future recommendations for this study is to use the Risk of Bias In Non-randomized Studies – of Interventions (ROBINS-I).

Another limitation in the literature is that studies often did not specify the type of ADM used as the comparator. Most studies, especially those investigating all-cause mortality, used 'non-metformin'. This grouping may influence the results as previous research has identified an association between hyperinsulinemia and increased cancer risk [78]. Therefore, the mixture of treatments, some which may have a greater or less effect of breast cancer mortality, may mask the overall treatment effect.

For studies that investigated breast cancer incidence, we pooled several studies from different countries with a variety cancer screening protocols. Therefore, the frequency of mammography screening is likely a confounding factor that many studies did not adjust for, except for Chlebowski *et al.* and Calip *et al.* [88,94]. The ethnic diversity of included studies may also affect heterogeneity of the overall estimate because of differences in treatment guidelines (i.e. when to prescribe first or second line therapy) in each country.

In our meta-analysis, we did not investigate the association of metformin in other oncologic outcomes such as cancer recurrence and breast cancer mortality. There was also an unknown degree of missing data in several studies, therefore a sensitivity analysis was not conducted. We were also unable to stratify by the type of breast cancer classification in our subgroup analyses because most studies did not specify by subtype. In our main analyses, we identified a strong presence of publication bias from the asymmetrical funnel plots. Possible sources of asymmetry beyond publication bias (reporting bias) includes: poor methodological design, true heterogeneity, and chance [81,116]. Both funnel plots for breast cancer incidence and all-cause mortality suggest that the missing studies are studies that show that metformin is harmful (OR > 1). Therefore, if it is true that these studies are not published, this could greatly affect the overall interpretation and dilute the validity of our results.

5.6 Strengths

Despite the limitations of this study, there are also several strengths worthy of mention. Firstly, a comprehensive review of the existing literature using a broad search strategy was conducted. The literature search also included grey literature to ensure the most up to date evidence is available. The screening of abstracts, full-text articles, and abstraction were completed in duplicate with a second author. Multiple secondary analyses to account for heterogeneity, presence of time-related biases, and time-dependent confounders were conducted. To our knowledge, this is also the first systematic review and meta-analysis to use the GRADE system.

5.7 Future Directions

Future directions for this systematic review and meta-analysis include evaluating the effect of metformin on breast cancer specific mortality and recurrence. Further prospective studies are warranted to confirm the use of metformin as a chemopreventative agent or as a supplement to adjuvant chemotherapy in the treatment of breast cancer.

5.7.1 Randomized Controlled Trials

While we did not pool any RCTs, a few trials have been published in regards to metformin use and mortality. Here we will highlight a few phase III trials that have been published or are currently ongoing.

A recently published substudy from the ALLTO study randomized control trial (RCT), investigated the association between diabetes and metformin in HER2+ breast cancer patients [117]. The authors reported that the use of metformin among diabetics and early hormone

receptor positive breast cancer patients may improve prognosis [117]. Patients with diabetes who had not been treated with metformin experienced worse disease-free survival (DFS) (HR: 1.40, 95%CI: 1.01-1.94), distant disease free survival (DDFS) (HR: 1.56, 95%CI: 1.10-2.22), and overall survival (OS) (HR: 1.87, 95%CI: 1.23 to 2.85). These results were reported after stratifying the timing of chemotherapy, central hormone receptor status, and lymph node status, as well as adjusting for treatment arm, tumor size, and BMI status. Most notably, this effect was limited to hormone receptor positive patients.

Study authors from this RCT further observed that metformin had protective effects in hormone receptor positive breast cancer patients compared to insulin's detrimental effects. This effect was observed in multivariable analysis in DFS (HR: 0.46, 95%CI: 0.24-0.89 vs. HR: 2.29, 95%CI: 1.15-4.58), DDFS (HR: 0.29, 95%CI: 0.14-0.63 vs. HR: 2.36, 95%CI: 1.10-5.07), and OS (HR: 0.27, 95%CI: 0.10-0.71 vs. HR: 3.34, 95%CI: 1.33-8.37). Findings from this RCT are supported by preclinical data and epidemiological studies for all-cause mortality.

Currently, there is an ongoing phase III RCT, the PLOTINA study, conducted at the Italian National Cancer Institute comparing metformin versus placebo in postmenopausal women at high risk of T2DM (n=16,000) [118]. The aim of this RCT is to evaluate the effect of metformin on the incidence of breast cancer incidence and cardiovascular diseases [118]. An additional second RCT with similar eligibility criteria will further add diet intervention (based on reduction of high caloric food, increase in vegetable intake) to evaluate the effects of metformin [118].

Another ongoing large multicenter phase III RCT is the NCIC CTG MA.32, for metformin versus placebo in early breast cancer patients (NCT01101438) [119]. This multicenter trial, NCIC CTG MA.32, will compare invasive DFS of patients with node-positive or high-risk

node negative breast cancer who are receiving standard therapy [119]. A total of 3582 participants will be randomized to receive metformin 850mg twice daily or placebo for 5 years [119]. Secondary outcomes include: OS, distant disease free survival, breast cancer free survival, BMI changes, adverse events, and quality of life [119].

It is important to highlight that this trial excluded women with diabetes or elevated glucose due to the use of placebo. Previous studies have raised concerns if the effect of metformin would only be limited to patients with impaired glucose homeostasis [53,120]. However, interim data monitoring and safety results from the MA.32 trial reported that metformin in breast cancer patients demonstrated beneficial effects on body weight, insulin, glucose, leptin, and C-reactive protein [121]. This further suggests that metformin can improve metabolic effects regardless of baseline insulin and body size [121]. Results from the interim analysis support the continuation of this large trial to determine the effects of metformin on cancer outcomes and non-cancer outcomes [121]. Currently, the study completed recruitment and the estimated study completion date is July 2020.

6.0 CONCLUSIONS

In conclusion, we observed a protective effect of metformin for all-cause mortality in breast cancer patients with diabetes. We did not observe a significant effect for the incidence of breast cancer. Results from our meta-analyses must be interpreted cautiously due to the presence of methodological bias, publication bias, and very low rating using the GRADE system. Observational studies in the context of metformin and cancer therapy are complex as management for diabetes changes over time, and presence of time-related biases are difficult to detect.

6.1 Knowledge Translation

Findings will be at global conferences such as the American Society of Clinical Oncology Annual Meeting, San Antonio Breast Cancer Symposium, and the World Diabetes Congress. The authors of this investigation will seek publication in journals with oncologist and endocrinologist readers such as: Breast Cancer Research and Treatment, and Diabetes Care.

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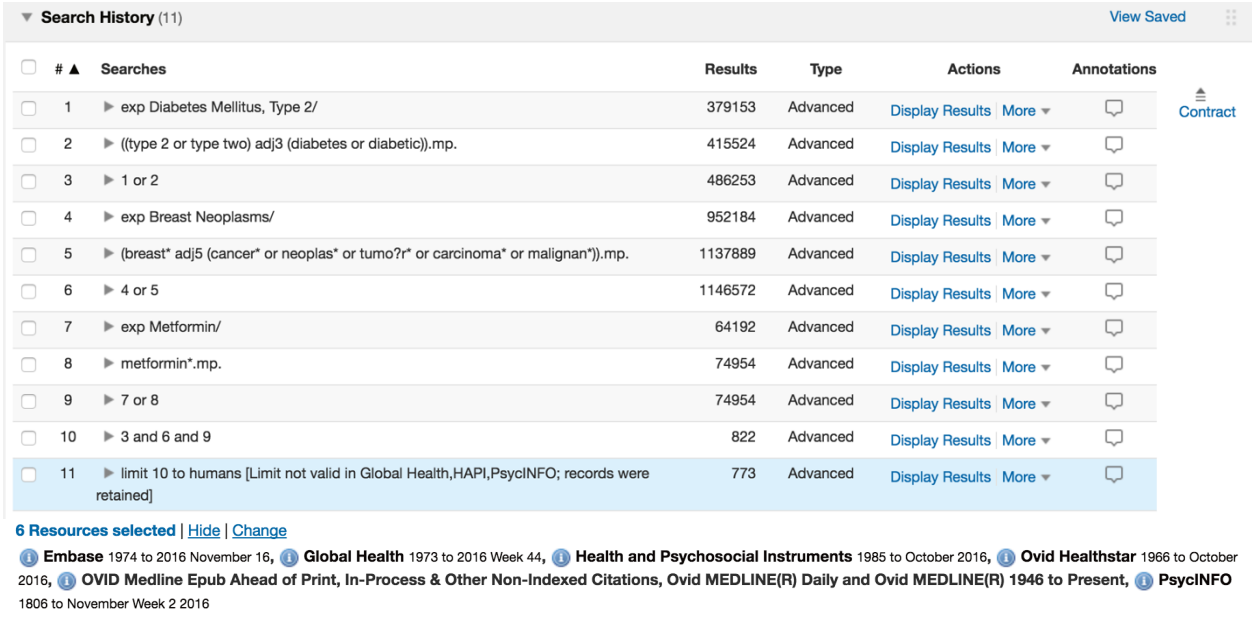
Appendix 1: Full Literature Search Strategies

Pubmed Search Strategy (1946 to November 2016)

Search	Add to builder	Query	Items found	Time
#5	Add	Search **(#4 not (animals/ not humans))**	264	15:03:29
#4	Add	Search **((#1) AND #2) AND #3**	269	15:01:13
#3	Add	Search **metformin***	14915	15:00:54
#2	Add	Search **((Breast Neoplasms) OR ((breast* and (cancer* or neoplas* or tumo?r* or carcinoma* or malignan*)))**	335976	15:00:23
#1	Add	Search **((Diabetes Mellitus, Type 2)) OR (((type 2 or type two) OR (diabetes or diabetic)))**	1550734	15:00:13

EMBASE Search Strategy (1966 to November 2016)



Search History (11) View Saved

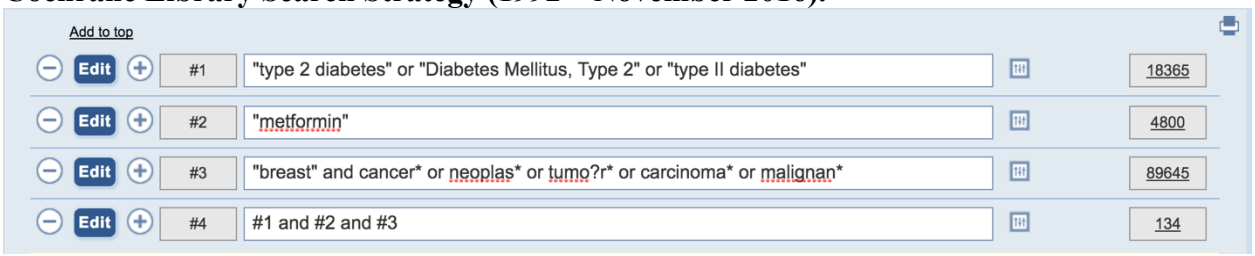
# ▲	Searches	Results	Type	Actions	Annotations
1	exp Diabetes Mellitus, Type 2/	379153	Advanced	Display Results | More	
2	((type 2 or type two) adj3 (diabetes or diabetic)).mp.	415524	Advanced	Display Results | More	
3	1 or 2	486253	Advanced	Display Results | More	
4	exp Breast Neoplasms/	952184	Advanced	Display Results | More	
5	(breast* adj5 (cancer* or neoplas* or tumo?r* or carcinoma* or malignan*)).mp.	1137889	Advanced	Display Results | More	
6	4 or 5	1146572	Advanced	Display Results | More	
7	exp Metformin/	64192	Advanced	Display Results | More	
8	metformin*.mp.	74954	Advanced	Display Results | More	
9	7 or 8	74954	Advanced	Display Results | More	
10	3 and 6 and 9	822	Advanced	Display Results | More	
11	limit 10 to humans [Limit not valid in Global Health,HAPI,PsycINFO; records were retained]	773	Advanced	Display Results | More	

Contract

6 Resources selected | Hide | Change

Embase 1974 to 2016 November 16, **Global Health** 1973 to 2016 Week 44, **Health and Psychosocial Instruments** 1985 to October 2016, **Ovid Healthstar** 1966 to October 2016, **OVID Medline Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to Present**, **PsycINFO** 1806 to November Week 2 2016

Cochrane Library Search Strategy (1992 – November 2016).



Add to top

Edit	#1	"type 2 diabetes" or "Diabetes Mellitus, Type 2" or "type II diabetes"	18365
Edit	#2	"metformin"	4800
Edit	#3	"breast" and cancer* or neoplas* or tumo?r* or carcinoma* or malignan*	89645
Edit	#4	#1 and #2 and #3	134

Appendix 2: Grey Literature Sources

Clinical Trial Databases:

1. Clinical trials.gov
2. EU Clinicals register

Conference Proceedings

1. American Society of Clinical Oncology – Meeting Library
2. American Diabetes Association – Meeting Library

Appendix 3: Template of Data Extraction Forms
Cohort Studies

Data Extraction Form – Cohort Study

Reviewer:
Date:

REF#:
Title:
Author
Journal:
Peer Reviewed journal (y/n):

Year of Publication:
Country of the population studied:

METHODS:
Study Period:
Retrospective or Prospective:
Follow-up Period:
Sample Size (total – breast cancer only):

Race/Ethnicity (if applicable):

Patient Age (range):

Exposure ascertainment (self-reported, database):

INTERVENTION

Type of ADM: Metformin Sample Size (in the arm): Dose: Duration:	Age (range): BMI: Weight:

Type of ADM: SU Sample Size: Dose (if applicable) Duration	Duration (if applicable) Age (range): BMI: Weight:

Type of ADM: TZD Sample Size: Dose (if applicable) Duration	Duration (if applicable) Age (range): BMI: Weight:

Type of ADM: Insulin Sample Size: Dose (if applicable) Duration	Duration (if applicable) Age (range): BMI: Weight:

COMPARATOR:

Type of ADM: Sample Size (in the arm): Dose: Duration:	Age (range): BMI: Weight:

Type of ADM: Sample Size (in the arm): Dose: Duration:	Age (range): BMI: Weight:

Type of ADM: Sample Size (in the arm): Dose: Duration:	Age (range): BMI: Weight:

Type of ADM: Sample Size (in the arm): Dose: Duration:	Age (range): BMI: Weight:

Primary outcome (INCIDENCE):
Outcome ascertainment (e.g. ICD-9 CODES)

Confounding factors controlled for:

Metformin vs. no metformin

Crude OR/HR:	Adjusted OR/HR:

SU vs no SU

Crude OR/HR:	Adjusted OR/HR:

TZD vs no TZD

Crude OR/HR:	Adjusted OR/HR:

Insulin vs. no insulin

Crude OR/HR:	Adjusted OR/HR:

Primary outcome (MORTALITY):
Outcome ascertainment (e.g. ICD-9 CODES)

Confounding factors controlled for:

Metformin vs. non-metformin

Overall Survival (years): Crude:	Overall Survival: Adjusted HR:

SU vs. no SU

Overall Survival (years): Crude:	Overall Survival: Adjusted HR:

TZD vs. no TZD

Overall Survival (years): Crude:	Overall Survival: Adjusted HR:

Insulin vs. no insulin

Overall Survival (years): Crude:	Overall Survival: Adjusted HR:

Case-Control Studies

Data Extraction Form – Case-Control Study

Reviewer:
Date:

REF#
Title:
Journal:
Peer Reviewed journal?
Author:
Year of Publication/Time of Period of Study:
Country of the population studied:

METHODS:
Study Period:
Follow-up Period:

Entire Study population:
Sample Size:
- Number of cases:
- Number of controls:

Population/Demographics/Database:

Race/Ethnicity (if applicable):

Patient Age Range:

Exposure ascertainment (self-reported, database):

Matching:

INTERVENTION:

Type of ADM: Metformin Sample Size (in the arm): Dose: Duration: Age: BMI

Type of ADM: SU Sample Size (in the arm): Dose: Duration: Age: BMI:

Type of ADM: TZD Sample Size (in the arm): Dose: Duration: Age: BMI

Type of ADM: INSULIN Sample Size (in the arm): Dose: Duration: Age: BMI

COMPARATOR:

Type of ADM: Sample Size (in the arm): Dose: Duration:	Age (range): BMI: Weight:

Type of ADM: Sample Size (in the arm): Dose: Duration:	Age (range): BMI: Weight:

Type of ADM: Sample Size (in the arm): Dose: Duration:	Age (range): BMI: Weight:

Type of ADM: Sample Size (in the arm): Dose: Duration:	Age (range): BMI: Weight:

Primary outcome (INCIDENCE):
Outcome ascertainment (e.g. ICD-9 CODES)

Confounding factors controlled for:

Metformin vs. non-use

Crude OR: 95% CI:	Adjusted OR: 95% CI:

SU vs. non-use

Crude OR: 95% CI:	Adjusted OR: 95% CI:

TZD vs. non-use

Crude OR: 95% CI:	Adjusted OR: 95% CI:

Insulin vs. non-use

Crude OR: 95% CI:	Adjusted OR: 95% CI:

Other vs. non use

Crude OR: 95% CI:	Adjusted OR: 95% CI:

Primary outcome (MORTALITY):
Confounding factors controlled for:

Metformin vs. nonuse

Overall Survival: Crude HR: 95% CI:	Overall Survival: Adjusted HR: 95% CI:

SU vs. non use

Overall Survival: Crude HR: 95% CI:	Overall Survival: Adjusted HR: 95% CI:

TZD vs. non-use

Overall Survival: Crude HR: 95% CI:	Overall Survival: Adjusted HR: 95% CI:

Insulin vs. nonuse

Overall Survival: Crude HR: 95% CI:	Overall Survival: Adjusted HR: 95% CI:

Other vs. nonuse

Overall Survival: Crude HR: 95% CI:	Overall Survival: Adjusted HR: 95% CI:

Notes:

Appendix 4: Summary of HR to RR to OR Conversions

For studies that report the hazard ratio obtained from a Cox proportional hazards model, we can calculate the relative risk (or risk ratio, RR) as follows:

$$RR = \frac{1 - e^{HR \cdot \ln(1 - P_0)}}{P_0},$$

where HR is the hazard ratio and P_0 is the proportion of patients in the control group who had an event by the desired follow-up time point. This assumes that the control group is in the denominator of the RR. For example, if your "groups" are exposure and no exposure, and the RR that you are wanting to calculate is exposed/nonexposed, then the event rate you would use in this formula is the event rate for the nonexposed patients.

To obtain an odds ratio (OR) from a relative risk, we can use the following formula:

$$OR = \frac{RR \cdot (1 - P_0)}{1 - RR \cdot P_0},$$

Again, P_0 is the proportion of patients in the control group who had an event by the desired follow-up time point.

Note – An alternative to P_0 is the anticipated incidence rate for the event.

Sample of HR to RR to OR Conversion:

For all conversions, a P_0 of 0.00341 will be used. This value is the anticipated incidence rate for breast cancer among T2DM patients as reported by Michels et al . This rate was observed in the Nurses' Health Study cohort of 116,488 patients between the ages of 30 to 55. The incidence was calculated using the number of breast cancer cases divided by the person-years.

$P_0 = \frac{Number\ of\ new\ breast\ cancer\ cases}{Person{-}years} = \frac{202}{59171}$
$= 0.00341$

Callip (2016)
Reported adjusted Cox PH HR for metformin vs. never use of metformin: 0.86
$P_0 = 0.00341$

$RR = \frac{1-e^{0.86*In(1-0.00341)}}{0.00341} = 0.86$

$OR = \frac{0.86(1-0.00341)}{1-0.86\ (0.00341)} = 0.859$

The conversion from Cox PH HR for metformin to OR is 0.86 using P_0 is 0.00341.

Appendix 5: PRISMA Diagram

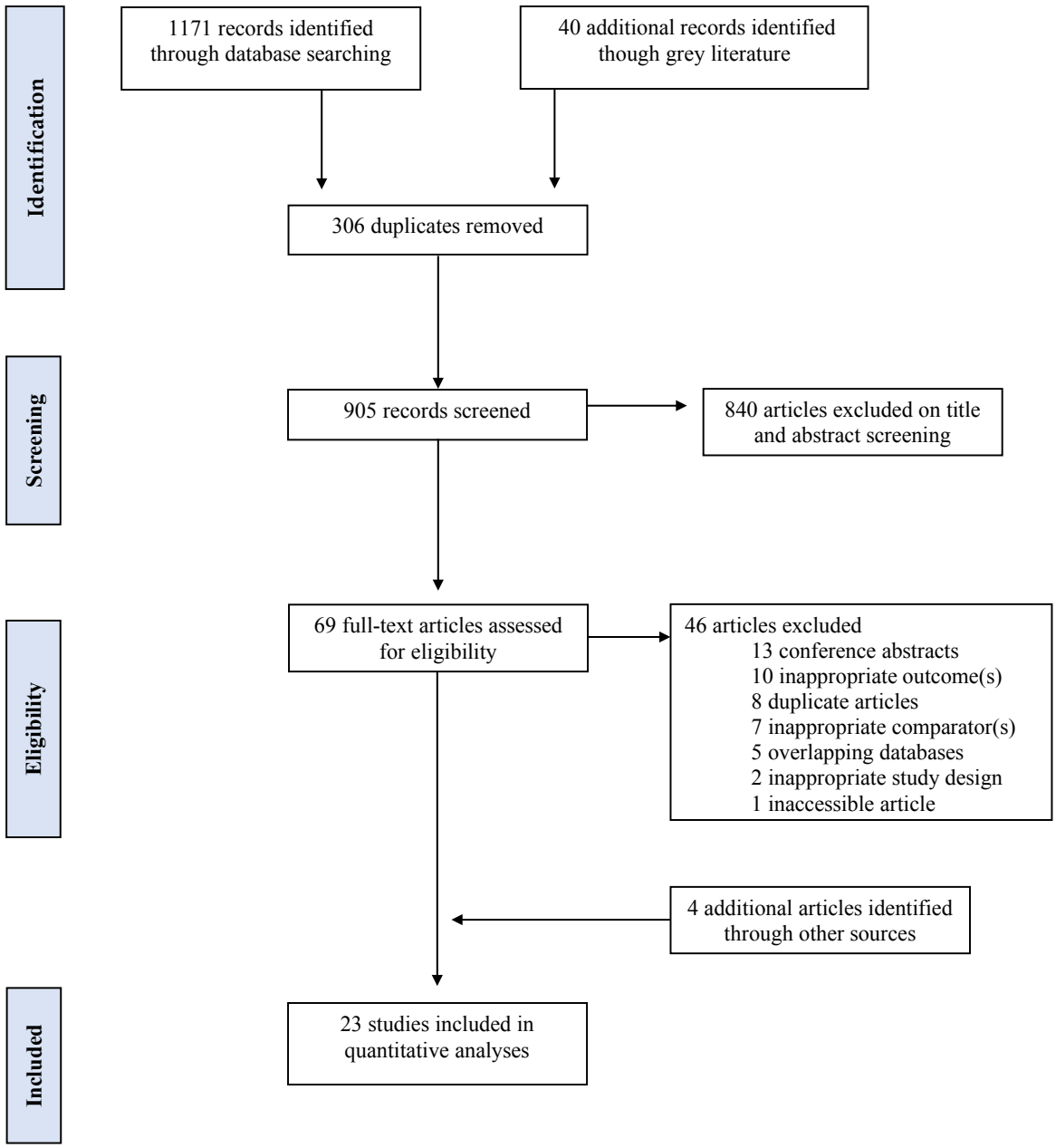


Figure 1. PRISMA diagram for study selection

Appendix 6: Summary of Characteristics of Included Studies for Breast Cancer Incidence

Table 1. Study Characteristics of the included studies for metformin and incidence of breast cancer

Study (Author, Year)	Location of Study	Study Design	Study duration; Follow up period (years)	Total Sample Size	Age (years)	Exposure ascertainment	Outcome Ascertainment; type of breast cancer	Inclusion Criteria
Currie, 2009	United Kingdom	Retrospective cohort; all cancers	≥ 2002; 152,065 person-years	62,809	Mean (SD): 62 (14.6)	The Health Information Network Database	First record of any solid tumor in database; all	Age ≥40, had received six or more sequential prescriptions for oral hypoglycemic agents
Libby, 2009	Scotland	Retrospective cohort; all cancers	1994-2003; NR	4085	NR	Scottish Care Information Diabetes Collaboration database	ICD9 and ICD10 codes; all	Diagnosed with T2DM, ≥ 35 years old, no previous diagnosis of cancer, no ADM previous use
Bosco, 2011	Denmark	Nested case-control; breast cancer specific	1989-2008; NR	4323	50 to ≥ 80	Danish National Registry of Patients; at diagnosis, by prescription	Danish Cancer Registry using ICD8 or ICD10 codes; all	Incident breast cancer cases; diagnosis of T2DM
Morden, 2011	USA	Retrospective cohort; all cancers	2006 to 2008; mean (SD): 23.1 months (10.5 months)	81,681	Mean: 77.4	Medicare Part D prescription database	ICD9 codes; all	Enrolled in the Part D prescription database, remained enrolled in the database for 4 months, ≥ 68 years old, at least 36 months of continuous fee-for-service
Chlebowski, 2012	USA	Prospective cohort; breast cancer specific	1993-2011; mean: 11.8	68,019	Mean, SD: 64 (6.7)	Women's Health Initiative Clinical Trials database	SEER; invasive	Medical history or reporting use of ADM at any time.
Hsieh, 2012	Taiwan	Retrospective cohort; all cancers	2000 to 2008; NR	10,189	Mean (SD): 61.2 (14.0)	Taiwan's National Health Insurance database	ICD9 codes; all	Continuous drug coverage for at least one year, no prior diagnosis of cancer
Ruiter, 2012	Netherlands	Retrospective cohort; all cancers	1998 to 2008; NR	85,259	≥18	PHARMA Record Linkage System	ICD9 codes; all	All individuals with more than one prescription for ADM

Table 1 (continued):

Study (Author, Year)	Location of Study	Study Design	Study duration; Follow up period (years)	Total Sample Size	Age (years)	Exposure ascertainment	Outcome Ascertainment; type of breast cancer	Inclusion Criteria
Soffer, 2014	USA	Retrospective cohort; breast and gynecological cancers	1998 to 2004; median: 6.5	66,778	≥18	Kaiser Permanete Southern California EMR	SEER; all	T2DM diagnosis, users of ADM, no previous use of ADM
Tsilidis, 2014	United Kingdom	Retrospective cohort; all cancers	1987-2010; median: 5.1	95,820	35 to 90	UK Clinical Practice Research Database	National Health Service Read Codes; post-menopausal	ADM prescription after 6 months of enrollment in database, no previous diagnosis of cancer
Chen, 2015	Taiwan	Retrospective cohort; all cancers	1998-2007; median: 2.5	7,325	Median, IQR: 62.6 (20.4)	Longitudinal Health Insurance Dataset	ICD9 Codes; all	New onset T2DM aged ≥ 30 years receiving a single hypoglycemic drug (monotherapy) for glycemic control without preexisting cancer at the index date
Kowall, 2015	United Kingdom and Germany	Retrospective cohort; all cancers	1995 to 2012; mean: 4.8	60,571 (UK); 19,692 (Germany)	30 to 89	Disease Analyzer database	ICD10 codes; all	T2DM diagnosis, prescription of ADM, ADM were not prescribed prior to first diagnosis of T2DM, no previous cancer diagnosis
Calip, 2016	USA	Retrospective cohort; breast cancer specific	1996-2011; median: 6.7	10,050	Mean, SD: 61.6 (12.3)	Group Health Cooperative database	SEER; invasive	T2DM diagnosis after index date, aged >40 years, enrolled in the database for at least 2 years

Abbreviations: USA = United States of America; SD = standard deviation; NR = not reported; ICD = International Classification of Disease; SEER = Surveillance, Epidemiology, and End Results; T2DM = type 2 diabetes mellitus; ADM = antidiabetic medication; IQR = interquartile range; EMR = electronic medical record

Table 2. Summary of Main Outcomes for Incidence of Breast Cancer

Study (Author, Year)	Users of metformin (n)	Comparator; number of users (n)	Breast cancer events (metformin; comparator(s))	Treatment duration	Adjusted variables	OR/HR, 95% CI
Currie, 2009*	NR	Sulfonylureas; NR Insulin; NR	305; 305; 305	NR	Age, sex, smoking status, diagnosis of prior cancer, HbA1c, diabetes duration	1.02 (0.71-1.45); 0.93 (0.69-1.27)
Libby, 2009*	4085	Non-metformin; 4085	24; 41	NR	Age, sex, smoking, deprivation, BMI, HbA1c, insulin use, and sulphonylurea use	0.60 (0.32-1.10)
Bosco, 2011	1250	Other ADM; 2197	≥ 1 year: 96; 1154 > 5 year: 35; 418	≥ 1 year > 5 years	Complications due to diabetes, clinical obesity, age at index date, post-menopausal hormone use, multiple imputations to impute missing parity	≥ 1 year: 0.81 (0.63-0.96) >5 years: 0.83 (0.56-1.22)
Morden, 2011*	15,286	Insulin; NR	NR	NR	Age category, race/ethnicity, diabetes complications, obesity diagnosis, oral estrogen use, Part D low-income subsidy (a poverty indicator), CCI and tobacco exposure diagnosis	1.28 (1.05-1.57)
Chlebowski, 2012	556	Other ADM; 2177	104; 129	NR	Age at menopause, parity, age at first birth, breastfeeding, smoking, alcohol, BMI, physical activity, use of estrogen + progesterone, mammogram, bilateral oophorectomy, mammogram, and race/ethnicity	0.75 (0.57-0.99)
Hsieh, 2012*	2048;	Sulfonylureas; 2804 Insulin; 338	19; 48; 5	≥ 1 year	Age	0.57 (0.33-0.97) 0.61 (0.22-1.67)
Ruiter, 2012*	52,698	Sulfonylurea; 32,591	207; 217	Cumulative exposure	Age at first ADM prescription, sex, year in which the first ADM prescription was dispensed, number of unique drugs used in the year, and number of hospitalizations in the year before the start of the ADM	0.95 (0.91-0.98)

Table 2 (continued):

Study (Author, Year)	Users of metformin (n)	Comparator; number of users (n)	Breast cancer events (metformin; comparator(s))	Treatment duration	Adjusted variables	OR/HR, 95% CI
Soffer, 2014*	4887	Non-metformin; 14,865	NR	≥1 year	Age, race/ethnicity, estrogen receptor therapy status, statin use, CCI, and outpatient utilization.	0.89 (0.74-1.09)
Tsilidis, 2014*	51,484	Sulfonylureas; 18,264	307; 153	≥1 year	Smoking status, BMI, alcohol consumption status, use of aspirin or NSAIDs, use of statins, use of exogenous hormones in women, diabetes duration (in days), and year of the first ADM prescription	1.03 (0.82-1.31)
Chen, 2015*	2223	Sulfonylureas; 3965	6; 14	NR	Age, sex, CCI, smoking-related diagnosis, alcohol use, morbidity, obesity, pancreatitis, hypertension, hyperlipidemia, monthly household income, and urbanization level	0.8 (0.3-2.12)
Kowall, 2015*	NR	Sulfonylureas; NR Insulin; NR	96; 24; 23; 14	≥ 1 year ≥ 5 year ≥ 1 year	Age at first diabetes medication, sex, country, time between diagnosis of T2DM and prescription of drug, obesity, hypertension, hyperlipidemia, prevalence of microcomplications (retinopathy, neuropathy, or nephropathy), CCI, use of anti-hypertensives, use of antithrombotic agents, use of aspirin, use of statins, use of nonsteroidal anti-inflammatory drugs, and use of contraceptives	≥ 1 year (sulfonylurea): 1.06 (0.65-1.72); ≥ 5 years (sulfonylurea): 0.50 (0.19-1.29); ≥ 1 year (insulin): 0.94 (0.51-1.72)
Calip, 2016	5700	Non-metformin (never use); 4350	135; NR	1-2.9 years, ≥ 3 years	Use of other ADM, age study entry year, smoking status, menopausal status, CCI, statin use, menopausal hormone therapy	1-2.9 years: 0.39 (0.19-0.80) ≥ 3 years: 1.14 (0.68-1.91)

* = studies that also included other cancers; Abbreviations: NR = not reported; OR = odds ratio; HR = hazard ratio; 95% CI = 95% confidence interval; CCI = Charleston Comorbidity Index; BMI = body mass index; T2DM = type 2 diabetes mellitus; HbA1c = hemoglobin A1c; ADM= antidiabetic medication; NSAID = nonsteroidal anti-inflammatory drug;

Appendix 7: Summary of Characteristics of Included Studies for All-Cause Mortality

Table 3. Study Characteristics of the included studies for metformin and all-cause mortality for breast cancer patients

Study (Author, Year)	Location of Study	Study Design	Study duration; Follow up period (years)	Total Sample Size	Age (years)	Exposure ascertainment	Outcome Ascertainment; type of breast cancer	Inclusion Criteria
He, 2011	USA	Retrospective; breast cancer specific	1998 to 2010; median 47.6 months (range 0.3 to 152.2 months)	1988	NR	MD Anderson Breast Cancer Management System Database	Tumor registry or through mailed questionnaires or Social Security Death Index; HER2+ breast cancer	Consecutive patients with stage ≥ 2 HER2+ breast cancer
Bayraktar, 2012	USA	Retrospective; breast cancer specific	1997 to 2007; median: 62 months (1 to 176 months)	1448	NR	MD Anderson Breast Cancer Management System Database	NR; triple negative breast cancer	Triple negative breast cancer patients who were receiving adjuvant chemotherapy
Lega, 2013	Canada	Retrospective; breast cancer specific	1997 to 2008; mean (SD): 4.5 (3.0)	2361	Mean (SD): 77.4 (6.3)	Ontario Diabetes Database	Ontario Cancer Registry or Registered Persons Database; all	Women with incident diabetes, aged 66 years or older
Peeters, 2013	Denmark	Retrospective; breast cancer specific	1996 to 2008; Metformin: median (IQR): 1.8 (0.8-3.8) Non-metformin: median (IQR): 2.6 (0.9-4.4)	1058	60 to 82	Denmark National Hospital Discharge Register and National Pharmacological Database	Death certificate register; all	Females (aged 18+) receiving treatment for diabetes mellitus who had a diagnostic code for breast cancer between 1997 and 2007
El-Benhaway, 2014	Egypt	Retrospective; breast cancer specific	Jan 2008 to Dec 2008; median 46 months (22-60)	439	NR	University of Alexandria records	University of Alexandria records; stage I to III breast cancer	Pathologically proved stage I to III breast cancer

Table 3 (continued):

Study (Author, Year)	Location of Study	Study Design	Study duration; Follow up period (years)	Total Sample Size	Age (years)	Exposure ascertainment	Outcome Ascertainment; type of breast cancer	Inclusion Criteria
Oppong, 2014	USA	Retrospective; breast cancer specific	2000 to 2005; median 87 months (range: 6.9 to 140.4)	141	38 to 80 years	EMR: Memorial Sloan Keating Cancer Centre	EMR; all	Patients who reported a diagnosis of T2DM and received systematic chemotherapy for stages I to III breast cancer
Xiao, 2014	China	Retrospective; breast cancer specific	2002 to 2006; median 70 months (10–120 months)	5785	NR	Tianjin Medical Database	EMR; luminal breast cancer	Luminal type breast cancer between 2002 to 2006
Calip, 2015	USA	Retrospective; breast cancer specific	1990-2008; median: 6.5	4216	Mean (SD): 6.8 (3.8)	Group Health Database	SEER database; stage I and II	≥ 18 years, residing in Washington State, incident, histologically confirmed stage I and II breast cancer (non-bilateral) between 1990 and 2008
Kim, 2015	South Korea	Retrospective; breast cancer specific	1997 to 2007; median 100.3 months	6967	NR	Asan Medical Centre Breast Cancer Database	EMR; all	Patients who were diagnosed with breast cancer and underwent surgery.
Xu, 2015	USA	Retrospective; all cancers	1995 to 2010; NR	Vanderbilt: 5796 Mayo: 8939	NR	EMR from Mayo Clinic and Vanderbilt	EMR from Vanderbilt University Medical Center and Mayo Clinic; all	≥18 years, incident cancer diagnosis excluding non-melanoma skin cancers between 1995 and 2010
Vissers, 2015	United Kingdom	Retrospective; breast cancer specific	1998 to 2009; mean 4.4 years	1057	Mean (SD): 70.6 (11.3)	Clinical Practice Research Datalink	National Cancer Data Repository and the Office of National Statistics; all	Cohort of female breast cancer patients, diagnosed between 1998 and 2009, type 2 diabetes was identified

Abbreviations: USA=United States of America; NR=not reported; HER2=human epidermal growth factor receptor 2; IQR=interquartile range; SEER=Surveillance, Epidemiology, and End Results; EMR = electronic medical record; T2DM=type 2 diabetes mellitus; SD=standard deviation

Table 4. Summary of Main Outcomes for all-cause Mortality

Study (Author, Year)	Users of metformin (n)	Comparator; number of users (n)	Deaths (metformin; comparator(s))	Treatment Duration	Adjusting variables	HR, 95% CI
He, 2011	88	Non-user of metformin; 66	NR; NR	At diagnosis and during follow-up	Age, BMI, ER/PR status, insulin therapy and insulin secretagogue therapy	0.52 (0.28-0.97)
Bayraktar, 2012	63	Non-metformin; 67	20;23	During adjuvant chemotherapy	Age, body weight, tumor size, nodal status, nuclear grade, lymphovascular invasion, type of adjuvant chemotherapy	0.82 (0.44-1.52)
Lega, 2013	1094	Non-metformin; 1267	175; 835	Cumulative metformin exposure	Sulfonylurea, insulin, TZD use, age at breast cancer diagnosis, duration of diabetes, before breast cancer, comorbidity score based on adjusted clinical group score at time of cohort entry, breast cancer treatments received within 1 year of diagnosis (surgery, radiotherapy, chemotherapy, aromatase inhibitor, tamoxifen), and exposure to glucose-lowering drugs before breast cancer diagnosis	0.97 (0.92-1.02)
Peeters, 2013	508	Non-metformin; 550	112; 176	Prescription of metformin in the past 3 months after cancer diagnosis	Age, CCI, number of years between January 1, 1997 and the date of breast cancer diagnosis, and use of concomitant medication during follow-up: metformin, sulfonylureas, thiazolidinediones, other antidiabetic drugs, hormone replacement therapy, and statins in the past 6 months	0.74 (0.58-0.96)
El-Benhaway, 2014	25	Non-metformin; 14	NR	During adjuvant chemotherapy	Age at presentation, ER and PR, lymph node status, tumor grade, clinical stage	0.11 (0.028-0.44);
Oppong, 2014	76	Non-metformin; 65	10; 12	Metformin use at baseline (at time of breast cancer diagnosis) or at the time of diabetes diagnosis if that occurred within 6 months	Age, hormone receptor status, and stage	0.80 (0.33-1.96)

Table 4 (continued):

Study (Author, Year)	Users of metformin (n)	Comparator; number of users (n)	Deaths (metformin; comparator(s))	Treatment Duration	Adjusting variables	HR, 95% CI
Xiao, 2014	Luminal A: 84; Luminal B (high ki67): 140; Luminal B (HER2+): 51	Non-metformin; 117; 201; 87;	NR	NR	Age, BMI, amenorrhea, the presence of cardiovascular and cerebrovascular disease, pathological stage, pathological type, lymph node involvement, vessel carcinoma embolus, and the chemotherapy and endocrine regimen	Luminal A 0.28 (0.11-0.66) Luminal B (high ki67) 0.31 (0.18-0.54) Luminal B (HER2+) 0.49 (0.25-0.98)
Calip 2015	381	Non-metformin; NR	NR	≥ 1 dispensing of medication during follow-up	Other medication classes of interest; age at diagnosis; diagnosis year; stage; hormone receptor; primary treatment for initial BC; endocrine therapy for the incident BC; BMI, smoking status, menopausal status, CCI; statin use; prescription non- steroidal anti-inflammatory medication use, Cox-2 inhibitors, aspirin; receipt of screening mammogram in the 12 months prior to events	0.55 (0.38-0.79)
Kim, 2015	202	Non-metformin; 184	NR	NR	Age, BMI, tumor size, lymph node metastasis, ER, PR, and HER2-neu status, and systemic treatment	0.53 (0.35-0.80)
Vissers, 2015	Prevalent diabetes: 688	Prevalent diabetes: Never use; 369	Prevalent 174; 174	<2 years use ≥ 2 years use	Age at BC diagnosis, diabetes duration before BC, year of BC diagnosis, BC treatment (surgery, radiotherapy, chemotherapy and hormone therapy within 6 months after BC diagnosis), hormone replacement therapy prior to BC diagnosis and comorbidity (stroke, chronic pulmonary disease, congestive heart disease, diabetes with complications, myocardial infarction, peptic ulcer disease, peripheral vascular disease and renal disease) prior to BC diagnosis	Prevalent: <2 years 0.9 (0.70-1.16) ≥ 2 years: 0.7 (0.49-0.99)

Table 4 (continued):

Study (Author, Year)	Users of metformin (n)	Comparator; number of users (n)	Deaths (metformin; comparator(s))	Treatment Duration	Adjusting variables	HR, 95% CI
Xu, 2015*	Vanderbilt: 9% of 2218 Mayo Clinic: 12% of 3029	Vanderbilt: Non-metformin: 4% of 903 Insulin: 3% of 377 Mayo Clinic: Non-metformin: 7% of 1629 Insulin: 7% of 1426	NR	After cancer diagnosis	Age at cancer diagnosis, sex, race, BMI, insulin use, tobacco use, tumor type, and tumor stage	Vanderbilt: Non-metformin: 0.47 (0.26-0.86) Insulin: 0.38 (0.13-1.05) Mayo Clinic: Non-metformin: 0.49 (0.31-0.77) Insulin: 0.57 (0.34-0.95)

* = studies that also included other cancers; Abbreviations: CCI = Charleston Comorbidity Index; BMI=body mass index; NR=not reported; ER= estrogen receptors; PR=progesterone receptors; HER2=human epidermal growth factor receptor; BC=breast cancer; COX2=cyclooxygenase-2; TZD=thiazolidinediones;

Appendix 8: Newcastle-Ottawa Scale for Breast Cancer Incidence Studies

Table 5. Newcastle-Ottawa Scale for Included Cohort Studies – Breast Cancer Incidence

Quality Assessment Criteria	Acceptable (*)	Currie (2009)	Hsieh (2009)	Libby (2009)	Morden (2011)	Chlebowski (2012)	Ruiter (2012)
SELECTION							
Representativeness of the exposed cohort	Truly and somewhat representative of average adult with T2DM in community	*	*	*	*	*	*
Selection of the non-exposed cohort	Drawn from same community as exposed cohort	*	*	*	*	*	*
Ascertainment of exposure	Secured records, structured interview	*	*	*	*	*	*
Demonstration that outcome of interest was not preset at start of study	Yes	*	*	*	*	*	*
COMPARABILITY							
Study controls for age?	Yes	*	*	*	*	*	*
Study controls for at least 3 additional risk factors	BMI, HbA1c levels, ethnicity, family history of cancer, physical activity, smoking, T2DM duration/severity	*		*		*	
OUTCOME							
Assessment of outcome	Independent blind assessment, record linkage		*	*	*	*	*
Was follow up long enough for outcomes to occur?	Follow up ≥ 3 years					*	
Adequacy of follow up of cohorts	Complete follow up or subjects lost to follow up unlikely to bias					*	
OVERALL SCORE (0 to 9)		**6**	**6**	**7**	**6**	**9**	**6**

Table 5 (continued):

Quality Assessment Criteria	Acceptable (*)	Soffer (2014)	Tsilidis (2014)	Chen (2015)	Kowall (2015)	Callip (2016)
SELECTION						
Representativeness of the exposed cohort	Truly and somewhat representative of average adult with T2DM in community	*	*	*	*	*
Selection of the non-exposed cohort	Drawn from same community as exposed cohort	*	*	*		*
Ascertainment of exposure	Secured records, structured interview	*	*	*	*	*
Demonstration that outcome of interest was not preset at start of study	Yes	*	*	*	*	*
COMPARABILITY						
Study controls for age?	Yes	*	*	*	*	*
Study controls for at least 3 additional risk factors	BMI, HbA1c levels, ethnicity, family history of cancer, physical activity, smoking, T2DM duration/severity		*		*	*
OUTCOME						
Assessment of outcome	Independent blind assessment, record linkage	*	*	*	*	*
Was follow up long enough for outcomes to occur?	Follow up ≥ 3 years	*	*			*
Adequacy of follow up of cohorts	Complete follow up or subjects lost to follow up unlikely to bias	*		*		
OVERALL SCORE (0 TO 9)		**8**	**8**	**7**	**6**	**8**

Abbreviations: T2DM = Type 2 Diabetes Mellitus, BMI = body mass index, HbA1c = hemoglobin A1c

Table 6 Newcastle-Ottawa Scale for Included Case-Control Studies – Breast Cancer Incidence

Quality Assessment Criteria	Acceptable (*)	Bosco (2011)
SELECTION		
Is the case definition adequate?	Requires some independent validation (e.g. >1 person/record/time/process to extract information, or reference to primary record source such as x-rays or medical/hospital records)	*
Representativeness of cases	Consecutive or obviously representative series of cases	*
Selection of controls	Community controls (i.e. same community as cases would be cases if had outcome)	*
Definition of controls	No history of disease	*
COMPARABILITY		
Study controls for age	Yes	*
Study controls for at least 3 additional factors	BMI, ethnicity, family history of cancer, physical activity, smoking, T2DM duration/severity, statin use, tumor size, nodal status, chemotherapy	*
OUTCOME		
Ascertainment of Exposure	Secure record, structured interview where blind to case/control status	*
Same method of ascertainment for cases and controls	Yes	*
Nonresponse rate	Same for both group	
OVERALL SCORE (0 TO 9)		**8**

Abbreviations: T2DM = Type 2 Diabetes Mellitus, BMI = body mass index, HbA1c = hemoglobin A1c

Appendix 9: Newcastle-Ottawa Scale for All-Cause Mortality studies

Table 7. Newcastle-Ottawa Scale for Included Cohort Studies – All-Cause Mortality

Quality Assessment Criteria	Acceptable (*)	He (2011)	Bayraktar (2012)	Lega (2013)	Peeters (2013)	El-Benhawy (2014)	Oppong (2014)
SELECTION							
Representativeness of the exposed cohort	Truly and somewhat representative of average adult with T2DM in community	*	*	*	*		*
Selection of the non-exposed cohort	Drawn from same community as exposed cohort	*	*	*	*	*	*
Ascertainment of exposure	Secured records, structured interview	*	*	*	*	*	*
Demonstration that outcome of interest was not preset at start of study	Yes	*	*	*	*	*	*
COMPARABILITY							
Study controls for age?	Yes	*	*	*	*	*	*
Study controls for at least 3 additional risk factors	BMI, HbA1c levels, ethnicity, family history of cancer, physical activity, smoking, T2DM duration/severity, tumor size, nodal status, chemotherapy	*	*	*			
OUTCOME							
Assessment of outcome	Independent blind assessment, record linkage	*		*	*		*
Was follow up long enough for outcomes to occur?	Follow up ≥ 3 years	*	*	*		*	*
Adequacy of follow up of cohorts	complete follow up or subjects lost to follow up unlikely to bias		*		*		
OVERALL SCORE (0 TO 9)		**8**	**8**	**8**	**7**	**5**	**7**

Table 7 (continued):

Quality Assessment Criteria	Acceptable (*)	Xiao (2014)	Callip (2015)	Kim (2015)	Xu (2015)	Vissers (2015)
SELECTION						
Representativeness of the exposed cohort	Truly and somewhat representative of average adult with T2DM in community	*	*	*	*	*
Selection of the non-exposed cohort	Drawn from same community as exposed cohort	*	*	*	*	*
Ascertainment of exposure	Secured records, structured interview	*	*	*	*	*
Demonstration that outcome of interest was not preset at start of study	Yes	*	*	*	*	*
COMPARABILITY						
Study controls for age?	Yes	*	*	*	*	*
Study controls for at least 3 additional risk factors	BMI, HbA1c levels, ethnicity, family history of cancer, physical activity, smoking, T2DM duration/severity, tumor size, nodal status, chemotherapy	*	*		*	*
OUTCOME						
Assessment of outcome	Independent blind assessment, record linkage	*	*	*	*	*
Was follow up long enough for outcomes to occur?	Follow up ≥ 3 years	*	*	*		
Adequacy of follow up of cohorts	Complete follow up or subjects lost to follow up unlikely to bias	*				
OVERALL SCORE (0 TO 9)		**9**	**8**	**7**	**7**	**7**

Abbreviations: T2DM = Type 2 Diabetes Mellitus, BMI = body mass index, HbA1c = hemoglobin A1c

Appendix 10: Meta-Analyses for Metformin and Incidence of Breast Cancer

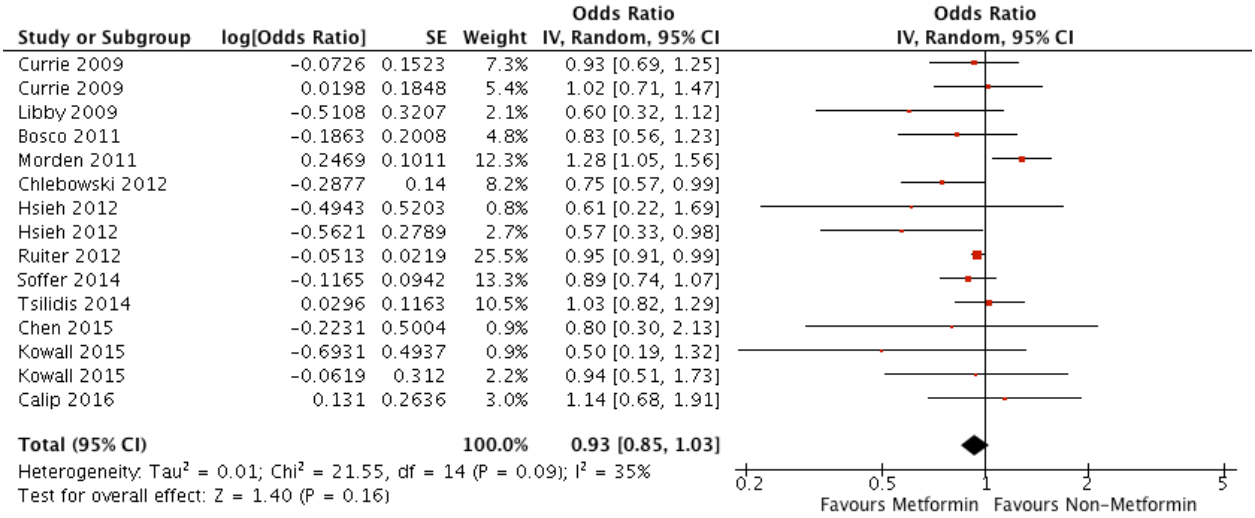


Figure 2. Forest Plot for Metformin and Incidence of Breast Cancer
Forest plot of the odds ratio (OR) of breast cancer incidence. Squares represent the OR of each single study (size of the square reflects the study specific statistical weight); horizontal lines represent 95% CI confidence intervals; diamonds represent the pooled estimates, based on the random effects model

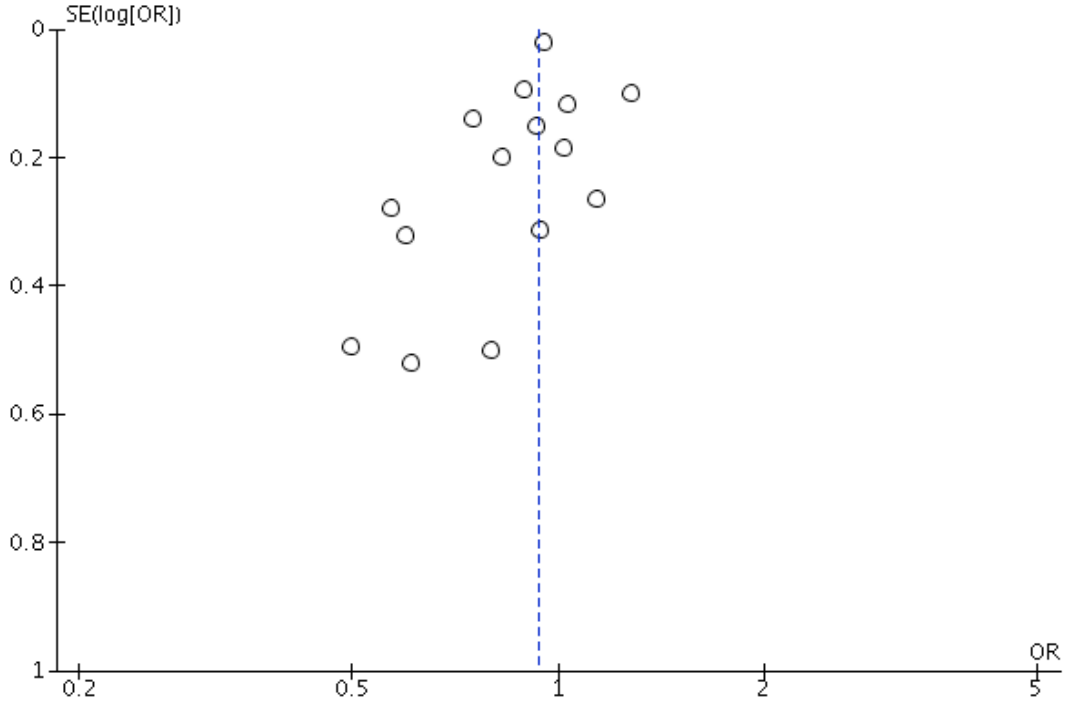


Figure 3. Egger's Funnel plot for metformin and incidence of breast cancer

Table 8. Summary of Subgroup Analyses for Metformin and Incidence of Breast Cancer

		n*	OR	95% CI	P value	I^2
Duration of Treatment	≥ 3 years**	4	0.95	0.91, 0.99	0.01	0%
	< 3 years	8	0.85	0.72, 1.00	0.05	35%
Comparators	Sulfonylureas**	6	0.94	0.85, 1.04	0.26	14%
	Insulin	4	1.06	0.83, 1.36	0.65	39%
	Non-Metformin	5	0.85	0.74, 0.97	0.01	0%
Time-Related Biases	No Bias**	8	0.94	0.91, 0.98	0.005	0%
	Bias	7	0.89	0.70, 1.13	0.34	59%

*number of effect sizes pooled; ** exploratory analysis conducted
Abbreviations: OR = odds ratio, 95%CI = 95% Confidence Interval;

Table 9. Summary of Sensitivity Analyses for Metformin and Incidence of Breast Cancer

		n*	OR	95% CI	P value	I^2
Quality of Studies	Low RoB	5	0.90	0.80, 1.02	0.09	1%
	High RoB	10	0.93	0.80, 1.09	0.38	46%
BMI/Obesity	Adjusted	8	0.90	0.73, 1.11	0.34	56%
	Unadjusted**	7	0.95	0.91,0.98	0.007	0%
All types	Breast cancer specific	3	0.82	0.67,1.01	0.07	0%
	All cancers	12	0.95	0.86, 1.06	0.38	38%
Fixed Effects**		15	0.95	0.91, 0.99	0.009	35%

*number of effect sizes pooled; ** exploratory analysis conducted
Abbreviations: OR = odds ratio, 95%CI = 95% Confidence Interval; RoB = risk of bias; BMI = body mass index

Appendix 11: Time-Related Biases

Table 10 Time-Related Biases in Metformin and Breast Cancer Incidence Studies

Author	Time-Related Bias	Explanation
Currie (2009)	Immortal Time and Time lag bias	Minimum exposure of 6 consecutive prescriptions to be included in the study, however, follow-up starts date of first prescription. Potentially presence of immortal bias as participants could interchange type of anti-diabetic medication during the 6-month period. This study also compared different cohorts which included second- and third-line therapies. Metformin monotherapy is a first line therapy, this introduces time-lag bias from different stages of diabetes. It also ignores that previous exposures may have affected future cancer risk.
Libby (2009)	Time Lag	Comparison of non-metformin user does not have a prescription date to define time zero, instead it was matched with first metformin prescription for the 'user' (i.e. index date). Time lag bias is introduced because 'non-users' who should have been excluded because of previous cancer before index date was included. This is further discussed by Suissa & Azoulay
Bosco (2011)	Time-window bias	Study participants were not matched on duration of follow-up or duration of exposure time.
Morden (2011)	Time-lag bias	Inclusion criteria for comparators included insulin prescription in the first 4 months of enrollment in the part D program. This implies that all have advanced T2DM at study entry, which may introduce time-lag bias.
Chlebowski (2012)	Unlikely	Study used Cox-regression models with a time-dependent categorical exposure variable that included information on diabetes diagnoses and medications used.
Hsieh (2012)	Time-lag	Study included monotherapy with metformin, sulphonylurea or insulin, and received continuous drug coverage for at least 1 year during study period. Time lag bias is introduced when metformin is compared against insulin therapy (typically 3rd line disease), implying advanced diabetes.
Ruiter (2012)	Unlikely	Cox proportional hazard model with time-varying determinant was used to account for metformin versus sulfonylurea.
Soffer (2014)	Unlikely	The study conducted multivariable Cox regression models with time-dependent drug use status to avoid immortal time bias.

Table 10 (continued):

Tsilidis (2014)	Unlikely	Intention-to-treat analysis was used in this study to avoid time-related biases.
Chen (2015)	Unlikely	Excluded any patients who crossed over to a different hypoglycemic drug or initiated the use of combination therapy to prevent misclassification. Patients developing cancer within the first year of follow-up were excluded from further study and the person-time was censored at the date of cancer diagnosis.
Kowall (2015)	Unlikely	Intention-to-treat analyses was used in this study; patients using metformin as first diabetes were compared with patients using sulfonylurea and insulin as first diabetes drug.
Callip (2016)	Unlikely	The study updated the exposure status daily for each study participant. Definition of 'user' of a medication class of interest had 2+ dispensing of medication during 6-month period prior to cohort entry to avoid misclassification.

Table 11. Time-Related Biases in Metformin and All-Cause Mortality

Author	Time-Related Bias	Explanation
He (2011)	Immortal time	Misclassification as 'exposed', the time between cancer diagnosis and first prescription of metformin during following up, when it is 'unexposed' to metformin. This is further described in Suissa and Azoulay's article.
Bayraktar (2012)	Immortal time bias	Exposure status of treatments unknown, time-dependent analysis was not conducted. Immortal time bias most likely present in this study.
El-Benhawy (2012)	Immortal Time and Time lag bias	Study did not specify duration of medications were taken, the study did not state a minimum exposure period, potentially introducing immortal time bias. The study also did not account for disease severity of diabetes (i.e. those who are taking insulin, third line therapy compared to metformin, first line therapy).
Lega (2013)	Unlikely	Time varying approach was used where a subject's exposure classification could vary over time in prescription was filled during follow up.
Peeters (2013)	Unlikely	Cumulative number of metformin prescriptions was updated and assessed as time-dependent variable.
Oppong (2014)	Immortal time bias	Patients were stratified on metformin use at baseline, defined as use at time of BC diagnosis. Immortal time bias may be present as the study did not exclude prior use of other anti-diabetic medications prior to study recruitment.
Xiao (2014)	Immortal time bias	Exposure status to metformin and non-metformin was not indicated in the study, immortal time bias most likely introduced.
Callip (2015)	Unlikely	Presence of diabetes and use of diabetes medication classes were modelled as time-varying covariates for the Cox PH regression model. This study also adjusted for disease duration.
Kim (2015)	Immortal time bias	Exposure status of treatments unknown, time-varying analysis was not conducted, immortal time bias likely present in this study.
Vissers (2015)	Unlikely	Time-dependent analyses were conducted to avoid time-related biases.
Xu (2015)	Immortal time	The study acknowledged that they may be subject to immortal bias due to inability to discern whether erroneous exposure time was assigned between cohort entry and mention of medication in clinical record.

Appendix 12: Analyses for Metformin and All-Cause Mortality

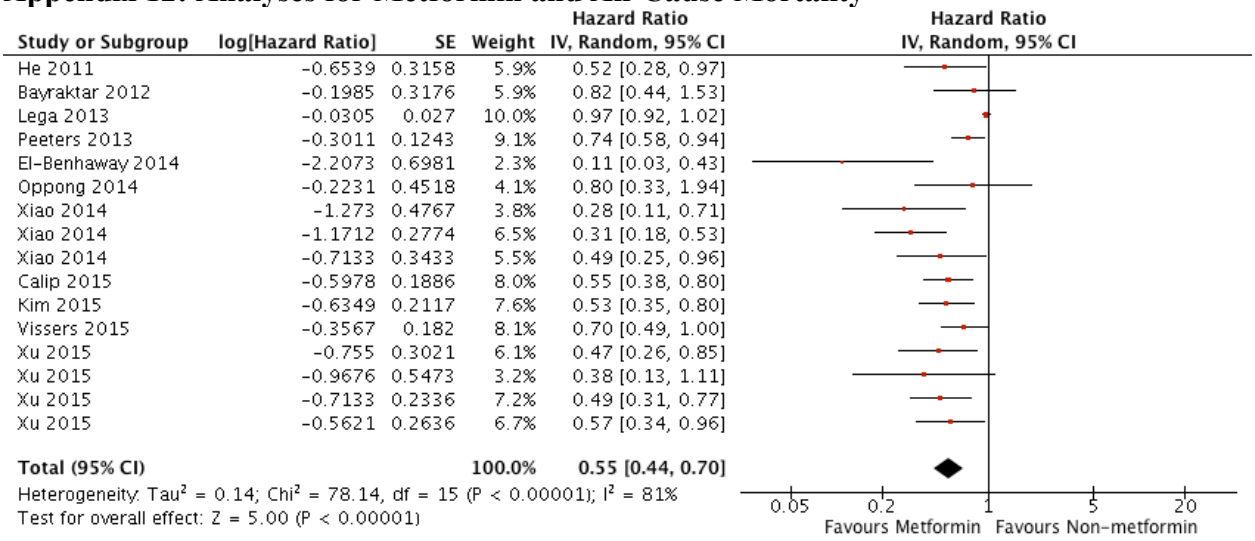


Figure 4. Forest Plot for Metformin and All-Cause Mortality

Forest plot of the hazard ratio (HR) of all-cause mortality. Squares represent the HR of each single study (size of the square reflects) the study specific statistical weight); horizontal lines represent 95% CI confidence intervals; diamonds represent the pooled estimates, based on the random effects model

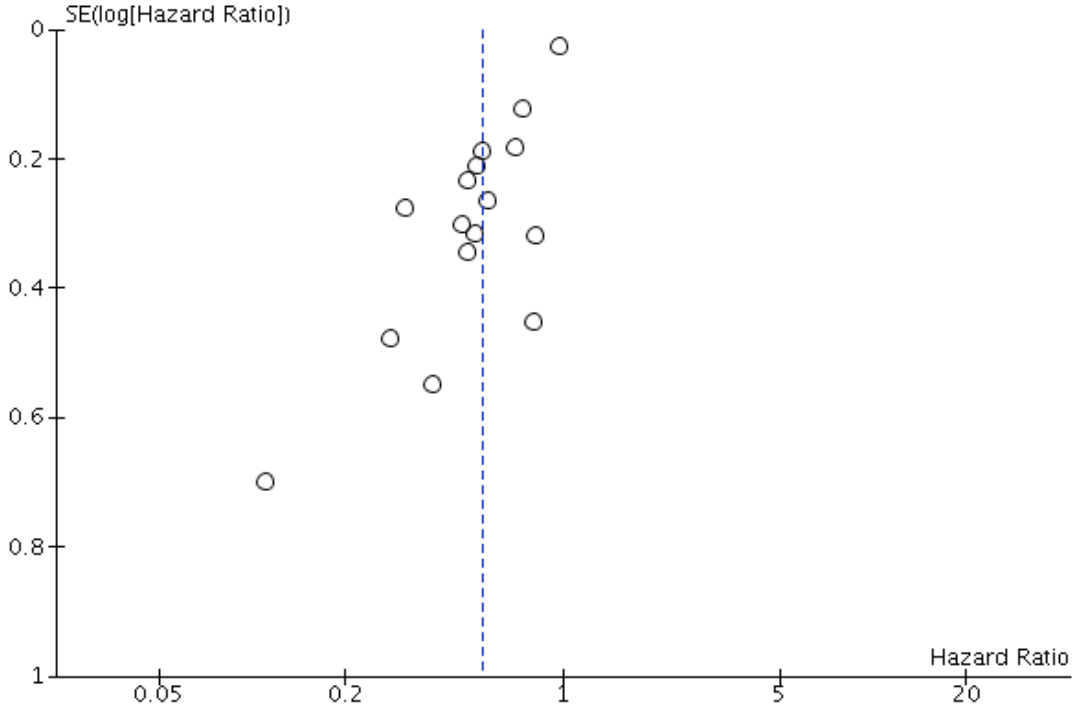


Figure 5. Egger's Funnel plot for metformin and all-cause mortality

Table 12. Summary of Subgroup Analyses for Metformin and All-Cause Mortality

		n*	HR	95% CI	P value	I^2
Time-related biases	No bias	4	0.75	0.58, 0.98	0.04	81%
	Bias	12	0.48	0.40, 0.59	< 0.00001	17%

*number of effect sizes pooled
Abbreviations: HR = hazard ratio, 95%CI = 95% Confidence Interval;

Table 13 Summary of Sensitivity Analyses for Metformin and All-Cause Mortality of Breast Cancer

		n*	HR	95% CI	P value	I^2
Quality of Studies	Low RoB	7	0.55	0.36, 0.83	0.004	85%
	High RoB	9	0.58	0.49, 0.71	< 0.00001	35%
BMI/Obesity	Adjusted	11	0.50	0.43, 0.59	<0.00001	0%
	Unadjusted	5	0.74	0.55, 1.01	0.06	77%
Fixed Effects**		17	0.90	0.85, 0.94	< 0.0001	81%

*number of effect sizes pooled; ** exploratory analysis conducted
Abbreviations: HR = hazard ratio, 95%CI = 95% Confidence Interval; RoB= risk of bias; BMI=body mass index

Appendix 13: GRADE Results

Table 14. Association of Metformin with Breast Cancer Incidence and All-Cause Mortality – Evidence Profile

Quality assessment							№ of patients		Effect		Quality
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Metformin	Non-metformin	Relative (95% CI)	Absolute (95% CI)	
Breast Cancer Incidence											
12	observational studies	serious [a,b,c]	not serious [d,e]	not serious	not serious [f]	publication bias strongly suspected [g]	1238/7506 (16.5%)	1673/8724 (19.2%)	OR 0.93 (0.85 to 1.03)	**11 fewer per 1,000** (from 5 more to 24 fewer)	⊕◯◯◯ VERY LOW
All-Cause Mortality											
11	observational studies	serious [h,i,j]	serious [k,l]	not serious	not serious [m]	publication bias strongly suspected [g]	491/3400 (14.4%)	1220/2987 (40.8%)	HR 0.55 (0.44 to 0.70)	**158 fewer per 1,000** (from 101 fewer to 202 fewer)	⊕◯◯◯ VERY LOW

CI: Confidence interval; **OR:** Odds ratio; **HR:** Hazard Ratio

Explanations

a. Presence of time-related biases in 42% (5/12) of the included studies
b. Five studies (Hsieh (2012), Morden (2011), Ruiter (2012), Soffer (2014), Chen (2015)) failed to adjust for additional confounding factors as described in the Newcastle-Ottawa Scale assessment
c. Of the included studies, 58% (7/12) were rated high risk of bias using the Newcastle-Ottawa Scale (i.e score <8)
d. Moderate degree of heterogeneity (I square = 35%)
e. p value is not statistically significant for heterogeneity (p=0.09)
f. Optional information size is met; summary RR does cross 1.0, upper and lower limit of the 95% CI does not include appreciable harm or benefit (RR 0.75)
g. Asymmetry identified in Egger's funnel plot
h. Four studies (Peeters (2013), El-Benhawy (2014), Oppong (2014), Kim (2015)) failed to adjust for additional confounding factors as described in the Newcastle-Ottawa Scale
i. Presence of time-related biases in 64% (7/11) of the included studies
j. Of the included studies, 55% (6/11) studies were rated high risk of bias using the Newcastle Ottawa Scale (i.e. score <8)
k. High degree of heterogeneity (I square = 81%)
l. p value is statistically significant for heterogeneity (p < 0.00001)
m. Optional information size is met; summary HR does not cross 1.0, upper and lower limit of the 95% CI does not include appreciable harm or benefit (RR 0.75)

Table 15. Association of Metformin with Breast Cancer Incidence and All-Cause Mortality – Summary of Findings

Summary of findings:

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)
	Risk with Non-metformin	Risk with Metformin			
Breast Cancer Incidence	192 per 1,000	181 per 1,000 (168 to 196)	**OR 0.93** (0.85 to 1.03)	16230 (12 observational studies)	⊕◯◯◯ VERY LOW [a,b,c,d,e,f,g]
All-Cause Mortality	408 per 1,000	251 per 1,000 (206 to 308)	**HR 0.55** (0.44 to 0.70)	6387 (11 observational studies)	⊕◯◯◯ VERY LOW [g,h,i,j,k,l,m]

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **OR:** Odds ratio; **HR:** Hazard Ratio

GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

Explanations

a. Presence of time-related biases in 42% (5/11) of the included studies
b. Five studies (Hsieh (2009), Morden (2011), Ruiter (2012), Soffer (2014), Chen (2015)) failed to adjust for additional confounding factors as described in the Newcastle-Ottawa Scale assessment
c. Of the included studies, 58% (7/12) were rated high risk of bias using the Newcastle-Ottawa Scale (i.e score <8)
d. Moderate degree of heterogeneity (I square = 35%)
e. p value is not statistically significant for heterogeneity (p=0.09)
f. Optional information size is met; summary RR does cross 1.0, upper and lower limit of the 95% CI does not include appreciable harm or benefit (RR 0.75)
g. Asymmetry identified in Egger's funnel plot
h. Four studies (Peeters (2013), El-Benhawy (2014), Oppong (2014), Kim (2015)) failed to adjust for additional confounding factors as described in the Newcastle-Ottawa Scale
i. Presence of time-related biases in 64% (7/11) of the included studies
j. Of the included studies, 55% (6/11) studies were rated high risk of bias using the Newcastle Ottawa Scale (i.e. score <8)
k. High degree of heterogeneity (I square = 81%)
l. p value is statistically significant for heterogeneity (p < 0.00001)
m. Optional information size is met; summary HR does not cross 1.0, upper and lower limit of the 95% CI does not include appreciable harm or benefit (RR 0.75)