University of Groningen

Oral glucose lowering agents and cancer in type 2 diabetes mellitus: focus on sulphonylurea derivatives

Schrijnders, Dennis

DOI:
[10.33612/diss.103517830](https://doi.org/10.33612/diss.103517830)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):
Schrijnders, D. (2019). *Oral glucose lowering agents and cancer in type 2 diabetes mellitus: focus on sulphonylurea derivatives*. [Thesis fully internal (DIV), University of Groningen]. Rijksuniversiteit Groningen. <https://doi.org/10.33612/diss.103517830>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

**ORAL GLUCOSE LOWERING AGENTS AND CANCER IN TYPE 2
DIABETES MELLITUS: FOCUS ON SULPHONYLUREA DERIVATIVES**

D. Schrijnders

D. Schrijnders

Oral glucose lowering agents and cancer in type 2 diabetes mellitus:
focus on sulphonylurea derivatives

ISBN: 978-94-034-2210-7

ISBN Electronic: 978-94-034-2209-1

Lay-out: Gildeprint, Enschede

Cover: Nathan Anderson, Dennis Schrijnders and Gildeprint, Enschede

Printing: Gildeprint, Enschede

The research in this thesis was financially supported by a research grant (grant number 836041017) of the research programme Good Use of Medication from the Netherlands Organization for Health Research and Development (ZonMw). Printing of this thesis was financially supported by University of Groningen, University Medical Center Groningen and Isala Diabetes Centre.

© 2019 D. Schrijnders

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any other means without prior written permission of the author.

rijksuniversiteit
 groningen

Oral glucose lowering agents and cancer in type 2 diabetes mellitus: focus on sulphonylurea derivatives

Proefschrift

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. C. Wijmenga
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

woensdag 18 december 2019 om 14.30 uur

door

Dennis Schrijnders

geboren op 18 oktober 1985
te Uitgeest

Promotores

Prof. dr. H.J.G. Bilo

Prof. dr. G.H. de Bock

Copromotores

Dr. G.W.D. Landman

Dr. N. Kleefstra

Beoordelingscommissie

Prof. dr. P. Denig

Prof. dr. B.H.R. Wolffenbuttel

Prof. dr. S. Siesling

Paranimfen

Dhr. M. Schrijnders

Mw. M. Pfeufer

TABLE OF CONTENTS

Chapter 1	Introduction	9
Chapter 2	Sulphonylurea derivatives and cancer, friend or foe?	31
Chapter 3	Within-Sulphonylurea-Class Evaluation of Time to Intensification with Insulin (ZODIAC-43)	63
Chapter 4	Addition of sulphonylurea to metformin does not relevantly change body weight: a prospective observational cohort study (ZODIAC-39)	75
Chapter 5	Body mass index and obesity-related cancer risk in men and women with type 2 diabetes: a cohort study (ZODIAC-56)	99
Chapter 6	Sex differences in obesity related cancer incidence in relation to type 2 diabetes diagnosis (ZODIAC-49)	115
Chapter 7	Within-class differences in cancer risk for sulfonylurea treatments in patients with type 2 diabetes (ZODIAC-55) – a study protocol	135
Chapter 8	Summarizing Discussion	147
Chapter 9	Nederlandse samenvatting	165
Appendices	Dankwoord	173
	Curriculum Vitae	179
	List of publications	183
	Previous dissertations	189

1

General introduction

DIABETES WORLDWIDE

An increasing group of people worldwide is known with diabetes mellitus. According to the World Health Organization (WHO), the number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014 (1). The global prevalence of diabetes based on this figure among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014, a rise with immense consequences (1). The International Diabetes Federation (IDF) estimated that the worldwide prevalence of diabetes will be somewhere between 521 and 829 million in 2040 (2).

Over 90% of the people who are known with diabetes are diagnosed with type 2 diabetes (T2D), and the prevalence of T2D is expected to double in the next decades, far outpacing both the relative and absolute growth of people known with type 1 diabetes mellitus (T1D) (3–6).

The majority of cases of T2D are characterised by insulin resistance in peripheral tissues often followed by a relative insulin insufficiency, since β -cells cannot keep up with the increased demand for insulin (7). Risk of developing T2D increases with age, obesity and lack of physical activity (7).

Diabetes is a failure of maintaining a normal glucose metabolism and is together with other risk factors an important cause of micro- and macrovascular complications. These risk factors together can eventually lead to vision impairment, decline in kidney function and eventually kidney failure, myocardial infarction, stroke and lower limb amputation (8).

INCIDENCE AND PREVALENCE OF DIABETES IN THE NETHERLANDS

In the Netherlands 1,084,100 people (men: 66.9/1,000, women: 60.5/1,000) were known with diabetes mellitus (both T1D and T2D) in 2016 (9). More than 90% of these patients were diagnosed with T2D (4,10). According to the RIVM estimates, in 2016, 73,900 (men: 4.8/1000, women: 3.9/1000) were newly diagnosed with diabetes mellitus (9). It is estimated that in 2025 more than 1.3 million people in the Netherlands will be known with diabetes (4,5). The prevalence and incidence of T2D are age-dependent (Fig 1-2) (9).

Figure 1: One-year prevalence of diabetes mellitus (type 1 and type 2) in 2016 in the Netherlands. Source: NIVEL Zorgregistraties eerste lijn (9)

Figure 2: Incidence of diabetes mellitus (type 1 and type 2) in 2016 in the Netherlands. Source: Nivel Zorgregistraties eerste lijn (9).

DIABETES TREATMENT

About 90% of the patients diagnosed with T2D in the Netherlands are treated in primary care (11). The first step in the 2013 Dutch primary care diabetes guideline is education concerning lifestyle and diet (12). When treatment goals are not met, pharmacological treatment is initiated, starting with metformin. A preferred second step is addition of a sulfonylurea (preferably gliclazide). A third step is the addition of NPH-insulin. In addition to the already mentioned biguanides (metformin), sulfonylureas and insulin, there are more treatment classes available including meglitinides, thiazolidinediones, α -glucosidase inhibitors, DPP-IV inhibitors, incretin mimetics and SGLT-2 inhibitors (12). Most of these classes consist of more than one individual drug. Differences in safety in renal impairment, cardiovascular event risk and cancer exist between these classes (13–16).

CANCER WORLDWIDE AND IN THE NETHERLANDS

The International Agency for Research on Cancer (IARC) estimated that in 2012 14.1 million people worldwide were diagnosed with cancer and 8.2 million people died as a result of cancer (17). The most common cancer types worldwide were cancer of the lung, breast, colorectal, prostate and stomach (17). In the Netherlands 102,744 people were diagnosed with cancer in 2012 (Fig 3, men: 5.1/1000 patient years, women: 4.3/1000 patient years) (18). In 2012, 43,660 patients died as the result of cancer (men: 1.5/1000 patient years, women: 1.9/1000 patient years) (18). The most common cancers in the Netherlands were skin cancer, colorectal cancer, breast cancer, lung cancer and prostate cancer.

CANCER RISK AND DIABETES

In recent years, it has been suggested that patients known with T2D are threatened not only by microvascular and macrovascular complications, but that their chances of developing malignancies are also higher, at least for some specific forms of cancer (19–21).

Studies have reported that patients with T2D are more frequently diagnosed with cancer than people without T2D (19). Cancer and T2D occur concomitantly and have also been associated with each other (19–21). A study has shown that T2D patients have an excess cancer risk (Standardized Incidence Rate (SIR) in men 1.08 (95%CI 1.07-1.09), women 1.22 (95%CI 1.20-1.23)) (22). The different phenomena explaining and linking diabetes to cancer are discussed elaborately in next two paragraphs.

Figure 3: Number of newly diagnosed cancer in 2012 (Source: Nederlandse Kanker Registratie, IKNL).

The strongest associations between specific cancer types and T2D have been reported for liver and pancreas cancer (relative risk (RR) ~ 2) (20,21,23). Endometrial, colorectal, breast and bladder cancer also have been associated with an increased incidence in T2D patients (RR of 1.2 – 1.5) (20,21,23). Recent studies have suggested that at least part of the association might be explained by reverse causality (in case of liver and pancreas cancer) or ascertainment bias (23,24). In reverse causality it seems that an association is in one direction, but in the true association is in reverse. Ascertainment bias is a systematic difference in how an outcome is determined.

OBESITY: MEDIATOR TOWARDS INCREASED CANCER RISK?

Obesity is a major health problem in the world. Obesity is defined by the World Health Organization (WHO) as a BMI ≥ 30 kg/m² (25). The WHO estimates that 1.9 billion adults (39%) were overweight and 650 million adults (13%) were obese in 2016 (26). In the Netherlands 49.2% of the adults aged 18 and older were overweight, and 14.2% were obese in 2016 (27). The prevalence of obesity increases with age (27). A higher than normal BMI has been associated with increased risk of cardiovascular diseases, T2D, osteoarthritis and some specific cancers (26). To further evaluate and differentiate between effects of T2D and obesity is of importance.

DIABETES, OBESITY AND CANCER RISK IN DETAIL

The relationships between hyperglycaemia in T2D, obesity and cancer are complicated (figure 4). In addition to T2D being associated with obesity and cancer, obesity itself is also associated with cancer (and T2D) (28,29). The cancers associated with T2D overlap to a large extent with the cancers associated with obesity. The continuous update project of the World Cancer Research Fund associates 11 cancers with obesity (28). These include liver, advanced prostate, ovarian, gallbladder, kidney, colorectal, oesophageal, postmenopausal breast, pancreatic, endometrial, and stomach (cardia) cancer (28). A viewpoint published by the International Agency for Research on Cancer (IARC) associates some additional cancer types with obesity. In this viewpoint, the IARC recognizes cancers of the gastric cardia, colorectal, liver, gallbladder, pancreas, corpus uteri, ovary, thyroid, breast (postmenopausal only), kidney (renal-cell), meningioma, multiple myeloma and adenocarcinoma of the oesophagus to be associated with obesity (29). According to the IARC the evidence concerning the relation with advanced prostate cancer is limited (29).

Figure 4: Relationship between T2D, Obesity and Cancer

There remains discussion whether the increased occurrence of cancer in persons with T2D is mediated through diabetes per se, or whether an increased BMI is a more predominant factor for the relationship found (30). A recent study showed that 5.6% of all incident cancers worldwide in 2012 could be attributed to a combination of diabetes (both T1D and T2D) and high BMI (30). Of all liver cancer and endometrial cancer cases, 24.5% and 38.4% could be attributed to this combination of risk factors, respectively (30).

If a causal relation between T2D, BMI and cancer risk exists, then weight loss could lead better glycaemic control and both to a lower cancer risk. Indeed, studies have shown that interventions leading to lower bodyweight are an effective treatment option to treat

T2D (31–34). However, whether weight loss in subjects with T2D not only provides a better glycaemic control, but also leads to a lower cancer risk remains to be seen. One meta-analysis of prospective and retrospective studies involving 236,955 cases and 3,963,367 controls showed that weight loss results in a lower breast cancer risk (35). A meta-analysis of 54 RCTs involving 30,206 patients failed to show an effect of weight loss on cancer risk (36).

PATHOPHYSIOLOGY OF INSULIN RESISTANCE

The pathophysiological process underlying insulin resistance and T2D is not entirely clear. Inflammation, mitochondrial dysfunction and lipotoxicity are frequently discussed hypotheses (37,38). Inflammation as a result of obesity has been shown to inhibit insulin signalling pathways in adipocytes and hepatocytes through several mechanisms (37). However, muscle insulin action is not sensitive to inflammation and thus not impaired (37). Mitochondria are responsible for oxidation and metabolism of free fatty acids and glucose. Mitochondrial dysfunction could lead to free fatty acid and lipid accumulation. Some recent evidence, however, showed that mitochondrial dysfunction is not the cause of insulin resistance but the result of insulin resistance (37). There is no consensus for a unifying explanation of insulin resistance (37,38).

It has also been shown that insulin resistance is associated with both low and high levels of IGF-1 in a U-shaped manner (39). IGF-1 has been identified as a growth factor for cancer and high IGF-1 levels in insulin resistance could therefore contribute to cancer risk and growth (40). Both high insulin levels and abnormally high glucose concentrations are potent growth factors for cancer. It has been shown in animal models that hyperinsulinemia stimulates the PI3K pathway in cells at risk of malignant transformation resulting in increased rates of carcinogenesis (41). The PI3K pathway is an intracellular signalling pathway that regulates the cell cycle. Continued activation of the pathway contributes to cell proliferation, survival, motility and angiogenesis (42). However, this effect has not yet been confirmed in clinical studies.

BIASES, CONFOUNDERS AND EFFECT MODIFIERS BETWEEN CANCER AND T2D

There are several factors complicating the evaluation of the relation between cancer and T2D and T2D related treatment.

Several risk factors can influence the relation between cancer risk and T2D related medication use (23). Poor diet and limited physical activity can influence cancer risk via food content but also through obesity and insulin resistance, independent of medication use. Also smoking has been associated with numerous cancers (23). It has been shown that persons with T2D smoke more often than those who don't have T2D (43). In addition, smoking has been associated with a higher probability of developing insulin resistance (44).

Since a higher body weight is associated with both T2D occurrence and cancer, oral glucose lowering agents that modify body weight can confound a presumed relation. Another confounding factor in assessing cancer risk of medications could be renal failure, which has been reported to be independently associated with increased cancer risk (45,46), but also with a higher BMI (47–49). If, for example, a certain medication can't be prescribed to patients with renal failure, but a comparator can, an observed difference in cancer risk could in theory also be explained by differences in renal function.

Several sources of bias can influence the outcome of cancer risk (23). The most important sources are as follows. A first type that is often described is detection bias, sometimes also referred to as ascertainment bias (50,51). Detection bias is a systematic difference in how an outcome is determined (52). It is known that this kind of bias occurs frequently in observational studies researching T2D and cancer risk (23,24,53,54). For example, a health care provider might be more likely to do more intense surveillance or screening in T2D patients. Because these patients have contact with a health care provider, is it more likely that they are diagnosed with other diseases in addition to the T2D.

Another important potential source of bias is diagnostic bias. This occurs when exposure to something, for example a certain medication, makes it more likely to be diagnosed. For example, a study investigating the relationship between endometrial cancer and oral contraceptives might suffer from this type of bias. Women who use oral contraceptives might be offered screening more often systematically or because of breakthrough bleeding. In other words, the use of oral contraceptives might trigger a diagnostic process for endometrial cancer (because patients without symptoms bleed), rather than causing it.

A third source is reverse causality. In this case it seems that factor A, for example hyperglycaemia, is associated with factor B, for example pancreatic cancer. However, the true association is in reverse, i.e. pancreatic cancer is the cause of the hyperglycaemia by a dysfunction in insulin secretion caused by the effect of the tumour on the pancreatic endocrine function. Recent studies have suggested that at least part of the association between increased cancer risk in case of liver and pancreas cancer and T2D might be explained by reverse causality.

Other sources of bias include prevalent-user bias and immortal time bias. Prevalent-users are patients who benefit from the treatment. Patient who do not benefit from, for example, SU use, are more likely to stop taking SUs and start something else. This could then result in overestimation of benefits of SUs and underestimation of harms of SUs. This is especially important when investigating within-class differences. If a patient using glibenclamide is more likely to experience side-effects or non-response and subsequently switch to another class than a patient using gliclazide, this could then result overestimation of the benefits of gliclazide and an underestimation of the harm of glibenclamide.

Immortal time bias happens when, by design, death or the study outcome cannot occur in a certain follow-up period (55). There can be either misclassified immortal time or excluded immortal time (56). For example, suppose one would want to investigate cancer occurrence before diabetes diagnosis in a diabetes cohort. Patient who were diagnosed with cancer, but did not survive could not have been included in the diabetes cohort. In other words, the patients included in the cohort are survivors.

DIABETES TREATMENT AND POSSIBLE MODIFICATION OF CANCER RISK

Since increased body weight is associated with both T2D occurrence and cancer, oral glucose lowering agents that potentially modify body weight and or glycaemic control could theoretically confound a presumed relationship between diabetes and cancer risk. There are glucose lowering agents that have been associated with weight changes. Metformin and GLP-1 agonists have been associated with weight loss, while insulin, TZDs and SUs have been associated with weight gain (38–41). It is unclear whether this increase is a class effect or can be attributed to specific drugs. Metformin is the only biguanide available and has been associated with a lower cancer mortality compared with non-use of metformin (57). Recent meta-analyses show that metformin is associated with improved survival (HR 0.78, 95% CI 0.66-0.92 (58) and HR 0.86, 95% CI 0.76-0.97 (59))

in pancreatic cancer, a reduction of overall mortality in women with endometrial cancer (adjusted HR [aHR] 0.64, 95% CI 0.45-0.89, $p=0.009$) (60), a reduced risk of colorectal adenoma (pooled OR = 0.76, 95%CI 0.63-0.92) (61) and a reduced overall prostate cancer risk (HR 0.81, 95% CI 0.69-0.95) (62). Next to metformin, other drugs and drug classes have been implicated to influence cancer risk.

However, within the classes that contain multiple drugs, differences exist in almost every class with regard to safety in renal impairment, cardiovascular risk and cancer risk. For example, pioglitazone use, not rosiglitazone, is possibly associated with developing bladder cancer (63,64) and in some studies insulin glargine has been associated with increased risk of breast cancer (65). However, recent studies could not confirm these associations (66,67).

SULFONYLUREAS: CLASS EFFECTS OR WITHIN CLASS DIFFERENCES?

SUs promote insulin release by binding to sulfonylurea receptors on the β -cell in the pancreas (68). The sulfonylurea receptors have several isoforms: SUR1 located on the pancreatic β -cell, SUR2A located on cardiac myocytes and SUR2B located on vascular smooth tissue (69). It has been shown that gliclazide and glipizide bind selectively to SUR1, tolbutamide is partially selective and glimepiride and glibenclamide are non-selective (70).

Within-class differences have been reported for the sulfonylureas. These within-class differences are especially important because, at least in the Netherlands, the SUs are the preferred oral glucose lowering agent added to metformin, gliclazide is the preferred SU within its class (12). Whether the possible influence on other processes than glucose lowering should be seen as a class effect, or whether there might be within class differences is discussed below.

Glibenclamide is associated with an increased hypoglycaemia risk (1.83, 95%CI 1.35–2.49), both non-severe and severe, compared to other SUs (71). Compared with glimepiride users, glibenclamide users have a higher incidence of hypoglycaemia (0.86/1000 person years vs 5.6/1000 person years) (72). A study investigating glibenclamide versus glimepiride showed that glibenclamide delays plasma glucose recovery and stimulates insulin secretion at low plasma glucose levels in contrast to glimepiride (73). A systematic review and network meta-analysis showed that gliclazide has the lowest risk of hypoglycaemia compared with glipizide (OR 0.22, 95% Credible Interval (CrI) 0.05-0.96), glimepiride (OR 0.40, 95%CrI 0.13-1.27) and glibenclamide (OR 0.21, 95%CrI 0.03-1.48) (74).

Several studies reported that the initiation of individual SUs is accompanied by weight increase (75–78). The UKPDS-34 study showed an increase of about 4 kilograms in the first 3 years after glibenclamide initiation compared to metformin (75). In the ADOPT glibenclamide use resulted in a 1.6 kg increase in the first year compared to baseline (78). Glimepiride was associated with a mean weight gain of 1.2 kg in 30 weeks compared to baseline (76). However, there are no studies directly comparing the different SUs head-to-head.

Within-class SU differences have also been reported for failure rate (i.e. when HbA1c treatment target is no longer met with a certain SU). Glipizide and glibenclamide are associated with a higher failure rate compared to other SUs (79,80). This finding suggests that the time until the need to start insulin might differ between the individual SUs; however, up to now, this has not been investigated.

Differences in all-cause mortality and cardiovascular mortality have also been suggested. Of the non-selective SUs, glibenclamide, but not glimepiride, prevents ischemic preconditioning, a cardioprotective phenomenon (81). Compared to glibenclamide, both gliclazide (Relative Risk (RR) 0.65, Credible Interval (CrI) 0.53-0.79) and glimepiride (RR 0.83, CrI 0.68-1.00) were associated with a lower risk of all-cause mortality, whilst glipizide (RR 0.98, 0.80-1.19), tolbutamide (RR 1.13, 0.72-1.43) and chlorpropamide (1.34, 0.98-1.86) were not (82). For cardiovascular mortality similar associations were found (82,83).

SULFONYLUREAS AND CANCER RISK

A meta-analysis of cohort studies has shown that use of SUs (as a group) is associated with an increased risk of developing cancer compared to non-SU use ($n = 278,291$, relative risk (RR) 1.55 (95%CI 1.48-1.63) (84). Unfortunately, most of the included studies have serious methodological shortcomings. The included studies showed substantial heterogeneity, and were limited by lack of details such as cancer type and stage, and whether cumulative dose was taken into account. (84,85). The previously mentioned study also performed a meta-analysis of RCTs, which failed to show a significant change in cancer risk. However, only two RCTs were available for inclusion, and cancer risk was not the primary endpoint of these RCTs (84). Whereas SUs as a group might thus be associated with an increased risk of developing cancer, gliclazide use compared to other SUs appears to be associated with a decreased cancer risk in several small cohort studies (86–89). However, also these studies have methodological limitations; cancer types were grouped together, and the authors did not perform head-to-head comparisons and only adjusted for potential confounders to a varying degree.

Combining this information leads to the tentative conclusion that gliclazide could be the most selective SU, associated with the lowest mortality risk in observational studies; furthermore, gliclazide has the lowest hypoglycaemia risk and might also have a superior safety with regards to cancer risk.

GENERAL AIMS AND OUTLINE

This thesis aims to investigate the within-class SU differences that can act as confounders in the relationship between T2D and specifically SUs used for treating T2D and cancer risk in the Dutch primary care diabetes population. The presence of within-class SU differences in cancer risk could influence SU preference and prescription behaviour as a consequence. This thesis is part of a larger scale ZonMW project which aims to investigate within-class SU differences in cancer risk in a Dutch T2D primary care population.

In **chapter 2** a systematic review is presented, in which the available literature on within-class SU differences is discussed and summarized. In this review, observational and pre-clinical studies are assessed and further potential mechanisms explored. The study in **chapter 3** investigates whether within-class differences in weight gain exist. In **chapter 4** the relationship between BMI and obesity-related cancers in men and women with T2D is investigated. In **chapter 5** within-class SU differences with regard to time to insulin initiation are investigated. Diagnostic bias and detection bias are two important sources of bias. The aim of **chapter 6** was to investigate cancer incidence for obesity-related cancer and all-cancer in a 10-year time period around diabetes diagnosis. In **chapter 7** a study protocol incorporating aspects of this thesis for further research is presented.

DATA SOURCES

ZODIAC

The Zwolle Outpatient Diabetes Integrating Available Care project was initiated in 1998 in the Zwolle region as an observational cohort study to investigate the effects of shared care for patients treated in primary care (90).

Analysis of longitudinal data showed improved clinical variables and quality of life, and shared care became the standard care in the Zwolle region. The project was expanded to other regions in the Netherlands in 2006, 2009 and 2012. Patients included

in the ZODIAC cohort are diagnosed with T2D and are treated in primary care. Patients with a short life expectancy or insufficient cognitive capabilities are excluded. Data on age, sex, date of T2D diagnosis, HbA1c, length, weight, estimated GFR, creatinine, urinary albumin-creatinine ratio (ACR), total cholesterol, HDL-cholesterol, total/HDL cholesterol ratio, triglycerides, blood pressure, macrovascular complications (myocardial infarction, transient ischemic attack, stroke), medication use (both diabetes-specific and other medication), smoking (yes/no) and alcohol use (yes/no) are recorded annually and collected by the Isala Diabetes Centre for benchmarking and research purposes. Especially the yearly recorded weight and HbA1c data are unique to this cohort. All patients participating in ZODIAC consented with the anonymous use of their data for research purposes. Informed consent was obtained by the general practitioner and noted in the ZODIAC database system.

National Cancer Registry

The National Cancer Registry (NCR) was founded in 1989 and records all cancer events based on notification by the National Pathology Archive (PALGA) and hospital discharge registries. Specially trained registration employees collect data on every cancer event recorded in hospital information systems in the Netherlands. The base dataset includes cancer type, diagnosis date, stage and primary treatment. Depending on the cancer type additional data may be recorded (91).

Combined ZODIAC-NCR

The ZODIAC dataset was linked to the NCR by a trusted third party using postal code, full name, date of birth and sex by the end of 2014. The first linkage resulted in a combined ZODIAC-NCR cohort containing of 71.648 patients, of which 10.717 were diagnosed with 12.617 cancer events between January 1st 1989 and December 31st 2012. The linkage was updated in April 2017 to include all cancer events between January 1st 1989 and May 31st 2017. The updated linkage includes 71.648 patients of which 14.144 were diagnosed with 17.404 cancer events. Seven thousand sixty-three all-cancer events and 3678 obesity-related cancer events occurred after patients started participating in ZODIAC. The NCR expects that the number of false-positive and the number false-negative for the ZODIAC-NCR linkage is both under 1%.

Municipal Personal Records Database

In order to obtain date of death the ZODIAC-NCR dataset was linked with the Municipal Personal Records Database (GBA) by the Isala Hospital using a combination of the

personal identification number and full name, postal code, date of birth and sex. The GBA is a system used by Dutch governmental organizations to record personal data of all inhabitants of the Netherlands, including date of death. Of the 71.648 patients in the ZODIAC-NCR dataset 68.910 could be linked; 14.045 patients were deceased and 54.865 patients were alive at the moment of linkage (16 December 2016). A total of 2.738 patients 3.8% could not be linked.

REFERENCES

1. WHO | Diabetes Fact Sheet [Internet]. WHO. World Health Organization; 2017 [cited 2018 Mar 10]. Available from: <http://www.who.int/mediacentre/factsheets/fs312/en/>
2. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract* [Internet]. 2017 Jun 1 [cited 2018 Mar 10];128:40–50. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28437734>
3. World Health Organization. Global Report on Diabetes. 2016; Available from: http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf
4. Baan CA, van Baal PHM, Jacobs-van der Bruggen MAM, Verkley H, Poos MJJC, Hoogenveen RT, et al. [Diabetes mellitus in the Netherlands: estimate of the current disease burden and prognosis for 2025]. *Ned Tijdschr Geneesk* [Internet]. 2009 [cited 2017 Oct 6];153:A580. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19785785>
5. Kleefstra N, Landman GWD, Van Hateren KJJ, Meulepas M, Romeijnders A, Rutten GEH, et al. Dutch diabetes prevalence estimates (DUDE-1). *J Diabetes* [Internet]. 2016 Nov [cited 2017 Oct 6];8(6):863–5. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26694523>
6. Shaw JEE, Sicree RAA, Zimmet PZZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pr* [Internet]. 2010 Jan [cited 2018 Feb 1];87(1):4–14. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19896746>
7. 2. Classification and Diagnosis of Diabetes. *Diabetes Care* [Internet]. 2016 Dec 15;40(Supplement 1):S11 LP-S24. Available from: http://care.diabetesjournals.org/content/40/Supplement_1/S11.abstract
8. Magliano DJ, Martin VJ, Owen AJ, Zomer E, Liew D. The Productivity Burden of Diabetes at a Population Level. *Diabetes Care* [Internet]. 2018 Feb 28 [cited 2018 Mar 10];dc172138. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/29490901>
9. RIVM. Diabetes Mellitus: Cijfers & Context - Huidige situatie [Internet]. 2017 [cited 2017 Oct 6]. Available from: <https://www.volksgezondheidenzorg.info/onderwerp/diabetes-mellitus/cijfers-context/huidige-situatie>
10. Holman N, Young B, Gadsby R. Current prevalence of Type 1 and Type 2 diabetes in adults and children in the UK. *Diabet Med* [Internet]. 2015 Sep [cited 2017 Oct 6];32(9):1119–20. Available from: <http://doi.wiley.com/10.1111/dme.12791>
11. Nijenhuis-Rosien L, Hendriks SH, Kleefstra N, Bilo HJG, Landman GWD. Nationwide diabetes-related lower extremity amputation rates in secondary care treated patients with diabetes in the Netherlands (DUDE-7). *J Diabetes Complications* [Internet]. 2017 Apr [cited 2017 Jul 3];31(4):675–8. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S105687271630650X>
12. Rutten G, De Grauw WJC, Nijpels G, Houweling ST, Van de Laar FA, Bilo HJ, et al. NHG-Standaard Diabetes mellitus type 2 (derde herziening). *Huisarts Wet*. 2013;56(10):512–25.
13. Gallagher EJ, LeRoith D. Diabetes, antihyperglycemic medications and cancer risk. *Curr Opin Endocrinol Diabetes Obes* [Internet]. 2013 Oct [cited 2018 Feb 4];20(5):485–94. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23974779>
14. Vora J, Ray K, Kosiborod M, Poulter NR, Rajagopalan S, Leiter LA. Cancer risks of anti-hyperglycemic drugs for type 2 diabetes treatment - a clinical appraisal. *J Diabetes Complications* [Internet]. 2017 Sep [cited 2018 Feb 4];31(9):1451–7. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S1056872717304245>
15. Arnouts P, Bolignano D, Nistor I, Bilo H, Gnudi L, Heaf J, et al. Glucose-lowering drugs in patients with chronic kidney disease: a narrative review on pharmacokinetic properties. *Nephrol Dial Transpl*. 2013;12/11. 2014;29(7):1284–300.
16. St Onge EL, Motycka CA, Miller SA. A review of cardiovascular risks associated with medications used to treat type-2 diabetes mellitus. *Pharm Ther* [Internet]. 2009 Jul [cited 2018 Feb 4];34(7):368–78. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20140101>

17. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J cancer* [Internet]. 2015 Mar 1 [cited 2017 Jul 3];136(5):E359-86. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25220842>
18. IKNL. Nederlandse Kankerregistratie, beheerd door IKNL [Internet]. 2018 [cited 2018 Jan 12]. Available from: cijfersoverkanker.nl
19. Tsilidis KK, Kasimis JC, Lopez DS, Ntzani EE, Ioannidis JPA. Type 2 diabetes and cancer: umbrella review of meta-analyses of observational studies. *BMJ* [Internet]. 2015 Jan 2;350(jan02 1):g7607–g7607. Available from: <http://www.bmj.com/content/350/bmj.g7607.abstract>
20. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, et al. Diabetes and cancer: a consensus report. *Diabetes Care*. 2010/07/01. 2010;33(7):1674–85.
21. Harding JL, Shaw JE, Peeters A, Cartensen B, Magliano DJ. Cancer risk among people with type 1 and type 2 diabetes: disentangling true associations, detection bias, and reverse causation [Correction]. *Diabetes Care* 2015;38:264-270. *Diabetes Care* [Internet]. 2015 Apr [cited 2016 Aug 19];38(4):734–5. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25805866>
22. Harding JL, Shaw JE, Peeters A, Cartensen B, Magliano DJ. Cancer risk among people with type 1 and type 2 diabetes: disentangling true associations, detection bias, and reverse causation. *Diabetes Care* [Internet]. 2015 Feb [cited 2016 Aug 19];38(2):264–70. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25488912>
23. Johnson JA, Carstensen B, Witte D, Bowker SL, Lipscombe L, Renehan AG, et al. Diabetes and cancer (1): Evaluating the temporal relationship between type 2 diabetes and cancer incidence. *Diabetologia* [Internet]. 2012/04/06. 2012;55(6):1607–18. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22476947>
24. Lega IC, Wilton AS, Austin PC, Fischer HD, Johnson JA, Lipscombe LL. The temporal relationship between diabetes and cancer: A population-based study. *Cancer* [Internet]. 2016;1–8. Available from: <http://doi.wiley.com/10.1002/cncr.30095>
25. WHO. BMI classification [Internet]. [cited 2018 Feb 4]. Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html
26. WHO. Obesity and overweight. 2017 [cited 2018 Feb 4]; Available from: <http://www.who.int/mediacentre/factsheets/fs311/en/>
27. RIVM. Overgewicht volwassenen [Internet]. 2016. Available from: <https://www.volksgezondheidenzorg.info/onderwerp/overgewicht/cijfers-context/huidige-situatie#node-overgewicht-volwassenen>
28. World Cancer Research Fund. Cancers linked with greater body fatness [Internet]. Vol. 2015. WCRF; 2015 [cited 2018 Jan 19]. Available from: <http://www.wcrf.org/int/cancer-facts-figures/link-between-lifestyle-cancer-risk/cancers-linked-greater-body-fatness>
29. Lauby-Secretan B, Scoccianti C, Loomis D, Grosse Y, Bianchini F, Straif K. Body Fatness and Cancer - Viewpoint of the IARC Working Group. *N Engl J Med* [Internet]. 2016 Aug 25 [cited 2017 Jul 3];375(8):794–8. Available from: <http://www.nejm.org/doi/10.1056/NEJMSr1606602>
30. Pearson-Stuttard J, Zhou B, Kontis V, Bentham J, Gunter MJ, Ezzati M. Worldwide burden of cancer attributable to diabetes and high body-mass index: a comparative risk assessment. *lancet Diabetes Endocrinol* [Internet]. 2018 Feb [cited 2018 Feb 22];6(2):95–104. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S2213858717303662>
31. McCombie L, Leslie W, Taylor R, Kennon B, Sattar N, Lean MEJ. Beating type 2 diabetes into remission. *BMJ* [Internet]. 2017 Sep 13 [cited 2017 Oct 6];358:j4030. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28903916>
32. UK Prospective Diabetes Study 7: response of fasting plasma glucose to diet therapy in newly presenting type II diabetic patients, UKPDS Group. *Metabolism* [Internet]. 1990 Sep [cited 2018 Jan 12];39(9):905–12. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/2392060>
33. Goldstein DJ. Beneficial health effects of modest weight loss. *Int J Obes Relat Metab Disord* [Internet]. 1992 Jun [cited 2018 Jan 12];16(6):397–415. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/1322866>

34. Pastors JG, Warshaw H, Daly A, Franz M, Kulkarni K. The evidence for the effectiveness of medical nutrition therapy in diabetes management. *Diabetes Care* [Internet]. 2002 Mar 1 [cited 2018 Jan 12];25(3):608–13. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11874956>
35. Hardefeldt PJ, Penninkilampi R, Ediramanne S, Eslick GD. Physical Activity and Weight Loss Reduce the Risk of Breast Cancer: A Meta-analysis of 139 Prospective and Retrospective Studies. *Clin Breast Cancer* [Internet]. 2018 Aug;18(4):e601–12. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/29223719>
36. Ma C, Avenell A, Bolland M, Hudson J, Stewart F, Robertson C, et al. Effects of weight loss interventions for adults who are obese on mortality, cardiovascular disease, and cancer: systematic review and meta-analysis. *BMJ* [Internet]. 2017 Nov 14;359:j4849. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/29138133>
37. Ye J. Mechanisms of insulin resistance in obesity. *Front Med* [Internet]. 2013 Mar [cited 2017 Oct 19];7(1):14–24. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23471659>
38. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* [Internet]. 2006/12/15. 2006;444(7121):840–6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17167471>
39. Friedrich N, Thuesen B, Jorgensen T, Juul A, Spielhagen C, Wallaschofski H, et al. The Association Between IGF-I and Insulin Resistance: A general population study in Danish adults. *Diabetes Care* [Internet]. 2012 Apr 1 [cited 2017 Dec 21];35(4):768–73. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22374641>
40. Gallagher EJ, LeRoith D. Minireview: IGF, Insulin, and Cancer. *Endocrinology* [Internet]. 2011/05/05. 2011;152(7):2546–51. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21540285>
41. Klil-Drori AJ, Azoulay L, Pollak MN. Cancer, obesity, diabetes, and antidiabetic drugs: is the fog clearing? *Nat Rev Clin Oncol* [Internet]. 2016 Aug 9 [cited 2017 Oct 19];14(2):85–99. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27502359>
42. Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov* [Internet]. 2009 Aug [cited 2018 Jan 9];8(8):627–44. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19644473>
43. Schipf S, Schmidt CO, Alte D, Werner A, Scheidt-Nave C, John U, et al. Smoking prevalence in Type 2 diabetes: results of the Study of Health in Pomerania (SHIP) and the German National Health Interview and Examination Survey (GNHIES). *Diabet Med* [Internet]. 2009 Aug [cited 2018 May 20];26(8):791–7. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19709149>
44. Chang SA. Smoking and type 2 diabetes mellitus. *Diabetes Metab J* [Internet]. 2012 Dec [cited 2018 May 20];36(6):399–403. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23275932>
45. Shebl FM, Warren JL, Eggers PW, Engels EA. Cancer risk among elderly persons with end-stage renal disease: a population-based case-control study. *BMC Nephrol* [Internet]. 2012/07/28. 2012;13:65. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22834953>
46. Matas AJ, Simmons RL, Kjellstrand CM, Buselmeier TJ, Najarian JS. Increased incidence of malignancy during chronic renal failure. *Lancet* [Internet]. 1975/04/19. 1975;1(7912):883–6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/47534>
47. Yun H-R, Kim H, Park JT, Chang TI, Yoo T-H, Kang S-W, et al. Obesity, Metabolic Abnormality, and Progression of CKD. *Am J Kidney Dis* [Internet]. 2018 Sep [cited 2018 Sep 9];72(3):400–10. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/29728317>
48. Othman M, Kawar B, El Nahas AM. Influence of obesity on progression of non-diabetic chronic kidney disease: a retrospective cohort study. *Nephron Clin Pract* [Internet]. 2009 [cited 2018 Sep 9];113(1):c16–23. Available from: <https://www.karger.com/Article/FullText/228071>
49. Foster MC, Hwang S-J, Larson MG, Lichtman JH, Parikh NI, Vasan RS, et al. Overweight, obesity, and the development of stage 3 CKD: the Framingham Heart Study. *Am J Kidney Dis* [Internet]. 2008 Jul [cited 2018 Sep 9];52(1):39–48. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0272638608005751>
50. Sedgwick P. Non-response bias versus response bias. *BMJ* [Internet]. 2014 Apr 9 [cited 2018 May 13];348(apr09 1):g2573–g2573. Available from: <http://www.bmj.com/cgi/doi/10.1136/bmj.g2573>

51. Sedgwick P. Bias in observational study designs: prospective cohort studies. *BMJ* [Internet]. 2014 Dec 19 [cited 2018 May 13];349:g7731. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25527114>
52. Cochrane. Assessing Risk of Bias in Included Studies [Internet]. [cited 2018 Apr 15]. Available from: <http://methods.cochrane.org/bias/assessing-risk-bias-included-studies>
53. Colmers IN, Majumdar SR, Yasui Y, Bowker SL, Marra CA, Johnson JA. Detection bias and overestimation of bladder cancer risk in type 2 diabetes: a matched cohort study. *Diabetes Care* [Internet]. 2013/08/31. 2013;36(10):3070–5. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23990517>
54. Bowker SL, Richardson K, Marra CA, Johnson JA. Risk of breast cancer after onset of type 2 diabetes: evidence of detection bias in postmenopausal women. *Diabetes Care* [Internet]. 2011/10/06. 2011;34(12):2542–4. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21972408>
55. Suissa S, Azoulay L. Metformin and the risk of cancer: time-related biases in observational studies. *Diabetes Care* [Internet]. 2012/11/23. 2012;35(12):2665–73. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23173135>
56. Lévesque LE, Hanley JA, Kezouh A, Suissa S. Problem of immortal time bias in cohort studies: example using statins for preventing progression of diabetes. *BMJ* [Internet]. 2010 Mar 12 [cited 2018 Feb 18];340:b5087. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20228141>
57. Landman GW, Kleefstra N, van Hateren KJ, Groenier KH, Gans RO, Bilo HJ. Metformin associated with lower cancer mortality in type 2 diabetes: ZODIAC-16. *Diabetes Care* [Internet]. 2009/11/18. 2010;33(2):322–6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19918015>
58. Dong Y-W, Shi Y-Q, He L-W, Cui X-Y, Su P-Z. Effects of metformin on survival outcomes of pancreatic cancer: a meta-analysis. *Oncotarget*. United States; 2017.
59. Li X, Li T, Liu Z, Gou S, Wang C. The effect of metformin on survival of patients with pancreatic cancer: a meta-analysis. *Sci Rep*. 2017 Jul;7(1):5825.
60. Perez-Lopez FR, Pasupuleti V, Gianuzzi X, Palma-Ardiles G, Hernandez-Fernandez W, Hernandez A V. Systematic review and meta-analysis of the effect of metformin treatment on overall mortality rates in women with endometrial cancer and type 2 diabetes mellitus. *Maturitas*. 2017 Jul;101:6–11.
61. Jung YS, Park CH, Eun CS, Park D II, Han DS. Metformin use and the risk of colorectal adenoma: A systematic review and meta-analysis. *J Gastroenterol Hepatol*. 2017 May;32(5):957–65.
62. Haring A, Murtola TJ, Talala K, Taari K, Tammela TLJ, Auvinen A. Antidiabetic drug use and prostate cancer risk in the Finnish Randomized Study of Screening for Prostate Cancer. *Scand J Urol* [Internet]. 2017 Feb;51(1):5–12. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28084175>
63. Lewis JD, Ferrara A, Peng T, Hedderson M, Bilker WB, Quesenberry CP, et al. Risk of bladder cancer among diabetic patients treated with pioglitazone: interim report of a longitudinal cohort study. *Diabetes Care* [Internet]. 2011/03/31. 2011 Apr;34(4):916–22. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21447663>
64. Tuccori M, Filion KB, Yin H, Yu OH, Platt RW, Azoulay L. Pioglitazone use and risk of bladder cancer: population based cohort study. *BMJ* [Internet]. 2016;352:i1541. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27029385>
65. Wu JW, Filion KB, Azoulay L, Doll MK, Suissa S. The Effect of Long-Acting Insulin Analogs on the Risk of Cancer: A Systematic Review of Observational Studies. *Diabetes Care* [Internet]. 2016/01/08. 2016; Available from: <http://care.diabetesjournals.org/content/early/2016/01/05/dc15-1816>
66. Lewis JD, Habel LA, Quesenberry CP, Strom BL, Peng T, Hedderson MM, et al. Pioglitazone Use and Risk of Bladder Cancer and Other Common Cancers in Persons With Diabetes. *JAMA* [Internet]. 2015 Jul 21 [cited 2017 Aug 22];314(3):265–77. Available from: <http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.2015.7996>
67. Korhonen P, Heintjes EM, Williams R, Hoti F, Christopher S, Majak M, et al. Pioglitazone use and risk of bladder cancer in patients with type 2 diabetes: retrospective cohort study using datasets from four European countries. *BMJ* [Internet]. 2016 Aug 16 [cited 2017 Aug 22];354:i3903. Available from: <http://www.bmj.com/lookup/doi/10.1136/bmj.i3903>

68. de Wet H, Proks P. Molecular action of sulphonylureas on KATP channels: a real partnership between drugs and nucleotides. *Biochem Soc Trans* [Internet]. 2015 Oct 1 [cited 2018 Feb 4];43(5):901–7. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26517901>
69. Burke MA, Mutharasan RK, Ardehali H. The sulfonylurea receptor, an atypical ATP-binding cassette protein, and its regulation of the KATP channel. *Circ Res* [Internet]. 2008;102(2):164–76. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18239147>
70. Abdelmoneim AS, Hasenbank SE, Seubert JM, Brocks DR, Light PE, Simpson SH. Variations in tissue selectivity amongst insulin secretagogues: a systematic review. *Diabetes Obes Metab* [Internet]. 2011/09/20. 2012 Feb [cited 2017 Aug 22];14(2):130–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21923736>
71. Gangji AS, Cukierman T, Gerstein HC, Goldsmith CH, Clase CM. A systematic review and meta-analysis of hypoglycemia and cardiovascular events: a comparison of glyburide with other secretagogues and with insulin. *Diabetes Care*. 2007/01/30. 2007;30(2):389–94.
72. Holstein A, Plaschke A, Egberts EH. Lower incidence of severe hypoglycaemia in patients with type 2 diabetes treated with glimepiride versus glibenclamide. *Diabetes Metab Res Rev*. 2002/01/05. 2001;17(6):467–73.
73. Szoke E, Gosmanov NR, Sinkin JC, Nihalani A, Fender AB, Cryer PE, et al. Effects of glimepiride and glyburide on glucose counterregulation and recovery from hypoglycemia. *Metabolism* [Internet]. 2006 Jan [cited 2017 Aug 22];55(1):78–83. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16324923>
74. Andersen SE, Christensen M. Hypoglycaemia when adding sulphonylurea to metformin: a systematic review and network meta-analysis. *Br J Clin Pharmacol* [Internet]. 2016 Nov 1 [cited 2017 Aug 22];82(5):1291–302. Available from: <http://doi.wiley.com/10.1111/bcp.13059>
75. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* [Internet]. 1998/09/22. 1998;352(9131):854–65. Available from: [http://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(98\)07037-8/abstract](http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(98)07037-8/abstract)
76. Arechavaleta R, Seck T, Chen Y, Krobot KJ, O'Neill EA, Duran L, et al. Efficacy and safety of treatment with sitagliptin or glimepiride in patients with type 2 diabetes inadequately controlled on metformin monotherapy: a randomized, double-blind, non-inferiority trial. *Diabetes Obes Metab* [Internet]. 2011/01/05. 2011;13(2):160–8. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1463-1326.2010.01334.x/abstract>
77. Hermann LS, Schersten B, Bitzen PO, Kjellstrom T, Lindgarde F, Melander A. Therapeutic comparison of metformin and sulfonylurea, alone and in various combinations. A double-blind controlled study. *Diabetes Care*. 1994/10/01. 1994;17(10):1100–9.
78. Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, et al. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* [Internet]. 2006;355(23):2427–43. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17145742>
79. Harrower AD. Comparison of efficacy, secondary failure rate, and complications of sulfonylureas. *J Diabetes Complicat*. 1994/10/01. 1994;8(4):201–3.
80. Harrower AD, Wong C. Comparison of secondary failure rate between three second generation sulphonylureas. *Diabetes Res*. 1990/01/01. 1990;13(1):19–21.
81. Rahmi Garcia RM, Rezende PC, Hueb W. Impact of hypoglycemic agents on myocardial ischemic preconditioning. *World J Diabetes* [Internet]. 2014 Jun 15 [cited 2017 Oct 6];5(3):258–66. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24936247>
82. Simpson SH, Lee J, Choi S, Vandermeer B, Abdelmoneim AS, Featherstone TR. Mortality risk among sulfonylureas: a systematic review and network meta-analysis. *Lancet Diabetes Endocrinol* [Internet]. 2014/12/04. 2015;3(1):43–51. Available from: [http://www.thelancet.com/journals/landia/article/PIIS2213-8587\(14\)70213-X/abstract](http://www.thelancet.com/journals/landia/article/PIIS2213-8587(14)70213-X/abstract)
83. Landman GW, de Bock GH, van Hateren KJ, van Dijk PR, Groenier KH, Gans RO, et al. Safety and efficacy of gliclazide as treatment for type 2 diabetes: a systematic review and meta-analysis of randomized trials. *PLoS One* [Internet]. 2014/02/18. 2014;9(2):e82880. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3922704/pdf/pone.0082880.pdf>

84. Thakkar B, Aronis KN, Vamvini MT, Shields K, Mantzoros CS. Metformin and sulfonylureas in relation to cancer risk in type II diabetes patients: a meta-analysis using primary data of published studies. *Metabolism* [Internet]. 2013/02/20. 2013;62(7):922–34. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23419783>
85. Walker JJ, Johnson JA, Wild SH. Diabetes treatments and cancer risk: The importance of considering aspects of drug exposure. *Lancet Diabetes Endocrinol* [Internet]. 2014/03/14. 2013;1(2):132–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24622319>
86. Yang X, So WY, Ma RC, Yu LW, Ko GT, Kong AP, et al. Use of sulphonylurea and cancer in type 2 diabetes-The Hong Kong Diabetes Registry. *Diabetes Res Clin Pr* [Internet]. 2010/10/05. 2010;90(3):343–51. Available from: <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed9&AN=2010652991>
87. Monami M, Lamanna C, Balzi D, Marchionni N, Mannucci E. Sulphonylureas and cancer: a case-control study. *Acta Diabetol* [Internet]. 2008/12/17. 2009;46(4):279–84. Available from: <http://link.springer.com/article/10.1007%2Fs00592-008-0083-2>
88. Monami M, Balzi D, Lamanna C, Barchielli A, Masotti G, Buiatti E, et al. Are sulphonylureas all the same? A cohort study on cardiovascular and cancer-related mortality. *Diabetes Metab Res Rev* [Internet]. 2007/03/27. 2007;23(6):479–84. Available from: <http://onlinelibrary.wiley.com/doi/10.1002/dmrr.736/abstract>
89. Bo S, Castiglione A, Ghigo E, Gentile L, Durazzo M, Cavallo-Perin P, et al. Mortality outcomes of different sulphonylurea drugs: the results of a 14-year cohort study of type 2 diabetic patients. *Eur J Endocrinol* [Internet]. 2013/05/11. 2013;169(1):117–26. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23660643>
90. Ubink-Veltmaat LJ, Bilo HJ, Groenier KH, Rischen RO, Meyboom-de Jong B. Shared care with task delegation to nurses for type 2 diabetes: prospective observational study. *Neth J Med* [Internet]. 2005/04/09. 2005;63(3):103–10. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15813422>
91. IKNL. Data collected by National Cancer Registry [Dutch] [Internet]. [cited 2018 Jan 9]. Available from: <http://www.cijfersoverkanker.nl/wat-wordt-geregistreerd--14.html>

2

Sulphonylurea derivatives and cancer, friend or foe?

Published as:

Hendriks AM*, Schrijnders D*, Kleefstra N, de Vries EGE, Bilo HJG, Jalving M*, Landman GWD*. "Sulphonylurea derivatives and cancer, friend or foe?". Eur J Pharmacol. 2019 Oct 15;861:172598

* contributed equally

ABSTRACT

Introduction:

Type 2 diabetes mellitus (T2DM) is associated with a higher risk of cancer and cancer-related mortality. Increased blood glucose and insulin levels in T2DM patients may be, at least in part, responsible for this effect. Indeed, lowering glucose and/or insulin levels pharmacologically appears to reduce cancer risk and progression, as has been demonstrated for the biguanide metformin in observational studies. Studies investigating the influence of sulfonylurea derivatives (SUs) on cancer risk have provided conflicting results, partly due to comparisons with metformin. Furthermore, little attention has been paid to within-class differences in systemic and off-target effects of the SUs. The aim of this systematic review is to discuss the available pre-clinical and clinical evidence on how the different SUs influence cancer development and risk.

Methods:

Databases including PubMed, Cochrane, Database of Abstracts on Reviews and Effectiveness and trial registers were systematically searched for available clinical and pre-clinical evidence on within-class differences of SUs and cancer risk.

Results:

The overall pre-clinical and clinical evidence suggest that the influence of SUs on cancer risk in T2DM patients differs between the various SUs. Potential mechanisms include differing affinities for the sulfonylurea receptor and thus differential systemic insulin exposure and off-target anti-cancer effects mediated for example through potassium transporters and drug export pumps.

Conclusion:

Pre-clinical evidence supports potential anti-cancer effects of SUs, which are of interest for further studies and potentially repurposing of SUs. At this time, the evidence on differences in cancer risk between SUs is not strong enough to guide clinical decision making.

CHEMICAL COMPOUNDS STUDIED IN THIS ARTICLE

Acetohexamide (PubChem ID: 1989); Chlorpropamide (PubChem ID: 2727); Tolazamide (PubChem ID: 5503); Tolbutamide (PubChem ID: 5505); Glyburide / Glibenclamide (PubChem ID: 3488); Glipizide (PubChem ID: 3478); Gliclazide (PubChem ID: 3475); Glimepiride (PubChem ID: 3476).

ABBREVIATIONS

Akt: protein kinase B; ALDH-3: aldehyde dehydrogenase 3; BMI: body-mass index; CI: confidence interval; HbA1c: glycated haemoglobin; HDL: high-density lipoprotein; HR: hazard ratio; IGF: insulin-like growth factor; K_{ATP}^{+} channel: ATP-sensitive potassium channel; LDL: low-density lipoprotein; MRP: multidrug-resistance protein; OR: odds ratio; RR: relative risk; SU: sulfonylurea derivative; T2DM: type 2 diabetes mellitus; TNF: tumour necrosis factor.

1. INTRODUCTION

Patients diagnosed with type 2 diabetes mellitus (T2DM) have an increased risk of cancer and cancer-related mortality (1,2). This increased risk is already present before diagnosis of T2DM (3–6). T2DM is characterized by insulin resistance, hyperglycaemia and hyperinsulinemia, which have all been associated with cancer development (7,8). Cancer cells have an altered energy metabolism characterized by high glucose consumption and high glycolysis rates. This provides energy to generate ATP as well as metabolic intermediates for production of biomass required for cellular proliferation (9). This so-called metabolic reprogramming is one of the hallmarks of cancer (10). The metabolic characteristics of tumours and their microenvironment are increasingly important for understanding cancer development, treatment resistance and for the identification of novel treatment targets (11). Therefore, investigation of factors associated with cancer development in T2DM patients is of particular interest.

Increased plasma glucose and insulin levels are, at least in part, responsible for the increased risk of cancer and cancer related mortality of T2DM patients (12). The various classes of glucose-lowering agents have different mechanisms of action, and thus differential effects on plasma glucose and insulin levels and different off-target effects.

Therefore, these classes of drugs may also differ in their influence on cancer risk and development. Pre-clinical studies in cancer models and observational clinical evidence indicate anti-cancer effects of the biguanide metformin and clinical trials testing effectivity of metformin in cancer patients are ongoing (8,13). For the other important class of oral glucose lowering drugs, the sulfonylurea derivatives (SUs), available data is conflicting (14–19). Several studies have reported an association between increased cancer risk and use of SUs in T2DM patients, in some cases potentially confounded by the use of metformin as a comparator (7,14,20). Other studies have shown that SU use did not increase cancer risk in T2DM patients (20,21) or even decreased cancer risk (22) compared to T2DM patients not using SUs. The conflicting data regarding the effects of SUs on cancer risk and development may be the result of differential effects of the individual SUs in terms of systemic or off-target effects.

The aim of this systematic review is to discuss the available pre-clinical and clinical evidence on differences in cancer risk and development between patients treated with different SU drugs. Understanding these so-called within-SU class differences is important to understand the conflicting data regarding cancer risk of SUs and to determine whether sufficient data is available to guide clinical selection of SUs for glycaemic control. In addition, the accumulated pre-clinical and clinical evidence of the differential effects of SUs on cancer risk in T2DM patients may help to identify novel cancer treatment targets.

2. SEARCH STRATEGY

For clinical studies, databases including Medline (using PubMed), Cochrane, Database of Abstracts on Reviews and Effectiveness and several trial registers (last search update March 21st, 2019, see supplementary file S1 for the complete search strategy) were searched for relevant meta-analyses, randomized trials, case-control studies and observational studies by two authors. Acetohexamide and tolazamide were excluded from the search, since these SUs are currently not registered in Europe or the United States of America. Studies that investigated cancer incidence in T2DM patients and compared individual SUs to each other were eligible for selection. Title and abstract were screened by two authors and full text articles were selected. See supplementary file S1 for detailed information on search strategy for clinical data and Fig. S1 for the flow chart of data extraction.

For pre-clinical data, separate searches were performed for the eight different SUs in Medline (using PubMed) combined with the terms “cancer OR tumor* OR tumour*”. Based on the abstracts, relevant articles on the effects of SUs on cancer cell growth

and intracellular mechanisms in pre-clinical models of cancer were selected by two authors. Relevant references of the selected articles were also searched. Articles written in languages other than English were excluded. Only original papers were included.

3. DIFFERENCES BETWEEN SUS IN GLUCOSE LOWERING CAPACITY

Increased glucose and insulin levels have cancer initiating and growth stimulatory effects in pre-clinical cancer models (12). Therefore, SUs that consistently normalize blood glucose levels with minimal systemic insulin exposure, e.g. by a specific meal dependent insulin release, are most likely to show benefit in terms of reducing relative cancer risk. SUs are grouped into three generations (Table 1), which differ with respect to strength of glucose-lowering capacity, side-effects and the presence of active metabolites. Target molecules for SUs are the sulfonylurea receptors, which are subunits of ATP-sensitive potassium channels (K^+_{ATP} channel) (Fig. 1) (23). The different SUs have varying affinities for the sulfonylurea receptor isoforms and differ in hypoglycaemia risk (Table 1). No severe hypoglycaemia cases have been reported for gliclazide users, in contrast to the other SUs (24–26).

Extra-pancreatic blood glucose lowering effects of SUs in humans have also been described (27–29), however the available studies are small and not all SUs have been investigated, nor were different SUs compared within clinical studies. In dogs, glibenclamide was shown to have a lower extra-pancreatic blood glucose-lowering capacity than glimepiride (30). Postulated mechanisms for the extra-pancreatic effects include effects on hepatic glycogen metabolism, gluconeogenesis and lipogenesis. However, these mechanisms have mostly been studied at varying, often supra-physiological, drug concentrations and have not been studied in humans (27,30,31).

Table 1: Mechanism of actions of SUs.

Sulfonylurea derivative	Generation	Mechanism of action (32–34)
Acetohexamide	1	<ul style="list-style-type: none">- Increases pancreatic insulin secretion.- Closes K_{ATP}^+ channels located on β-cells.
Chlorpropamide	1	<ul style="list-style-type: none">- Increases pancreatic insulin secretion.- Closes K_{ATP}^+ channels located on β-cells.
Tolazamide	1	<ul style="list-style-type: none">- Increases pancreatic insulin secretion.- Closes K_{ATP}^+ channels located on β-cells.- Increases peripheral insulin sensitivity.
Tolbutamide	1	<ul style="list-style-type: none">- Increases pancreatic insulin secretion.- Closes K_{ATP}^+ channels located on β-cells.
Glibenclamide	2	<ul style="list-style-type: none">- Increases pancreatic insulin secretion.- Closes K_{ATP}^+ channels located on β-cells.
Glipizide	2	<ul style="list-style-type: none">- Increases pancreatic insulin secretion.- Closes K_{ATP}^+ channels located on β-cells.
Gliclazide	2	<ul style="list-style-type: none">- Increases pancreatic insulin secretion.- Closes K_{ATP}^+ channels located on β-cells.
Glimepiride	3	<ul style="list-style-type: none">- Increases pancreatic insulin secretion.- Closes K_{ATP}^+ channels located on β-cells.

HR: hazard ratio; K_{ATP}^+ channels: ATP-sensitive potassium channels; OR: odds ratio; RR: relative risk; SUR: sulfonylurea receptor. *: study in patients aged 65 years or older; this is relevant because this patient group has a higher hypoglycemia risk than younger patients. §: HR, OR, and RR were all adjusted.

Point of action	Hypoglycemia risk §
SUR1 (34)	No reliable data
SUR1 (35)	Only as comparator group
SUR1 (34)	Versus chlorpropamide: RR 0.6 (0.4-1.0)* (36)
SUR1 (33)	Versus chlorpropamide: RR 0.2 (0.1-0.4)* (36)
SUR1 (33) SUR2A (33) SUR2B (33)	Versus other SUs: HR 1.83 (1.35–2.49) (37) Versus metformin: HR 3.95 (3.66-4.26) (38) Versus glipizide: HR 1.04 (0.18-6.85) (39) Versus chlorpropamide: RR 1.0 (0.8-1.3)* (36) Incidence: 5.6/1000 (25)
SUR1 (33)	Versus metformin: HR 2.57 (2.38-2.78) (38) Versus chlorpropamide RR 0.6 (0.4-0.9)* (36)
SUR1 (33)	Versus other SUs: RR 0.47 (0.27-0.79) (26) Versus glipizide: OR 0.22 (0.05 to 0.96) (39) Versus glimepiride OR 0.40 (0.13 to 1.27) (39) Versus glibenclamide: OR 0.21 (0.03 to 1.48) (39)
SUR1 (33) SUR2 (33)	Versus metformin: 3.28 (2.98-3.62) (38) Versus glibenclamide: OR 0.51 (0.09-2.83) (39) Versus glipizide: OR 0.54 (0.18-1.64) (39) Incidence: 0.86/1000 (25)

Table 2: Overview of selected clinical studies.

Author	Design	Mean follow-up*	Size (n)	Diabetes duration*	Baseline HbA1c (%)
Bo, 2013 (40)	Retrospective	14.0 ^b	1,277	9 (11) ^a	6.7 (1.2) ^b
Monami, 2007 (41)	Retrospective	5.0 ^b	568	7.7 (5.1), gliclazide 13.2 (10.6), glibenclamide	7.4 8.4
Tuccori, 2015 (42)	Prospective	5.3 ^b	52,600	0.3 (0.9), glibenclamide 1.3 (2.0), other	8.7 8.5
Chang, 2012 (43)	Retrospective	7.4 ^a	108,920	3.6 (2.3) ^b	NR
Yang, 2010 (7)	Prospective	4.8 ^b	6,103	6 (9) ^b , controls 8 (10) ^b , cases	7.2 7.4
Monami, 2009 (44)	Prospective	6.5 ^b	390	9.3, cases 9.3, controls	7.7 7.7

DDD: daily defined dose; HbA1c: glycated hemoglobin; HR: hazard ratio; NR: not reported; OR: odds ratio. ^a: median (interquartile range), ^b: mean (standard deviation), *: in years. All outcome measures presented in the table are adjusted values if provided. All studies presented in the table investigated any site of cancer.

Fig 1 - Schematic representation of the mechanism of action of sulfonylurea derivatives (SUs) on a β-cell of the pancreas. ADP: adenosine diphosphate; ATP: adenosine triphosphate; Ca²⁺: calcium; GLUT-2: glucose transporter 2; K⁺: potassium; SUR-1: sulfonylurea receptor 1. The numbered boxes describe the mechanism of action of SUs in sequential order.

Specification of SU treatment	Comparator	Outcome
Gliclazide Tolbutamide	Glibenclamide Glibenclamide	HR 0.30 (0.16-0.55) (Mortality) HR 0.48 (0.29-0.79) (Mortality)
Glibenclamide	Gliclazide	OR 3.6 (1.1-11.9) (Mortality)
Glibenclamide Glibenclamide >1096 DDD	Other 2 nd generation SU Other 2 nd generation SU	HR 1.09 (0.98-1.22) (Cancer risk) HR 1.27 (1.06-1.51) (Cancer risk)
SU (first/second generation) SU (glimepiride)	Non-use of SUs Non-use of SUs	OR 1.08 (1.01-1.15) (Cancer risk) OR 1.00 (0.93-1.08) (Cancer risk)
Ever glibenclamide Ever gliclazide	Never glibenclamide Never gliclazide	HR 0.67 (0.51-0.89) (Cancer risk) HR 0.65 (0.49-0.83) (Cancer risk)
Glibenclamide (12 months) Glibenclamide (36 months) Gliclazide (12 months) Gliclazide (36 months)	Non-use of glibenclamide Non-use of glibenclamide Non-use of gliclazide Non-use of gliclazide	OR 2.24 (1.21-4.14) (Cancer risk) OR 2.62 (1.26-5.42) (Cancer risk) OR 0.39 (0.21-0.74) (Cancer risk) OR 0.40 (0.23-0.69) (Cancer risk)

4. DIFFERENCES BETWEEN SUS IN CANCER RISK: CLINICAL EVIDENCE

The search strategy for the clinical studies yielded six eligible studies (Fig. S1) (7,40–44), of which three retrospective (40–42) and three prospective (7,43,44) (). All studies included patients with varying durations of diabetes and the mean follow-up periods ranged from 4.8 to 14 years. Five of the six studies focused on all-cancer incidence (7,40–43) and one reported both all-cancer and site-specific cancer incidence (44). Two of the six studies had all-cancer mortality as primary outcome (40,41) and the other four had cancer incidence as primary outcome. Three of the six studies investigated dose-response relationships for individual SUs (7,42,43) and one of the six studies investigated treatment duration-response differences for individual SUs (44). One study incorporated a time-varying design, taking changes in covariates during follow-up into account (42). In these six studies, data on cancer risk was found for gliclazide, glimepiride, glibenclamide and tolbutamide use; no data was found for the other SUs of interest (chlorpropamide and glipizide).

4.1 Gliclazide

Clinical evidence regarding effects of gliclazide use on cancer risk and development was described in three studies. In a retrospective observational study (n=1,277) with a

mean follow-up of 14 years, T2DM patients treated with gliclazide had a lower cancer mortality risk (hazard ratio (HR) 0.30, 95% confidence interval (CI) 0.16-0.55) compared to glibenclamide treated patients (40). The analyses were adjusted for metformin and insulin use, age, sex, body-mass index (BMI), smoking, diabetes duration, anti-hypertensive therapy, glycated haemoglobin (HbA1c), presence of retinopathy, neuropathy and cardiovascular diseases.

The second study had a prospective design including 6,103 patients and showed that ever use compared to never use of gliclazide was associated with a lower cancer risk (HR 0.65, 95%CI 0.49-0.83) in a dose dependent manner in the ever group (7). Ever use was defined as use of gliclazide at, or within 2.5 years before, enrolment or during follow-up period. Outcomes were adjusted for age, sex, BMI, smoking, alcohol use, baseline HbA1c, systolic blood pressure, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, and ever use of statins, renin-angiotensin system inhibitor, metformin and insulin. This study did not correct for HbA1c levels of patients during follow-up.

The third study was a prospective, case-control study including 195 cases with cancer and 195 cases without cancer matched for age, sex, duration of diabetes, BMI, HbA1c, comorbidity, smoking and alcohol use (44). This study showed that T2DM patients using gliclazide for at least 12 months (odds ratio (OR) 0.39, 95%CI 0.21-0.74) and for at least 36 months (OR 0.40, 95%CI 0.23-0.69) had a lower cancer risk compared to patients who had never used gliclazide. The analyses were adjusted for other glucose lowering treatments. Due to the limit cohort sizes, no separate HRs for specific cancer types were calculated.

4.2 Glibenclamide

Clinical evidence regarding effects of glibenclamide use on cancer risk and development was described in four studies. The first study had a prospective design including 6,103 patients and showed that ever compared to never use was associated with a lower cancer risk (HR 0.67, 95%CI 0.51-0.89) in a dose-dependent manner in the ever group (7). Ever use was defined as use of glibenclamide at, or within 2.5 years before, enrolment or during the follow-up period. Outcomes were adjusted for age, sex, BMI, smoking, alcohol use, baseline HbA1c, systolic blood pressure, LDL-cholesterol related risks, HDL-cholesterol, triglyceride, and ever use of statins, renin-angiotensin system inhibitor, metformin and insulin. This study did not correct for HbA1c levels of patients during follow-up.

In contrast, the second study described a retrospective analysis involving 52,600 SU-naïve T2DM patients starting SU use between 1 January 1988 and 31 July 2013 with a mean follow-up was 5.3 years (42). Glibenclamide use was not associated with a change

in risk of developing cancer compared to other second-generation SUs (HR 1.09, 95%CI 0.98-1.22) (42). However, for a high cumulative dose (>1,096 drug consumptions of the daily defined dose of glibenclamide, a dose-dependent higher cancer risk was found compared to other second-generation SUs (HR 1.27, 95%CI 1.06-1.51) (42). Results were adjusted for year of cohort entry, age, sex, BMI, smoking, alcohol use, HbA1c before study cohort entry, duration of treated diabetes before study cohort entry, ever use of other anti-diabetic drugs before study cohort entry (including first-generation SUs), and ever use of aspirin, statins and non-steroidal anti-inflammatory drugs. Strong points of this study were that both duration of SU use and the dose were taken into account, and a time-dependent analysis of exposure to SUs were incorporated.

Similarly, another study also described an association between glibenclamide use and higher cancer risk. This study was a prospective, case-control study including 195 cases with cancer and 195 cases without cancer matched for age, sex, duration of diabetes, BMI, HbA1c, comorbidity, smoking and alcohol use (44). This study showed that the OR for malignancies after exposure to glibenclamide for at least 12 and at least 36 months was 2.24 (95%CI 1.21-4.14) and 2.62 (95%CI 1.26-5.42), respectively, compared to no exposure to glibenclamide (44). The analyses were adjusted for other oral glucose lowering treatments. The number of patients in this study is relatively small.

Lastly, a retrospective study (n=568) with a mean follow-up of 5 years, described a higher all-cancer mortality in glibenclamide users compared to gliclazide users (OR 3.6, 95%CI 1.1-11.9) (41). This analysis was corrected for age, sex, BMI, insulin and metformin treatment. No dose response or cumulative duration analyses performed and the analysis was not adjusted for HbA1c.

4.3 Glimepiride

In a case-control study, 108,920 newly diagnosed T2DM patients were identified (43). From this cohort, 8,194 cancer cases and 32,776 age- and sex-matched diabetic controls with a mean diabetes duration of 3.6 years and a median follow-up of 7.4 years were included in the analyses (43). Use of glimepiride compared to 'non-use' did not increase overall cancer risk (OR 1.00, 95%CI 0.93-1.08) in newly-diagnosed diabetes patients (43). It is not clear whether the non-use group included patients using no medication at all or patients not using glimepiride. The remaining non-glimepiride SUs were grouped together as first/second generation SUs and showed an increased cancer risk compared to 'non-use' (OR 1.08, 95%CI 1.01-1.15) (43). No dose-response or duration response relationships for any SUs were found (43). The analyses were adjusted for many covariates including insulin, SUs, glinides, metformin, thiazolidinediones, α -glucosidase inhibitors, statins,

β -blocker, calcium channel blockers, angiotensin-converting enzyme inhibitors, aspirin, chronic liver disease, nephropathy, cerebrovascular disease, and chronic kidney disease. However, it was not described whether the analysis was also adjusted for other diabetes related covariates, for example HbA1c or BMI.

4.4 Tolbutamide

In a retrospective observational study (n=1,277) with a mean follow-up of 14 patients treated with tolbutamide had a lower cancer mortality compared to patients treated with glibenclamide (HR 0.48, 95%CI 0.29-0.79) (40). The analyses were adjusted for metformin and insulin use, age, sex, BMI, smoking, diabetes duration, anti-hypertensive therapy, HbA1c, presence of retinopathy, neuropathy and cardiovascular diseases.

4.5 Conclusions clinical evidence

In the majority of the studies described, gliclazide use was either associated with a lower risk of developing cancer (7,44) or a lower risk of cancer-related mortality (40,41) compared to other SUs or never use. Moreover, one study showed that the reduced cancer risk in gliclazide users was dose-dependent (7). The majority of studies point to a higher cancer risk and all-cancer mortality risk for glibenclamide users compared to T2DM patients using other SUs or never use and this effect was also dose-dependent (40–42,44). Only two studies directly compared SUs to one another (glibenclamide vs tolbutamide and glibenclamide vs gliclazide (40), and glibenclamide vs gliclazide (41)), confirming differences in cancer-related mortality of SUs. However, the evidence should be interpreted with caution since all studies included were either small and grouped all cancers together or did not account for duration or dose-response relationships. Grouping cancer types might introduce bias, since certain tissue types and malignancies may be more sensitive to effects of increased blood glucose and insulin levels. Furthermore, the methodology used in each study differed substantially; different study designs and different statistical analyses were used. All studies adjusted for potential confounders but to a varying degree. Importantly, all included studies corrected for at least metformin and insulin use. In case patients used other glucose lowering agents, the analyses were either corrected for or these patients were excluded from the study. It is uncommon to use two different SUs at the same time, so it is unlikely that the effect seen is influenced by concurrent SU use. Taking these limitations into account, T2DM patients using gliclazide may have a lower cancer risk and T2DM patients using glibenclamide may have a higher cancer risk than T2DM patients using other SUs.

5. PRE-CLINICAL EVIDENCE OF THE EFFECTS AND WORKING MECHANISMS OF SUS ON CANCER CELLS

To gain insight in possible systemic or off-target effects explaining the different clinical cancer risks of varying SUs, available evidence of growth inhibitory effects and potential anti-cancer mechanisms of SUs in pre-clinical models of cancer is discussed in this section. This evidence is summarized in Fig. 2. Most data are available for glibenclamide, which is frequently used in cell line models as a pharmacological tool to block transporters such as the potassium transporter channel. This provides data on *in vitro* effects of glibenclamide on cancer cell growth from studies not primarily designed to study SUs.

5.1 Effect of SUs on cancer cell growth

Glibenclamide inhibited growth of human prostate (45), hepatocellular (46–48), breast (49,50), gastric (51), bladder (52), glioma (53) and colon (54) cancer cell lines *in vitro*. In human prostate, glioma and colon cancer cells this effect was dose-dependent and occurred at doses around 0.1 mM (50 µg/mL) (45,53,54), which is at least 100-fold higher than the plasma concentrations achieved clinically in daily practice with doses used in

T2DM patients (55). In bladder and breast cancer cells, reduced cellular proliferation has also been reported to be dose-dependent and was seen at concentrations which did not differ greatly from clinically achievable plasma concentrations (50,52). In human ovarian cancer cell line models, cell growth was not inhibited by glibenclamide (56,57). Proliferation of glioma cells was inhibited by 7 days tolbutamide treatment at 100 µM (58), this is about 2600 times the clinically achievable range of tolbutamide plasma concentrations (59).

Glipizide, but not glimepiride, suppressed tumour growth and metastases *in vivo* in breast cancer and melanoma xenografts and in transgenic mouse models of breast cancer (60). The doses of glipizide and glimepiride used in the study resulted in concentrations consistent with clinically achievable plasma concentrations (61).

In general, it appears that in certain conditions, treatment with glibenclamide can reduce growth of cancer cells. However, in the majority of studies only one or two cancer cell lines were studied and glucose concentration of cell culture media used varied widely or were not mentioned. No studies compared the influence of glibenclamide on cell growth in a large range of different cell lines in the same conditions. Furthermore, the potential mechanisms of growth inhibition of glibenclamide are not elucidated. Therefore, the results should be interpreted with caution and effects and mechanisms may be cell line specific. The possible mechanisms by which SUs might influence cancer development are discussed in the following paragraphs.

Fig 2 - Potential anti-cancer effects of sulfonylurea derivatives (SUs). ABC: adenosine triphosphate (ATP) binding cassette transporter; Akt: protein kinase B; Chemo: chemotherapy; IGF: insulin-like growth factor; IGFR: insulin-like growth factor receptor; IR: insulin receptor; IKK: I-kappaB-kinase; K⁺_{ATP}: ATP-sensitive potassium channel; MMP: matrix metalloproteinase; PI3K: phosphoinositide 3-kinase; TNF: tumor necrosis factor; TNFR: tumor necrosis factor receptor; VEGF: vascular endothelial growth factor. SUs have been shown to inhibit VEGF in vitro and in vivo resulting in impaired vasculoneogenesis and angiogenesis. Degradation of the extracellular matrix is impaired by the SU glibenclamide by inhibitions of MMPs, which results in less proliferation and invasiveness of cancer cells. Sensitivity of cancer cells might increase due to inhibited cellular export of chemotherapeutics due to inhibitory effects of the SU glibenclamide on ABC transporters. The SU chlorpropamide selectively inhibits the Akt pathway activity in cancer cells, resulting in cell growth arrest and apoptosis of cancer cells in vitro and in vivo. SUs have also been shown to inhibit TNF levels and TNFRs in cancer cell, which might have pro-cell death effects. Inhibition of K⁺_{ATP} by SUs reduces cancer cell growth in vitro and in vivo, possibly by inducing apoptosis.

5.2 Effects of SUs on growth factors and growth factor pathways

Insulin and insulin-like growth factor (IGF) have been shown to stimulate cancer cell proliferation. Exposing a human neuroblastoma cell line to glibenclamide resulted in an increase of mRNA expression of insulin and IGF-1 receptors (62). This resulted in increased binding of insulin and IGF-1 to the neuroblastoma cells, but not in increased glucose uptake. Only short-term experiments, up to 24 hours duration, were described. Studies with longer drug incubation times are required to determine whether this results in enhanced cancer cell growth.

Gliclazide inhibited levels of tumour necrosis factor (TNF) production by human peripheral blood mononuclear cells and inhibited TNF bioactivity and immunoreactivity in mouse serum (63–65). Chlorpropamide inhibited levels of TNF receptors in peripheral blood mononuclear cells (66). TNF is a cytokine, which can have both pro-survival and

pro-cell death effects, mediated through the TNF receptors. More detailed mechanistic studies are required to determine how different SUs influence this delicate balance in cancer cells.

The protein kinase B (Akt) pathway is a signalling pathway, often activated in cancer and downstream of the insulin receptor. This pathway is involved in many cellular processes, including cell survival, cell cycle progression and cellular growth (67). A chlorpropamide analogue API-2 selectively inhibited the Akt pathway activity in cancer cells with elevated Akt expression without influencing other pathways, resulting in cell growth arrest and apoptosis of cancer cells *in vitro* and *in vivo* (68). This is of interest since the Akt pathway is hyperactivated in many cancer types and therefore an attractive therapeutic target. However, clinical development of such inhibitors has been hampered by drug-toxicity (69).

5.3 Effects of SUs on ATP-binding cassette transporters and solute channels

Sulfonylurea receptors belong to the ATP-binding cassette transporter superfamily, which are transmembrane proteins that transport substrates across cellular membranes. Multidrug-resistance proteins (MRPs) are ATP-binding cassette transporters involved in the cellular export of several drugs, including chemotherapeutic drugs, and can therefore protect cancer cells from anti-cancer drugs (70). Glibenclamide is an inhibitor of various ATP-binding cassette transporter proteins (71–74). Glibenclamide inhibited MRP1 in human lung cancer cells resulting in enhanced sensitivity of these cells to the anti-cancer drug and MRP substrate vincristine (75). However, in this study, the dose of glibenclamide required for this effect was at least 10-fold higher than the therapeutic plasma concentration currently achieved clinically (55). Glibenclamide also inhibited the transport of the drug alpha-tocopheryl-phosphate across the cell membrane into cells in a leukaemia cell line, thereby reducing the anti-proliferative effect of this drug (76). However, the transporter involved was not identified. If this effect is indeed confirmed at physiological doses for certain drug transporters then caution may be required when glibenclamide use is combined with anti-cancer drugs. However, although the efficacy of efflux pump inhibitors to improve the effect of chemotherapeutics has been extensively studied clinically, results have so far been disappointing (77).

Some potassium channels are regulated by sulfonylurea receptors and can be (potently) inhibited by, for example, glibenclamide (48,78,79). Deregulated expression of all four classes of potassium channels has been demonstrated in human cancers and overexpression has been correlated with increased cell proliferation (80–83). Pharmacological inhibition of potassium channels has been shown to reduce cancer cell

growth *in vitro* and *in vivo* in cancers with overexpression of these channels (84). Voltage-gated potassium channels were blocked by glibenclamide in a hepatocellular carcinoma cell line, resulting in reduced proliferation (48). However, in another study, no effect was seen in an ovarian cancer cell line (56). Potassium channel blockers mediate depolarization of membranes; this can result in programmed cell-death (apoptosis) and therefore this may also be expected for SUs. Indeed, induction of apoptosis by glibenclamide was described in several human cancer cell line models (45,46,51,53,85,86). In addition, both glibenclamide and tolbutamide enhanced apoptosis induced by other drugs (87,88). In contrast, gliclazide protected cancer cells from hydrogen peroxide induced apoptosis (89–91). In general, it appears that influence of glibenclamide on potassium channels and apoptosis is likely to be dose dependent and potentially cell-line dependent.

5.4 Effects of SUs on angiogenesis and metastasis

Angiogenesis plays a major role in cancer progression and metastasis, and is mediated by growth factors such as vascular endothelial growth factor (92). Inhibition of neovascularization can be useful as anti-cancer therapy by controlling tumour growth and metastasis. Bevacizumab, a monoclonal anti-body against vascular endothelial growth factor A, and several tyrosine kinase inhibitors with anti-angiogenic properties are registered as anti-cancer therapy in selected tumour types.

Glibenclamide inhibited cellular invasion and migration in a human ovarian cancer cell line model through inhibition of components of the angiogenic pathway (93). *In vivo* studies in early chick embryo chorioallantoic membrane and yolk sac membrane models and in prostate cancer mouse models showed that glipizide indeed inhibited vasculoneogenesis and angioneogenesis (60,92,94).

Degradation of the extracellular matrix is essential for tumour invasion and metastasis. Among the essential proteins for this process of degradation are various types of matrix metalloproteinase. Growth, proliferation and invasiveness of breast cancer in TA2 mice was inhibited by a combined treatment of glibenclamide and cobalt chloride through inhibition of matrix metalloproteinases (95). In contrast, in ovarian cancer cells, glibenclamide had no effect on cell adhesion, cell invasion or migration (96). Lymphatic spread is frequently a first step in cancer metastasis. Acetohexamide dose-dependently inhibited breast cancer intravasation into lymph ducts in a three-dimensional cell co-culture assay (97). This effect of acetohexamide was identified during a screening procedure of hundreds of drugs registered for non-cancer indications. This observation is yet to be confirmed in other *in vitro* and *in vivo* cancer models and the exact mechanism has not been elucidated.

5.5 The effects of SUs on anti-cancer treatment

SUs might not only affect cancer initiation and progression but also the efficacy of anti-cancer treatment. Glibenclamide has been shown to protect human glioblastoma, primary astrocytes and normal lung tissue cell lines from radiation-induced cell death (98). Mice injected with glibenclamide before total body irradiation lived longer compared to mice injected with glibenclamide after radiation therapy or without glibenclamide treatment (98). The specific underlying mechanisms of the radio-protective effect of glibenclamide pre-administration are unknown (98). Furthermore, in liver and lung cancer cell lines glibenclamide has been shown to increase the cytotoxicity of the chemotherapeutic drug doxorubicin (99). This study shows altering DNA conformation effects of glibenclamide possibly explaining its synergistic effects on doxorubicin treatment (99).

Analogues of chlorpropamide inhibited human aldehyde dehydrogenase 3 (ALDH-3) in cancer cells and sensitize breast cancer cells to chemotherapy (100,101). ALDH-3 can be overexpressed in cancer cells and is involved in detoxification of certain types of chemotherapy and may therefore be involved in resistance to these therapies. Thus, inhibition of ALDH-3 is of interest to enhance tumour sensitivity to these chemotherapeutic drugs, however this has yet to be investigated in *in vivo* models.

Cancer cell resistance to chemo- and radiotherapy is, among others, enhanced by upregulation of the Akt-pathway (102,103). A chlorpropamide analogue, API-2, inhibited this pathway and reduced chemo- and radioresistance (102–104). Furthermore, API-2 sensitized immune resistant tumours for CD8⁺ T-cell mediated apoptosis by Akt inhibition and might therefore improve immunotherapy (105). API-2 can induce either apoptosis or metastasis depending on the nuclear β -catenin expression, which differs between cancer cells suggesting pleiotropic effects of API-2 (106). It is unknown whether use of chlorpropamide itself results in the same effects.

The pre-clinical evidence suggests numerous potential anti-cancer mechanisms of SUs. However, systemic studies in cancer cell line panels and relevant model systems at clinically achievable drug concentrations are lacking.

6. DISCUSSION

The overall clinical evidence suggests that the influence of SUs on cancer risk in T2DM patients differs between the various SUs. Potential mechanisms include differing affinities for the sulfonylurea receptor resulting in differential systemic insulin exposure and off-target anti-cancer effects mediated for example through potassium transporters or drug export pumps.

Observational studies demonstrated that the use of the second generation SU gliclazide was associated with a lower risk of developing cancer compared to never use of gliclazide (7,44). Gliclazide users also had a lower risk of cancer-related mortality compared to glibenclamide users (40,41). Glibenclamide use was associated with a higher risk of developing cancer compared to other second-generation SUs and to no use of glibenclamide (42,44). All studies had important methodological limitations and used different study designs and statistical methods. Interpretation of results and direct comparisons of the retrospective clinical studies were hampered by low numbers of cancer cases, grouping together of different cancer types and lack of data on dose or cumulative exposure of SUs.

Higher insulin levels have tumour promoting effects *in vitro* in cell line models, *in vivo* in animal models and in human epidemiological studies (12). Metformin reduces peripheral insulin resistance resulting in lower blood insulin and glucose levels (Fig. 3). This could, at least in part, explain the potential anti-cancer effects of this drug (8,13). The different effects of gliclazide and glibenclamide on cancer risk and mortality may also be explained by differential influences on blood insulin levels of both agents (Fig. 3). Glibenclamide has been associated with an increased and pro-longed hypoglycaemia risk compared to other SUs. This can be explained by the relatively long half-life time of glibenclamide due to high affinity for the sulfonylurea receptor 1 and the slow reversibility of the sulfonylurea receptor 1 binding (107,108). The accumulation of active metabolites due to impaired renal function may also contribute to the hypoglycaemia risk of glibenclamide (32,37,109). Glibenclamide is not selective for just sulfonylurea receptor 1 located on the pancreatic β -cells of the pancreas, but also binds sulfonylurea receptor 2A and B which are widely expressed, amongst others, on cardiac muscle (107,110). In contrast, gliclazide binds more selectively with rapid reversibility to sulfonylurea receptor 1 located on pancreatic β -cells (107,110) and is hepatically metabolised into inactive metabolites before renal elimination (111).

SUs with the most selective meal dependent insulin release are expected to be associated with the lowest cancer risks due to lower overall insulin exposure and this may explain the observed clinical differences. Differential insulin responses of glibenclamide and gliclazide have indeed been demonstrated. The physiological pancreatic insulin response is biphasic, the first phase concerns rapid insulin exocytosis within 5-10 minutes after stimulation whereas the second phase can sustain for hours in case of persisting elevated glucose levels (112). In an *in vitro* model measuring insulin release in isolated rat pancreases, a biphasic insulin response to gliclazide treatment

was demonstrated which was in contrast to the delayed monophasic insulin response to glibenclamide treatment (113). In two small randomized double-blind trials, containing 24 and 12 patients respectively, glibenclamide was shown to have a stimulatory effect on the second but not on the first phase of insulin secretion (114,115). Furthermore, inappropriate insulin secretion in case of low blood glucose levels has been shown in T2DM patients and healthy volunteers using glibenclamide (116–119). The biphasic glucose-dependent insulin response of gliclazide was confirmed in four diabetic patients and four healthy controls (120). In conclusion, gliclazide appears to result in a selective glucose dependent insulin release which in turn results in a more physiological insulin response and a corresponding lower average blood insulin level than for example glibenclamide (Fig. 3).

Pre-clinical studies suggest that SUs could have direct inhibitory effects on cancer cell growth (45–54). Especially glibenclamide had anti-proliferative effects in some pre-clinical cancer models, potentially through blockade of potassium channels thus inducing apoptosis (45,46,71–74,47–54). However, glibenclamide is often studied due to its pharmacological effect on the potassium channels and not systematically with the goal of studying the anti-cancer effects of the drug. No large systematic comparison of growth inhibitory effects of the different SUs in panels of cancer cell lines have been performed. Furthermore, thorough investigation of effects of SUs on signal-transduction routes important to cancer cell growth have not been performed, and no studies on cancer cell metabolism were identified. Extra-pancreatic, organ specific effects of SUs on glucose metabolism exist and may differ between individual SUs as has been described for glibenclamide and glimepiride (27,30,31). The anti-cancer effects of SUs may be considered a novel extra-pancreatic effect. There is insufficient data available to determine whether the anti-cancer effects of SUs are correlated to their known extra-pancreatic effects on glucose metabolism.

The reported concentrations of SUs used in the reviewed *in vitro* studies appear to be high compared to the achievable therapeutic range in patients, which is measured as total (free plus protein-bound) plasma concentrations. Several factors complicate interpretation of these reported *in vitro* concentrations. Firstly, SUs bind to proteins in the cell culture medium (121–124) and, therefore, the actual free drug concentration available at the target may be lower than the reported SU concentration. Consequently, it is unknown at which actual drug concentrations the described anti-cancer effects of SUs at the cellular levels occurred. Secondly, SU binding affinities to albumin used for cell culture versus human albumin differ and SUs may bind to other proteins than only albumin (121). Thus translation of the *in*

vitro data is seriously hampered due to the probable differing degrees of protein binding between *in vitro* models and patients. Furthermore, there are multiple factors besides protein binding that determine the free drug concentrations surrounding the therapeutic target in the tumour in patients (125).

◀ **Figure 3 – Schematic representation of blood insulin and glucose interactions in relation to oral glucose lowering agents.** GLUT-4: glucose transporter 4; IR: insulin receptor; T2DM: type 2 diabetes mellitus. **A)** In non-diabetic patients, binding of insulin to peripheral tissues leads to translocation of glucose transporter 4 (GLUT-4) to the cell membrane enabling glucose influx. Glucose stimulates insulin excretion by β -cells of the pancreas. Insulin inhibits gluconeogenesis by the liver. **B)** In T2DM patients, peripheral tissues and liver tissue are insulin resistant. This results in hyperglycemia due to reduced cellular influx of glucose, increased insulin secretion by β -cells of the pancreas and stimulation of gluconeogenesis. **C1)** T2DM patients suffer from hyperglycemia and hyperinsulinemia. **C2)** Gliclazide treatment results in a slightly higher basal blood insulin level than in untreated T2DM patients and in a selective glucose-dependent insulin release. **C3)** Glibenclamide results in a less specific glucose-dependent insulin release than gliclazide, resulting in higher basal blood insulin levels and prolonged insulin secretion after a meal. **C4)** Metformin reduces insulin resistance of peripheral tissues and liver tissue resulting in less insulin secretion.

Clinical and pre-clinical data are conflicting regarding influence of glibenclamide on cancer risk and development. This is likely due to differential systemic versus intra-cellular effects of SUs. The association of glibenclamide use and increased cancer risk may be explained by the relatively high insulin exposure during glibenclamide treatment compared to other SUs. This is clearly not compensated by cancer inhibiting effects at the cellular level in patients. The pre-clinically described potential anti-cancer effects of SUs are of interest from a mechanistic point of view and deserve further investigation. This may result in novel anti-cancer drug targets and/or potential repurposing of SUs.

In conclusion, it appears that T2DM patients using gliclazide may have a lower cancer risk than those using glibenclamide. However, the evidence on differences in cancer risk between the different SUs and SU classes is, at this time, not strong enough to guide clinical decision-making.

SUPPLEMENTARY DATA

Search strategy

- PubMed: ((tumor OR tumour OR tumou*) OR (malignancy) OR (malignan*) OR (cancer) OR ("neoplasms"[MeSH Terms]) OR (neoplasm) OR (neoplas*)) AND ((sulfonylu*) OR (glicl*) OR (tolbut*) OR (glimepir*) OR (glybur*) OR (gliben*) OR (glipiz*) OR (Chlorprop*) OR ("Sulfonylurea Compounds"[MeSH Terms]) OR ("gliclazide"[MeSH Terms]) OR ("Chlorpropamide"[MeSH Terms]) OR ("Glipizide"[MeSH Terms]) OR ("Glyburide"[MeSH Terms]) OR ("Tolbutamide"[MeSH Terms]))
- Cochrane, Database of Abstracts on Reviews and Effectiveness, Current Controlled Trials (<http://controlled-trials.com>), ClinicalTrials.gov (<http://clinicaltrials.gov/>), Netherlands Trial Register (<http://www.trialregister.nl>), NHS Centre for Reviews and Dissemination, NHS Economic Evaluation Database, Health Technology Assessment Database, Health Economic Evaluation Database (<http://www.ohe-heed.com/>), Emerging Care Research Institute database (<http://www.ecri.org/>):
 - Sulfonylurea
 - Gliclazide

Figure S1 - Flow chart showing the selection of clinical studies for review. SU = sulfonylurea derivative.

REFERENCES

1. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, et al. Diabetes and cancer: a consensus report. *Diabetes Care*. 2010;33(7):1674–85.
2. Chen Y, Wu F, Saito E, Lin Y, Song M, Luu HN, et al. Association between type 2 diabetes and risk of cancer mortality: a pooled analysis of over 771,000 individuals in the Asia Cohort Consortium. *Diabetologia* [Internet]. 2017 [cited 2017 Jul 5];60(6):1022–32. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28265721>
3. Redaniel MT, Jeffreys M, May MT, Ben-Shlomo Y, Martin RM. Associations of type 2 diabetes and diabetes treatment with breast cancer risk and mortality: a population-based cohort study among British women. *Cancer Causes Control* [Internet]. 2012;23(11):1785–95. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22971998>
4. Yu F, Guo Y, Wang H, Feng J, Jin Z, Chen Q, et al. Type 2 diabetes mellitus and risk of colorectal adenoma: a meta-analysis of observational studies. *BMC Cancer* [Internet]. 2016 [cited 2018 Apr 8];16(1):642. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27535548>
5. de Kort S, Masclee AAM, Sanduleanu S, Weijenberg MP, van Herk-Sukel MPP, Oldenhof NJJ, et al. Higher risk of colorectal cancer in patients with newly diagnosed diabetes mellitus before the age of colorectal cancer screening initiation. *Sci Rep* [Internet]. 2017 [cited 2018 Apr 8];7:46527. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28436468>
6. Schrijnders D, Hendriks SH, Kleefstra N, Vissers PAJ, Johnson JA, de Bock GH, et al. Sex differences in obesity related cancer incidence in relation to type 2 diabetes diagnosis (ZODIAC-49). *PLoS One*. 2018;13(1):1–17.
7. Yang X, So WY, Ma RC, Yu LW, Ko GT, Kong AP, et al. Use of sulphonylurea and cancer in type 2 diabetes-The Hong Kong Diabetes Registry. *Diabetes Res Clin Pr* [Internet]. 2010;90(3):343–51. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20889221>
8. Jalving M, Gietema JA, Lefrandt JD, de Jong S, Reyners AK, Gans RO, et al. Metformin: taking away the candy for cancer? *Eur J Cancer* [Internet]. 2010;46(13):2369–80. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20656475>
9. Liberti M V, Locasale JW. The Warburg effect: How does it benefit cancer cells? *Trends Biochem Sci* [Internet]. 2016 [cited 2016 Nov 25];41(3):211–8. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0968000415002418>
10. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Vol. 144, *Cell*. 2011. p. 646–74.
11. Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. *Cell Metab* [Internet]. 2016;23(1):27–47. Available from: <http://dx.doi.org/10.1016/j.cmet.2015.12.006>
12. Arianas G, de Jong S, Gietema JA, Lefrandt JD, de Vries EG, Jalving M. Cancer-drug induced insulin resistance: innocent bystander or unusual suspect. *Cancer Treat Rev* [Internet]. 2015;41(4):376–84. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25724262>
13. Soranna D, Scotti L, Zamboni A, Bosetti C, Grassi G, Catapano A, et al. Cancer risk associated with use of metformin and sulfonylurea in type 2 diabetes: a meta-analysis. *Oncologist* [Internet]. 2012;17(6):813–22. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22643536>
14. Chen Y, Du L, Li L, Ma J, Geng X, Yao X, et al. Cancer risk of sulfonylureas in patients with type 2 diabetes mellitus: A systematic review. *J Diabetes* [Internet]. 2017;9(5):482–94. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27273872>
15. Hsieh MC, Lee TC, Cheng SM, Tu ST, Yen MH, Tseng CH. The influence of type 2 diabetes and glucose-lowering therapies on cancer risk in the Taiwanese. *Exp Diabetes Res* [Internet]. 2012;2012. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22719752>
16. Currie CJ, Poole CD, Evans M, Peters JR, Morgan CL. Mortality and other important diabetes-related outcomes with insulin vs other antihyperglycemic therapies in type 2 diabetes. *J Clin Endocrinol Metab* [Internet]. 2013;98(2):668–77. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23372169>

17. Mamtani R, Pfanzer N, Haynes K, Finkelman BS, Wang X, Keefe SM, et al. Incidence of bladder cancer in patients with type 2 diabetes treated with metformin or sulfonylureas. *Diabetes Care* [Internet]. 2014;37(7):1910–7. Available from: <http://care.diabetesjournals.org/content/37/7/1910.long>
18. Morgan CL, Poole CD, Evans M, Barnett AH, Jenkins-Jones S, Currie CJ. What next after metformin? A retrospective evaluation of the outcome of second-line, glucose-lowering therapies in people with type 2 diabetes. *J Clin Endocrinol Metab* [Internet]. 2012;10/19. 2012;97(12):4605–12. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23076348>
19. Qiu H, Rhoads GG, Berlin JA, Marcella SW, Demissie K. Initial metformin or sulphonylurea exposure and cancer occurrence among patients with type 2 diabetes mellitus. *Diabetes Obes Metab* [Internet]. 2013;15(4):349–57. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23137378>
20. Thakkar B, Aronis KN, Vamvini MT, Shields K, Mantzoros CS. Metformin and sulfonylureas in relation to cancer risk in type II diabetes patients: a meta-analysis using primary data of published studies. *Metabolism* [Internet]. 2013;62(7):922–34. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23419783>
21. Calip GS, Yu O, Elmore JG, Boudreau DM. Comparative safety of diabetes medications and risk of incident invasive breast cancer: a population-based cohort study. *Cancer Causes Control* [Internet]. 2016 [cited 2017 Aug 23];27(5):709–20. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27053250>
22. Häggström C, Van Hemelrijck M, Zethelius B, Robinson D, Grundmark B, Holmberg L, et al. Prospective study of Type 2 diabetes mellitus, anti-diabetic drugs and risk of prostate cancer. *Int J Cancer* [Internet]. 2017 [cited 2017 Aug 23];140(3):611–7. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27770555>
23. Nichols CG. KATPchannels as molecular sensors of cellular metabolism. *Nature*. 2006;440(7083):470–6.
24. Holstein A, Hammer C, Hahn M, Kulamadayil N-S-A, Kovacs P. Severe sulfonylurea-induced hypoglycemia: a problem of uncritical prescription and deficiencies of diabetes care in geriatric patients. *Expert Opin Drug Saf* [Internet]. 2010 [cited 2017 Feb 24];9(5):675–81. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20553106>
25. Holstein A, Plaschke A, Egberts EH. Lower incidence of severe hypoglycaemia in patients with type 2 diabetes treated with glimepiride versus glibenclamide. *Diabetes Metab Res Rev* [Internet]. 2001;17(6):467–73. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/11757083>
26. Chan SP, Colagiuri S. Systematic review and meta-analysis of the efficacy and hypoglycemic safety of gliclazide versus other insulinotropic agents. *Diabetes Res Clin Pract* [Internet]. 2015 [cited 2018 Feb 25];110(1):75–81. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26361859>
27. Beck-Nielsen H, Hother-Nielsen O, Pedersen O. Mechanism of Action of Sulphonylureas with Special Reference to the Extrapancreatic Effect: An Overview. *Diabet Med*. 1988;5:613–20.
28. Kolterman O. The impact of sulfonylureas on hepatic glucose metabolism in type II diabetics. *Diabetes Metab Rev*. 1987;3(2):399–414.
29. DeFronzo R, Simonson D. Oral sulfonylurea agents suppress hepatic glucose production in non-insulin-dependent diabetic individuals. *Diabetes Care*. 1984;7:Suppl 1:72–80.
30. Müller G, Satoh Y, Geisen K. Extrapancreatic effects of sulfonylureas - a comparison between glimepiride and conventional sulfonylureas. 1995;8227(95).
31. Feldman JM, Lebovitz HE. Appraisal of the Extrapancreatic Actions of Sulfonylureas. *Arch Intern Med*. 1969;123(3):314–22.
32. Arnouts P, Bolignano D, Nistor I, Bilo H, Gnudi L, Heaf J, et al. Glucose-lowering drugs in patients with chronic kidney disease: a narrative review on pharmacokinetic properties. *Nephrol Dial Transpl*. 2014;29(7):1284–300.
33. Proks P, Reimann F, Green N, Gribble F, Ashcroft F. Sulfonylurea Stimulation of Insulin Secretion. *Diabetes* [Internet]. 2002;51(suppl 3):S368–76. Available from: <https://doi.org/10.2337/diabetes.51.2007.S368>

34. Gopalakrishnan M, Molinari EJ, Shieh CC, Monteggia LM, Roch JM, Whiteaker KL, et al. Pharmacology of human sulphonylurea receptor SUR1 and inward rectifier K(+) channel Kir6.2 combination expressed in HEK-293 cells. *Br J Pharmacol* [Internet]. 2000 Apr [cited 2019 Mar 21];129(7):1323–32. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10742287>
35. Gribble FM, Reimann F. Sulphonylurea action revisited: the post-cloning era. *Diabetologia* [Internet]. 2003 Jul 1 [cited 2019 Mar 21];46(7):875–91. Available from: <http://link.springer.com/10.1007/s00125-003-1143-3>
36. Shorr RI, Ray WA, Daugherty JR, Griffin MR. Individual sulfonylureas and serious hypoglycemia in older people. *J Am Geriatr Soc* [Internet]. 1996;44(7):751–5. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/8675920>
37. Gangji AS, Cukierman T, Gerstein HC, Goldsmith CH, Clase CM. A systematic review and meta-analysis of hypoglycemia and cardiovascular events: a comparison of glyburide with other secretagogues and with insulin. *Diabetes Care*. 2007;30(2):389–94.
38. Leonard CE, Han X, Brensinger CM, Bilker WB, Cardillo S, Flory JH, et al. Comparative risk of serious hypoglycemia with oral antidiabetic monotherapy: A retrospective cohort study. *Pharmacoepidemiol Drug Saf* [Internet]. 2018 [cited 2018 Feb 25];27(1):9–18. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/29108130>
39. Andersen SE, Christensen M. Hypoglycaemia when adding sulphonylurea to metformin: a systematic review and network meta-analysis. *Br J Clin Pharmacol* [Internet]. 2016 [cited 2018 Feb 25];82(5):1291–302. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27426428>
40. Bo S, Castiglione A, Ghigo E, Gentile L, Durazzo M, Cavallo-Perin P, et al. Mortality outcomes of different sulphonylurea drugs: the results of a 14-year cohort study of type 2 diabetic patients. *Eur J Endocrinol* [Internet]. 2013;169(1):117–26. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23660643>
41. Monami M, Balzi D, Lamanna C, Barchielli A, Masotti G, Buiatti E, et al. Are sulphonylureas all the same? A cohort study on cardiovascular and cancer-related mortality. *Diabetes Metab Res Rev* [Internet]. 2007;23(6):479–84. Available from: <http://onlinelibrary.wiley.com/doi/10.1002/dmrr.736/abstract>
42. Tuccori M, Wu JW, Yin H, Majdan A, Azoulay L. The use of glyburide compared with other sulfonylureas and the risk of cancer in patients with type 2 diabetes. *Diabetes Care* [Internet]. 2015;38(11):2083–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26341130>
43. Chang CH, Lin JW, Wu LC, Lai MS, Chuang LM. Oral insulin secretagogues, insulin, and cancer risk in type 2 diabetes mellitus. *J Clin Endocrinol Metab* [Internet]. 2012;97(7):E1170–5. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22563104>
44. Monami M, Lamanna C, Balzi D, Marchionni N, Mannucci E. Sulphonylureas and cancer: a case-control study. *Acta Diabetol* [Internet]. 2009;46(4):279–84. Available from: <http://link.springer.com/article/10.1007/s00592-008-0083-2>
45. Abdul M, Hoosein N. Expression and activity of potassium ion channels in human prostate cancer. *Cancer Lett* [Internet]. 2002;186(1):99–105. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12183081>
46. Kim JA, Kang YS, Lee SH, Lee EH, Yoo BH, Lee YS. Glibenclamide induces apoptosis through inhibition of cystic fibrosis transmembrane conductance regulator (CFTR) Cl(-) channels and intracellular Ca(2+) release in HepG2 human hepatoblastoma cells. *Biochem Biophys Res Commun* [Internet]. 1999;261(3):682–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10441486>
47. Malhi H, Irani AN, Rajvanshi P, Suadicani SO, Spray DC, McDonald T V, et al. KATP channels regulate mitogenically induced proliferation in primary rat hepatocytes and human liver cell lines. Implications for liver growth control and potential therapeutic targeting. *J Biol Chem* [Internet]. 2000;275(34):26050–7. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10862612>
48. Zhou Q, Kwan HY, Chan HC, Jiang JL, Tam SC, Yao X. Blockage of voltage-gated K+ channels inhibits adhesion and proliferation of hepatocarcinoma cells. *Int J Mol Med* [Internet]. 2003;11(2):261–6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12525889>

49. Nunez M, Medina V, Cricco G, Croci M, Cocca C, Rivera E, et al. Glibenclamide inhibits cell growth by inducing G0/G1 arrest in the human breast cancer cell line MDA-MB-231. *BMC Pharmacol Toxicol* [Internet]. 2013;14:6. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3558386/pdf/2050-6511-14-6.pdf>
50. Woodfork KA, Wonderlin WF, Peterson VA, Strobl JS. Inhibition of ATP-sensitive potassium channels causes reversible cell-cycle arrest of human breast cancer cells in tissue culture. *J Cell Physiol* [Internet]. 1995;162(2):163–71. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/7822427>
51. Qian X, Li J, Ding J, Wang Z, Duan L, Hu G. Glibenclamide exerts an antitumor activity through reactive oxygen species-c-jun NH2-terminal kinase pathway in human gastric cancer cell line MGC-803. *Biochem Pharmacol* [Internet]. 2008;76(12):1705–15. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18840412>
52. Wondergem R, Cregan M, Strickler L, Miller R, Suttles J. Membrane potassium channels and human bladder tumor cells: II. Growth properties. *J Membr Biol* [Internet]. 1998;161(3):257–62. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9493131>
53. Ru Q, Tian X, Wu Y-X, Wu R-H, Pi M-S, Li C-Y. Voltage-gated and ATP-sensitive K⁺ channels are associated with cell proliferation and tumorigenesis of human glioma. *Oncol Rep* [Internet]. 2014 [cited 2016 Aug 4];31(2):842–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24284968>
54. Abdul M, Hoosein N. Voltage-gated potassium ion channels in colon cancer. *Oncol Rep* [Internet]. 2002;9(5):961–4. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12168055>
55. Coppock SW, Lant AF, McIntosh CS, Rodgers AV. Pharmacokinetic and pharmacodynamic studies of glibenclamide in non-insulin dependent diabetes mellitus. *Br J Clin Pharmacol* [Internet]. 1990;29(6):673–84. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/2116159>
56. Zhanping W, Xiaoyu P, Na C, Shenglan W, Bo W. Voltage-gated K⁺ channels are associated with cell proliferation and cell cycle of ovarian cancer cell. *Gynecol Oncol* [Internet]. 2007;104(2):455–60. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17014896>
57. Han X, Wang F, Yao W, Xing H, Weng D, Song X, et al. Heat shock proteins and p53 play a critical role in K⁺ channel-mediated tumor cell proliferation and apoptosis. *Apoptosis* [Internet]. 2007 [cited 2016 Aug 4];12(10):1837–46. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17624594>
58. Huang L, Li B, Li W, Guo H, Zou F. ATP-sensitive potassium channels control glioma cells proliferation by regulating ERK activity. *Carcinogenesis* [Internet]. 2009 [cited 2016 Aug 4];30(5):737–44. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19176641>
59. Andreasen PB, Vesell ES. Comparison of plasma levels of antipyrine, tolbutamide, and warfarin after oral and intravenous administration. *Clin Pharmacol Ther* [Internet]. 1974 [cited 2017 Dec 18];16(6):1059–65. Available from: <http://doi.wiley.com/10.1002/cpt19741661059>
60. Qi C, Zhou Q, Li B, Yang Y, Cao L, Ye Y, et al. Glipizide, an antidiabetic drug, suppresses tumor growth and metastasis by inhibiting angiogenesis. *Oncotarget*. 2014;5(20):9966–79.
61. Simonson DC, Kourides IA, Feinglos M, Shamon H, Fischette CT. Efficacy, safety, and dose-response characteristics of glipizide gastrointestinal therapeutic system on glycemic control and insulin secretion in NIDDM. Results of two multicenter, randomized, placebo-controlled clinical trials. *The Glipizide Gastrointest*. *Diabetes Care*. 1997;20(4):597–606.
62. Ota A, Shen-Orr Z, LeRoith D. Insulin and IGF-I receptors in neuroblastoma cells: increases in mRNA and binding produced by glyburide. *Neuropeptides* [Internet]. 1989;14(3):171–5. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/2559356>
63. Desfaits AC, Serri O, Renier G. Normalization of plasma lipid peroxides, monocyte adhesion, and tumor necrosis factor- α production in NIDDM patients after glizalide treatment. *Diabetes Care* [Internet]. 1998;21(4):487–93. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9571329>
64. Qiang X, Satoh J, Sagara M, Fukuzawa M, Masuda T, Miyaguchi S, et al. Glizalide inhibits diabetic neuropathy irrespective of blood glucose levels in streptozotocin-induced diabetic rats. *Metabolism* [Internet]. 1998 [cited 2016 Aug 4];47(8):977–81. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0026049598903547>

65. Fukuzawa M, Satoh J, Qiang X, Miyaguchi S, Sakata Y, Nakazawa T, et al. Inhibition of tumor necrosis factor- α with anti-diabetic agents. *Diabetes Res Clin Pract.* 1999;43(3):147–54.
66. Nunes FB, Alves-Filho JCF, Alves Bastos CM, Tessele PM, Caberlon E, Moreira KB, et al. Effect of the chlorpropamide and Fructose-1,6-bisphosphate on soluble TNF receptor II levels. *Pharmacol Res.* 2004;49(5):449–53.
67. Fresno Vara J, Casado E, de Castro J, Cejas P, Belda-Iniesta C, González-Barón M. PI3K/Akt signalling pathway and cancer. *Cancer Treat Rev* [Internet]. 2004;30(2):193–204. Available from: papers://b994deed-0281-4413-a671-0b6c113f7809/Paper/p2269
68. Yang L, Dan HC, Sun M, Liu Q, Sun X, Feldman RI, et al. Akt / protein kinase B signaling inhibitor-2, a selective small molecule inhibitor of Akt signaling with antitumor activity in cancer cells overexpressing Akt. *Genes Dev.* 2004;2:4394–9.
69. Mundi PS, Sachdev J, McCourt C, Kalinsky K. AKT in cancer: new molecular insights and advances in drug development. *Br J Clin Pharmacol.* 2016;110:943–56.
70. Lautier D, Canitrot Y, Deeley RG, Cole SP. Multidrug resistance mediated by the multidrug resistance protein (MRP) gene. *Biochem Pharmacol* [Internet]. 1996;52(7):967–77. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/8831715>
71. Golstein PE, Boom A, van Geffel J, Jacobs P, Masereel B, Beauwens R. P-glycoprotein inhibition by glibenclamide and related compounds. *Pflugers Arch* [Internet]. 1999;437(5):652–60. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10087141>
72. Hamon Y, Luciani MF, Becq F, Verrier B, Rubartelli A, Chimini G. Interleukin-1 β secretion is impaired by inhibitors of the Atp binding cassette transporter, ABC1. *Blood* [Internet]. 1997;90(8):2911–5. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9376570>
73. Sheppard DN, Robinson KA. Mechanism of glibenclamide inhibition of cystic fibrosis transmembrane conductance regulator Cl-channels expressed in a murine cell line. *J Physiol* [Internet]. 1997;503:333–46. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9306276>
74. Stieger B, Fattinger K, Madon J, Kullak-Ublick GA, Meier PJ. Drug- and estrogen-induced cholestasis through inhibition of the hepatocellular bile salt export pump (Bsep) of rat liver. *Gastroenterology* [Internet]. 2000;118(2):422–30. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10648470>
75. Payen L, Delugin L, Courtois A, Trinquart Y, Guillouzo A, Fardel O. The sulphonylurea glibenclamide inhibits multidrug resistance protein (MRP1) activity in human lung cancer cells. *Br J Pharmacol* [Internet]. 2001;132(3):778–84. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11159731>
76. Negis Y, Meydani M, Zingg J-M, Azzi A. Molecular mechanism of α -tocopheryl-phosphate transport across the cell membrane. *Biochem Biophys Res Commun.* 2007;359(2):348–53.
77. Ughachukwu P, Unekwe P. Efflux pump-mediated resistance in chemotherapy. *Ann Med Health Sci Res* [Internet]. 2012;2(2):191. Available from: <http://www.amhsr.org/text.asp?2012/2/2/191/105671>
78. Burke MA, Mutharasan RK, Ardehali H. The sulfonylurea receptor, an atypical ATP-binding cassette protein, and its regulation of the KATP channel. *Circ Res* [Internet]. 2008;102(2):164–76. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18239147>
79. Reeve HL, Vaughan PFT, Peers C. Glibenclamide inhibits a voltage-gated K⁺ current in the human neuroblastoma cell line SH-SY5Y. *Neurosci Lett.* 1992;135(1):37–40.
80. Huang X, Jan LY. Targeting potassium channels in cancer. *J Cell Biol* [Internet]. 2014;206(2):151–62. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25049269>
81. Wang Z. Roles of K⁺ channels in regulating tumour cell proliferation and apoptosis. Vol. 448, *Pflugers Arch.* 2004. p. 274–86.
82. Hemmerlein B, Weseloh RM, Mello de Queiroz F, Knotgen H, Sanchez A, Rubio ME, et al. Overexpression of Eag1 potassium channels in clinical tumours. *Mol Cancer* [Internet]. 2006;5:41. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17022810>
83. Pardo LA, Stuhmer W. Eag1: an emerging oncological target. *Cancer Res* [Internet]. 2008;68(6):1611–3. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18339837>

84. Downie BR, Sanchez A, Knotgen H, Contreras-Jurado C, Gymnopoulos M, Weber C, et al. Eag1 expression interferes with hypoxia homeostasis and induces angiogenesis in tumors. *J Biol Chem* [Internet]. 2008;283(52):36234–40. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18927085>
85. Yan B, Peng Z, Xing X, Du C. Glibenclamide induces apoptosis by activating reactive oxygen species dependent JNK pathway in hepatocellular carcinoma cells. *Biosci Rep* [Internet]. 2017;37(5):BSR20170685. Available from: <http://bioscirep.org/lookup/doi/10.1042/BSR20170685>
86. Suzuki Y, Inoue T, Murai M, Suzuki-Karasaki M, Ochiai T, Ra C. Depolarization potentiates TRAIL-induced apoptosis in human melanoma cells: role for ATP-sensitive K⁺ channels and endoplasmic reticulum stress. *Int J Oncol* [Internet]. 2012/05/23. 2012;41(2):465–75. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22613960>
87. Mohapatra S, Chu B, Zhao X, Djeu J, Cheng JQ, Pledger WJ. Apoptosis of metastatic prostate cancer cells by a combination of cyclin-dependent kinase and AKT inhibitors. *Int J Biochem Cell Biol*. 2009;41(3):595–602.
88. Paíno T, Gangoso E, Medina JM, Tabernero A. Inhibition of ATP-sensitive potassium channels increases HSV-tk/GCV bystander effect in U373 human glioma cells by enhancing gap junctional intercellular communication. *Neuropharmacology*. 2010;59(6):480-91.
89. O'Brien RC, Luo M, Balazs N, Mercuri J. In vitro and in vivo antioxidant properties of gliclazide. *J Diabetes Complicat* [Internet]. 2000;14(4):201–6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11004429>
90. Sliwinska A, Rogalska A, Szwed M, Kasznicki J, Jozwiak Z, Drzewoski J. Gliclazide may have an antiapoptotic effect related to its antioxidant properties in human normal and cancer cells. *Mol Biol Rep* [Internet]. 2012;39(5):5253–67. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22183301>
91. Kimoto K, Suzuki K, Kizaki T, Hitomi Y, Ishida H, Katsuta H, et al. Gliclazide protects pancreatic beta-cells from damage by hydrogen peroxide. *Biochem Biophys Res Commun* [Internet]. 2003;303(1):112–9. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/12646174>
92. Gu Q, Wang C, Wang G, Han Z, Li Y, Wang X, et al. Glipizide suppresses embryonic vasculogenesis and angiogenesis through targeting natriuretic peptide receptor A. *Exp Cell Res* [Internet]. 2015;333(2):261–72. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25823921>
93. Yasukagawa T, Niwa Y, Simizu S, Umezawa K. Suppression of cellular invasion by glybenclamide through inhibited secretion of platelet-derived growth factor in ovarian clear cell carcinoma ES-2 cells. *FEBS Lett* [Internet]. 2012;586(10):1504–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22673517>
94. Qi C, Li B, Yang Y, Yang Y, Li J, Zhou Q, et al. Glipizide suppresses prostate cancer progression in the TRAMP model by inhibiting angiogenesis. *Sci Rep* [Internet]. 2016;6(May):1–12. Available from: <http://dx.doi.org/10.1038/srep27819>
95. Rong Z, Li L, Fei F, Luo L, Qu Y, Kaufmann M, et al. Combined treatment of glibenclamide and CoCl₂ decreases MMP9 expression and inhibits growth in highly metastatic breast cancer. *J Exp Clin Cancer Res* [Internet]. 2013 [cited 2016 Aug 4];32(1):32. Available from: <http://jeccr.biomedcentral.com/articles/10.1186/1756-9966-32-32>
96. Li M, Wang Q, Lin W, Wang B. Regulation of ovarian cancer cell adhesion and invasion by chloride channels. *Int J Gynecol Cancer* [Internet]. 2009 May [cited 2016 Aug 4];19(4):526–30. Available from: <http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00009577-200905000-00008>
97. Kretschy N, Teichmann M, Kopf S, Atanasov AG, Saiko P, Vonach C, et al. In vitro inhibition of breast cancer spheroid-induced lymphendothelial defects resembling intravasation into the lymphatic vasculature by acetohexamide, isoxsuprine, nifedipin and proadifen. *Br J Cancer*. 2013;108(3):570–8.
98. Jiang J, McDonald PR, Dixon TM, Franicola D, Zhang X, Nie S, et al. Synthetic protection short interfering RNA screen reveals glyburide as a novel radioprotector. *Radiat Res* [Internet]. 2009 [cited 2016 Aug 4];172(4):414–22. Available from: <http://www.bioone.org/doi/10.1667/RR1674.1>

99. Subramaniam N, Arumugam S, Babu P, Ariraman S, Biswas I, Kumar S, et al. Unveiling anticancer potential of glibenclamide: Its synergistic cytotoxicity with doxorubicin on cancer cells. *J Pharm Biomed Anal* [Internet]. 2018;154:294–301. Available from: <https://doi.org/10.1016/j.jpba.2018.03.025>
100. Rekha GK, Devaraj VR, Sreerama L, Lee MJ, Nagasawa HT, Sladek NE. Inhibition of human class 3 aldehyde dehydrogenase, and sensitization of tumor cells that express significant amounts of this enzyme to oxazaphosphorines, by chlorpropamide analogues. *Biochem Pharmacol* [Internet]. 1998;55(4):465–74. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/9514081>
101. Sládek NE, Rekha GK, Lee MJC, Nagasawa HT. Inhibition of ALDH3A1-catalyzed oxidation by chlorpropamide analogues. Vol. 138, *Chem Biol Interact*. 2001. p. 201–15.
102. Shimura T, Noma N, Sano Y, Ochiai Y, Oikawa T, Fukumoto M, et al. AKT-mediated enhanced aerobic glycolysis causes acquired radioresistance by human tumor cells. *Radiother Oncol*. 2014;112(2):302–7.
103. Yang J-Y, Chang C-J, Xia W, Wang Y, Wong K-K, Engelman JA, et al. Activation of FOXO3a is sufficient to reverse mitogen-activated protein/extracellular signal-regulated kinase kinase inhibitor chemoresistance in human cancer. *Cancer Res* [Internet]. 2010 [cited 2016 Aug 4];70(11):4709–18. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20484037>
104. Williams TM, Flecha AR, Keller P, Ram A, Karnak D, Galbán S, et al. Cotargeting MAPK and PI3K signaling with concurrent radiotherapy as a strategy for the treatment of pancreatic cancer. *Mol Cancer Ther* [Internet]. 2012 [cited 2016 Aug 4];11(5):1193–202. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22411900>
105. Noh KH, Kang TH, Kim JH, Pai SI, Lin KY, Hung C-F, et al. Activation of Akt as a mechanism for tumor immune evasion. Vol. 17, *Mol Ther*. 2009. p. 439–47.
106. Tenbaum SP, Ordóñez-Morán P, Puig I, Chicote I, Arqués O, Landolfi S, et al. β -catenin confers resistance to PI3K and AKT inhibitors and subverts FOXO3a to promote metastasis in colon cancer. *Nat Med* [Internet]. 2012 [cited 2016 Aug 4];18(6):892–901. Available from: <http://www.nature.com/doi/10.1038/nm.2772>
107. Gribble FM, Reimann F. Differential selectivity of insulin secretagogues: mechanisms, clinical implications, and drug interactions. *J Diabetes Complications* [Internet]. 2003/03/08. 2003;17(2 Suppl):11–5. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12623163>
108. Melander A. Kinetics-effect relations of insulin-releasing drugs in patients with type 2 diabetes: brief overview. *Diabetes* [Internet]. 2004;53(Suppl 3):S151–5. Available from: https://doi.org/10.2337/diabetes.53.suppl_3.S151
109. Harrower ADB. Comparative tolerability of sulphonylureas in diabetes mellitus. *Drug Saf*. 2000;22(4):313–20.
110. Seino S, Takahashi H, Takahashi T, Shibasaki T. Treating diabetes today: A matter of selectivity of sulphonylureas. *Diabetes, Obes Metab*. 2012;14(1 SUPPL. 1):9–13.
111. The electronic Medicines Compendium. Summary of Product Characteristics - Gliclazide [Internet]. [cited 2019 Mar 1]. Available from: https://www.medicines.org.uk/emc/product/5883/smpc#PHARMACOLOGICAL_PROPS
112. Wang Z, Thurmond DC. Mechanisms of biphasic insulin-granule exocytosis - roles of the cytoskeleton, small GTPases and SNARE proteins. *J Cell Sci*. 2009;122(7):893–903.
113. Gregorio F, Ambrosi F, Cristallini S, Pedetti M, Filipponi P, Santeusano F. Therapeutical concentrations of tolbutamide, glibenclamide, gliclazide and gliquidone at different glucose levels: in vitro effects on pancreatic A- and B-cell function. *Diabetes Res Clin Pract*. 1992;18(3):197–206.
114. Cozma LS, Luzio SD, Dunseath GJ, Langendorg KW, Pieber T, Ownes DR. Comparison of the effects of three insulinotropic drugs on plasma insulin levels after a standard meal. *Diabetes Care* [Internet]. 2002;25(8):1271–6. Available from: <https://doi.org/10.2337/diacare.25.8.1271>
115. Ligtenberg JJM, Venker CE, Sluiter WJ, Reitsma WD, van Haeften TW. Effect of glibenclamide on insulin release at moderate and high blood glucose levels in normal man. *Eur J Clin Invest*. 1997;27(8):685–9.

116. Szoke E, Gosmanov NR, Sinkin JC, Nihalani A, Fender AB, Cryer PE, et al. Effects of glimepiride and glyburide on glucose counterregulation and recovery from hypoglycemia. *Metabolism* [Internet]. 2006 Jan [cited 2019 Feb 27];55(1):78–83. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0026049505002982>
117. Riefflin A, Ayyagari U, Manley SE, Holman RR, Levy JC. The effect of glibenclamide on insulin secretion at normal glucose concentrations. *Diabetologia* [Internet]. 2015 Jan 9 [cited 2019 Feb 27];58(1):43–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25297572>
118. Draeger K, Wernicke-Panten K, Lomp H, Schüler E, Roskamp R. Long-term treatment of type 2 diabetic patients with the new oral antidiabetic agent glimepiride (amaryl): a double-blind comparison with glibenclamide. *Horm Metab Res*. 1996;28(9):419–25.
119. Hollander PA, Schwartz SL, Gatlin MR, Haas SJ, Zheng H, Foley JE, et al. Importance of early insulin secretion: Comparison of nateglinide and glyburide in previously diet-treated patients with type 2 diabetes. *Diabetes Care*. 2001;24(6):983–8.
120. Chiasson J, Hamet P, Verdy M. The effect of Diamicron on the secretion and action of insulin. *Diabetes Res Clin Pr* [Internet]. 1991;14(1991):S47–51. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/1794265>
121. Proks P, Kramer H, Haythorne E, Ashcroft FM. Binding of sulphonylureas to plasma proteins – A KATP channel perspective. *PLoS One*. 2018;13(5):e0197634.
122. Seedher N, Kanojia M. Mechanism of interaction of hypoglycemic agents glimepiride and glipizide with human serum albumin. *Cent Eur J Chem*. 2009;7(1):96–104.
123. Seedher N, Kanojia M. Reversible binding of antidiabetic drugs, repaglinide and gliclazide, with human serum albumin. *Chem Biol Drug Des*. 2008;72(4):290–6.
124. Hsu P, Ma J, Luzzi L. Interactions of sulfonylureas with plasma proteins. *J Pharm Sci*. 1974;63(4):570–3.
125. Smith DA, Di L, Kerns EH. The effect of plasma protein binding on in vivo efficacy: Misconceptions in drug discovery. *Nat Rev Drug Discov*. 2010;9(12):929–39.

3

Within-Sulfonylurea-Class Evaluation of Time to Intensification with Insulin (ZODIAC-43)

Published as:

Schrijnders D, Hartog LC, Kleefstra N, Groenier KH, Landman GWD, et al. (2016) Within-Sulfonylurea-Class Evaluation of Time to Intensification with Insulin (ZODIAC-43). PLOS ONE 11(6): e0157668.

ABSTRACT

Background:

Previous studies have shown that many within-class differences exist between sulfonylureas (SUs), however, whether differences exist regarding the time it takes between initiating an SU and the need to intensify treatment with insulin is unclear. The aim of this study was to investigate the relationships between the three frequently used sulphonylureas, prescribed as dual therapy next to metformin, and the time needed to treatment intensification with either insulin or oral triple therapy in patients with type 2 diabetes mellitus.

Methods:

Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) is a prospective observational cohort study set in primary care in the Netherlands. Annually collected data on diabetes medication and clinical variables within ZODIAC are used to evaluate the primary outcome, time to insulin and secondary outcome, time to either insulin or triple oral therapy. For statistical analysis a time-dependent cox proportional hazard model was used.

Results:

3507 patients were included in the analysis, with a mean age of 61 (SD 11.4) and a median HbA1c of 6.8% [IQR 6.4-7.4] (50.8 mmol/mol [IQR 46.4-57.4]). The hazard ratio (HR) for the primary endpoint was 1.10 (95% CI 0.78-1.54) for metformin/glimepiride and 0.93 (95% CI 0.67-1.30) for metformin/tolbutamide with metformin/gliclazide as reference group. The HR for the secondary outcome was 1.04 (95% CI 0.78-1.40) and 0.85 (95% CI 0.64-1.13), respectively.

Conclusion:

In this large Dutch primary care cohort, new users of neither gliclazide, glimepiride nor tolbutamide as dual therapy with metformin, resulted in differences in the time needed for further treatment intensification.

INTRODUCTION

Many important within-class SU differences have been described, mostly in favour of gliclazide. For example; gliclazide is considered the safest SU in patients with renal impairment(1). Furthermore, there is also a clear benefit of prescribing gliclazide for reasons of hypoglycaemia risk(2) and possibly cardiovascular safety(3). Within-class SU differences have also been described for SU failure rate. For example, glipizide and glibenclamide are associated with a higher failure rate compared to gliclazide(4). In the 2013 Dutch type 2 diabetes mellitus (T2DM) guideline, gliclazide specifically became the preferred sulphonylurea (SU) and the first intensification step after metformin (5).

When dual oral therapy fails, next to lifestyle interventions, a switch to or addition of, once daily insulin or possibly a third oral agent are advised steps for regaining adequate glycaemic control. This moment in time could be considered a turning point for patients and is regarded as disease progression for patients and therefore is a relevant surrogate endpoint.

Whether within-class differences exist for “time needed to intensification” for SUs remains unclear. We hypothesized that a difference in time to intensification could be present, since the subsequent generations of SUs have become increasingly more potent (4, 6). The presence of a within-class difference concerning time to insulin could benefit both patient related outcomes as well as aid in controlling health care costs since treatment with oral glucose lowering agents is more cost-effective than treatment with insulin (7).

Using a new-user design, the aim of this study was to investigate the relationships between the three most frequently prescribed SUs in the Netherlands, prescribed as dual therapy next to metformin and the time needed to treatment intensification with either insulin or oral triple therapy in patients with T2DM.

METHODS

The study is reported according to the STROBE (Strengthening the reporting of observational studies in epidemiology) recommendations (8).

Study design and data collection

This study is part of the prospective ZODIAC (Zwolle Outpatient Diabetes project Integrating Available Care) cohort study. This ongoing study started in 1998 in the Zwolle region, and has since expanded to more than 600 general practices in the north-east and western part of the Netherlands. Following two major expansions of the cohort in 2006 and 2009, the majority of patients were included from 2006 onwards. Patients included in the study are diagnosed with T2DM and are exclusively treated in primary care. Data are collected annually by general practitioners and sent to the diabetes centre annually.

Patient selection

A “new-user” design (9) was used. Patients who used metformin monotherapy for at least one year and subsequently intensified for the first time with gliclazide, glimepiride or tolbutamide were selected. Patients were also required to be included in the ZODIAC study between 1998 and 2012. Patients with an eGFR below 30mL/min/1.73m² or when eGFR at baseline was missing were excluded. We hypothesized that including these patients could lead to selection bias because gliclazide is the preferred SU in renal impairment (1).

Outcome measures

The primary outcome was the first receipt of insulin and secondary outcome was first receipt of either insulin or triple oral therapy.

Statistical analysis

Quantitative variables are presented as means with standard deviation for normally distributed values, and median and interquartile range (IQR) for skewed variables. Time-dependent cox proportional hazard analyses, adjusted for HbA1c, age, gender, diabetes duration, updated mean creatinine and updated mean BMI were used to evaluate the primary and secondary outcome. All analyses were performed with IBM SPSS Statistics (Version 22.0).

ETHICS STATEMENT

In the ZODIAC study, patients consented to anonymous use of their data for study purposes. The medical ethics committee of Isala, Zwolle, The Netherlands approved the ZODIAC study (METC reference numbers 03.0316 and 07.0335).

RESULTS

From the complete cohort (N = 82.167), 25.183 patients used metformin monotherapy. From these, 4096 received some form of intensification. Patients receiving insulin as second step on top of metformin (n=83) and 506 patients with an eGFR < 30 ml/min/1.73m² or missing eGFR were excluded (Fig 1). From the 3507 selected patients, 47% was female, the mean age was 61 (SD 11.4), median HbA1c 6.8% [IQR 6.4-7.4] (50.8 mmol/mol [IQR 46.4-57.4]), median BMI 29.7 [IQR 26.8-33.3], median diabetes duration 6.8 years [IQR 4.5-9.4], mean eGFR 83.2 (SD 20.1) and median creatinine 76.0 [IQR 65.0-89.0] (Table 1). There were no baseline differences in median HbA1c between the different treatment groups; metformin/gliclazide 6.9 [IQR 6.4-7.4], metformin/glimepiride 6.8 [IQR 6.3-7.4] and metformin/tolbutamide 6.9 [IQR 6.4-7.5]. Two-and-half and 5 years after intensification 13.0% and 32.0% of patients were using insulin, respectively. Two-and-half and 5 years after intensification 17.5% and 39.7% of patients were using either insulin or triple oral therapy, respectively.

Table 1: Baseline characteristics.

Variables	n=3507
Gender ^a	Female: 47%, Male: 53%
Age at diagnosis (years) ^b	57.8 [17.9]
Age (years) ^b	61.0 [11.4]
eGFR ^b	83.2 [20.1]
Creatinine (μmol/L) ^c	76.0 [65.0-89.0]
Diabetes duration (years) ^c	6.8 [4.5-9.4]
HbA1c (%) / HbA1c (mmol/mol) ^c	6.8 [6.4-7.4] / 50.8 [46.4-57.4]
BMI ^c	29.7 [26.8-33.3]

^a%, ^b mean with SD, ^c median with interquartile range.

PRIMARY ENDPOINT

In the unadjusted model the HR for metformin/glimepiride was 1.15 (95% CI 0.83-1.61) and for metformin/tolbutamide 0.97 (95% CI 0.70-1.34), with metformin/gliclazide as a reference category. There were no significant differences between the three groups in primary outcome (Fig 2). The pairwise HR's for the primary endpoint in the unadjusted model are shown in Table 2.

Fig 1. Flow of selected patients.

Fig 2. Cumulative survival. Panel A shows the cumulative survival for gliclazide, glimepiride and tolbutamide and time to insulin using the unadjusted model. Panel B shows the cumulative survival for gliclazide, glimepiride and tolbutamide and time to insulin or triple oral therapy using the unadjusted model.

Table 2: unadjusted model. *Reference category.

	Time to insulin			Time to insulin or third oral agent		
	HR	95% CI	p	HR	95% CI	p
Medication			0.429			0.161
Gliclazide* vs Glimepiride	1.151	0.825-1.605	0.407	1.111	0.834-1.480	0.470
Gliclazide* vs Tolbutamide	0.965	0.695-1.340	0.831	0.878	0.660-1.168	0.371
Glimepiride* vs Tolbutamide	0.838	0.637-1.103	0.207	0.790	0.620-1.006	0.056

The HR for time to insulin using a fully corrected model for metformin/glimepiride was 1.10 (95% CI 0.78-1.54) and for metformin/tolbutamide 0.93 (95% CI 0.67-1.30), with metformin/gliclazide as a reference category. The pairwise HR's for the primary endpoint in the fully corrected model are shown in Table 3. There were no significant differences between the three groups in primary outcome. HbA1c and age are significantly associated with the primary endpoint with HRs of 1.08 (95%CI 1.00-1.15) and 0.99 (95%CI 0.97-1.00) respectively. Creatinine, BMI, gender, and diabetes duration are not significantly associated with the primary endpoint (Table 3).

Table 3: Covariates included in Cox proportional hazard model. * reference category

	Time to insulin			Time to insulin or third oral agent		
	HR	95% CI	p	HR	95% CI	p
HbA1c	1.077	1.008-1.151	0.028	1.082	1.022-1.145	0.007
Creatinine	0.998	0.990-1.006	0.688	0.992	0.985-1.000	0.410
BMI	1.007	0.983-1.033	0.585	1.021	1.000-1.043	0.510
Gender	1.012	0.768-1.334	0.934	0.896	0.701-1.145	0.380
Diabetes duration	0.988	0.951-1.026	0.545	0.994	0.969-1.020	0.644
Age	0.986	0.974-0.998	0.026	0.984	0.974-0.995	0.003
Medication			0.541			0.234
Gliclazide* vs Glimepiride	1.095	0.780-0.537	0.602	1.044	0.780-1.397	0.772
Glicazide* vs Tolbutamide	0.933	0.668-1.304	0.684	0.848	0.635-1.134	0.266
Glimepiride* vs Tolbutamide	0.852	0.643-1.131	0.268	0.813	0.634-1.041	0.101

SECONDARY ENDPOINT

In the unadjusted model the HR for metformin/glimepiride was 1.11 (95% CI 0.83-1.48) and for metformin/tolbutamide 0.88 (95% CI 0.66-1.17), with metformin/gliclazide as a reference category. There were no significant differences between the three groups in primary outcome (Fig 2). The pairwise HR's for the primary endpoint in the unadjusted model are shown in Table 2.

The HRs for time to insulin or triple oral therapy for metformin/glimepiride was 1.04 (95% CI 0.78-1.40) and for metformin/tolbutamide 0.85 (95% CI 0.64-1.13) with metformin/gliclazide as a reference category. The pairwise HR's for the secondary endpoint are shown in Table 3. HbA1c (HR 1.08, 95% CI 1.02-1.15) and age (HR 0.98, 95% CI 0.97 – 1.00) were significantly associated with the need for intensification therapy with insulin of a third oral agent. Risk for time to insulin or triple oral therapy by increased 8% per 1% increase in HbA1c and decreased 2% per 1-year increase in age. Creatinine, BMI, Gender and diabetes duration were not significantly associated with the secondary endpoint (Table 3).

DISCUSSION

In this primary care cohort of patients who used metformin monotherapy and intensified with gliclazide, glimepiride or tolbutamide, the so-called new users, there were no statistically significant differences in time to insulin, or time to either insulin or a third oral agent. After 2.5 and 5 years respectively 13.0% and 32.0% of patients were using insulin and respectively 17.5% and 39.7% of patients were using insulin or triple therapy. The numbers are in accordance with the numbers found previous studies (10).

No studies were found investigating within-class differences in time to insulin in patients using metformin and a SU as dual therapy. A retrospective cohort study conducted in Colombia showed a higher risk of insulin treatment (OR=3.49, 95%CI 2.61-4.66) in patients treated with metformin and glibenclamide (11). In a Swedish retrospective cohort study the probability of insulin use was increased (HR = 2.71, 95%CI: 2.15-3.43) in patients using more than one oral antidiabetic agent (12). However, the risk of insulin use was not evaluated in individual agents within the SU class.

Based on the results of this study, that in daily primary care practice, the time to intensification with insulin or a third oral agent is not influenced by the choice of a specific SU but rather by specific patient characteristics despite the differences in potency between the three SUs (4, 6). In this study, HbA1c and age appeared to be more important factors.

The strengths of this study were the inclusion of patients from a daily care setting and the new-user design. The daily care setting increases the generalizability of our results. Excluding prevalent users allowed us to avoid under ascertainment of insulin prescription that occurs early after the start of SUs and also allowed us to avoid that confounders are influenced by previous treatment (9). A limitation was that the data is collected annually; changes in medication within this year might not be reflected in our database. A second limitation is the possibility that patients who required insulin could have been referred to secondary care. Patients within ZODIAC are lost to follow-up when referred to secondary care for their diabetes treatment. However, in the Netherlands insulin treatment is usually initiated in primary care and thus expect the underestimation of time to insulin to be limited. Furthermore, patients could have refused intensification of therapy like the addition of insulin, leading to an underestimation of the primary endpoint which was the reason for also analysing the time needed to a third oral agent. However, we don't expect a between-group difference in motivation to use insulin.

CONCLUSION

In conclusion, there were no differences found in this large Dutch primary care cohort with respect to the time needed for further treatment intensification with neither insulin nor a third oral agent when treated with gliclazide, glimepiride or tolbutamide on top of metformin. In this study higher HbA1c and lower age are associated with a shorter time needed to insulin and a shorter time to a third oral agent.

REFERENCES

1. Arnouts P, Bolignano D, Nistor I, Bilo H, Gnudi L, Heaf J, et al. Glucose-lowering drugs in patients with chronic kidney disease: a narrative review on pharmacokinetic properties. *Nephrol Dial Transplant*. 2014;29(7):1284-300.
2. Landman GW, de Bock GH, van Hateren KJ, van Dijk PR, Groenier KH, Gans RO, et al. Safety and efficacy of gliclazide as treatment for type 2 diabetes: a systematic review and meta-analysis of randomized trials. *PLoS One*. 2014;9(2):e82880.
3. Schramm TK, Gislason GH, Vaag A, Rasmussen JN, Folke F, Hansen ML, et al. Mortality and cardiovascular risk associated with different insulin secretagogues compared with metformin in type 2 diabetes, with or without a previous myocardial infarction: a nationwide study. *Eur Heart J*. 2011;32(15):1900-8.
4. Harrower AD. Comparison of efficacy, secondary failure rate, and complications of sulfonylureas. *J Diabetes Complications*. 1994;8(4):201-3.
5. Rutten G, De Grauw W, Nijpels G, Houweling S, Van de Laar F, Bilo H, et al. NHG-Standaard Diabetes mellitus type 2 (derde herziening). *Huisarts Wet*. 2013;56(10):512-25.
6. Dearden JC, Hewitt M, Rowe PH. QSAR study of some anti-hyperglycaemic sulphonylurea drugs. *SAR QSAR Environ Res*. 2015;26(6):439-48.
7. Grzeszczak W, Czupryniak L, Kolasa K, Sciborski C, Lomon ID, McEwan P. The cost-effectiveness of saxagliptin versus NPH insulin when used in combination with other oral antidiabetes agents in the treatment of type 2 diabetes mellitus in Poland. *Diabetes Technol Ther*. 2012;14(1):65-73.
8. von Elm E, Altman DG, Egger M, Pocock SJ, Gotsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol*. 2008;61(4):344-9.
9. Ray WA. Evaluating medication effects outside of clinical trials: new-user designs. *Am J Epidemiol*. 2003;158(9):915-20.
10. Rascati K, Richards K, Lopez D, Cheng LI, Wilson J. Progression to insulin for patients with diabetes mellitus on dual oral antidiabetic therapy using the US Department of Defense Database. *Diabetes Obes Metab*. 2013;15(10):901-5.
11. Machado-Alba JE, Machado-Duque ME, Moreno-Gutierrez PA. Time to and factors associated with insulin initiation in patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract*. 2015;107(3):332-7.
12. Ringborg A, Lindgren P, Yin DD, Martinell M, Stalhammar J. Time to insulin treatment and factors associated with insulin prescription in Swedish patients with type 2 diabetes. *Diabetes Metab*. 2010;36(3):198-203.

4

Addition of sulphonylurea to metformin does not relevantly change body weight: a prospective observational cohort study (ZODIAC-39)

Published as:

Schrijnders D, Wever R, Kleefstra N, Houweling ST, van Hateren KJJ, de Bock GH, Bilo HJG, Groenier KH, Landman GWD. Addition of sulphonylurea to metformin does not relevantly change body weight: a prospective observational cohort study (ZODIAC-39), Diabetes Obes Metab 2016, 18:973–979. DOI:10.1111/dom.12700

ABSTRACT

Aim:

To investigate changes in body weight trajectories after the addition of individual sulphonylureas (SUs) to metformin in patients with type 2 diabetes.

Methods:

Prospective observational cohort study, set in primary care, the Netherlands. Patients with type 2 diabetes, included in the ZODIAC cohort between 1998 and 2012, aged 18 years and older, and who received metformin monotherapy at inclusion (n=29,195), and used metformin as monotherapy for at least one year before receiving dual therapy by the addition of a SU for at least one year. Primary outcome was within-drug yearly change in body weight after receiving add-on therapy with individual SUs during 5-years of follow-up. Secondary outcome was within-drug yearly change in HbA1c. Annual changes in weight and HbA1c were estimated with linear mixed models, adjusted for age, gender and diabetes duration.

Results:

A total of 2,958 patients were included. No significant weight changes were observed within and between any of the individual SUs after treatment intensification ($p = 0.24$). In addition, no significant difference in weight between the add-on therapy combinations was observed ($p = 0.26$). The average HbA1c the year before intensification was 7.2% (55 mmol/mol) and dropped below 7.0% (53 mmol/mol) the year after.

Conclusions:

In patients with type 2 diabetes treated in primary care, strict glycaemic control can be maintained with SUs used as add-on therapy to metformin, without the offset of relevant weight changes.

INTRODUCTION

Avoiding relevant increases in body weight, also specifically after starting new glucose lowering agents, is an important treatment target in type 2 diabetes (1). Weight increase in patients with type 2 diabetes is problematic because it contributes to increased insulin resistance and disease progression (1-3). In trials investigating new glucose lowering agents, bodyweight is used as a separate end-point in studies investigating new glucose lowering agents. Except for lifestyle factors, the different glucose lowering agents have been reported to have either decreasing, neutral or increasing effects on bodyweight. From the available agents, metformin is reported to have no effect on weight (4-6). Several other glucose lowering agents were reported to cause small increases in weight (7-12). The UK Prospective Diabetes Study (UKPDS) reported an increase in weight of about 4 kg in the first 3 years after initiation of glibenclamide compared to metformin, after which weight remains relatively stable (7). The A Diabetes Outcome Progression Trial (ADOPT) showed that glibenclamide monotherapy causes a 1.6kg increase in weight in the first year compared to baseline, but remains stable after that for the next 5 years (13).

The magnitude of weight changes in daily practice after starting sulphonylureas (SUs) is not entirely clear. Only a few studies investigated the effects of initiation of glucose lowering agents on weight in observational cohort studies; a meta-analysis of only 2 prospective studies reported an increase of 2.06 kg (95% CI: 1.15-2.96) for SUs as a group compared to placebo (14). A retrospective cohort study showed that monotherapy with any SU was associated with a 1.05 kg/m² (95% CI: 0.90–1.20) higher BMI 12 months after initiation compared to metformin use (15).

It is unclear whether this weight increase is a class-effect or should be attributed to specific SU drugs. As a consequence, there is a growing interest in within-class SU differences. From the SUs, especially gliclazide has a remarkable safety profile: it's use is associated with exceptionally few hypoglycaemic events (16). Furthermore, gliclazide can even be used in patients with renal impairment without dose adjustment (17, 18) and it is possibly beneficial with respect to cardiovascular outcomes compared to other SUs (19). Together, this led to the incorporation of gliclazide as the preferred SU in the Dutch 2013 diabetes guideline when treatment intensification next to metformin is required (20). Evidence for within-class differences in weight change after the start of individual SUs, could have consequences regarding the preferred agent when treatment intensification is needed. The aim of this study was to investigate within and between SU-group weight trajectories, in a prospective primary care cohort of patients with type 2 diabetes, after starting add-on therapy with individual SUs, in addition to metformin.

METHODS

The study is reported according to the STROBE (Strengthening the reporting of observational studies in epidemiology) recommendations (21).

Study design and data collection

The here presented study (part of Clinicaltrials.gov #NCT02133118) concerns an analysis of patients included in the prospective ZODIAC (Zwolle Outpatient Diabetes project Integrating Available Care) study (22). The ZODIAC study started in 1998 and included patients diagnosed with type 2 diabetes who were treated exclusively in primary care. Since then, the ZODIAC study expanded to more than 600 general practices in the north-eastern and western part of the Netherlands. In the Netherlands, all inhabitants have a general practitioner and over 80% of patients with type 2 diabetes are treated exclusively in primary care (23).

In the ZODIAC study, the following data are collected by general practitioners and sent to the diabetes centre annually: the presence of macrovascular complications, diabetes duration, medication use (diabetes medication, insulin (including type), and all other medication), bodyweight, height (only at baseline), blood pressure, HbA1c, serum creatinine, urinary albumin-creatinine ratio, and lipid profiles. All values are measured once at the yearly check-up with the general practitioner. Any potential changes within the year were not recorded. All laboratory measurements were determined using standard laboratory procedures.

Patient selection

Eligible for selection were patients with type 2 diabetes, included in the ZODIAC cohort between 1998 and 2012, aged 18 years and older, who received metformin monotherapy during participation in the ZODIAC cohort, and who used metformin as monotherapy for at least one year before receiving dual therapy by the addition of a SU in the next year (new-user design) (24). Patients were not excluded on specific patient characteristics other than age (< 18 years). Data were censored when follow-up ended or when patients switched medication (including other SUs) during follow-up; for example, monotherapy with a SU or metformin, or when they subsequently received triple oral therapy or insulin therapy, or at the end of follow-up, or death, or after five year of follow-up. For each patient the baseline year was defined as the year before treatment intensification with a SU when they were using metformin monotherapy. Patients who achieved their HbA1c targets ($\text{HbA1c} < 7\% / 53 \text{ mmol/mol}$) on metformin monotherapy were not included in the analysis as a comparator group, because this comparator group could have been subject to selection bias.

Outcome measures

Primary outcome was mean yearly change in body weight compared to weight at baseline for each add-on group separately for 5 years. Secondary outcome was mean change in HbA1c compared to baseline.

Statistical analysis

Quantitative variables were presented as means with standard deviation for normally distributed values, and median and interquartile range (IQR) for skewed variables. Pairwise comparisons with one-way ANOVA or Kruskal-Wallis tests were Bonferroni adjusted. Changes in body weight and HbA1c during follow-up were estimated using linear mixed models. The use of linear mixed models allowed us to account for missing data. Patients with missing data on weight or HbA1c were not excluded; for example, if data on weight is missing in year 2 but available at year 1 and in year 3, the patient does not participate in the analysis in year 2 but does at year 1 and year 3. The changes in weight and HbA1c were modelled with a random intercept and slope, adjusted for baseline age, gender and diabetes duration. Age, gender and diabetes duration were modelled as fixed effects with age and diabetes duration as continuous variables, while patients were modelled as random effect. The model was adjusted for age, gender and diabetes duration because we hypothesized that these factors could have influenced both body weight and the choice for a specific SU. All analyses were performed with SPSS 20.0 software (SPSS Inc, Chicago, Illinois, USA).

Ethics statement

In the ZODIAC study, patients consented to anonymous use of their data for study purposes. The medical ethics committee of Isala, Zwolle, the Netherlands approved the ZODIAC study (METC reference numbers 03.0316 and 07.0335).

Patient involvement

Patients were not involved in the design of the study, the development of outcome measures and in the recruitment of patients.

RESULTS

From all metformin monotherapy users at inclusion (n=29,195), 2,958 (10.1%) patients were included in the analysis. The number of patients at each selection step are shown in supplemental figure S1. Baseline characteristics are shown in table 1. Mean age (years) in the 4 treatment groups ranged from 62.1 to 63.5. Median weight was 91.9 kg in the gliclazide group, 92.2 kg in the glibenclamide group, 91.3 kg in the glimepiride group and 89.4 kg in the tolbutamide group.

Fig 1. (A) Estimated mean body weight (kg) during follow-up. Adjusted for age, gender and diabetes duration. (B) Estimated mean HbA1c (%) during follow-up. Adjusted for age, gender, diabetes duration. Horizontal line indicates the treatment target at the time the study was performed.

Baseline weight and HbA1c were available for 520 (99.6%) and 521 (99.8%) patients in the gliclazide add-on group, for 43 (97.7%) and 43 (97.7%) patients in the glibenclamide add-on group, for 963 (99.8%) and 964 (99.9%) patients in the glimepiride add-on group and for 1415 (99.2%) and 1427 (100.0%) patients in the tolbutamide add-on group, respectively.

Weight changes during follow-up.

The overall results of the linear mixed model analysis showed a non-significant ($p=0.24$) linear change in weight during the follow-up period and a non-significant ($p=0.26$) difference in weight between the add-on therapy combinations. In addition, the change in weight did not significantly differ between the add-on therapy combinations (p value for interaction= 0.67). The regression parameters of the fixed effects of the mixed model analysis are presented in supplemental table S1. Estimated weights for the different add-on therapy combinations at yearly time points are presented in table 2 and figure 1, panel A. After 5 years a non-significant increase in weight was observed in the gliclazide and glibenclamide groups, in the glimepiride group no change was observed.

Glucose control—HbA1c

The HbA1c results showed a non-linear trend over time. A quadratic trend was introduced in the mixed model analysis for the changes in HbA1c during follow-up. The results showed a significant overall quadratic trend ($p<0.005$) in the HbA1c value during follow-up. There was no significant ($p=0.37$) difference between the different add-on therapy combinations, and a non-significant interaction effect ($p=0.14$). In supplemental table S2 the regression parameters of the fixed effects of the mixed model analysis are presented. Estimated HbA1c for the different add-on therapy combinations at different time periods are presented in table 3 and figure 1, panel B. In the gliclazide, glimepiride and tolbutamide add-on groups the average HbA1c levels steadily decreased from 7.1% (54 mmol/mol) and remained below 7.0% (53 mmol/mol) during the first four years following intensification, and rose above 7.0% (53 mmol/mol) in the fifth year.

Table 1: Demographic and clinical characteristics of patients at baseline.

	M+Glicazide (n=521)
Age Start (Years)	63.1 (11.7)
Gender (Women) (%)	221 (42.5%)
HbA1c % (mmol/mol)	7.1 [6.6 - 7.6] (54, [49 - 60]) (n=522)
Weight (kg)	91.9 [90.3, 93.4] (n=520)
BMI	30.9 (5.7)
Systolic Blood pressure (mmHg)	140 (17)
Diastolic Blood pressure (mmHg)	81(10)
Cholesterol-HDL ratio	3.7 [3.1 - 4.7]
Creatinine (μmol/L)	78 [66 - 90]
Albumin Creatinine Ratio	1.0 [0.5 - 2.9]
MVC at start	17.8%
Diabetes duration (years)	3.9 [1.8 - 5.9]

Data are mean (standard deviation), median [interquartile range] and %. MVC=Macro vascular complication.

Table 2: Estimated mean weight values (kilogram) [95% CI] corrected for age, gender and diabetes duration.

Treatment regimen	M+Glicazide	M+Glibenclamide	M+Glimepiride	M+Tolbutamide
Year 0 (Baseline)	91.8 [90.3, 93.4] (n = 520)	92.2 [86.2, 98.2] (n = 43)	91.3 [90.2, 92.4] (n = 963)	89.4 [88.5, 90.3] (n = 1415)
Year 1	91.9 [90.3, 93.5] (n = 510)	92.4 [85.7, 99.1] (n = 39)	91.3 [90.2, 92.4] (n = 953)	89.7 [88.8, 90.6] (n = 1451)
Year 2	92.2 [90.4, 93.9] (n = 373)	91.6 [84.6, 98.6] (n = 36)	90.6 [89.4, 91.9] (n = 681)	89.9 [88.9, 91.0] (n = 1009)
Year 3	92.1 [90.0, 94.1] (n = 243)	92.7 [84.2, 101.2] (n = 26)	91.0 [89.4, 92.6] (n = 434)	89.6 [88.3, 90.8] (n = 657)
Year 4	93.0 [90.2, 95.8] (n = 147)	92.4 [81.7, 103.2] (n = 16)	90.8 [88.6, 93.0] (n = 244)	89.9 [88.2, 91.7] (n = 364)
Year 5	95.7 [92.1, 99.3] (n = 94)	95.5 [84.5, 106.4] (n = 13)	91.4 [88.4, 94.4] (n = 148)	90.8 [88.3, 93.3] (n = 206)

M+Glibenclamide (n=43)	M+Glimepiride (n=964)	M+Tolbutamide (n=1427)
63.5 (12.3)	62.1 (11.3)	63.5 (11.5)
21 (50.0%)	449 (46.6%)	674 (47.2%)
7.0 [6.6 - 7.7] (53 [54 - 61]) (n=44)	7.0 [6.6 - 7.5] (53 [49 - 58]) (n=965)	7.0 [6.6 - 7.5] (53, [49 - 58]) (n=1427)
92.2 [86.2, 98.2] (n=43)	91.3 [90.2, 92.4] (n=963)	89.4 [88.5, 90.3] (n=1415)
31.4 (6.1)	30.7 (5.6)	30.2 (5.0)
140 (14)	139 (17)	138 (16)
83 (9)	82 (10)	80 (9)
3.9 [2.8 - 4.8]	3.7 [3.1 - 4.6]	3.7 [3.0 - 4.6]
70 [57 - 83]	76 [65 - 88]	76 [64 - 88]
0.8 [0.5 - 1.0]	1.0 [0.4 - 2.1]	0.8 [0.5 - 2.0]
9.1%	14.4%	16.7%
4.7 [1.3 - 6.8]	4.0 [2.0 - 6.3]	4.0 [1.9 - 6.8]

Table 3: Estimated mean HbA1c values (%) [95% CI] corrected for age, gender and diabetes duration.

Treatment regimen	M+Glicazide	M+Glibenclamide	M+Glimepiride	M+Tolbutamide
Year 0 (Baseline)	7.21 [7.18, 7.24] (n = 521)	7.19 [7.08, 7.29] (n = 43)	7.18 [7.16, 7.20] (n = 964)	7.15 [7.14, 7.17] (n = 1427)
Year 1	7.02 [6.99, 7.05] (n = 512)	7.12 [7.00, 7.24] (n = 39)	6.99 [6.97, 7.02] (n = 953)	7.02 [6.99, 7.03] (n = 1459)
Year 2	6.92 [6.88, 6.95] (n = 374)	7.02 [6.88, 7.15] (n = 36)	6.89 [6.86, 6.92] (n = 681)	6.93 [6.90, 6.95] (n = 1014)
Year 3	6.90 [6.85, 6.95] (n = 243)	7.06 [6.88, 7.23] (n = 26)	6.89 [6.85, 6.92] (n = 435)	6.91 [6.88, 6.94] (n = 659)
Year 4	6.92 [6.84, 6.99] (n = 147)	7.11 [6.85, 7.36] (n = 16)	6.98 [6.92, 7.04] (n = 245)	6.90 [6.86, 6.95] (n = 364)
Year 5	7.12 [6.99, 7.24] (n = 94)	7.31 [6.97, 7.65] (n = 13)	7.18 [7.10, 7.26] (n = 149)	7.01 [6.94, 7.08] (n = 206)

DISCUSSION

In this study, there was no evidence for a significant weight change within and between the four SU add-on groups in the years after starting a SU. When used as add-on therapy to metformin, increases in weight with comparable glycaemic efficacy have been reported in randomized studies (8, 10, 14, 25-27) investigating glibenclamide (7, 26, 28), gliclazide (29, 30), glimepiride (9), and glipizide (31). Most studies showed only modest increases in bodyweight (ranging from 0.5 to 3 kg) and most had a maximum follow-up time of 12 months. One study with 10 years of follow-up, showed that weight was gained only in the first 3 years after initiation of a SU (26).

Our results are in contrast with results from RCT's. The differences in weight change between this study and previous RCT's could partly have resulted from differences in design and baseline factors. Compared to RCT's, the patients included in this study differed with respect to glycaemic control; the average baseline HbA1c level in most RCT's (ranging from 7.3% (56 mmol/mol) to 8.5% (69 mmol/mol)) was higher than the HbA1c 7.2% (55 mmol/mol) before treatment intensification in this cohort (9, 26-32). In addition, patients not only differed with respect to glycaemic control but probably also according to intensity of patient visits and patient counselling regarding life-style; factors possibly responsible for the lower average glycaemic control.

These results indicate that it is possible to maintain strict glycaemic control in the majority of patients in a real life setting without relevant weight increases when starting an SU. Effects of SUs on weight appear to be different from effects found in observational studies. In these studies, significant or relevant weight increases have only been reported in studies with unfavourable glycaemic control at baseline and only when SUs were analysed as one group (32, 33). However, based on the confidence intervals the possibility of a small weight increase could not be excluded in our study. In contrast with previous observational studies, the glycaemic control of patients included in the ZODIAC cohort was excellent (34). Moreover, when interpreting weight changes in observational cohort studies, there are several other possible explanations for weight increase besides medication effects. In the general population aged between 55 and 65 an increase of several ounces per year is a phenomenon that is observed in several population studies performed in middle- and high-income countries and therefore is not necessarily related to diabetes medication (35, 36).

In the Netherlands a form of protocol-based care is deployed, which probably could be an explanation for the on average small and seemingly non-relevant decrease in HbA1c. At the time the study was performed, patients treated in the Netherlands are checked

at least once a year by their general practitioner and 3 times a year by practise nurses. During these visits there is a strong focus on life-style advices as well as glycaemic control (20). When patients are above their HbA1c target treatment is immediately intensified, even when patients are slightly above their target. In the RCTs there were no structured life-style advices described in their methods section. Non-adherence to life-style advices has been associated with worsening glycaemic control and weight gain (37-39), with subsequent increases in HbA1c leading to treatment intensification (40).

This study sample has a high degree of generalizability, and represents the majority of primary care treated patients in the Netherlands. Large regions of the Netherlands are participating in projects similar to this study project, and all patients with type 2 diabetes have the opportunity for opting out. In the Netherlands all patients have a primary care physician and from all patients with type 2 diabetes, more than 80% of patients with (T2) DM are treated exclusively in primary care(23). In contrast to the observational design in the current study, RCTs often have strict selection criteria, limiting the number of patients eligible for inclusion, possibly leading to sample selection bias and therefor complicating the generalizability to daily practise (41).

Strengths of this study were the number of patients, the daily care setting and the new-user design; thereby avoiding two potential types of bias; under ascertainment of weight changes that occur early after the start of add-on therapy and the inability to control for disease risk factors that may be altered by the study drugs themselves (24). Furthermore, over 97% of all observations on weight and 99% of all observations on HbA1c from any study visit was complete at baseline and during follow-up. The outcome measures weight and HbA1c were missing in a small percentage but this posed no problem when using linear mixed models, because all missing values are caused by random omissions in the registration and are consequently missing completely at random (MCAR). The decrease in number of patients can mostly be explained by the study design. Patients entered the ZODIAC cohort at different time points and also entered into the study at different time points. A patient who, for example, entered the study in 1998 can have the maximum follow-up of 6 years. However, a patient who entered in 2009 can only have 3 years of follow-up since the study cohort is censored at 31 December 2012. In addition, when a patient was included in the ZODIAC cohort the maximum length of the follow-up was also shortened when this patient has for example three years of metformin monotherapy before receiving an addon, because in the current study follow-up starts in the year before receiving the addon. Table S5 shows the number of people censored because of changes in medication during follow-up.

This study also had important limitations. Although there were no significant within-class differences in weight change after starting SUs in the first 5 years after treatment intensification, the number of patients in the gliclazide and glibenclamide group were small and a potentially relevant increase in weight could not be excluded. Furthermore, the decrease in number of patients in the tolbutamide and glibenclamide groups was relatively high compared to the other groups.

Secondly, baseline macrovascular complications (MVC) differed between the SU groups. Although the Dutch diabetes guideline did not base the choice of an oral glucose lowering agent on the presence of MVC, this theoretically could have influenced the choice of SU. However, the post-hoc sensitivity analysis (see table S3 and table S4) showed that the addition of MVC does not relevantly change the results. Third, the quality and reliability of our data were dependent on the accuracy of the data provided by practice nurses and GP's as part of a yearly benchmark. Information on doses and adherence to medication were not recorded within the ZODIAC cohort. Since data on medication adherence was not available, patients could have stopped medications during the following year which possibly could have resulted in misclassification. In addition, since patients may have been prescribed a SU at different time points during the year, the time between the first prescription of a SU and the measurement of bodyweight varied and was not always one year. However, there was no reason to assume that adherence and time of initiation within the year would substantially differ between groups. Furthermore, it is possible that patients who started to gain weight while using an SU, wanted to try a different drug. However, we have no evidence that this is the case (see supplementary table S6). The rapid decrease in number of patients after year 2 could complicate the interpretation of the results. However, in our study the confidence intervals remain narrow. A common side-effect in SUs as a group are severe hypoglycaemic events, which are not recorded in our database. However, the hazard of severe hypoglycaemia is very limited in gliclazide users (16), the most prescribed SU in the Netherlands. Furthermore, since no significant differences in weight were found, a dose response analysis was not performed.

CONCLUSION

There was no evidence for relevant within-class SU differences in weight during follow-up. The results of this study support the view that strict glycaemic control can be maintained in a substantial part of the primary care patients in the Netherlands with type 2 diabetes without clinically relevant weight change when adding SUs to metformin. In real life, when weight gain does occur, other factors than medication effects, for example life-style associated factors, are likely to be more important factors.

REFERENCES

1. Eng C, Kramer CK, Zinman B, Retnakaran R. Glucagon-like peptide-1 receptor agonist and basal insulin combination treatment for the management of type 2 diabetes: a systematic review and meta-analysis. *Lancet*. 2014.
2. Wiecek A, Rys P, Skrzekowska-Baran I, Malecki M. The role of surrogate endpoints in the evaluation of efficacy and safety of therapeutic interventions in diabetes mellitus. *The review of diabetic studies : RDS*. 2008;5(3):128-35.
3. Russell-Jones D, Khan R. Insulin-associated weight gain in diabetes--causes, effects and coping strategies. *Diabetes, obesity & metabolism*. 2007;9(6):799-812.
4. Mitri J, Hamdy O. Diabetes medications and body weight. *Expert opinion on drug safety*. 2009;8(5):573-84.
5. DeFronzo RA, Goodman AM. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. *The New England journal of medicine*. 1995;333(9):541-9.
6. United Kingdom Prospective Diabetes Study 24: a 6-year, randomized, controlled trial comparing sulfonylurea, insulin, and metformin therapy in patients with newly diagnosed type 2 diabetes that could not be controlled with diet therapy. United Kingdom Prospective Diabetes Study Group. *Annals of internal medicine*. 1998;128(3):165-75.
7. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet*. 1998;352(9131):854-65.
8. Arechavaleta R, Seck T, Chen Y, Krobot KJ, O'Neill EA, Duran L, et al. Efficacy and safety of treatment with sitagliptin or glimepiride in patients with type 2 diabetes inadequately controlled on metformin monotherapy: a randomized, double-blind, non-inferiority trial. *Diabetes, obesity & metabolism*. 2011;13(2):160-8.
9. Ferrannini E, Fonseca V, Zinman B, Matthews D, Ahren B, Byiers S, et al. Fifty-two-week efficacy and safety of vildagliptin vs. glimepiride in patients with type 2 diabetes mellitus inadequately controlled on metformin monotherapy. *Diabetes, obesity & metabolism*. 2009;11(2):157-66.
10. Hermann LS, Schersten B, Bitzen PO, Kjellstrom T, Lindgarde F, Melander A. Therapeutic comparison of metformin and sulfonylurea, alone and in various combinations. A double-blind controlled study. *Diabetes care*. 1994;17(10):1100-9.
11. Kaku K. Efficacy and safety of therapy with metformin plus pioglitazone in the treatment of patients with type 2 diabetes: a double-blind, placebo-controlled, clinical trial. *Current medical research and opinion*. 2009;25(5):1111-9.
12. Kooy A, de Jager J, Leher P, Bets D, Wulffele MG, Donker AJ, et al. Long-term effects of metformin on metabolism and microvascular and macrovascular disease in patients with type 2 diabetes mellitus. *Archives of internal medicine*. 2009;169(6):616-25.
13. Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, et al. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *The New England journal of medicine*. 2006;355(23):2427-43.
14. Phung OJ, Scholle JM, Talwar M, Coleman CI. Effect of noninsulin antidiabetic drugs added to metformin therapy on glycemic control, weight gain, and hypoglycemia in type 2 diabetes. *Jama*. 2010;303(14):1410-8.
15. Huizinga MM, Roumie CL, Greevy RA, Liu X, Murff HJ, Hung AM, et al. Glycemic and weight changes after persistent use of incident oral diabetes therapy: a Veterans Administration retrospective cohort study. *Pharmacoepidemiology and drug safety*. 2010;19(11):1108-12.
16. Landman GW, de Bock GH, van Hateren KJ, van Dijk PR, Groenier KH, Gans RO, et al. Safety and efficacy of gliclazide as treatment for type 2 diabetes: a systematic review and meta-analysis of randomized trials. *PloS one*. 2014;9(2):e82880.

17. Arnouts P, Bolignano D, Nistor I, Bilo H, Gnudi L, Heaf J, et al. Glucose-lowering drugs in patients with chronic kidney disease: a narrative review on pharmacokinetic properties. *Nephrology, dialysis, transplantation* : official publication of the European Dialysis and Transplant Association - European Renal Association. 2014;29(7):1284-300.
18. National Kidney Foundation. KDOQI Clinical Practice Guideline for Diabetes and CKD: 2012 Update. *American journal of kidney diseases* : the official journal of the National Kidney Foundation. 2012;60(5):850-86.
19. Schramm TK, Gislason GH, Vaag A, Rasmussen JN, Folke F, Hansen ML, et al. Mortality and cardiovascular risk associated with different insulin secretagogues compared with metformin in type 2 diabetes, with or without a previous myocardial infarction: a nationwide study. *European heart journal*. 2011;32(15):1900-8.
20. Rutten GEHM DGW, Nijpels G, Houweling ST, Van de Laar FA, Bilo HJ, Holleman F, Burgers JS, Wiersma TI, Janssen PGH. NHG-Standaard Diabetes mellitus type 2 (derde herziening). *Huisarts Wet*. 2013;56(10):512-25.
21. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Journal of clinical epidemiology*. 2008;61(4):344-9.
22. Ubink-Veltmaat LJ, Bilo HJ, Groenier KH, Houweling ST, Rischen RO, Meyboom-de Jong B. Prevalence, incidence and mortality of type 2 diabetes mellitus revisited: a prospective population-based study in The Netherlands (ZODIAC-1). *European journal of epidemiology*. 2003;18(8):793-800.
23. Edgar P, Sprangers N, van der Galiën O. Integrale bekostiging diabetes duur. *Medisch Contact*. 2012(16):991-2.
24. Ray WA. Evaluating medication effects outside of clinical trials: new-user designs. *American journal of epidemiology*. 2003;158(9):915-20.
25. Belsey J, Krishnarajah G. Glycaemic control and adverse events in patients with type 2 diabetes treated with metformin + sulphonylurea: a meta-analysis. *Diabetes, obesity & metabolism*. 2008;10 Suppl 1:1-7.
26. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet*. 1998;352(9131):837-53.
27. Ristic S, Collober-Maugeais C, Pecher E, Cressier F. Comparison of nateglinide and gliclazide in combination with metformin, for treatment of patients with Type 2 diabetes mellitus inadequately controlled on maximum doses of metformin alone. *Diabetic medicine : a journal of the British Diabetic Association*. 2006;23(7):757-62.
28. Marre M, Howlett H, Leheret P, Allavoine T. Improved glycaemic control with metformin-glibenclamide combined tablet therapy (Glucovance) in Type 2 diabetic patients inadequately controlled on metformin. *Diabetic medicine : a journal of the British Diabetic Association*. 2002;19(8):673-80.
29. Charbonnel B, Schernthaner G, Brunetti P, Matthews DR, Urquhart R, Tan MH, et al. Long-term efficacy and tolerability of add-on pioglitazone therapy to failing monotherapy compared with addition of gliclazide or metformin in patients with type 2 diabetes. *Diabetologia*. 2005;48(6):1093-104.
30. Filozof C, Gautier JF. A comparison of efficacy and safety of vildagliptin and gliclazide in combination with metformin in patients with Type 2 diabetes inadequately controlled with metformin alone: a 52-week, randomized study. *Diabetic medicine : a journal of the British Diabetic Association*. 2010;27(3):318-26.
31. Goke B, Gallwitz B, Eriksson J, Hellqvist A, Gause-Nilsson I. Saxagliptin is non-inferior to glipizide in patients with type 2 diabetes mellitus inadequately controlled on metformin alone: a 52-week randomised controlled trial. *International journal of clinical practice*. 2010;64(12):1619-31.
32. Morgan CL, Jenkins-Jones S, Evans M, Barnett AH, Poole CD, Currie CJ. Weight change in people with type 2 diabetes: secular trends and the impact of alternative antihyperglycaemic drugs. *Diabetes, obesity & metabolism*. 2012;14(5):424-32.

33. Kostev K, Rex J, Rockel T, Heilmaier C. Effects of selected antidiabetics on weight loss--a retrospective database analysis. *Primary care diabetes*. 2015;9(1):74-7.
34. van Hateren KJ, Drion I, Kleefstra N, Groenier KH, Houweling ST, van der Meer K, et al. A prospective observational study of quality of diabetes care in a shared care setting: trends and age differences (ZODIAC-19). *BMJ open*. 2012;2(4).
35. Dahl AK, Reynolds CA, Fall T, Magnusson PK, Pedersen NL. Multifactorial analysis of changes in body mass index across the adult life course: a study with 65 years of follow-up. *International journal of obesity* (2005). 2014;38(8):1133-41.
36. Wills AK, Hardy RJ, Black S, Kuh DJ. Trajectories of overweight and body mass index in adulthood and blood pressure at age 53: the 1946 British birth cohort study. *Journal of hypertension*. 2010;28(4):679-86.
37. Chiu CJ, Wray LA. Factors predicting glycemic control in middle-aged and older adults with type 2 diabetes. *Preventing chronic disease*. 2010;7(1):A08.
38. Khattab M, Khader YS, Al-Khawaldeh A, Ajlouni K. Factors associated with poor glycemic control among patients with type 2 diabetes. *Journal of diabetes and its complications*. 2010;24(2):84-9.
39. Bjorntorp P. Biochemistry of obesity in relation to diabetes. *International Textbook of Diabetes Mellitus*. Chichester: Wiley; 1992. p. 551-68.
40. Stanford J, Kaiser M, Ablah E, Dong F, Paull-Forney B, Early J. The effect of weight loss on fasting blood sugars and hemoglobin A1c in overweight and obese diabetics and non diabetics. *Journal of Diabetes Mellitus*. 2012(1):126-30.
41. Saunders C, Byrne CD, Guthrie B, Lindsay RS, McKnight JA, Philip S, et al. External validity of randomized controlled trials of glycaemic control and vascular disease: how representative are participants? *Diabetic medicine : a journal of the British Diabetic Association*. 2013;30(3):300-8.

SUPPLEMENTAL DATA

Fig S1: Selection of patients. *The number of patients during follow-up differ due to missing data.

Table S1: Parameter estimates of the fixed effects for the mixed model analysis of the change in weight.

Parameter	Estimate	p-value	95% Confidence Interval	
			Lower Bound	Upper Bound
Intercept	96.52	<0.001	95.36	97.67
Female	-9.43	<0.001	-10.53	-8.33
Male	0 ^b	.	.	.
Age	-0.49	<0.001	-0.54	-0.44
Diabetes duration	-0.13	0.095	-0.28	0.02
Follow-up year	0.00	0.920	-0.11	0.12
[Metformine + gliclazide]	1.43	0.074	-0.14	2.99
[Metformine + glibenclamide]	2.14	0.364	-2.49	6.77
[Metformine + glimepiride]	.74	0.259	-0.55	2.03
[Metformine + tolbutamide]	0 ^b	.	.	.
[Metformine + gliclazide] * Follow-up year	-0.00	0.946	-0.23	0.21
[Metformine + glibenclamide] * Follow-up year	-0.39	0.215	-1.01	0.23
[Metformine + glimepiride] * Follow-up year	-0.03	0.785	-0.21	0.16
[Metformine + tolbutamide] * Follow-up year	0 ^b	.	.	.

b. This parameter is the reference category

Table S2: Parameter estimates of the fixed effects for the mixed model analysis of the change in HbA1c.

Parameter	Estimate	p-value	95% Confidence Interval	
			Lower Bound	Upper Bound
Intercept	7.45	<0.001	7.35	7.54
Female	-0.02	0.337	-0.07	0.02
Male	0 ^b	.	.	.
Age	-0.01	<0.001	-0.01	-0.01
Diabetes duration	-0.01	0.010	-0.02	-0.00
Follow-up year	-0.24	<0.001	-0.30	-0.17
Follow-up year * Follow-up year	0.03	<0.001	0.02	0.04
[Metformin + gliclazide]	0.12	0.157	-0.05	0.29
[Metformin + glibenclamide]	-0.00	0.999	-0.49	0.49
[Metformin + glimepiride]	0.10	0.147	-0.04	0.24
[Metformin + tolbutamide]	0 ^b	.	.	.
[Metformin + gliclazide] * Follow-up year	-0.09	0.173	-0.21	0.04
[Metformin + glibenclamide] * Follow-up year	0.02	0.914	-0.33	0.36
[Metformin + glimepiride] * Follow-up year	-0.11	0.032	-0.21	-0.01
[Metformin + tolbutamide] * Follow-up year	0 ^b	.	.	.
[Metformin + gliclazide] * Follow-up year * Follow-up year	0.01	0.269	-0.01	0.03
[Metformin + glibenclamide] * Follow-up year * Follow-up year	0.01	0.840	-0.05	0.06
[Metformin + glimepiride] * Follow-up year * Follow-up year	0.02	0.021	0.00	0.04
[Metformin + tolbutamide] * Follow-up year * Follow-up year	0 ^b	.	.	.

b. This parameter is the reference category

Table S3: Parameter estimates of the fixed effects for the sensitivity analysis of the change in weight.

Parameter	Estimate	p-value	95% Confidence Interval	
			Lower Bound	Upper Bound
Intercept	96.05	<0.001	94.29	97.81
Female	-9.48	<0.001	-10.59	-8.38
Male	0 ^b	.	.	.
Age	-0.48	<0.001	-0.53	-0.44
Diabetes duration	-0.12	0.108	-0.27	0.03
Macrovascular complications (No)	0.52	0.524	-1.08	2.13
Macrovascular complications (Yes)	0 ^b	.	.	.
Follow-up year	0.00	0.947	-0.11	0.12
[Metformin + Gliclazide]	1.40	0.080	-0.17	2.97
[Metformin + Glibenclamide]	2.11	0.371	-2.56	6.74
[Metformin + Glimepiride]	0.78	0.238	-0.51	2.06
[Metformin + Tolbutamide]	0 ^b	.	.	.
[Metformin + Gliclazide] * Follow-up year	-0.01	0.914	-0.23	0.21
[Metformin + Glibenclamide] * Follow-up year	-0.39	0.216	-1.00	0.23
[Metformin + Glimepiride] * Follow-up year	-0.03	0.728	-0.21	0.15
[Metformin + Tolbutamide] * Follow-up year	0 ^b	.	.	.

b. This parameter is the reference category

Table S4: Parameter estimates of the fixed effects for the sensitivity analysis of the change in HbA1c.

Parameter	Estimate	Sig.	95% Confidence Interval	
			Lower Bound	Upper Bound
Intercept	7.42	<0.001	7.30	7.53
Female	-0.03	0.293	-0.08	0.02
Male	0 ^b	.	.	.
Age	-0.01	<0.001	-0.01	-0.01
Diabetes Duration	-0.01	0.011	-0.02	-0.00
Macrovascular complications (No)	0.03	0.385	-0.04	0.10
Macrovascular complications (Yes)	0 ^b	.	.	.
Follow-up year	-0.23	<0.001	-0.30	-0.17
Follow-up year * Follow-up year	0.03	<0.001	0.02	0.04
[Metformin + Gliclazide]	0.13	0.138	-0.04	0.30
[Metformin + Glibenclamide]	0.00	0.993	-0.49	0.50
[Metformin + Glimepiride]	0.11	0.134	-0.03	0.25
[Metformin + Tolbutamide]	0 ^b	.	.	.
[Metformin + Gliclazide] * Follow-up year	-0.09	0.150	-0.22	0.03
[Metformin + Glibenclamide] * Follow-up year	0.01	0.936	-0.33	0.36
[Metformin + Glimepiride] * Follow-up year	-0.12	0.027	-0.21	-0.01
[Metformin + Tolbutamide] * Follow-up year	0 ^b	.	.	.
[Metformin + Gliclazide] * Follow-up year * Follow-up year	0.01	0.233	-0.01	0.03
[Metformin + Glibenclamide] * Follow-up year * Follow-up year	0.01	0.816	-0.05	0.06
[Metformin + Glimepiride] * Follow-up year * Follow-up year	0.02	0.017	0.00	0.04
[Metformin + Tolbutamide] * Follow-up year * Follow-up year	0 ^b	.	.	.

b. This parameter is the reference category

Table S5. Reasons for censoring

	Number of patients
Deceased	77
Switch to metformin monotherapy	191
Switch to other monotherapy	79
Switch to metformin + insulin	60
Switch to insulin	6
Other medication	429

Table S6. Weight and HbA1c in year prior to censoring¹. Continued on next page.

Addon	Reason for censoring	Weight	HbA1c
Gliclazide	metformin monotherapy	87.11 (18.45)	6.53 (0.72)
		81.04 – 93.17	6.29 – 6.77
		N = 38	N = 37
	other monotherapy	91.25 (20.26)	6.87 (1.06)
		74.31 – 108.19	5.60 – 7.75
		N = 8	N = 8
	metformin + insulin	100.2 (22.53)	8.62 (0.83)
		72.22 – 128.18	7.59 – 9.65
		N = 5	N = 5
	insulin	74	6.7
		N = 1	N = 1
	other medication	95.24 (20.41)	6.93 (1.21)
		90.67 – 99.81	6.66 – 7.20
		N = 79	N = 80
Glibenclamide	metformin monotherapy	73.60 (11.30)	6.82 (1.96)
		59.56 – 87.64	4.39 – 9.25
		N = 5	N = 5
	other monotherapy	78.00 (4.24)	6.90 (0.28)
		39.88 – 116.12	4.36 – 9.44
		N = 2	N = 2
	metformin + insulin	96.80 (26.68)	8.28 (0.29)
		63.68 – 129.92	7.91 – 8.65
		N = 5	N = 5
	insulin	67	7.4
		N = 1	N = 1
	other medication	97.91 (12.20)	7.76 (1.21)
		89.71 – 106.11	6.95 – 8.58
		N = 11	N = 11

Table S6. Continued

Addon	Reason for censoring	Weight	HbA1c
Glimepiride	metformin monotherapy	87.98 (16.43)	6.63 (0.82)
		83.85 – 92.12 N = 63	6.43 – 6.84 N = 63
	other monotherapy	89.55 (21.04)	6.68 (0.78)
		80.22 – 98.88 N = 22	6.33 – 7.02 N = 22
	metformin + insulin	97.76 (20.44)	7.35 (0.72)
		87.25 – 108.28 N = 17	6.97 – 7.73 N = 16
	insulin	82 N = 1	11 N = 1
	other medication	96.18 (20.17) 93.03 – 99.32 N = 160	7.24 (1.24) 7.04 – 7.43 N = 154
Tolbutamide	metformin monotherapy	89.56 (16.35)	6.67 (0.75)
		85.92 – 93.20 N = 80	6.50 – 6.83 N = 83
	other monotherapy	86.87 (15.17)	6.55 (0.70)
		82.31 – 91.42 N = 45	6.34 – 6.75 N = 46
	metformin + insulin	95.73 (12.43)	7.79 (1.04)
		91.09 – 100.38 N = 30	7.39 – 8.20 N = 28
	insulin	91.00 (9.64) 67.04 – 114.96 N = 3	7.45 (0.21) 5.54 – 9.36 N = 2
	other medication	90.98 (19.94) 87.87 – 94.10 N = 160	7.24 (1.02) 7.08 – 7.40 N = 164

1) Mean (SD), 95% CI, N of patients.

5

The association between body mass index and obesity-related cancer risk in men and women with type 2 diabetes in primary care in the Netherlands: a cohort study (ZODIAC-56)

Published as:

Hendriks SH, Schrijnders D, van Hateren KJ, et al Association between body mass index and obesity-related cancer risk in men and women with type 2 diabetes in primary care in the Netherlands: a cohort study (ZODIAC-56) *BMJ Open* 2018;8:e018859. doi: 10.1136/bmjopen-2017-018859

ABSTRACT

Objective:

to investigate the relationship between BMI and obesity-related cancers in men and women with type 2 diabetes.

Design:

observational cohort study.

Setting:

Primary care.

Participants:

A total of 52,044 patients with type 2 diabetes who participated in the ZODIAC study between 1998 and 2012 was included (49% women). A dataset of these patients was linked to available information of the Netherlands Cancer Registry (NKR) to obtain data on cancer incidents.

Primary outcome measures:

Analyses were performed for the total group of obesity-related cancers and for non sex-specific and sex-specific obesity-related cancers (in men: advanced prostate cancer, in women: ovarian, endometrial and postmenopausal breast cancer).

Results:

The median follow-up period in all analyses was 3.1 (1.7 – 5.0) years in men and 3.1 (1.7 – 5.1) in women. During follow-up, 689 men and 914 women were diagnosed with an obesity-related cancer. In men, BMI was associated with a higher risk of the total group of obesity-related cancers and non sex-specific obesity-related cancers (HR (per 5 kg/m² increase) 1.12 (95%CI 1.02–1.23) and HR 1.18 (95%CI 1.06–1.31)). No association was found with prostate cancer. In women, an association between BMI and all obesity-related cancers combined and sex-specific obesity-related cancers was present (HR 1.15 (95%CI 1.08–1.22) and HR 1.22 (95%CI 1.14–1.32)). No association with non sex-specific cancers was found in women.

Conclusions:

BMI is associated with obesity-related cancers in men with type 2 diabetes, except with advanced prostate cancer. The results of this study provide reason to reconsider the classification of advanced prostate cancer as an obesity-related cancer, at least in type 2 diabetes. In women, BMI is associated with the total group of obesity-related cancers and with sex-specific obesity-related cancers.

INTRODUCTION

Type 2 diabetes is associated with an increased risk for cardiovascular morbidity and mortality (1,2). Furthermore, type 2 diabetes is also related to a higher cancer risk (3,4). This increased cancer risk appears to be site-specific; a higher risk for liver, pancreas, endometrial, colorectal, breast and bladder cancer has been reported in patients with type 2 diabetes (3). Whether this increased risk is directly related to type 2 diabetes, caused by longer periods of hyperglycaemia and/or elevated insulin levels, or indirectly, due to common risk factors like obesity, is not clear (3).

According to the World Cancer Research Fund, being overweight or obese is related to an increased incidence of oesophageal, stomach (cardia), colorectal, liver, gallbladder, pancreatic and kidney cancer in both sexes in the general population. Furthermore, being overweight or obese is related to an increased incidence of ovarian, endometrial and postmenopausal breast cancer in women and to advanced prostate cancer in men (5).

Whether there is an association between body mass index (BMI) and excess risk of developing obesity-related cancers in patients with type 2 diabetes is unclear. One previous study did not find an association between BMI and obesity-related cancers in type 2 diabetes (6). Another study investigated this association with colon cancer and found only a significant relationship in patients who suffered from obesity for a total duration of 4 years or more (7).

Even more unclear is whether there is a sex difference in the association between BMI and obesity-related cancers in patients with type 2 diabetes. A large prospective study from Sweden reported an association between being overweight and obesity with all cancers and gastrointestinal cancers in men. However, in women only an association with obesity and both cancer groups were found (8). This could indicate that the threshold for BMI to act as a risk factor for obesity-related cancer is lower in men compared to women with type 2 diabetes. Therefore, the aim of the present study was to investigate the relationship between BMI and obesity-related cancers in men and women with type 2 diabetes and specifically non sex-specific and sex-specific obesity-related cancers.

MATERIALS AND METHODS

Study group

The study population consisted of patients who were included in the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) study. This project started in 1998 and was part of a study at that time that primarily investigated the effects of shared care in patients with type 2 diabetes treated in primary care in the Zwolle region of the Netherlands (9). This shared care initiative became the standard care for the Zwolle region in 2002 and expanded to other regions in the Netherlands in the years thereafter. In these regions, general practitioners (GPs) provide data on an annual basis to the Diabetes Centre for benchmark and research purposes. Only type 2 diabetes patients treated in primary care are included in the ZODIAC project. The diagnosis of type 2 diabetes was based on the guidelines of the Dutch College of General Practitioners (twice a fasting plasma glucose level ≥ 7 mmol/l or once a non-fasting plasma glucose level ≥ 11.1 mmol/l accompanied by symptoms of hyperglycemia). Patients with a very short life expectancy or insufficient cognitive capabilities are excluded from participation. At the start in 1998, 53 GPs participated in this project, and this number increased to more than 700 GPs in 2012.

For the current study, available information on clinical variables from patients participating for at least one year in the ZODIAC project between 1998 and 2012 was linked to available information of the Netherlands Cancer Registry (NKR) to obtain data on cancer incidents in the years between 1989 and 2012. Information from the NKR in the period before 1998 was used to exclude patients who were diagnosed with an obesity-related cancer before entering the ZODIAC study. The NKR started in 1989 and records all malignancies based on notification by the National Pathology Archive (PALGA) and hospital discharge registries in the Netherlands. Basal cell carcinoma of the skin, carcinoma in situ of the cervix, myelodysplastic syndrome, myeloproliferative disorders are not registered in the NCR database. Data on patient characteristics, tumor type and grade, and treatment are collected by specially trained data-managers directly from the patients' files in all hospitals in the Netherlands. The linking was performed by a trusted third party using postal code, full name, date of birth and sex. The NKR expects that the number of false-positive and the number false-negative for the ZODIAC-NKR linkage is both under 1%. The combined ZODIAC-NKR database contains 71.634 patients, of which 10717 (15%) were diagnosed with cancer.

Data collection

For the current study a database was constructed which consisted of both the variables of the ZODIAC project and the variables of the NKR. The demographic and clinical data which were used in the present study were collected as part of ZODIAC project and included: sex, age, diabetes duration, HbA1c, serum creatinine, BMI, smoking status and the use of metformin, sulfonylurea derivatives and insulin. Information on smoking was collected during the check-up of the patient by the general practitioner or practice nurse as part of the ZODIAC project whereby smoking was documented as yes or no. No data concerning previous smoking was available. Baseline was defined as the first year that a patient was included in the ZODIAC project. Cancer characteristics provided and collected by the NKR were: cancer origin, incidence date, TNM stage, morphology and type of therapy.

Study procedure

The association between BMI and obesity-related cancer was investigated for three groups; total group of obesity-related cancers, non sex-specific obesity-related cancers and sex-specific obesity-related cancers. The non sex-specific cancer group consisted of gastric-cardia (ICD C16.0), colorectal (ICD C18,19,20), liver (ICD C22), gall bladder (ICD C23), pancreas (ICD C25) and kidney cancer (ICD C64) and adenocarcinoma of the oesophagus (ICD C15.5 + morphological codes 8140 or 8560). In men, the sex-specific cancer group was formed by advanced prostate cancers only (ICD C61 + TNM 3 or 4 or GLEASON > 7) (further referred to as prostate cancer). In women, the sex-specific cancer group consisted of ovarian (ICD C56), endometrial (ICD C54.1) and postmenopausal breast cancer (ICD C50) (further referred to as ovarian, endometrial and breast cancer). The total group of obesity-related cancers consisted of non sex-specific cancers and prostate cancer in men and of non sex-specific cancers and ovarian, endometrial and breast cancer in women. Post-menopausal breast cancer was in the present study defined as breast cancer in women ≥ 55 years of age (10). All included cancer cases were histologically confirmed by a pathologist.

Patients with a BMI < 18.5 kg/m² were excluded in all analyses, as undiagnosed cancer may lead to becoming underweight ($n = 130$ (0.2%)). Furthermore, patients were excluded if they had only baseline data recorded (only one check-up by their care provider) ($n = 18.643$ (26.0%)). For the analyses of the total group of obesity-related cancers, patients diagnosed with a non sex-specific or sex-specific obesity-related cancer before entering the ZODIAC study were excluded ($n = 2061$ (2.9%)). For the non sex-specific analysis, only patients with a non sex-specific cancer before entering the ZODIAC study were excluded ($n = 817$ (1.1%)). For the sex-specific cancer analysis, only patients with a sex-specific cancer before entering the ZODIAC study were excluded ($n = 1278$ (1.8%)) (Fig. 1).

Fig. 1. Flowchart of inclusion. * For the analyses of the total group of obesity-related cancers, patients diagnosed with a non sex-specific or sex-specific obesity-related cancer before entering the ZODIAC study were excluded. For the non sex-specific analysis, only patients with a non sex-specific cancer before entering the ZODIAC study were excluded. For the sex-specific cancer analysis, only patients with a sex-specific cancer before entering the ZODIAC study were excluded.

Clinical endpoint in all analyses was the first obesity-related cancer after baseline. Patients were not censored if they were diagnosed with a non-obesity-related cancer during follow-up. The end of follow up for patients who were not diagnosed with cancer was based on the last check up by their general practitioner.

Statistical analyses

Statistical analyses were performed using SPSS version 23 for Windows (SPSS Inc., Chicago, Illinois, USA) and Stata version 14.0 for Windows (StataCorp, College Station, Texas, USA). Multiple imputation analysis was performed for missing data on the independent variables, assuming that data was missing at random (MAR) or completely at random (MCAR). All variables of model 2 were included in the imputation model and the fully conditional specification model in SPSS was used to predict the missing data. Ten imputed datasets were created and the pooled results are presented. Baseline data are expressed as mean with standard deviation (SD) or median with interquartile range (IQR) for normally distributed and non-normally distributed data, respectively. Categorical variables are described in numbers and percentages. Normal distribution was

assessed on the basis of histograms and QQ-plots. A two-sided $p < 0.05$ was considered significant. Cox proportional hazard analyses were used to investigate the association between BMI as a continuous and categorical variable and obesity-related cancers in men and women with type 2 diabetes, separately. Hazard ratios refer to a BMI increase of 5 m²/kg in the analyses for BMI as a continuous variable. For the analyses of BMI as a categorical variable, four BMI categories were used ($\geq 18.5 - < 25.0$ kg/m², $\geq 25.0 - < 30.0$ kg/m², $\geq 30.0 - < 35.0$ kg/m², ≥ 35.0 kg/m²). The lowest category was used as reference category. Age was used as the time-scale in all analyses, because the risk of cancer is not proportional with age. Patients entered the analysis at their baseline age and exited at their event/censoring age. Two models were used: an age-adjusted model (model 1) and a model additionally adjusted for diabetes duration, HbA1c, serum creatinine, smoking status and the use of metformin, SU-derivatives and/or insulin (model 2). Furthermore, the analyses for model 2 were stratified according to the year of inclusion in the ZODIAC cohort. The assumption of proportional hazards for baseline predictors was investigated by visual inspection of the Schoenfeld residuals.

RESULTS

Total group of obesity-related cancers

Baseline results for the men and women who are included in the analyses for the total group obesity-related cancers are described in table 1. In these analyses, 25.811 men and 24.989 women were included. Mean age was 64.0 (SD 11.2) years in men and 66.6 (12.1) years in women. The median diabetes duration was higher in women. More men than women were current smokers and men had a lower BMI than women. A higher percentage of men used metformin whereas insulin use was more frequent in women. 30.4% of the patients did not use metformin, SU-derivates or insulin. These patients were on dietary interventions or other diabetes medication. The median follow-up period for these patients was 3.1 (1.7 – 5.0) years in men and 3.1 (1.7 – 5.1) in women. During follow-up, 689 (2.7%) men and 914 women (3.7%) were diagnosed with an obesity-related cancer.

Table 1. Baseline characteristics of men and women.

Variable	Men	Women	P-value*
N	25811 (49.2)	24989 (50.8)	
Age (years)	64.0 (± 11.2)	66.6 (± 12.1)	< 0.001
Diabetes duration (years)	2.5 (0.7 – 5.8)	2.8 (0.8 – 6.3)	< 0.001
HbA1c (mmol/mol)	49 (43 – 55)	49 (43 – 54)	0.091
Creatinine ($\mu\text{mol/L}$)	83 (73 – 95)	68 (59 – 79)	< 0.001
Smoking (yes)	8029 (31)	4857 (19)	< 0.001
BMI (kg/m^2)	29.2 (4.6)	30.4 (5.7)	< 0.001
Use of metformin (yes)	14813 (57)	13539 (54)	< 0.001
Use of SU-derivatives (yes)	7977 (31)	7638 (31)	0.408
Use of Insulin (yes)	2135 (8)	2512 (10)	< 0.001

* P-value for the difference between men and women. Values are depicted as n (%), mean ($\pm$ SD), or median (IQR). Continuous data were analysed using independent t-tests or the Mann-Whitney U test. Categorical variables were analysed using chi square tests.

The results of the association between BMI as a continuous variable and the total group of obesity-related cancers are described in table 2 for men and women separately. In both men and women, BMI was associated with a higher risk of obesity-related cancer in the age and fully adjusted analyses (in men: HR 1.11 (95% CI 1.01 – 1.22) and HR 1.12 (95% CI 1.02 – 1.23), in women: HR 1.14 (95% CI 1.07 – 1.21) and HR 1.15 (95% CI 1.08 – 1.22)). Analysis of BMI as a categorical variable showed that in men a BMI of 30.0 - 35.0 kg/m^2 was associated with a higher risk of obesity-related cancer compared to the reference group. In women, a BMI of 30.0 - 35.0 kg/m^2 and a BMI ≥ 35.0 kg/m^2 were both associated with a higher risk of obesity-related cancer compared to the reference group (table 3).

Non sex-specific obesity related cancers

In the analyses for non-sex specific obesity-related cancers, 25.945 men and 26.099 women were included. Baseline results for these patients did not significantly differ from the baseline results described in table 1 (data not shown). The median follow-up period was 3.1 (1.7 – 5.0) years in men and 3.1 (1.7 – 5.1) in women. During follow-up, 533 (2.1%) men and 385 (1.5%) women were diagnosed with a non-sex specific obesity-related cancer.

In men, BMI was associated with a higher risk of non-sex specific obesity-related cancer in the age and fully adjusted analyses (HR 1.17 (95% CI 1.05 – 1.30) and HR 1.18 (95% CI 1.06 – 1.31)) (table 4). In the categorical analyses, a BMI of 30.0 - 35.0 kg/m^2 and a BMI ≥ 35.0

kg/m² were also associated with a higher risk of non-sex specific obesity-related cancer in men (table 3). In women, no significant associations between BMI as a continuous or categorical variable and non-sex specific obesity-related cancers were found.

Sex-specific obesity-related cancers

In the analyses for sex-specific obesity-related cancers, 26.226 men and 25.357 women were included. Baseline results for these patients did not significantly differ from the baseline results described in table 1 (data not shown). The median follow-up period for these patients was 3.1 (1.7 – 5.0) years in men and 3.1 (1.7 – 5.1) in women. During follow-up, 170 (0.7%) men and 575 (2.3%) women were diagnosed with a sex-specific obesity-related cancer.

Table 2. Regression analyses for all obesity related cancers in men and women[#].

Variable	Men		Women	
	Crude HR (95% CI)	Adjusted HR (95% CI)	Crude HR (95% CI)	Adjusted HR (95% CI)
BMI (per 5 kg/m ²)	1.11 (1.01 – 1.22)	1.12 (1.02 – 1.23)	1.14 (1.07 – 1.21)	1.15 (1.08 – 1.22)
Diabetes duration (years)		0.99 (0.97 – 1.01)		0.99 (0.97 – 1.00)
HbA1c (mmol/mol)		1.00 (0.99 – 1.01)		1.00 (0.99 – 1.00)
Creatinine (μmol/L)		1.00 (0.99 – 1.00)		1.00 (1.00 – 1.00)
Use of metformin		0.94 (0.80 – 1.10)		0.98 (0.85 – 1.22)
Use of SU-derivatives		1.16 (0.98 – 1.37)		1.03 (0.88 – 1.19)
Use of insulin		1.14 (0.84 – 1.54)		1.09 (0.85 – 1.40)
Smoking		1.02 (0.86 – 1.22)		1.11 (0.93 – 1.33)

[#] gastric-cardia (ICD C16.0) (events in men: 18, events in women: 3), colorectal (ICD C18,19,20) (events in men: 330, events in women: 247), liver (ICD C22) (events in men: 15, events in women: 8), gall bladder (ICD C23) (events in men: 2, events in women: 7), pancreas (ICD C25) (events in men: 59, events in women: 41), kidney cancer (ICD C64) (events in men: 60, events in women: 38) and adenocarcinoma of the oesophagus (ICD C15.5 + morphological codes 8140 or 8560) (events in men: 46, events in women: 7). In men supplemented with advanced prostate cancers (C61 + TNM 3 or 4 or GLEASON > 7) (events in men: 159). In women supplemented with ovarian (ICD C56) (events in women: 39), endometrial (ICD C54.1) (events in women: 117) and postmenopausal breast cancer (ICD C50 and age ≥ 55 years) (events in women: 407).

Table 3. Regression analyses with BMI categories for all obesity related cancer groups in men and women.

Variable	Men		Non-sex specific		Sex-specific	
	All					
BMI (kg/m ²)	events	HR (95% CI)	events	HR (95% CI)	events	HR (95% CI)
≥ 18.5 - < 25.0	110	Reference	83	Reference	31	Reference
≥ 25.0 - < 30.0	333	1.12 (0.89– 1.40)	248	1.12 (0.86 – 1.46)	90	1.07 (0.69 – 1.66)
≥ 30.0 - < 35.0	194	1.34 (1.04 – 1.73)	152	1.42 (1.05 – 1.90)	48	1.15 (0.71 – 1.86)
≥ 35.0	52	1.24 (0.86 – 1.78)	50	1.61 (1.10 – 2.36)	1	- *

*Analyses were adjusted for age, diabetes duration, HbA1c, serum creatinine, smoking status and the use of metformin, SU-derivatives and/or insulin. * Only one event.*

Table 4. Regression analyses for non sex-specific obesity related cancers in men and women[#].

Variable	Men		Women	
	Crude HR (95% CI)	Adjusted HR (95% CI)	Crude HR (95% CI)	Adjusted HR (95% CI)
BMI (per 5 kg/m ²)	1.17 (1.05 – 1.30)	1.18 (1.06 – 1.31)	1.01 (0.92 – 1.12)	1.02 (0.92 – 1.13)
Diabetes duration (years)		1.00 (0.97 – 1.02)		0.98 (0.96 – 1.01)
HbA1c (mmol/mol)		1.00 (0.99 – 1.01)		1.00 (0.99 – 1.01)
Creatinine (μmol/L)		1.00 (0.99 – 1.00)		1.00 (0.99 – 1.01)
Use of metformin		0.88 (0.74 – 1.06)		1.16 (0.94 – 1.44)
Use of SU-derivatives		1.07 (0.88 – 1.30)		1.15 (0.92 – 1.44)
Use of insulin		1.04 (0.73 – 1.49)		1.19 (0.82 – 1.74)
Smoking		1.09 (0.90 – 1.33)		1.33 (1.01 – 1.76)

[#] gastric-cardia (ICD C16.0) (events in men: 19, events in women: 3), colorectal (ICD C18,19,20) (events in men: 330, events in women: 270), liver (ICD C22) (events in men: 15, events in women: 8), gall bladder (ICD C23) (events in men: 2, events in women: 8), pancreas (ICD C25) (events in men: 59, events in women: 44), kidney cancer (ICD C64) (events in men: 62, events in women: 45) and adenocarcinoma of the esophagus (ICD C15.5 + morphological codes 8140 or 8560) (events in men: 46, events in women: 7).

Women					
All		Non-sex specific		Sex-specific	
events	HR (95% CI)	events	HR (95% CI)	events	HR (95% CI)
118	Reference	62	Reference	63	Reference
337	1.24 (1.00 – 1.55)	141	0.98 (0.71 – 1.36)	210	1.46 (1.05 – 2.02)
280	1.31 (1.03 – 1.65)	120	1.06 (0.77 – 1.46)	176	1.51 (1.08 – 2.12)
179	1.61 (1.26 – 2.07)	62	1.07 (0.73 – 1.56)	126	2.09 (1.48 – 2.93)

Table 5. Regression analyses for sex-specific obesity related cancers in men and women[#].

Variable	Men		Women	
	Crude HR (95% CI)	Adjusted HR (95% CI)	Crude HR (95% CI)	Adjusted HR (95% CI)
BMI (per 5 kg/m ²)	0.92 (0.74 – 1.15)	0.93 (0.75 – 1.16)	1.22 (1.13 – 1.31)	1.22 (1.14 – 1.32)
Diabetes duration (years)		0.98 (0.95 – 1.02)		0.98 (0.96 – 1.01)
HbA1c (mmol/mol)		0.99 (0.98 – 1.01)		1.00 (0.99 – 1.01)
Creatinine (μmol/L)		1.00 (0.99 – 1.00)		1.00 (1.00 – 1.00)
Use of metformin		1.17 (0.85 – 1.61)		0.86 (0.73 – 1.03)
Use of SU-derivatives		1.50 (1.07 – 2.09)		0.93 (0.77 – 1.13)
Use of insulin		1.34 (0.74 – 2.42)		1.05 (0.76 – 1.44)
Smoking		0.81 (0.55 – 1.19)		1.00 (0.79 – 1.27)

[#] In men supplemented with advanced prostate cancers (C61 + TNM 3 or 4 or GLEASON > 7) (events in men: 170). In women supplemented with ovarian (ICD C56) (events in women: 39), endometrial (ICD C54.1) (events in women: 119) and postmenopausal breast cancer (ICD C50 and age ≥ 55 years) (events in women: 417).

In men, no significant associations between BMI as a continuous or categorical variable and prostate cancer was found (table 2 and table 5). In women, BMI was associated with a higher risk of ovarian, endometrial and breast cancer in the age and fully adjusted analyses (HR 1.22 (95% CI 1.13 – 1.31) and HR 1.22 (95% CI 1.14 – 1.32)). In the categorical analyses, a BMI of 25.0 - 30.0 kg/m², a BMI of 30.0 - 35.0 kg/m² and a BMI ≥ 35.0 kg/m² were associated with a higher risk of ovarian, endometrial and breast cancer in women (table 2).

DISCUSSION

The results of the present study showed that BMI was associated with the total group of obesity-related cancers in both men and women with type 2 diabetes. Important differences between men and women were found; BMI was associated with non sex-specific obesity-related cancers in men, but not in women. Results concerning the sex-specific cancers showed that BMI was associated with ovarian, endometrial and postmenopausal breast cancer in women. No association between BMI and advanced prostate cancer was found in men.

This is the first study which describes the association between BMI and the total group of obesity-related cancers in a large cohort of men and women with type 2 diabetes. A study from Japan could not find an association between BMI and all obesity-related cancers (6). However, this was a study that included 2334 patients of whom only 35 patients developed cancer during follow-up (6).

The absence of a significant association between BMI and non sex-specific obesity-related cancers in women may indicate that BMI (in contrast to men) is not a risk factor for this group of cancers. Only one study has previously described sex differences in the association between BMI and non-sex-specific obesity-related cancers in patients with type 2 diabetes (8). A study from Sweden found that being overweight or obese was associated with a higher risk of gastrointestinal cancers in men, whereas in women only obesity but not overweight was found to be associated with gastrointestinal cancers (8). The sex difference in the association between BMI and non-sex specific obesity-related cancers could partly be the result of a sex difference in distribution of fat. In general, obese men have more intra-abdominal fat compared to obese premenopausal women. Although the sex difference in intra-abdominal fat is less apparent in patients with type 2 diabetes, the amount of intra-abdominal fat is probably still higher in men (11). Especially this abdominal adiposity is associated with many metabolic abnormalities which may increase

cancer risk. It might be that BMI was not accurate enough to represent the amount of abdominal fat especially in women with type 2 diabetes. Using waist circumference as a marker of abdominal fat might have been more accurate.

In the present study, obese women had a 51% higher risk for ovarian, endometrial and, post-menopausal breast cancer compared to lean women with type 2 diabetes during follow-up. Only one previous study has described an association between BMI and sex-specific cancers in women with type 2 diabetes. Jonasson et al. described a 39% higher risk for postmenopausal breast cancer compared to lean women (8). The association of BMI with these types of cancer might be attributed to an increase in oestrogen production in adipose tissue in overweight and obese women with type 2 diabetes (12). Not only BMI but also type 2 diabetes itself has been found to be related to a higher risk of endometrial and breast cancer (3). Taken this together, this indicates that especially obese women with type 2 diabetes are at risk for the development of endometrial cancer and postmenopausal breast cancer. On the other hand, some researchers have questioned the relation between type 2 diabetes itself and breast cancer. They have suggested that the higher risk of postmenopausal breast cancer in women with type 2 diabetes could be completely explained by residual confounding by overweight and not by having a problem with metabolic disturbance related to glucose control (13).

In line with the results of the present study, Jonasson et al. could not find an association between BMI and prostate cancer in men with type 2 diabetes (8). However, they included all prostate cancers, whereas only advanced prostate cancer is described by the World Cancer Research Fund to be related with BMI in the general population (5). Nevertheless, the International Agency for Research on Cancer (IARC) Working Group has recently described that evidence for a preventive effect of the absence of excess body fat for fatal prostate cancer is limited (14). The present study adds to the literature that a relation between BMI and advanced prostate cancer is not present, at least not in men with type 2 diabetes. Advanced prostate cancer should therefore be reconsidered as being obesity-related.

The strengths of the present study were the prospective design and the use of a large cohort of type 2 diabetes patients of which data on BMI was available. Some limitations of the present study should also be mentioned. Firstly, the study population consisted only of patients with T2D who are treated in primary care. Patients in secondary care often have worse manifestations of T2D and might therefore have a higher cancer risk. Although this limitation implies that the generalisability of our results is limited to primary care, it is still representative for a large part of the T2D population due to the fact that the majority (>85%) of the patients with T2D are treated in primary care in the Netherlands (15).

Secondly, the clinical data and the data on medication use of the ZODIAC cohort were collected annually by practice nurses and general practitioners. The reliability of the data is therefore dependent on their accuracy. Thirdly, data on BMI was missing for 9% of the patients. BMI values for these patients were estimated using multiple imputations. Fourthly, the grouping of obesity-related cancers could be discussed. All different cancers were taken together as the number of total events would be too small when investigating each of the obesity-related cancers separately. Nevertheless, separate analyses would have been better. Finally, the follow-up period was relatively short for cancer epidemiology and therefore the number of events is relatively small.

CONCLUSIONS

In men with type 2 diabetes mellitus BMI is associated with the total group of obesity-related cancers and with non sex-specific obesity-related cancers. However, BMI is not related to advanced prostate cancer. The present study provides an extra argument for questioning advanced prostate cancer being obesity-related, at least in men with type 2 diabetes. In women, BMI is associated with the total group of obesity-related cancers and with ovarian, endometrial and postmenopausal breast cancer. BMI is not related to non sex-specific obesity-related cancers in women with type 2 diabetes.

REFERENCES

1. Peters SAE, Huxley RR, Woodward M. Diabetes as risk factor for incident coronary heart disease in women compared with men: a systematic review and meta-analysis of 64 cohorts including 858,507 individuals and 28,203 coronary events. *Diabetologia*. 2014 Aug;57(8):1542–51.
2. Peters SAE, Huxley RR, Woodward M. Diabetes as a risk factor for stroke in women compared with men: a systematic review and meta-analysis of 64 cohorts, including 775,385 individuals and 12,539 strokes. *Lancet*. 2014 Jun 7;383(9933):1973–80.
3. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, et al. Diabetes and cancer: a consensus report. *Diabetes Care*. 2010 Jul;33(7):1674–85.
4. Johnson JA, Carstensen B, Witte D, Bowker SL, Lipscombe L, Renehan AG, et al. Diabetes and cancer (1): evaluating the temporal relationship between type 2 diabetes and cancer incidence. *Diabetologia*. 2012 Jun;55(6):1607–18.
5. World Cancer Research Fund. Cancers linked with greater body fatness [Webpage] [Internet]. 2015 [cited 2016 Mar 23]. Available from: <http://www.wcrf.org/int/cancer-facts-figures/link-between-lifestyle-cancer-risk/cancers-linked-greater-body-fatness>
6. Yamamoto-Honda R, Takahashi Y, Yoshida Y, Kwazu S, Iwamoto Y, Kajio H, et al. Body mass index and the risk of cancer incidence in patients with type 2 diabetes in Japan: Results from the National Center Diabetes Database. *J Diabetes Investig*. 2016 Mar 25;
7. Peeters PJHL, Bazelier MT, Leufkens HGM, de Vries F, De Bruin ML. The risk of colorectal cancer in patients with type 2 diabetes: associations with treatment stage and obesity. *Diabetes Care*. 2015 Mar;38(3):495–502.
8. Miao Jonasson J, Cederholm J, Gudbjornsdottir S. Excess body weight and cancer risk in patients with type 2 diabetes who were registered in Swedish National Diabetes Register—register-based cohort study in Sweden. *PloS One*. 2014;9(9):e105868.
9. Ubink-Veltmaat LJ, Bilo HJG, Groenier KH, Rischen RO, Meyboom-de Jong B. Shared care with task delegation to nurses for type 2 diabetes: prospective observational study. *Neth J Med*. 2005 Mar;63(3):103–10.
10. Bowker SL, Richardson K, Marra CA, Johnson JA. Risk of breast cancer after onset of type 2 diabetes: evidence of detection bias in postmenopausal women. *Diabetes Care*. 2011 Dec;34(12):2542–4.
11. Wannamethee SG, Papacosta O, Lawlor DA, Whincup PH, Lowe GD, Ebrahim S, et al. Do women exhibit greater differences in established and novel risk factors between diabetes and non-diabetes than men? The British Regional Heart Study and British Women's Heart Health Study. *Diabetologia*. 2012 Jan;55(1):80–7.
12. Gallagher EJ, LeRoith D. Obesity and Diabetes: The Increased Risk of Cancer and Cancer-Related Mortality. *Physiol Rev*. 2015 Jul;95(3):727–48.
13. La Vecchia C, Giordano SH, Hortobagyi GN, Chabner B. Overweight, obesity, diabetes, and risk of breast cancer: interlocking pieces of the puzzle. *The Oncologist*. 2011;16(6):726–9.
14. Lauby-Secretan B, Scoccianti C, Loomis D, Grosse Y, Bianchini F, Straif K, et al. Body Fatness and Cancer—Viewpoint of the IARC Working Group. *N Engl J Med*. 2016 Aug 25;375(8):794–8.
15. InEen. Transparante ketenzorg diabetes mellitus, COPD en VRM rapportage zorggroepen over 2014. 2015 Jun.

6

Sex differences in obesity related cancer incidence in relation to type 2 diabetes diagnosis (ZODIAC-49)

Published as:

Schrijnders D, Hendriks SH, Kleefstra N, Vissers PAJ, Johnson JA, et al. (2018) Sex differences in obesity related cancer incidence in relation to type 2 diabetes diagnosis (ZODIAC-49). PLOS ONE 13(1): e0190870.

ABSTRACT

Background:

Diabetes and obesity seem to be partly overlapping risk factors for the development of obesity-related cancer (mainly breast, prostate and colorectal cancer) in patients with type 2 diabetes (T2DM). In the general population, women have a lower risk for obesity-related cancer compared to men. Previous studies involving cardiovascular disease have shown that T2DM eliminates a female advantage of lower CVD risk in the general population compared to men. It is unclear whether the same could be true for obesity-related cancer. This study aimed to investigate obesity-related cancer incidence in women and men known with T2DM as compared to the Dutch general population.

Methods:

This study included 69,583 patients with T2DM selected from a prospective primary care cohort, which was linked to the Dutch National Cancer Registry to obtain cancer specific data. Obesity-related cancers included liver, kidney, colorectal, gallbladder, pancreas, ovarian, endometrial, advanced prostate cancer, post-menopausal breast cancer and oesophageal adenocarcinoma. Primary outcome was sex-stratified, age and year of cancer diagnosis adjusted standardized incidence ratios (SIRs) for three time periods: 5 years before, the year after diagnosis and the next 4 years after T2DM diagnosis. The Dutch general population was used as reference group.

Results:

Women with T2DM were at an increased risk for obesity-related cancer compared to women in the general population already 5 years before diabetes diagnosis (SIR 1.77; 95%CI: 1.63-1.91). In both men and women, there was a peak in obesity-related cancer incidence following diabetes diagnosis (SIR: 1.38; 95%CI 1.11-1.64 and SIR: 2.21; 95%CI 1.94-2.30, respectively). From the second to the fifth year after diabetes diagnosis the obesity-related cancer incidence was higher in women compared to women in the general population (SIR: 2.12; 95%CI 1.94-2.30).

Conclusions:

Women with T2DM seem to have a substantially higher obesity-related cancer risk. As opposed to men, in women this risk was already increased years before diabetes diagnosis. These results could imply that a relative advantage of women in the general population with regard to cancer risk is lost in women with T2DM.

INTRODUCTION

Patients with type 2 diabetes (T2DM) have an increased risk of developing specific types of cancer compared to the general population(1). An increased risk has been reported for the development of cancers of the liver, pancreas, endometrium, colon, rectum, breast and bladder(1-4). The increased risk of these specific types of cancer in patients with T2DM largely overlap with the so called ‘obesity-related cancers’. The World Cancer Research Fund acknowledges liver, kidney, colorectal, gallbladder, pancreas, ovarian, endometrial, advanced prostate cancer, post-menopausal breast cancer and oesophageal adenocarcinoma as obesity-related cancer (4, 5). A large portion of patients with T2DM are overweight or obese (6, 7). So, diabetes and obesity appear to be partly overlapping risk factors for the development of cancer in patients with T2DM (8, 9). The development of diabetes is mostly a progressive process (10). It is well-known that being overweight is a very important factor in type 2 diabetes (11). Patients who are diagnosed with T2DM are already different with respect to i.e. body mass index, compared to persons in the general population also in the years prior to diabetes diagnosis (12, 13). Furthermore, these patients will for example have a more frequent impaired glucose tolerance and/or impaired fasting glucose, accompanied by higher glucose and insulin levels (14, 15). Both are growth factors important in tumour development (16, 17). In addition, it has been suggested that sex hormones increase cancer risk (18).

In the general population, risk for obesity-related cancer, like the risk for cardiovascular disease (CVD), is lower in women than in men (19). However, previous studies investigating risk of CVD have shown that T2DM eliminates this female advantage (20, 21). It is unclear if the same could be true for (obesity-related) cancer. Indeed, several small studies reported sex differences in cancer risk in patients with pre-diabetes or T2DM. Reports show that in the pre-diabetes phase, the risk to develop breast cancer is increased compared to women without pre-diabetes (22, 23). In addition, a significant increase in colon cancer incidence before T2DM diagnosis has been reported in men, where this was not observed in women (24, 25). The increased incidence of cancer among T2DM patients has only partly been observed in larger studies that investigated the distribution of overall incidence of cancer over the years before and after the diagnosis of diabetes (2, 26). However, those studies did not stratify the risk to develop cancer for males and females (2, 26). T2DM and adiposity have been associated with a significant additive effect of obesity-related cancer (27). This increased cancer incidence seems to be different in the periods before the diagnosis of diabetes, shortly after (1 year) and the period after 1 year after the diagnosis of diabetes (28). Thus far, only few studies investigated the overall

and obesity-related cancer risk in T2DM stratified for males and females to answer the question whether there are sex differences in cancer risk.

The primary aim of this study was to investigate the incidence of obesity-related cancers in women and men in relationship to the time of diabetes diagnosis. The obesity-related cancer incidence was analysed in the periods 5 years before, the year after and 2 to 5 years after the diagnosis of diabetes. A secondary aim was to investigate the incidence of all-cancer incidence in patients with a diagnosis of diabetes.

METHODS

This study is reported according to the STROBE (Strengthening the reporting of observational studies in epidemiology) recommendations (29).

Study design and data collection

For this study, two prospective cohorts were combined. A T2DM specific cohort, the observational ZODIAC (Zwolle Outpatient Diabetes project Integrating Available Care) cohort study, was merged with the Netherlands Cancer Registry (NCR). To obtain cancer specific data for patients included in the ZODIAC cohort, all cancer events that occurred between 1989 and 2012 were linked to the data of the ZODIAC cohort via a trusted third party using postal code, full name, date of birth and sex. The NCR expects that the number of false-positive and the number false-negative for the ZODIAC-NCR linkage is both under 1%.

ZODIAC is a prospective primary care cohort study, which was initiated in 1998 in the Zwolle region. The cohort has been expanded to different regions several times and now includes almost all patients in the north-eastern part of the Netherlands, part of Flevoland province and part of the province of North Holland. As such, the cohort represents the majority of primary care treated patients in the Netherlands and has a high degree of generalizability. In the ZODIAC study, clinical data were sent annually to the Diabetes Centre by general practitioners (GPs) for benchmarking and research purposes. Patients included in the ZODIAC study were diagnosed with T2DM and were at inclusion treated exclusively in primary care. There was no age restriction for participation. Patients were not eligible for participation if they had a short life expectancy, a cognitive impairment or were treated in secondary care. From all patients included in ZODIAC, diabetes specific data was electronically collected annually. Data includes date of birth, sex, date of diabetes diagnosis, HbA1c, height, weight, serum creatinine, albuminuria, cholesterol/

HDL ratio, blood pressure, micro- and macrovascular complications, medication use (both diabetes specific and other medication), smoking status and alcohol use. Entire primary care practises participated in ZODIAC and the project has been expanded to new regions several times and therefore included newly diagnosed and previously diagnosed T2DM patients. All patients were followed prospectively. All T2DM patients within participating primary care practises who were treated in primary care for their diabetes could participate.

The primary care provider in the Netherlands has a very important role in the health care system, for example as a mandatory gatekeeper to secondary care. All inhabitants of the Netherlands have a GP and health insurance. Since the late nineties the majority of patients (around 88%) with T2DM are treated exclusively in primary care (30) and benchmarking has become a mandatory part of receiving health care reimbursements. The quality of the Dutch diabetes primary care is regarded to be high and for example the average HbA1c is 6.7% (6).

The NCR is a population based mandatory national registry, founded in 1989, and records all malignancies based on notification by the National Pathology Archive (PALGA) and hospital discharge registries. Data is gathered by specially trained data-managers directly from the patients' files in hospitals in the Netherlands. Data recorded includes incidence date, TNM stage, morphology, location and primary cancer treatment. Basal cell carcinoma of the skin, carcinoma in situ of the cervix, myelodysplastic syndrome, myeloproliferative disorders were not registered in the NCR database. Benign and borderline tumours were excluded, with the following exceptions; benign brain tumours (included from 1999), carcinoids of the appendix (included from 2001), borderline tumours of the ovaries (included from 2001), thymoma (included from 2001), phyloides tumours (included from 2001) and T-cell leukaemia (included from 2004). Comorbidities, including diabetes, were also not registered in this database.

Patient selection

The combined ZODIAC-NCR database contained 71,648 patients of which 10,717 were diagnosed with 12,617 cancer events between 1 January 1998 and 31 December 2012. Only the first obesity-related cancer event (for the obesity-related cancer analyses) or first cancer event (in the all-cancers combined analyses) were included in the analysis. From the 71,648, a total of 2,065 (2.9%) patients were excluded because a cancer event occurred before the study period of interest, i.e. more than 5 years before diabetes diagnosis.

Baseline (time zero) was set at the date of diabetes diagnosis. The date of diabetes diagnosis was based on the electronically documented date of diabetes diagnosis derived from the medical history for patients with newly diagnosed diabetes. As previously mentioned, the date of diabetes diagnosis could very well be different to the time of entry into the ZODIAC study.

OUTCOME MEASURES

The primary outcome was the sex-stratified, age and year of cancer diagnosis adjusted standardized incidence ratios (SIR) of obesity-related cancers in three time frames; the 5 years before diabetes diagnosis, the first year following diabetes diagnosis and year 2 to 5 after diabetes diagnosis. The comparator group was the Dutch general population (including patients with diabetes). The following cancers (ICD-10 codes) were defined as obesity-related cancer: liver (C22), kidney (C64), colorectal (C18, C19, C20), gallbladder (C23), pancreas (C25), ovarian (C56), endometrial (54.1) and advanced prostate cancer (C61 with TNM 3 or 4 or Gleason > 7), post-menopausal breast cancer (C50 and age > 54) and oesophageal adenocarcinoma (C15.5) (5).

The secondary outcome was the same as the primary outcome but, instead of obesity-related cancer, combined all-cancers (non-melanoma skin cancer was excluded).

Statistical analysis

Using the date of diabetes diagnosis and the date of the first cancer event, age and year of cancer diagnosis standardized obesity-related and overall cancer incidence were calculated for one-year intervals from 5 years prior to 5 years after the diagnosis of T2DM. Patients from whom only the year (and not the month) of diabetes diagnosis was available, the first of July was chosen as date of diabetes diagnosis. The cancer incidence rate in the ZODIAC cohort was calculated first. The NCR provided incidence rates of the general population from the same time points that were included in the ZODIAC cohort. The NCR is unable to identify diabetes status, and thus the comparator group also includes patients with diabetes. Standardized incidence ratios with 95% confidence intervals stratified for sex and adjusted for age and year of cancer (in 5-year intervals) were calculated to compare the cancer incidence in the ZODIAC cohort to the general population (31). The SIR is analogous to the commonly used standardized mortality ratios (32). Incidence ratios were calculated by dividing the total number of cancer events in one-year time periods by the amount of contributed time in this one-year time period.

Reference data for all-cancer and for obesity-related cancer incidence of the whole Dutch population were provided by the NCR.

Both all-cancer and obesity-related SIR were stratified for sex. A separate analysis stratifying by BMI was performed. This analysis only includes patients who have a BMI recorded at diabetes diagnosis. Advanced prostate cancer and post-menopausal breast cancer were also examined separately. Furthermore, to make obesity-related cancers somewhat comparable in men and women T2DM an analysis excluding sex-specific cancers was performed. Sex-specific obesity-related cancers were ovarian, endometrial and post-menopausal breast cancer in women and advanced prostate cancer in men.

A sensitivity analysis excluding pancreas cancer was performed to investigate the effect of pancreatic cancer on T2DM diagnosis. Analyses were performed in STATA v14.

Ethics Statement

The medical ethics committee of Isala, Zwolle, the Netherlands approved the procedure of this study including the anonymous linking of the ZODIAC with the NCR (METC reference number 13.0765). No written informed consent was required. Patients consented with the anonymous use of their data for study purposes in the ZODIAC study.

RESULTS

Baseline characteristics are shown in Table 1. Among the 69,583 patients included, 49% were female and 40,417 patients had a follow-up of at least 5 years. Women were older and had a higher BMI compared to men.

Obesity-related cancers

Incidence ratios for obesity-related cancers are shown in Fig 1, S1 and S2 Table. Compared to the general population, in men and women combined the incidence of obesity-related cancer were elevated in the 5 years prior to, the first year after and year 2 to 5 after diabetes diagnosis, with the highest cancer incidence in the first year after diabetes diagnosis (SIR 1.80, 95%CI 1.59 – 2.01).

In women, the 5 years prior to diabetes diagnosis, there was a significantly increased obesity-related cancer incidence in all years compared to women in the general population. The SIR gradually increased from 1.86 (95%CI 1.37 – 2.00) at 5 years prior to

diabetes diagnosis to 2.06 (95%CI 1.74 – 2.39) at 2 to 1 year prior to diabetes diagnosis but decreases in the year before diagnosis of diabetes (SIR 1.44, 95%CI 1.17-1.70). The first year after diabetes diagnosis the SIR was the highest (SIR 2.21, 95%CI 1.88-2.54) and remained increased in the 5 years after diabetes diagnosis.

Table 1: Patient characteristics

	Women (n=34,312, 49.5%)	Men (n = 35,271, 50.5%)	P-value *
Age at diabetes diagnosis (SD), years	63.0 (12)	60.5 (11)	< 0.001
Diabetes duration (IQR), years	2.5 (0.6 – 6.3)	2.1 (0.5 – 5.7)	
HbA1c at diabetes diagnosis (IQR), mmol/mol	49 (43 – 55),	49 (43 – 55),	0.32
HbA1c at diabetes diagnosis (IQR), %	6.6 (6.1 – 7.2)	6.6 (6.1 – 7.2)	
BMI at diabetes diagnosis§ (IQR)	29.5 (26.3 – 33.7)	28.4 (26.0 – 31.5)	< 0.001
Creatinine at diabetes diagnosis (IQR), μmol/L	68 (59 – 80)	87 (74 – 95)	< 0.001
Cancer events			
All-cancer (n, %)	2130 (6.2)	2139 (6.1)	
Obesity-related cancer (n, %)	1344 (3.9)	709 (2.0)	
Non sex-specific obesity-related cancer (n, %)	374 (1.1)	479 (1.4)	
BMI categories§			< 0.001
18 to 25 (%)	16.5	16.8	
25 to 30 (%)	36.6	47.1	
>30 (%)	46.9	36.1	

SD: Standard deviation, IQR: Inter-quartile range, BMI: Body mass index. § Includes only patients who had a BMI recorded at diabetes diagnosis. Continuous data were analysed using independent t-tests or the Mann-Whitney U test. Categorical variables were analysed using chi square tests.

*P-value for the difference between men and women at baseline.

In men, no significantly increased cancer risk was observed in 5 to 1 year prior to diagnosis of diabetes. In contrast, in the year prior and the year after diabetes diagnosis the SIR was significantly increased cancer risk compared to men in the general population, 1.32 (95%CI 1.06 – 1.58) and 1.38 (95%CI 1.11 – 1.64), respectively.

An analysis where patients were stratified by BMI below or above 30 at diabetes diagnosis were in line with the main analyses (S7 Table and S3 to S4 Figs). The SIR for obesity-related cancers was higher in women compared to men for all years, with non-overlapping confidence intervals, except the one year before diagnosis of diabetes.

Fig 1. Standardized Incidence Ratio with 95%CI bars of obesity-related cancer. The horizontal line is 1.00 and means that there was no difference between the ZODIAC population and the general population.

Sex-specific influences on obesity-related cancer incidence

Fig 2, S1 and S3 Table show the SIR for obesity-related cancer when the sex-specific obesity-related cancer types (breast, ovarian and endometrial cancer in women and advanced prostate cancer in men) were excluded. In women a significantly increased SIR for obesity-related non-sex specific cancer before diabetes diagnosis was only observed for year minus 2 to minus 1 year before diabetes diagnosis (SIR 1.64, 95%CI 1.14-2.14). In the year following diabetes diagnosis a significantly increased SIR was observed (SIR 2.61, 95%CI 2.00-3.23) for all years after diabetes diagnosis. A separate analysis which included only breast cancer (S6 Table and S1 Fig) showed that the SIR was increased for all time points, and also showed an apparent decrease the year before diabetes diagnosis.

In men, there was a significant difference in cancer incidence at minus 3 years (SIR 1.50, 95%CI 1.06 – 1.94) and minus 1 year (SIR 1.96, 95%CI 1.49 – 2.42) prior to diabetes diagnosis compared to the general population. Cancer incidence was highest in the first year after diabetes diagnosis (SIR 2.04, 95%CI 1.56 – 2.51). A separate analysis investigating specifically advanced prostate cancer showed a decreased incidence in advance prostate cancer the 5 year prior to diabetes diagnosis and no significant difference compared to the general population in the years after diabetes diagnosis.

The SIRs for non-sex-specific obesity-related cancers were, based on overlapping confidence intervals, not significantly different between sexes.

Fig 2. Standardized Incidence Ratio with 95%CI bars of obesity-related cancer excluding sex-specific cancers. The horizontal line is 1.00 and means that there was no difference between the ZODIAC population and the general population.

Fig 3. Standardized Incidence Ratio with 95%CI bars of all-cancer combined (excluding non-melanoma skin cancer). The horizontal line is 1.00 and means that there was no difference between the ZODIAC population and the general population.

Standardized incidence ratios - all-cancers combined

The SIR for all-cancers combined for men and for women are shown in Fig 3, and S1 and S4 Table. Over the years, there was a gradual increase in SIR in men and women combined, becoming significantly from 3 to 2 years prior to diagnosis of diabetes (see S4 Table). A rise in cancer incidence was present for men and women combined the year prior to and around diabetes diagnosis, see S4 Table. There were increased cancer incidences in year 2 to 5 after diabetes diagnosis.

In women, there was an increased cancer incidence that started to increase from 5 years prior to diabetes diagnosis, see Fig 3 and S4 Table. In men, only the year prior to diabetes diagnosis and 5 years thereafter were increased, see Fig 3 and S4 Table. A sensitivity analysis excluding pancreas cancer did not change results significantly (S5 Table). An analysis where patient were stratified by a BMI below or above 30 at diabetes diagnosis were in line with in line with the main analyses (S8 Table and S3 to S4 Fig).

DISCUSSION

This study is the first to investigate standardized obesity-related cancer incidence ratios in three time periods (5 years before, the first year after and 2 to 5 years after diabetes diagnosis) and accounting for sex-differences.

In women there was an increase in obesity-related cancer incidence already 5 years before T2DM diagnosis and this was also increased after diabetes diagnosis compared to women in the general population. This was not observed in men. Also, from year 2 to 5 after diabetes diagnosis, women had a higher obesity-related cancer incidence compared to women in the general population.

The observed increase in obesity-related cancer incidence in women with T2DM compared to women in general population was explained to a large part by the contribution of sex-specific cancers, for example breast cancer. The exclusion of sex-specific cancers in women mitigated the increased obesity-related cancer incidence before diabetes diagnosis, highlighting the importance of sex-specific cancers in women. Furthermore, the differential effects do not seem to be confounded by obesity. It seems to be that after diabetes diagnosis, irrespective of the presence of obesity or not, there was an increased cancer incidence compared to the general population.

The analysis that included only breast cancer was in line with the obesity-related and all-cancer analyses and showed an increased breast cancer incidence in women with diabetes compared to the general population. This is in accordance with previous studies

that investigated breast cancer incidence also showed that breast cancer risk was already elevated in the pre-diabetes phase (23, 33). It could be that women put on relative more weight in the pre-diabetes phase. From all obesity-related cancers, breast cancer appears to show the strongest correlation with weight increases (10, 34). It has been suggested that the increased incidence could be due to increased aromatization of androgens to oestrogens locally in fat tissue (35). Eventually, this could lead to increased ER α signalling in breast cancer and possibly a greater risk of oestrogen-dependent breast cancer (35). Indeed, especially oestrogen receptor positive breast cancer risk has been reported to be increased in women with T2DM (36).

There was a decrease in obesity-related SIR in women 1 year before diabetes diagnosis for which we have no obvious explanation. There is some evidence that especially breast cancer is strongly associated with obesity and weight gain as such (37, 38). There are reports suggesting that obese patients could also be less likely to respond to screening programs (38, 39). During the study period, there were no national screening programs involving men. Another possible explanation could be related to the care provided. It could be that there is some degree of clinical distraction or pre-occupation with metabolic parameters that results in fewer diagnosis of cancer (40). An incidental cancer diagnosis is perhaps delayed until the diagnosis of diabetes is made. It could also be that during the year prior to diagnosis of diabetes cancer screening tests are performed but the definitive cancer diagnostic test takes longer to complete, thus delaying the cancer diagnosis until after diabetes is diagnosed. However, most cancer diagnostic tests don't take long to complete. A fourth possible explanation is that there is an issue with the registration of cancer events. However, this seemed unlikely since all cancer diagnoses in the Netherlands are automatically registered with the Cancer Registry. None of these factors seemed plausible. A final explanation could be that this finding is a coincidence for which we have no causal explanation. Cancer screening programmes in place in the Netherlands at the time of the study period are for breast cancer and cervical cancer. Nationwide, there was an attendance rate of 79% and 65%, respectively (41, 42). Previous studies have shown that women with comorbidity, for example obesity (39), and lower social economic status are less likely to attend screening (43-45). The incidence of diabetes is increased in people with lower socio-economic status (46-48). It would thus be more likely that there would be a decreased screening attendance instead of an increased screening attendance in the prediabetes phase. In addition, women with diabetes are also less likely to undergo a mammogram than women without diabetes (43). Some studies have shown more favourable survival rate in screen detected breast cancers and emphasized the importance of cancer screening in patients with diabetes (49). In the analyses excluding sex-specific

cancers this decline disappeared, which could point to breast cancer as a likely causative cancer. The decline was not present in men, which likely excludes a methodological error as an explanation. Overall, we have no causal explanation for this finding.

There was a pronounced increase in cancer incidence around the time of diabetes diagnosis. This increased detection has been reported in previous studies (26, 50). However, it seems questionable that detection bias is an important plausible explanation before diabetes diagnosis and in the years after the first year following diabetes diagnosis (2, 26, 28, 49). Another possible explanation for the differential effects observed is diagnostic bias. Patients diagnosed with diabetes are more likely to have additional diagnostic tests (50-52). The first year after diabetes diagnosis, obesity-related cancer incidence increased 121% for women and 38% for men compared to the general population. No previous studies combining obesity-related cancer incidence were found. However, an Austrian cohort study ($n=5709$, T2DM patients) also reported elevated SIRs for cancers of the pancreas (1.78, 95%CI 1.02 – 2.89) and corpus uteri (1.79, 95%CI 1.15 – 2.66) in women and for cancers of the liver (2.71, 95%CI 1.65 – 4.18) and pancreas (1.87, 95%CI 1.11 – 2.96) in men in the year after diagnosis of diabetes (53).

The peak in cancer incidence around diabetes diagnosis could partly be directly explained by diabetes developing as a result of for example pancreas cancer (49, 51). A higher cancer mortality has been reported in female pancreatic cancer patients compared to men (54). However, exclusion of pancreas cancer in a sensitivity analyses did not relevantly change results (S5 Table). Increased detection of diabetes in a cancer work-up or cancer in a diabetes work-up could also play a role in the rise in cancer incidence immediately after diagnosis of diabetes in both men and women (50-52). There is evidence from previous studies that detection bias is partly responsible for the increase in cancer incidence patients with T2DM, at least for the excessive peak that has been observed (2, 26).

In the period from 2 to 5 years after diabetes diagnosis obesity-related and all-cancer incidence was substantially and significantly higher in women and remained increased throughout year 2 to 5 after diabetes diagnosis. In men, there was a less obvious increase in obesity-related and all-cancer incidence.

In men, as opposed to women, exclusion of sex-specific obesity-related cancer (i.e. advanced prostate cancer) substantially increased obesity-related cancer incidence the years after diabetes diagnosis. This could point to a protective effect of T2DM to prostate cancer. Previous meta-analyses already showed a decreased risk of low stage prostate cancer in T2DM patients (49, 55, 56). The analysis which included only advanced prostate cancer showed a decreased cancer incidence in the 5 years prior to diabetes diagnosis,

and no difference in cancer risk after diabetes diagnosis. Our results could suggest that advanced prostate cancer might thus not be positively related to obesity. These results are in contrast to the definition used by the WCRF (5). A recent viewpoint of the International Agency for the Research of Cancer (IARC) also stated that there is limited evidence for fatal prostate cancer being related to obesity (57).

Strengths and Limitations

This study sample represents the majority of primary care treated T2DM patients in the Netherlands and has a high degree of generalizability. Another strength of this study was the size of the ZODIAC cohort. Thirdly, the long follow-up periods made it possible to estimate incidence ratios as early as five years before diagnosis of diabetes and study cancer incidence before diabetes onset.

There were also limitations. Some patients were selected with a date of diabetes diagnosis occurring before 1994. In theory, taking into account the 5-year window before diabetes diagnosis used in this study, patients could already have had a cancer event while diagnosed with diabetes before the NCR commenced in 1989. This cancer event would not have been registered in the NCR. However, we expect this effect to be fairly negligible, since only 4.4% of patients in our data were diagnosed with diabetes before 1994. A second limitation was that cancer incidence before diabetes diagnosis could be influenced by survival bias. Since the ZODIAC cohort only consists of patients diagnosed with T2DM, patients who died from, amongst others, cancer, could never have been included in this cohort since they did not have time to develop diabetes. Thirdly, the general population also included people diagnosed with diabetes; around 10 % of the general population in this age category in the Netherlands are diagnosed with diabetes (58, 59). It has been shown that with increasing age the proportion of people diagnosed with diabetes increases (59). Furthermore, as opposed to our cohort, the general population also included people with a history of cancer. If these two factors would be accounted for, they both would likely lead to an underestimation of the effect seen. The reported higher cancer incidence rates in patients with diabetes would in reality be more pronounced (58). In addition, we were unable to adjust for confounders other than age and sex. For example, BMI data in the general population were not available. Fourthly, the ZODIAC cohort expanded to different regions in the Netherlands at several time points and both patients with known diabetes and newly diagnosed diabetes were included. The date of diabetes diagnosis was based on an electronic health record and verified by a practice nurse. This could have been subject to recall bias. For example, some patients could not remember the month of diabetes diagnosis. In this case the first of July was

used as month of diabetes diagnosis in an attempt to minimize this bias. Unfortunately, we could not exclude a relevant effect of this recall bias, however, we have no reason to assume that this would be different in men or women.

It is plausible that detection bias is also present in the current study, explaining the peak in cancer incidence at diabetes diagnosis. This could in turn also explain the slight decrease in cancer risk seen in the 2 to 3 years after diabetes diagnosis in the present study which would be almost absent if there would not have been a peak. Increased detection does not necessarily lead to bias nor is it always a negative effect, but it is a phenomenon that partly results from increased contact with health care professionals (26).

Although no specific indications exist which suggests that people in the ZODIAC regions have a different cancer risk compared to the general population in the Netherlands, we do not know that for sure. It would have been better if we could have used a comparator group from the ZODIAC regions, but unfortunately such a group was not available. Lastly, hormone receptor status of breast cancer was not available in the current study, making it impossible to investigate the influence of oestrogen-dependent breast cancer. This will be subject for further studies.

In summary, in men and women with T2DM, cancer incidence peaked the year after diabetes diagnosis. A clear difference in cancer risk was observed between men and women. Women, not men, with T2DM had an increased obesity-related standardised cancer incidence compared to women in the general population, as early as 5 years before diabetes diagnosis. After diabetes diagnosis, both men and women had an increased SIR but the effect was more pronounced in women.

Exclusion of sex-specific obesity-related cancers resulted in a lower incidence in women and a higher incidence in men compared to the SIR in all obesity-related cancers. The latter might suggest that, in addition to low risk prostate cancer, advanced prostate cancer is not associated with obesity in patients diagnosed with T2DM.

Next to relative cancer risk, absolute obesity-related cancer risk in the general population show that women have a lower risk than men (19). It appears to be that the relative advantage in women disappears in the presence of diabetes and exemplify that the relative protective effect in women is lost when diabetes develops, which is comparable to CVD. The difference in women with T2DM compared to women in the general population and the difference of women compared to men with T2DM could mean that in the general population women have an advantage concerning cancer risk but when diabetes develops this advantage disappears. The impact of this should be subject of further investigations.

SUPPORTING INFORMATION (AVAILABLE ONLINE)

S1 Table. Pooled standardized incidence ratio

S2 Table. Standardized incidence ratio of obesity-related cancers. Cancers included: liver, kidney, colorectal, gallbladder, pancreas, ovarian, endometrial and advanced prostate cancer, post-menopausal breast cancer and esophageal adenocarcinoma.

S3 Table. Standardized incidence ratio of obesity-related cancer excluding sex-specific cancers. Cancers included: liver, kidney, colorectal, gallbladder, pancreas and esophageal adenocarcinoma

S4 Table. Standardized incidence ratio all cancers combined.

S5 Table. Standardized incidence ratio all cancers combined (excluding pancreas)

S6 Table. Standardized incidence ratio breast cancer (women) and advanced prostate cancer (men).

S7 Table. Standardized incidence ratio of obesity-related cancers in patients with a BMI below 30 and 30 and above. Cancers included: liver, kidney, colorectal, gallbladder, pancreas, ovarian, endometrial and advanced prostate cancer, post-menopausal breast cancer and esophageal adenocarcinoma.

S8 Table. Standardized incidence ratio all cancers combined in patients with a BMI below 30 and 30 and above.

S1 Fig. Standardized Incidence Ratio of postmenopausal breast cancer in women.

S2 Fig. Standardized Incidence Ratio of advanced prostate cancer in men.

S3 Fig. Standardized Incidence Ratio of all and obesity-related cancer stratified to BMI < 30 in men

S4 Fig. Standardized Incidence Ratio of all and obesity-related cancer stratified to BMI < 30 in women

S1 File. Cancer incidence rate general population.

S2 File. SIR Data.

REFERENCES

1. Dankner R, Boffetta P, Balicer RD, Boker LK, Sadeh M, Berlin A, et al. Time-Dependent Risk of Cancer After a Diabetes Diagnosis in a Cohort of 2.3 Million Adults. *Am J Epidemiol*. 2016;183(12):1098-106.
2. Johnson JA, Bowker SL, Richardson K, Marra CA. Time-varying incidence of cancer after the onset of type 2 diabetes: evidence of potential detection bias. *Diabetologia*. 2011;54(9):2263-71.
3. Carstensen B, Jorgensen ME, Friis S. The epidemiology of diabetes and cancer. *Curr Diab Rep*. 2014;14(10):535.
4. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, et al. Diabetes and cancer: a consensus report. *Diabetes Care*. 2010;33(7):1674-85.
5. World Cancer Research Fund. Cancers linked with greater body fatness: WCRF; 2015 [Available from: <http://www.wcrf.org/int/cancer-facts-figures/link-between-lifestyle-cancer-risk/cancers-linked-greater-body-fatness>.
6. van Hateren KJ, Drion I, Kleefstra N, Groenier KH, Houweling ST, van der Meer K, et al. A prospective observational study of quality of diabetes care in a shared care setting: trends and age differences (ZODIAC-19). *BMJ Open*. 2012;2(4).
7. Hendriks SH, van Hateren KJ, Groenier KH, Houweling ST, Maas AH, Kleefstra N, et al. Sex Differences in the Quality of Diabetes Care in the Netherlands (ZODIAC-45). *PLoS One*. 2015;10(12):e0145907.
8. Garg SK, Maurer H, Reed K, Selagamsetty R. Diabetes and cancer: two diseases with obesity as a common risk factor. *Diabetes Obes Metab*. 2014;16(2):97-110.
9. De Pergola G, Silvestris F. Obesity as a major risk factor for cancer. *J Obes*. 2013;2013:291546.
10. Moon JH, Kwak SH, Jung HS, Choi SH, Lim S, Cho YM, et al. Weight Gain and Progression to Type 2 Diabetes in Women With a History of Gestational Diabetes Mellitus. *J Clin Endocrinol Metab*. 2015;100(9):3548-55.
11. Franssens BT, van der Graaf Y, Kappelle JL, Westerink J, de Borst GJ, Cramer MJ, et al. Body Weight, Metabolic Dysfunction, and Risk of Type 2 Diabetes in Patients at High Risk for Cardiovascular Events or With Manifest Cardiovascular Disease: A Cohort Study. *Diabetes Care*. 2015.
12. Rahmanian K, Shojaei M, Jahromi AS, Madani A. The Association Between Pre-Diabetes With Body Mass Index and Marital Status in an Iranian Urban Population. *Glob J Health Sci*. 2016;8(4):95-101.
13. Haghighatdoost F, Amini M, Feizi A, Iraj B. Are body mass index and waist circumference significant predictors of diabetes and prediabetes risk: Results from a population based cohort study. *World J Diabetes*. 2017;8(7):365-73.
14. Olefsky JM, Reaven GM. Insulin Binding in Diabetes: Relationships with Plasma Insulin Levels and Insulin Sensitivity. *Diabetes*. 1977;26(7):680-8.
15. Johnson JL, Duick DS, Chui MA, Aldasouqi SA. Identifying prediabetes using fasting insulin levels. *Endocr Pract*. 2010;16(1):47-52.
16. Orgel E, Mittelman SD. The Links Between Insulin Resistance, Diabetes, and Cancer. *Curr Diab Rep*. 2013;13(2):213-22.
17. Peeters K, Van Leemputte F, Fischer B, Bonini BM, Quezada H, Tsytlonok M, et al. Fructose-1,6-bisphosphate couples glycolytic flux to activation of Ras. *Nat Commun*. 2017;8(1):922.
18. Teoh SL, Das S. Tumour biology of obesity-related cancers: understanding the molecular concept for better diagnosis and treatment. *Tumour Biol*. 2016;37(11):14363-80.
19. Moore LL, Chadi S, Singer MR, Kreger BE, Denis GV. Metabolic health reduces risk of obesity-related cancer in framingham study adults. *Cancer Epidemiol Biomarkers Prev*. 2014;23(10):2057-65.
20. Huxley R, Barzi F, Woodward M. Excess risk of fatal coronary heart disease associated with diabetes in men and women: meta-analysis of 37 prospective cohort studies. *Bmj*. 2006;332(7533):73-8.

21. Lee WL, Cheung AM, Cape D, Zinman B. Impact of diabetes on coronary artery disease in women and men: a meta-analysis of prospective studies. *Diabetes Care*. 2000;23(7):962-8.
22. Salinas-Martinez AM, Flores-Cortes LI, Cardona-Chavarria JM, Hernandez-Gutierrez B, Abundis A, Vazquez-Lara J, et al. Prediabetes, diabetes, and risk of breast cancer: a case-control study. *Arch Med Res*. 2014;45(5):432-8.
23. Onitilo AA, Stankowski RV, Berg RL, Engel JM, Glurich I, Williams GM, et al. Breast cancer incidence before and after diagnosis of type 2 diabetes mellitus in women: increased risk in the prediabetes phase. *Eur J Cancer Prev*. 2014;23(2):76-83.
24. Campbell PT, Deka A, Jacobs EJ, Newton CC, Hildebrand JS, McCullough ML, et al. Prospective study reveals associations between colorectal cancer and type 2 diabetes mellitus or insulin use in men. *Gastroenterology*. 2010;139(4):1138-46.
25. Onitilo AA, Berg RL, Engel JM, Glurich I, Stankowski RV, Williams G, et al. Increased risk of colon cancer in men in the pre-diabetes phase. *PLoS One*. 2013;8(8):e70426.
26. Lega IC, Wilton AS, Austin PC, Fischer HD, Johnson JA, Lipscombe LL. The temporal relationship between diabetes and cancer: A population-based study. *Cancer*. 2016.
27. Drake I. Type 2 diabetes, adiposity and cancer morbidity and mortality risk taking into account competing risk of noncancer deaths in a prospective cohort setting. 2017;141(6):1170-80.
28. Colmers IN, Majumdar SR, Yasui Y, Bowker SL, Marra CA, Johnson JA. Detection bias and overestimation of bladder cancer risk in type 2 diabetes: a matched cohort study. *Diabetes Care*. 2013;36(10):3070-5.
29. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol*. 2008;61(4):344-9.
30. Nijenhuis-Rosien L, Hendriks SH, Kleefstra N, Bilo HJ, Landman GW. Nationwide diabetes-related lower extremity amputation rates in secondary care treated patients with diabetes in the Netherlands (DUDE-7). *Journal of diabetes and its complications*. 2017;31(4):675-8.
31. Boyle P, Parkin D. Statistical methods for registries [Available from: <http://www.iarc.fr/en/publications/pdfs-online/epi/sp95/sp95-chap11.pdf>].
32. Naing NN. Easy Way to Learn Standardization : Direct and Indirect Methods. *Malays J Med Sci*. 2000;7(1):10-5.
33. Lipscombe LL, Goodwin PJ, Zinman B, McLaughlin JR, Hux JE. Increased prevalence of prior breast cancer in women with newly diagnosed diabetes. *Breast Cancer Res Treat*. 2006;98(3):303-9.
34. Colditz GA, Willett WC, Rotnitzky A, Manson JE. Weight gain as a risk factor for clinical diabetes mellitus in women. *Annals of Internal Medicine*. 1995;122(7):481-6.
35. Gallagher EJ, LeRoith D. Obesity and Diabetes: The Increased Risk of Cancer and Cancer-Related Mortality. *Physiol Rev*. 2015;95(3):727-48.
36. Michels KB, Solomon CG, Hu FB, Rosner BA, Hankinson SE, Colditz GA, et al. Type 2 diabetes and subsequent incidence of breast cancer in the Nurses' Health Study. *Diabetes Care*. 2003;26(6):1752-8.
37. Protani M, Coory M, Martin JH. Effect of obesity on survival of women with breast cancer: systematic review and meta-analysis. *Breast Cancer Res Treat*. 2010;123(3):627-35.
38. Chan DS, Vieira AR, Aune D, Bandera EV, Greenwood DC, McTiernan A, et al. Body mass index and survival in women with breast cancer-systematic literature review and meta-analysis of 82 follow-up studies. *Ann Oncol*. 2014;25(10):1901-14.
39. Maruthur NM, Bolen S, Brancati FL, Clark JM. Obesity and mammography: a systematic review and meta-analysis. *J Gen Intern Med*. 2009;24(5):665-77.
40. Friedman AM, Hemler JR, Rossetti E, Clemow LP, Ferrante JM. Obese women's barriers to mammography and pap smear: the possible role of personality. *Obesity (Silver Spring)*. 2012;20(8):1611-7.
41. Screening CfP. Breast Cancer Screening Factsheet. In: RIVM, editor.
42. RIVM. Landelijke Evaluatie Bevolkingsonderzoek Baarmoederhalskanker - LEBA rapportage tot en met 2014. In: RIVM, editor.

43. Chan W, Yun L, Austin PC, Jaakkimainen RL, Booth GL, Hux J, et al. Impact of socio-economic status on breast cancer screening in women with diabetes: a population-based study. *Diabet Med*. 2014;31(7):806-12.
44. Louwman WJ, van de Poll-Franse LV, Fracheboud J, Roukema JA, Coebergh JW. Impact of a programme of mass mammography screening for breast cancer on socio-economic variation in survival: a population-based study. *Breast Cancer Res Treat*. 2007;105(3):369-75.
45. Aarts MJ, Voogd AC, Duijm LE, Coebergh JW, Louwman WJ. Socioeconomic inequalities in attending the mass screening for breast cancer in the south of the Netherlands--associations with stage at diagnosis and survival. *Breast Cancer Res Treat*. 2011;128(2):517-25.
46. Derks IP, Koster A, Schram MT, Stehouwer CD, Dagnelie PC, Groffen DA, et al. The association of early life socioeconomic conditions with prediabetes and type 2 diabetes: results from the Maastricht study. *Int J Equity Health*. 2017;16(1):61.
47. Puolakka E, Pahkala K, Laitinen TT, Magnussen CG, Hutri-Kahonen N, Tossavainen P, et al. Childhood Socioeconomic Status in Predicting Metabolic Syndrome and Glucose Abnormalities in Adulthood: The Cardiovascular Risk in Young Finns Study. *Diabetes Care*. 2016;39(12):2311-7.
48. Tao X, Li J, Zhu X, Zhao B, Sun J, Ji L, et al. Association between socioeconomic status and metabolic control and diabetes complications: a cross-sectional nationwide study in Chinese adults with type 2 diabetes mellitus. *Cardiovasc Diabetol*. 2016;15:61.
49. Harding JL, Shaw JE, Peeters A, Cartensen B, Magliano DJ. Cancer risk among people with type 1 and type 2 diabetes: disentangling true associations, detection bias, and reverse causation. *Diabetes Care*. 2015;38(2):264-70.
50. De Bruijn KMJ, Ruiter R, de Keyser CE, Hofman A, Stricker BH, van Eijck CHJ. Detection bias may be the main cause of increased cancer incidence among diabetics: Results from the Rotterdam Study. *European journal of cancer*. 2014;50(14):2449-55.
51. Johnson JA, Carstensen B, Witte D, Bowker SL, Lipscombe L, Renehan AG, et al. Diabetes and cancer (1): evaluating the temporal relationship between type 2 diabetes and cancer incidence. *Diabetologia*. 2012;55(6):1607-18.
52. Suissa S, Azoulay L. Metformin and the risk of cancer: time-related biases in observational studies. *Diabetes Care*. 2012;35(12):2665-73.
53. Oberaigner W, Ebenbichler C, Oberaigner K, Juchum M, Schonherr HR, Lechleitner M. Increased cancer incidence risk in type 2 diabetes mellitus: results from a cohort study in Tyrol/Austria. *BMC Public Health*. 2014;14:1058.
54. Verlato G, Zoppini G, Bonora E, Muggeo M. Mortality from site-specific malignancies in type 2 diabetic patients from Verona. *Diabetes Care*. 2003;26(4):1047-51.
55. Tsilidis KK, Kasimis JC, Lopez DS, Ntzani EE, Ioannidis JPA. Type 2 diabetes and cancer: umbrella review of meta-analyses of observational studies. *BMJ : British Medical Journal*. 2015;350.
56. Bansal D, Bhansali A, Kapil G, Undela K, Tiwari P. Type 2 diabetes and risk of prostate cancer: a meta-analysis of observational studies. *Prostate Cancer Prostatic Dis*. 2013;16(2):151-8, s1.
57. Lauby-Secretan B, Scoccianti C, Loomis D, Grosse Y, Bianchini F, Straif K. Body Fatness and Cancer — Viewpoint of the IARC Working Group. *New England Journal of Medicine*. 2016;375(8):794-8.
58. Kleefstra N, Landman GW, Van Hateren KJ, Meulepas M, Romeijnnders A, Rutten GE, et al. Dutch diabetes prevalence estimates (DUDE-1). *J Diabetes*. 2016;8(6):863-5.
59. Baan CA, van Baal PH, Jacobs-van der Bruggen MA, Verkley H, Poos MJ, Hoogenveen RT, et al. [Diabetes mellitus in the Netherlands: estimate of the current disease burden and prognosis for 2025]. *Ned Tijdschr Geneesk*. 2009;153:A580.

7

Within-class differences in cancer risk for sulfonylurea treatments in patients with type 2 diabetes (ZODIAC-55) – a study protocol

Published as:

Schrijnders, D, de Bock GH, Houweling ST, van Hateren KJJ, Groenier KH, Johnson JA, Bilo HJG, Kleefstra N, Landman GWD. (2017). Within-class differences in cancer risk for sulfonylurea treatments in patients with type 2 diabetes (ZODIAC-55) - a study protocol. *BMC cancer*, 17(1), 444. doi:10.1186/s12885-017-3433-z

ABSTRACT

Background:

Patients with type 2 diabetes (T2D) are at increased risk for developing cancer. As approximately 8% of the world's population is living with T2D, even a slight increase in cancer risk could result in an enormous impact on the number of persons developing cancer. In addition, several glucose lowering drug classes for treating patients with T2D have been associated with a difference in risk of cancer overall, and especially for obesity related cancers. In what way and to what degree cancer risk is modified by the use of different sulfonylureas (SU) is unclear. The primary aim of this study will be to evaluate within-class SU differences in obesity related cancer risk. Secondary aims will be to investigate within-class SU differences in risk for all cancers combined and site-specific cancers separately (i.e. breast, colorectal, prostate, bladder and lung cancer) and to account for duration-response relationships between individual SU use and cancer risk.

Methods:

Patients will be selected from a Dutch primary care cohort of patients with T2D linked with the Dutch Cancer Registration (ZODIAC-NCR). Within this cohort study annually collected clinical data (e.g. blood pressure, weight, HbA1c) and nationwide data on cancer incidence are available. Time-dependent cox proportional hazard analyses will be performed to evaluate SU cancer risk, adjusted for potential confounders.

Discussion:

This study will be the first prospective cohort study investigating within-class SU differences in cancer risk and could contribute to improved decision making regarding the individual drugs within the class of SUs, and possibly improve quality of life and result in an increased cost-effectiveness of healthcare in patients with T2D.

Trial Registration:

Nederlands Trialregister (www.trialregister.nl), NTR6166, 6 Jan 2017.

BACKGROUND

Patients diagnosed with type 2 diabetes (T2D) are at increased risk for developing cancer; especially the risk of obesity-related cancers (1-5). According to the most recent World Cancer Research Fund (WRCF) definitions obesity related cancers include oesophageal cancer, liver cancer, kidney cancer, stomach cardia cancer, colorectal cancer, advanced prostate cancer, post-menopausal breast cancer, gallbladder cancer, pancreatic cancer, ovarian cancer and endometrial cancer (6).

In addition to T2D and obesity, glucose-lowering agents used in the treatment of T2D have also been associated with cancer risk and some studies have reported that these relations can be drug specific. For example, the use of pioglitazone, not rosiglitazone, has been linked to the development of bladder cancer in some studies (7, 8), although the robustness of the evidence underlying this possible relationship remains unclear and was absent in recent reports (9, 10). Also, insulin glargine has been linked to higher breast cancer risk in some studies (11), although - again - several studies reported no or even an inverse association (11-13). Metformin has more consistently been associated with a decreased cancer risk (14), however concerns have been raised that this association might have been influenced by several types of bias (15, 16).

The sulfonylureas (SUs) are one out of six classes of oral glucose-lowering agents advised by the EASD and ADA as a second step when the glycaemic treatment targets are not reached with metformin mono-therapy (17). Sulfonylureas have been available for many years and are highly efficacious at low costs. In the Dutch primary care treatment guideline for T2D, gliclazide is the preferred SU, as opposed to both the ADA and EASD which do not recommend a specific SU (17). Previous studies have shown that within the class of SUs differences exist with regard to hypoglycaemia risk (18), for example there have been no reports of severe hypoglycaemia events in gliclazide users (18). In addition, within-class differences in risk of cardiovascular events and safety when prescribed to patients with renal failure have been reported (19, 20).

An association between the class of SUs and increased overall cancer risk has also been reported (21-24). Most previous studies are limited by methodological issues, for example many studies reported baseline SU use and did not account for duration of SU use (22-24). There is also evidence suggesting within-class SU differences in cancer risk, where gliclazide use has been associated with a lower cancer risk (21-24). There are several potential mechanistic explanations, one of which could be that gliclazide leads

to a more selective glucose dependent insulin response and lower insulin levels. In what way and to what degree cancer risk is modified by different SUs is unclear and requires further investigation and confirmation.

Most evidence, however, is derived from small observational cohort studies and substantial knowledge gaps exist. This also holds true for the presumed favourable long-term cancer safety profile of gliclazide in particular. The relations between use of glucose lowering agents and cancer are complex and there is overlap in risk factors; for example, several glucose lowering agents have been associated with weight gain, which in itself has also been related to an increased cancer risk.

The primary aim of this study is to evaluate within-class SU differences in risk for obesity-related cancer (excluding non-melanoma skin cancers) accounting for weight changes during follow-up and drug exposure (6). Secondary aims are to evaluate within-class SU differences concerning all cancers combined and the cancer risks of the five largest groups of site-specific cancers (breast, colorectal, prostate, bladder and lung cancer) accounting for duration of drug use.

METHODS/DESIGN

Data source

This study will be conducted using a combined database of the ZODIAC (Zwolle Outpatient Diabetes project Integrating Available Care) study and NCR (Dutch National Cancer Registration).

ZODIAC

The ZODIAC cohort is part of an ongoing primary care prospective study initiated in 1998, in which annually collected data are used for care improvement, benchmarking and research (25). Patients consented with the anonymous use of their data for study purposes. Patients included are diagnosed with T2D and are exclusively treated in primary care in a shared care setting. Data on age, sex, date of T2D diagnosis, HbA1c, length, weight, estimated GFR, creatinine, albumin-creatinine ratio (ACR), cholesterol/HDL ratio, blood pressure, macrovascular complications (myocardial infarction, TIA, CVA) medication use (both diabetes-specific and other medication), smoking (yes/no) and alcohol use (yes/no) are recorded.

NCR

The Netherlands Cancer Registration (NCR) was founded in 1989 and has since recorded almost every cancer event in the Netherlands, and includes incidence date, TNM (tumour, node, metastasis) stage, morphology, location and the therapy received (26). Basal cell carcinoma of the skin, carcinoma in situ of the cervix, myelodysplastic syndrome and myeloproliferative disorders are all excluded for the NCR database. Benign and borderline tumours are excluded with the following exceptions; benign brain tumours (included from 1999), carcinoids of the appendix (included from 2001), borderline tumours of the ovaries (included from 2001), thymoma (included from 2001), phyllodes tumours (included from 2001) and T-cell leukaemia (included from 2004).

Study population

Combined ZODIAC-NCR cohort

All cancer events that occurred between 1 January 1989 and 2012 were linked to the data of the ZODIAC study via a trusted third party using postal code, full name, date of birth and sex. The medical ethics committee of Isala, Zwolle, the Netherlands approved the linkage of ZODIAC and NCR (METC reference number 13.0765). The NCR expected that the number of false-positive and the number false-negative linkage is both under 1%. By combining the two databases a cohort composed of patients diagnosed with T2D between 1 January 1998 and 31 December 2012 was assembled.

Patient selection

The study cohort entry date and baseline date will be the date the patients started participation in the ZODIAC cohort.

Inclusion

All patients included will be participating in the ZODIAC-NCR cohort on or after January 1998 and will be users of SUs.

Exclusion

Patients treated with long-acting or mixed insulin before oral glucose lowering agents and those receiving insulin on top of SU at study entry will be excluded. Patients who received a cancer diagnosis before receiving a SU will be excluded. For the main analyses patients who switch medication within the class of SUs will be excluded at the time the switch occurs.

Follow-up

All patients will be followed from the year of cohort entry until a diagnosis of cancer (3-5). Patients with no diagnosis of cancer will be censored at the time of death, end of registration within the ZODIAC cohort or end of the study period (31 December 2012), whichever occurred first.

Study endpoints

The primary outcome will be within-class SU difference in obesity-related cancer risk (see table 1 for included cancers). The secondary outcomes will be all cancer risk (table 2), site-specific cancer risk and the presence of a duration response relationship between SU use and cancer. Cancer sites of special interest will be specific cancers of the breast, colorectal, bladder, advanced prostate and lung cancer. Study endpoints will be evaluated for men and women separately.

Table 1. Cancers included in primary endpoint obesity related cancer.

Men	Women
Oesophageal (adenocarcinoma)	Oesophageal (adenocarcinoma)
Stomach cardia	Stomach cardia
Kidney	Kidney
Gallbladder	Gallbladder
Liver	Liver
Pancreatic	Pancreatic
Colorectal	Colorectal
Prostate cancer	Ovarian
	Breast
	Endometrial

Table 2. Cancer excluded from secondary endpoint all cancer risk

Men (cancers excluded)	Women (cancers excluded)
Non-melanoma skin cancer	Non-melanoma skin cancer
Breast	Prostate
Ovarian	Male genital organs
Endometrial	
Female genital organs	

Exposure

Patients will be considered unexposed to SUs until the time of the first SU prescription within ZODIAC. A one-year lag period will be accounted for. A lag period is necessary to take into account a latency time window and to minimise possible detection bias around the time of treatment initiation. Exposure to a SU will be classified according to one of the following, mutually exclusive, categories: gliclazide use, glimepiride use, tolbutamide use, glibenclamide use, non-SU use.

We aim to determine whether there are duration-response relationships between the use of SUs and obesity-related cancer incidence. Duration-response will be assessed in terms of cumulative duration of use, defined as the total number of years of use calculated by summing the durations of yearly prescriptions received between cohort entry and the time of the event and will be used as a time-dependent covariate.

Co-variables

Co-variables collected at cohort entry and annually thereafter are: age, sex, year of cohort entry, HbA1c (continuous), diabetes duration (time between diabetes diagnosis and cohort entry, continuous), BMI (continuous), serum creatinine (continuous), metformin use (yes, no), insulin use (yes/no), history of cancer (no non-melanoma skin cancer) (yes, no) and smoking (ever, never, unknown).

Primary analysis

Descriptive statistics will be used to characterize the patients at cohort entry. Time dependent cox proportional hazard analyses will be used to estimate the adjusted hazard ratio of developing obesity-related cancer when using gliclazide compared to other SUs (both individual and grouped as non-gliclazide SU). Exposure to SUs will be included as the cumulative number of years exposed to a specific SUs. Exposure status for SU will be updated annually. The primary analyses will be corrected for the previously mentioned confounders measured at baseline.

Secondary analyses

In a secondary analyses changes diabetes medication during follow-up will be accounted for. Time dependent cox proportional hazard analyses will be used to estimate the adjusted hazard ratio of developing all cancer when using gliclazide compared to other SUs (both individual and grouped as non-gliclazide SU). Exposure to confounders (including concurrent metformin and insulin use) will be handled as time varying variables where

follow-up is available. The updated mean method will be used for HbA1c, BMI and serum creatinine. These analyses will be repeated to investigate the adjusted hazard ratio of breast, colorectal, prostate, bladder and lung cancer.

Missing data

When appropriate, in case of missing data multiple imputation will be used. In case multiple imputation cannot be used (e.g. data are not missing at random or missing completely at random), the updated means method will be used. The updated mean method averages the baseline values with the mean annual values (27). The updated mean method is similar to the technique used in the UKPDS (28). When calculating the updated means, we will allow a maximum of 2 consecutive years to be missing, with a maximum of 3 years in the complete follow-up.

Subgroup analyses

In subgroup analyses effects of exposure to BMI and HbA1c during follow-up and the relation between SUs and cancer will be investigated and interaction will be tested. A second subgroup analysis will investigate cancer risk in patients who do and do not use metformin in combination with an SU.

Sensitivity analyses

Six sensitivity analyses will be planned for supporting the main analyses. Firstly, because the latency window is uncertain, the primary analyses for within-class differences will be repeated with lag periods of zero and two years. Secondly, the primary analysis will be repeated but the adjusting confounders will be measured at the year before first SU prescription. Thirdly, the main analysis will be repeated but with the exclusion of cancer events 1 year after initiation of a SU. Fourthly, to investigate the accuracy of our results the analysis will be repeated in patients in whom all data on medication are complete. Fifthly, to investigate the accuracy of our results the analysis will be repeated in patients who have no missing data on HbA1c, BMI and serum creatinine. Sixthly, to quantify the effect of patients switching medication within the class of SUs, an intention-to-treat analysis for patients who switch SUs will be performed.

DISCUSSION

This study will be the first large observational cohort study investigating differences in cancer risk within the class of SUs. An estimated 8% of the global population is known with T2D (29) and this could translate into an increased risk of cancer for a substantial amount of people. The prevalence of T2DM is expected to rise evermore, at least for the next decades (30). A minimal change in cancer risk could result in a substantial change in the relative and even absolute number of patients diagnosed with cancer. If this study confirms the presence of within-class SU cancer risk differences, it could help patients and physicians in making a shared decision for a specific SU. This could contribute to quality of life of the patients as well as contribute to increasing effective care and cost-effectiveness of healthcare. If no differences are present, the safety, efficacy, and cost of SU will remain the only criteria for selecting the best SU.

REFERENCES

1. Carstensen B, Jorgensen ME, Friis S. The epidemiology of diabetes and cancer. *Current diabetes reports*. 2014;14(10):535.
2. Johnson JA, Bowker SL, Richardson K, Marra CA. Time-varying incidence of cancer after the onset of type 2 diabetes: evidence of potential detection bias. *Diabetologia*. 2011;54(9):2263-71.
3. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, et al. Diabetes and cancer: a consensus report. *Diabetes care*. 2010;33(7):1674-85.
4. Tobias DK, Pan A, Jackson CL, O'Reilly EJ, Ding EL, Willett WC, et al. Body-mass index and mortality among adults with incident type 2 diabetes. *The New England journal of medicine*. 2014;370(3):233-44.
5. Vazquez G, Duval S, Jacobs DR, Jr., Silventoinen K. Comparison of body mass index, waist circumference, and waist/hip ratio in predicting incident diabetes: a meta-analysis. *Epidemiologic reviews*. 2007;29:115-28.
6. World Cancer Research Fund. Cancers linked with greater body fatness [Webpage]. 2015 [Available from: <http://www.wcrf.org/int/cancer-facts-figures/link-between-lifestyle-cancer-risk/cancers-linked-greater-body-fatness>].
7. Lewis JD, Ferrara A, Peng T, Hedderson M, Bilker WB, Quesenberry CP, Jr., et al. Risk of bladder cancer among diabetic patients treated with pioglitazone: interim report of a longitudinal cohort study. *Diabetes care*. 2011;34(4):916-22.
8. Tuccori M, Filion KB, Yin H, Yu OH, Platt RW, Azoulay L. Pioglitazone use and risk of bladder cancer: population based cohort study. *BMJ (Clinical research ed)*. 2016;352:i1541.
9. Lewis JD, Habel LA, Quesenberry CP, Strom BL, Peng T, Hedderson MM, et al. Pioglitazone Use and Risk of Bladder Cancer and Other Common Cancers in Persons With Diabetes. *Jama*. 2015;314(3):265-77.
10. Korhonen P, Heintjes EM, Williams R, Hoti F, Christopher S, Majak M, et al. Pioglitazone use and risk of bladder cancer in patients with type 2 diabetes: retrospective cohort study using datasets from four European countries. *BMJ (Clinical research ed)*. 2016;354:i3903.
11. Wu JW, Filion KB, Azoulay L, Doll MK, Suissa S. The Effect of Long-Acting Insulin Analogs on the Risk of Cancer: A Systematic Review of Observational Studies. *Diabetes care*. 2016.
12. Warren H, Raison N, Dasgupta P. Pioglitazone and bladder cancer. *BJU international*. 2016;118(1):16-7.
13. Peeters PJ, Bazelier MT, Leufkens HG, Auvinen A, van Staa TP, de Vries F, et al. Insulin glargine use and breast cancer risk: Associations with cumulative exposure. *Acta oncologica*. 2016;55(7):851-8.
14. Thakkar B, Aronis KN, Vamvini MT, Shields K, Mantzoros CS. Metformin and sulfonylureas in relation to cancer risk in type II diabetes patients: a meta-analysis using primary data of published studies. *Metabolism: clinical and experimental*. 2013;62(7):922-34.
15. Mamtani R, Pfanzer N, Haynes K, Finkelman BS, Wang X, Keefe SM, et al. Incidence of bladder cancer in patients with type 2 diabetes treated with metformin or sulfonylureas. *Diabetes care*. 2014;37(7):1910-7.
16. Suissa S, Azoulay L. Metformin and cancer: mounting evidence against an association. *Diabetes care*. 2014;37(7):1786-8.
17. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, et al. Management of hyperglycaemia in type 2 diabetes, 2015: a patient-centred approach. Update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetologia*. 2015;58(3):429-42.
18. Landman GW, de Bock GH, van Hateren KJ, van Dijk PR, Groenier KH, Gans RO, et al. Safety and efficacy of gliclazide as treatment for type 2 diabetes: a systematic review and meta-analysis of randomized trials. *PloS one*. 2014;9(2):e82880.

19. Schramm TK, Gislason GH, Vaag A, Rasmussen JN, Folke F, Hansen ML, et al. Mortality and cardiovascular risk associated with different insulin secretagogues compared with metformin in type 2 diabetes, with or without a previous myocardial infarction: a nationwide study. *European heart journal*. 2011;32(15):1900-8.
20. Arnouts P, Bolignano D, Nistor I, Bilo H, Gnudi L, Heaf J, et al. Glucose-lowering drugs in patients with chronic kidney disease: a narrative review on pharmacokinetic properties. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*. 2014;29(7):1284-300.
21. Bo S, Castiglione A, Ghigo E, Gentile L, Durazzo M, Cavallo-Perin P, et al. Mortality outcomes of different sulphonylurea drugs: the results of a 14-year cohort study of type 2 diabetic patients. *European journal of endocrinology / European Federation of Endocrine Societies*. 2013;169(1):117-26.
22. Monami M, Balzi D, Lamanna C, Barchielli A, Masotti G, Buiatti E, et al. Are sulphonylureas all the same? A cohort study on cardiovascular and cancer-related mortality. *Diabetes/metabolism research and reviews*. 2007;23(6):479-84.
23. Monami M, Lamanna C, Balzi D, Marchionni N, Mannucci E. Sulphonylureas and cancer: a case-control study. *Acta diabetologica*. 2009;46(4):279-84.
24. Yang X, So WY, Ma RC, Yu LW, Ko GT, Kong AP, et al. Use of sulphonylurea and cancer in type 2 diabetes-The Hong Kong Diabetes Registry. *Diabetes research and clinical practice*. 2010;90(3):343-51.
25. van Hateren KJ, Drion I, Kleefstra N, Groenier KH, Houweling ST, van der Meer K, et al. A prospective observational study of quality of diabetes care in a shared care setting: trends and age differences (ZODIAC-19). *BMJ open*. 2012;2(4).
26. NCR. About the registration [Available from: <http://www.cijfersoverkanker.nl/about-the-registration-37.html>].
27. van Hateren KJ, Landman GW, Kleefstra N, Groenier KH, Kamper AM, Houweling ST, et al. Lower blood pressure associated with higher mortality in elderly diabetic patients (ZODIAC-12). *Age and ageing*. 2010;39(5):603-9.
28. Adler AI, Stratton IM, Neil HA, Yudkin JS, Matthews DR, Cull CA, et al. Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes (UKPDS 36): prospective observational study. *BMJ (Clinical research ed)*. 2000;321(7258):412-9.
29. WHO. Global status report on noncommunicable diseases 2014. WHO; 2014.
30. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes research and clinical practice*. 2010;87(1):4-14.

8

Summarizing discussion

SUMMARY OF MAIN FINDINGS

Type 2 diabetes (T2D) affects many people worldwide and its prevalence is expected to increase in the next decades ¹⁻³. Cancer, also highly prevalent ^{4,5}, occurs more frequently in patients with T2D (Standardized incidence ratio (SIR) men 1.08, 95%CI 1.07-1.09, women 1.22 95%CI 1.20-1.23) ⁶. Insulin resistance is an important characteristic of T2D. Age, obesity and hyperinsulinemia are related to insulin resistance. Insulin resistance is one of the possible etiologic mechanisms in the relationship between T2D and (obesity-related) cancer.

In recent years, an increasing amount of studies have been conducted investigating the effect of glucose-lowering medication on cancer risk. There is a possible positive relationship between the class of sulphonylureas (SU) as a whole and the risk of cancer ⁷. Results from several small observational studies suggested that within-class differences in cancer risk could exist within the SU class ⁸⁻¹¹.

The general aim of this thesis was to investigate cancer risk in a Dutch primary care diabetes population, with special attention for possible within-class differences when using a SU derivate. For this purpose, a large prospective Dutch primary care treated diabetes population was studied.

A systematic literature review on potential mechanisms behind the relationship between SU use and cancer was performed. In an observational cohort, potential factors that potentially can confound the relationship between SU and cancer were explored in patients diagnosed with T2D.

In **chapter 2**, a systematic review was conducted reviewing clinical and preclinical literature regarding the relationship between the various SUs and cancer (risk).

Results from preclinical studies showed potential anti-cancer effects of SUs on cancer cell growth, cell transporters and channels, growth factor pathways, DNA damage, blood vessel formation and metastases. When assessing the association between different SUs and cell growth in different cell lines, the results lead to unequivocal conclusions in the various preclinical studies.

Out of 228 initially identified records of clinical studies, only six were eligible for further analysis and comparison. The clinical studies showed that T2D patients using gliclazide seemed to have a lower cancer risk compared to T2D patients using other SUs. In addition, patients using glibenclamide had an increased cancer risk compared to patients using tolbutamide and gliclazide. Unfortunately, no meta-analysis could be performed because the methodology of the studies varied too much to allow pooling of the data; different SUs were compared, different study designs were used, and different

statistical methods were applied. Pre-clinical evidence for gliclazide was limited, and most evidence was found for glibenclamide. The anti-cancer effects of glibenclamide reported in the preclinical studies was not confirmed in observational clinical and epidemiological studies. Results of three of the four studies investigating glibenclamide suggested that the use of glibenclamide was associated with an increased risk of cancer¹². For other SUs, no conclusive data was available, thus precluding firm conclusions with regards to gliclazide, glimepiride, and tolbutamide. Part of this thesis addresses the effects of these molecules.

In **Chapter 3**, the time between SU initiation and the need for treatment intensification with insulin was investigated. The moment that glycaemic control worsens and treatment intensification is needed, the addition of once-daily insulin could be regarded as an indicator of disease progression and increasing insulin resistance^{13–15}. This assessment was performed for a variety of reasons. First, even when starting SU treatment with the lowest effective dose, dose increases will be necessary, thus contributing to exposure to higher cumulative doses. Ultimately, after reaching the maximum allowed daily dose, insulin can be added. In this study, insulin initiation was partly considered to be the surrogate endpoint for use (at that point) of the maximum (tolerated) SU dose. This would translate into a lower cumulative SU exposure; the exact consequences of such a finding are unclear. Moreover, insulin treatment will (in general) lead to higher insulin concentrations compared to oral treatment. Higher insulin concentrations are associated with a higher cancer risk¹⁶. In addition, when SU treatment failure occurs, the earlier the failure, the earlier there will be exposure to higher HbA1c levels. Higher HbA1cs have also been associated with increased cancer risk^{17,18}.

In total, 3507 patients starting a SU in addition to metformin were included (gliclazide 643, glimepiride 1178, tolbutamide 1686). Using metformin/gliclazide as a reference, there were neither differences in time to insulin in the metformin/glimepiride group (HR 1.10, 95%CI 0.78-1.54) nor in the metformin/tolbutamide group (HR 0.93, 95%CI 0.67-1.30).

Therefore, and with some hesitation, it might be concluded that, since there were no differences in time to insulin initiation between SUs, factors like exposure to the effects of higher HbA1cs, and changes in insulin concentrations can be considered comparable between the different SUs. Of course, the total cumulative dose has not been assessed, and since equipotency of SU is related to glucose levels attained and not to total milligram dose of use, this point remains open. The results, as described in this study, suggest that at least the duration of SU exposure and insulin treatment-induced changes in insulin concentrations are comparable within SUs.

In **chapter 4**, the presence of within-class differences in body weight trajectories in new users of SUs in a Dutch T2D cohort was investigated. Being overweight and obese has been associated with the development of certain specific cancer types¹⁹. Within-class SU differences in body weight trajectories after the start of SUs can therefore confound a possible relationship between SU and cancer. Cancers of the oesophagus, stomach (cardia), colorectal, liver, gallbladder, pancreas and kidney have been associated with obesity in men and women in the general population¹⁹. Significant weight changes were observed neither within individual SUs ($p = 0.24$) nor between the different SUs ($p = 0.26$). Baseline weight (kilogram) was 91.8 (90.3-93.4) for the gliclazide group, 92.2 (86.2-98.2) for glibenclamide group, 91.3 (90.2-92.4) for the glimepiride group and 89.4 (88.5-90.3) for the tolbutamide group. The year after addition of a SU weights were 91.9 (90.3-93.5), 92.4 (85.7-99.1), 91.3 (90.2-92.4) and 89.7 (88.8-90.6), respectively. None of these weight changes was significant. The average HbA1c decreased from 55 mmol/mol before intensification to 53 mmol/mol after intensification with a SU. It was thus possible to maintain good glycaemic control with SU as second-line therapy without the offset of relevant weight changes in this Dutch Cohort.

Therefore, in this cohort, there was no indication that the relationship between different SUs and cancer is potentially confounded by differences in weight changes after SU initiation because new use of any SU was not associated with relevant weight changes.

In the study in **chapter 5**, the relationship between BMI and obesity-related cancer in men and women was investigated. In 25,811 men, 689 obesity-related cancers were identified. In this study, BMI was associated with obesity-related cancers in men with T2D (HR 1.12 95%CI 1.02 to 1.23), except for advanced prostate cancer (HR 0.93 95%CI 0.75 to 1.16). In women, 24,989 patients were included and 914 were diagnosed with obesity-related cancer. BMI was associated with obesity-related cancers (HR (per 5 kg/m² increase) 1.15 95%CI 1.08 to 1.22) and with sex-specific obesity-related cancers in women with T2D (HR (per 5 kg/m² increase) 1.22, 95%CI 1.14 to 1.32).

In conclusion, this study further enhances earlier findings in other studies with regards to the relation of various cancers with BMI. As such, one has also to keep an open eye for the possibility, that the increased cancer occurrence in patients with T2D is to a large extent due to the higher BMIs in patients with T2D. Moreover, because of the findings in this study, there are reasons to reconsider the assumption that advanced prostate cancer is obesity-related. It also shows that individual cancers provide individual cancer risk and should also be analysed separately.

The study confirms the association between BMI and obesity-related cancer and sex-specific cancers (ovarian, endometrial, postmenopausal breast cancer) in women with T2D. However, in women, the group of non-sex specific obesity-related cancers was not

associated with BMI. This could imply that in women with T2D, BMI is not a risk factor for this group of cancers, in contrast to men with T2D. No proper explanation for this finding could be formulated.

Previous studies have suggested that the incidence of cancer around the diabetes diagnosis is increased compared to cancer incidence in the general public. In **Chapter 6**, the results show that in both men and women a peak in excess obesity-related cancer incidence exists at the time of diabetes diagnosis (SIR 1.38; 95%CI 1.11 to 1.64 and SIR 2.21; 95%CI 1.94 to 2.30, respectively). Women, as opposed to men, already had an excess obesity-related cancer incidence five years before diabetes diagnosis (SIR 1.77; 95%CI: 1.63 to 1.91). After the diabetes diagnosis, women continue to have an excess obesity-related cancer incidence. In men, obesity-related cancer incidence decreases after diabetes diagnosis to a non-significant elevated incidence compared to the general public. These results could imply that at least part of the relative advantage of women in the general population concerning cancer risk is lost in women with T2D; a comparable phenomenon has been observed for cardiovascular disease ^{20,21}.

GENERAL DISCUSSION

The relationship between T2D, cancer and diabetes medication is complicated, and there are many different possible pathophysiological mechanisms, confounding factors and biases.

The general aim of this thesis was to investigate cancer risk in a Dutch primary care diabetes population, with special attention for possible within-class differences when using a SU derivate. For this purpose, a large prospective Dutch primary care treated diabetes population was studied.

Methodological reflection: confounders

Describing the relationship between SU, T2D and cancer risk in a primary care cohort with T2D patients can be complicated by confounding and bias. Cancer risk, as well as T2D, can be influenced by an unhealthy diet and insufficient physical activity, through food content, but also through insulin resistance. This can be independent of medication use but also mediated by medication. Only a few studies evaluating the relationships between SU use and cancer risk accounted for important confounding factors and bias ^{22–24}. Especially time-dependent adjusting for confounding factors is lacking in most previous studies in this field ^{22–24}.

Body weight

One of the most important confounders in the relationship between T2D and cancer is body weight, in particular obesity ¹⁶. It has been hypothesised that certain specific subtypes of cancer are responsive to the metabolic conditions found in T2D ¹⁶. A majority of patients diagnosed with T2D are either overweight or obese, and obesity is strongly related to insulin resistance syndrome. Furthermore, it has been shown that insulin resistance is associated with both low and high levels of IGF-1 in a U-shaped manner ²⁵. IGF-1 has been identified as a growth factor for cancer and high IGF-1 levels in insulin resistance could, therefore, contribute to cancer risk and growth ²⁶. Especially visceral (abdominal) fat has been associated with increased insulin resistance ^{27–29}. Previous studies reported that SU use is associated with weight gain ^{30,31}.

The results of **chapter 4** show that - in a primary care Dutch group of patients with T2D - SU use (on top of metformin) was associated with neither a clinically important nor significant weight gain, and that at the same time adequate glycaemic control can be maintained in a primary care setting. This suggests that when weight gain does occur, other causes like lifestyle factors might be more important to explain weight gain than SU initiation ³².

This observation is in contrast with previous assumptions and observations in other studies ^{33,34}. Up until now, it has been generally reported that SU use is associated with clinically significant weight gain ^{33,34}. The fact that weight gain does not occur in this primary care cohort is important when investigating cancer risk. Visceral obesity has been associated with adipose tissue inflammation, production of cytokines and changes in adipokines ¹⁶. Both can contribute to insulin resistance in fat, liver and skeletal muscle. The World Cancer Research Fund (WCRF) recognises that oesophageal, stomach (cardia), colorectal, liver, gallbladder, pancreatic, and kidney cancer are related to being overweight or obese in both men and women ¹⁹. Additionally, in women, ovarian, endometrial and postmenopausal breast cancer, and advanced prostate cancer in men are also associated with an increased body weight ¹⁹. Obviously, when a specific SU causes weight gain, this could confound a possible relationship between cancer risk and SU use via alterations in body weight.

Sex

Differences in fat distribution between men and women might explain the reported differences in cancer risks ³⁵. In people without diabetes, fat distribution between men and women is different, with men generally having relatively more visceral fat, and women having more subcutaneous fat ³⁵. Specifically, visceral fat is associated with metabolic

abnormalities that could increase cancer risk ¹⁶. It could be that in women with T2D, BMI does not accurately depict the amount of visceral obesity. In patients with T2D, the difference in visceral fat mass between men and women is reduced, but it is probably still higher in men ³⁶.

Furthermore, adiposity is also associated with increased levels of oestrogen. Insulin resistance has been shown to decrease the synthesis of sex hormone binding globulin ^{37,38}. Moreover, obesity has been associated with an increased aromatisation of androgens to oestrogen. The interaction of these two phenomena leads to higher bioavailability of oestrogen. This could partly contribute to the risk of hormone-dependent breast cancer and endometrial cancer ^{39,40}.

It has been shown that weight loss after bariatric surgery decreases obesity-related cancer risk by 38% ⁴¹. All cancer risk decreased by 27% in women. Intentional weight loss of more than 9kg has also been associated with a decreased obesity-related cancer risk in women (RR 0.79, 95%CI 0.65-0.97).

In conclusion, abnormally increased body weight is a significant factor in modifying obesity-related cancer risk and differs between the sexes.

Methodological reflection on the results reported in this thesis: Bias

In all studies, it is essential to search for and identify bias and try to avoid bias in the design of the study or to adjust for bias in the analysis of the data. Especially observational studies are prone to bias since the selection of patients and the treatments are not at random.

Detection and diagnostic bias are two important types of bias and can influence the interpretation of results. This kind of bias might explain at least partly the findings in chapters 4 and 5. Different studies have shown that the epidemiological association between cancer and diabetes can – at least partly – be explained by detection bias around the time of diabetes diagnosis ^{6,42}. At the time of diagnosis, there is more contact with health care professionals, e.g. to educate patients and evaluate the effects of medication ²³. By having more contact, there are more opportunities for patients to express complaints and for the health care professional to identify (signs of) other diseases. It could also be that patients on e.g. glibenclamide experience more hypoglycaemic events than patients on other SUs ⁴³. This could lead to more encounters with healthcare professionals and possibly more routine diagnostic work-ups in patients using glibenclamide.

Reverse causality can also play a role in falsely assuming a relation between increased cancer risk and individual sulphonylurea use ⁴⁴. Reverse causality needs to be considered when it seems that factor A, for example hyperglycaemia, is associated with factor B, for

example pancreatic cancer. However, the real association is in reverse, i.e. pancreatic cancer is the cause of the hyperglycaemia by a dysfunction in insulin secretion caused by the effect of the tumour on the pancreatic endocrine function. Reverse causality might at least partly explain the findings in the study presented in chapter 6. For example, when pancreatic cancer develops, some of its symptoms might be related to hyperglycaemia. This might then be regarded as T2D, which in due course is accompanied by a diagnosis of pancreatic cancer, which in its turn is (to a large extent) responsible for the hyperglycaemia. It is not caused by the insulin resistance or beta cell deficiency caused by increasing age.

In all analyses, we chose not to include prevalent users but instead applied a new user design. The ZODIAC cohort, however, does include not only new users but also prevalent users. Including **prevalent users** in a study can introduce two kinds of bias. The first is **selection bias**, as prevalent users can be seen as survivors of a previous period of pharmacotherapy. This can be problematic if, as is the case with cancer, the risk varies with time. Also, at study entry covariates of users of a specific drug could be affected by the drug. This might, for example, lead to a different treatment choice in patients with chronic kidney disease. The European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) advises using new-user designs to avoid the biases introduced by using prevalent users ⁴⁵.

Immortal time bias, also known as survivor bias, is a type of selection bias. It occurs when, during a period of time, a certain outcome cannot occur. It has been shown that this type of bias often occurs in observational studies ^{46,47}, and can lead to an illusion of treatment effectiveness. In a review investigating metformin and cancer risk showed that 13 out of the 26 observational studies suffered from immortal time bias ⁴⁸.

The ZODIAC cohort also included patients with previously diagnosed diabetes. In other words, patients could have been diagnosed with diabetes years before they entered the cohort. In Chapter 6, a retrospective analysis into cancer risk is performed prior to diabetes diagnosis. Patients who previously died before the diagnosis could, are not included in the analysis and could lead to an underestimation of cancer-risk. Diabetes-related data was only collected from the moment patients were included in the cohort. Any SU use before inclusion in the cohort can have an effect on cancer risk. However, the date of diabetes diagnosis was reported at inclusion.

Strengths and limitations

A major strength of the studies as presented in this thesis is the inclusion of a large population of patients with T2D treated for their diabetes in primary care. 90% of the patients diagnosed with T2D in the Netherlands are treated in primary care ⁵⁰. Diabetes-

related data was gathered in one prospective database (ZODIAC database: Zwolle Outpatient Diabetes project Integrating Available Care). The diagnosis of T2D was made according to well-defined criteria ^{51,52}, and care is well organised ⁵³. All patients treated in primary care diagnosed with T2D except those with a very short life expectancy were included at baseline (baseline data of specific patients could be in different years, depending on the year of inclusion in the cohort) ⁵³. Data collection on T2D was standardized according to the Dutch Primary Care guideline on T2D ⁵², and the vast majority of data considered to be relevant was rather complete; of those items on which data were collected, data were complete in the majority of items for over 95%; exceptions were information on foot control and eye control, with information available on 89% of the participants). Date of diabetes diagnosis was reported at baseline. BMI was collected over several years and the date of diagnosis of diabetes was recorded by practice nurses at inclusion.

Data on cancer history, incidence and prevalence were obtained through the national cancer database, the IKNL (Integraal Kankercentrum Nederland) registry; this database contains a plethora of relevant information concerning cancer with regard to type, severity, duration, treatment, and outcomes (both morbidity and mortality) of the included patients. However, this database also has limitations. For example, pancreas carcinoma is likely underreported as it is also recorded by the Dutch Pancreatic Cancer Group.

Matching these databases and blinding of individual patients in the new database was performed by an independent organization, resulting in a database with information over a long time-span (IKNL data from 1989, ZODIAC data from 1997, merging of data of both databases until 2014), partly also with information on individuals with regard to cancer events both before and after the diagnosis of T2D.

Strengths can also be limitations at the same time. Including in a study a large, mainly unselected population of patients with T2D with a reasonable life expectancy treated in primary care means, that patients with a short life expectancy and patients with T2D who need very intensive counselling or have a difficult to treat T2D could have been treated in secondary care and therefore were excluded from analysis. The results probably are only generalizable to about 90% of the population and patients with a reasonable to well-regulated glycaemic control. Furthermore, the average HbA1c of this population was below 53 mmol/mol ⁵⁴. Even though about 90% of the patients diagnosed with T2D in the Netherlands are treated in primary care ⁵⁰, this could obviously have some impact. The effect could be an imprecise estimate with regards to actual cancer incidence and prevalence within the total population of patients

with T2D. In general, patients with T2D treated in secondary care will have a more complex disease and multimorbidity, often necessitating a treatment approach different from the standard treatment. This also may be reflected in a different incidence or prevalence of cancer compared to patients with T2D treated in primary care. However, when assessing within-class differences, this impact probably will be limited, unless other – up to now unknown – factors play a role. Part of such a bias can be alleviated by including a well-matched control group in the analyses; this was not possible within the scope of this thesis. Although the ZODIAC dataset is quite comprehensive, no data about socio-economic status (SES) was recorded. It is conceivable that this could play a role in developing T2D, obesity and cancer ^{55–57}.

Another possible limitation is the lack of complete and extensive information with regards to medication, which was collected annually, better would have been monthly, weekly or even daily. The dose of diabetes medication was not available in the ZODIAC dataset. This might hamper a proper evaluation of the effects of specific medication on cancer incidence since cumulative dose might be one of the factors which need to be taken into account.

Another factor which is important for evaluating the relationship between drugs and cancer is exposure time irrespective of dose ^{58,59}. Data on medication were extracted from the primary care electronic records once yearly only, thus leading to exposure times within that specific year theoretically varying from 1 to 364 days. A switch in medication is not noticed until the next annual check-up. A last factor concerning medication to be taken into account is exposure and dosing of other (chronic) medication used for other conditions throughout the years.

In recent years, attention has grown with regards to the effects of lifestyle on the occurrence of both diabetes and cancer. Although BMI and smoking habits were part of the dataset (also longitudinally), no further information on exercise, alcohol and eating habits (for example red meat consumption) was available. In recent analyses, such lifestyle factors were found to have an impact on cardiovascular disease and especially T2D and possibly also cancer occurrence ^{32,60}. In conclusion, data on exercise, alcohol consumption and eating habits could help improve analyses in cancer risk.

Implications for care and possible further research

The results of the various studies presented in this thesis, and specifically the confounding factors described in this thesis, should be taken into account when designing future research investigating the relationship between different treatment modalities and (the development of) cancer. The inclusion of BMI and BMI changes as a confounder in these

relationships is important. Moreover, confounding factors should be adjusted for in a time-dependent manner. To avoid immortal time bias, a Cox proportional hazard model with time-dependent covariates could be used.

The points as described under limitations could be further addressed. Extending research to a secondary line population might add knowledge. Combining data of ZODIAC and IKNL with data derived from a cause-specific mortality registry (CBS) or national reimbursement data information (Vektis) would further enhance the detailing of the available information. Implementing some of the linkages is impossible at the moment in light of the interpretation of the existing privacy law. Taking the existing privacy law into account, in **Chapter 7**, a detailed research proposal is suggested for a possible first follow-up step.

Still, the results again emphasise, that with overweight and obesity combined with diabetes, attention should not only focus on preventing or delaying the risk of microvascular and macrovascular diabetes complications, but there should also be awareness of the relationship between weight and cancer in patients with diabetes. The latter fact might also be incorporated into patient education by physicians and patient associations.

Furthermore, the finding that in a large primary care T2D cohort, weight gain does not occur when starting a SU as an add-on to metformin, is also important to incorporate in both patient and physician education.

In most larger databases, no systematic information is available on lifestyle factors like eating habits and exercise activity. Adding such information in already existing databases is a challenge but should be considered imperative in order to gain more insight.

As a final point at this stage, when (re)evaluating data, another important factor which should be taken into account is socio-economic status (SES). In the lowest SES range, the chance of developing diabetes is higher and life expectancy lower. Such information should elicit the use of SES when assessing data and matching populations.

REFERENCES

1. World Health Organization. Global Report on Diabetes. 2016. http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf.
2. Baan CA, van Baal PHM, Jacobs-van der Bruggen MAM, et al. [Diabetes mellitus in the Netherlands: estimate of the current disease burden and prognosis for 2025]. *Ned Tijdschr Geneesk*. 2009;153:A580. <http://www.ncbi.nlm.nih.gov/pubmed/19785785>. Accessed October 6, 2017.
3. Kleefstra N, Landman GWD, Van Hateren KJJ, et al. Dutch diabetes prevalence estimates (DUDE-1). *J Diabetes*. 2016;8(6):863-865. doi:10.1111/1753-0407.12370
4. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J cancer*. 2015;136(5):E359-86. doi:10.1002/ijc.29210
5. IKNL. Nederlandse Kankerregistratie, beheerd door IKNL. cijfersoverkanker.nl. Published 2018. Accessed January 12, 2018.
6. Harding JL, Shaw JE, Peeters A, Cartensen B, Magliano DJ. Cancer risk among people with type 1 and type 2 diabetes: disentangling true associations, detection bias, and reverse causation [Correction]. *Diabetes Care* 2015;38:264-270. *Diabetes Care*. 2015;38(4):734-735. doi:10.2337/dc15-er04a
7. Thakkar B, Aronis KN, Vamvini MT, Shields K, Mantzoros CS. Metformin and sulfonylureas in relation to cancer risk in type II diabetes patients: a meta-analysis using primary data of published studies. *Metabolism*. 2013;62(7):922-934. doi:10.1016/j.metabol.2013.01.014
8. Yang X, So WY, Ma RC, et al. Use of sulphonylurea and cancer in type 2 diabetes-The Hong Kong Diabetes Registry. *Diabetes Res Clin Pr*. 2010;90(3):343-351. doi:10.1016/j.diabres.2010.08.022
9. Monami M, Lamanna C, Balzi D, Marchionni N, Mannucci E. Sulphonylureas and cancer: a case-control study. *Acta Diabetol*. 2009;46(4):279-284. doi:10.1007/s00592-008-0083-2
10. Monami M, Balzi D, Lamanna C, et al. Are sulphonylureas all the same? A cohort study on cardiovascular and cancer-related mortality. *Diabetes Metab Res Rev*. 2007;23(6):479-484. doi:10.1002/dmrr.736
11. Bo S, Castiglione A, Ghigo E, et al. Mortality outcomes of different sulphonylurea drugs: the results of a 14-year cohort study of type 2 diabetic patients. *Eur J Endocrinol*. 2013;169(1):117-126. doi:10.1530/EJE-13-0299
12. Tuccori M, Wu J, Yin H, Majdan A, Azoulay L. The Use of Glyburide Compared With Other Sulfonylureas and the Risk of Cancer in Patients With Type 2 Diabetes. *Diabetes Care*. 2015;Conference(November):28th International Conference on Pharmacoepidemiol. doi:10.2337/dc15-1358
13. Fonseca VA. Defining and Characterizing the Progression of Type 2 Diabetes. *Diabetes Care*. 2009;32(Suppl 2):S151. doi:10.2337/DC09-S301
14. Rolla AR. Progression of type 2 diabetes and insulin initiation. *J Natl Med Assoc*. 2011;103(3):241-246. <http://www.ncbi.nlm.nih.gov/pubmed/21671527>. Accessed May 15, 2019.
15. Inzucchi SE, Tunceli K, Qiu Y, et al. Progression to insulin therapy among patients with type 2 dual therapy. *Diabetes Obes Metab*. 2015:1-9.
16. Gallagher EJ, LeRoith D. Obesity and Diabetes: The Increased Risk of Cancer and Cancer-Related Mortality. *Physiol Rev*. 2015;95(3):727-748. doi:10.1152/physrev.00030.2014
17. de Beer JC, Liebenberg L. Does cancer risk increase with HbA1c, independent of diabetes? *Br J Cancer*. 2014;110(9):2361-2368. doi:10.1038/bjc.2014.150
18. Goto A, Noda M, Sawada N, et al. High hemoglobin A1c levels within the non-diabetic range are associated with the risk of all cancers. *Int J Cancer*. 2016;138(7):1741-1753. doi:10.1002/ijc.29917
19. World Cancer Research Fund. Cancers linked with greater body fatness. <http://www.wcrf.org/int/cancer-facts-figures/link-between-lifestyle-cancer-risk/cancers-linked-greater-body-fatness>. Published 2015. Accessed January 19, 2018.

20. Huxley R, Barzi F, Woodward M. Excess risk of fatal coronary heart disease associated with diabetes in men and women: meta-analysis of 37 prospective cohort studies. *BMJ*. 2006;332(7533):73-78. doi:10.1136/bmj.38678.389583.7C
21. Lee WL, Cheung AM, Cape D, Zinman B. Impact of diabetes on coronary artery disease in women and men: a meta-analysis of prospective studies. *Diabetes Care*. 2000;23(7):962-968. <http://www.ncbi.nlm.nih.gov/pubmed/10895847>. Accessed April 11, 2019.
22. Harding JL, Shaw JE, Peeters A, Cartensen B, Magliano DJ. Cancer risk among people with type 1 and type 2 diabetes: disentangling true associations, detection bias, and reverse causation. *Diabetes Care*. 2015;38(2):264-270. doi:10.2337/dc14-1996
23. Johnson JA, Bowker SL, Richardson K, Marra CA. Time-varying incidence of cancer after the onset of type 2 diabetes: evidence of potential detection bias. *Diabetologia*. 2011;54(9):2263-2271. doi:10.1007/s00125-011-2242-1
24. Carstensen B, Witte DR, Friis S. Cancer occurrence in Danish diabetic patients: Duration and insulin effects. *Diabetologia*. 2012;55(4):948-958. doi:10.1007/s00125-011-2381-4
25. Friedrich N, Thuesen B, Jorgensen T, et al. The Association Between IGF-I and Insulin Resistance: A general population study in Danish adults. *Diabetes Care*. 2012;35(4):768-773. doi:10.2337/dc11-1833
26. Gallagher EJ, LeRoith D. Minireview: IGF, Insulin, and Cancer. *Endocrinology*. 2011;152(7):2546-2551. doi:10.1210/en.2011-0231
27. Gabriely I, Ma XH, Yang XM, et al. Removal of visceral fat prevents insulin resistance and glucose intolerance of aging: an adipokine-mediated process? *Diabetes*. 2002;51(10):2951-2958. doi:10.2337/DIABETES.51.10.2951
28. Banerji MA, Faridi N, Atluri R, Chaiken RL, Lebovitz HE. Body Composition, Visceral Fat, Leptin, and Insulin Resistance in Asian Indian Men ¹. *J Clin Endocrinol Metab*. 1999;84(1):137-144. doi:10.1210/jcem.84.1.5371
29. Gastaldelli A, Cusi K, Pettiti M, et al. Relationship Between Hepatic/Visceral Fat and Hepatic Insulin Resistance in Nondiabetic and Type 2 Diabetic Subjects. *Gastroenterology*. 2007;133(2):496-506. doi:10.1053/J.GASTRO.2007.04.068
30. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet*. 1998;352(9131):854-865. [http://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(98\)07037-8/abstract](http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(98)07037-8/abstract).
31. Kahn SE, Haffner SM, Heise MA, et al. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med*. 2006;355(23):2427-2443. doi:10.1056/NEJMoa066224
32. Jalving AC, Gant CM, Binnenmars SH, et al. Glycaemic control in the diabetes and Lifestyle Cohort Twente: A cross-sectional assessment of lifestyle and pharmacological management on HbA1c target achievement. *Diabetes, Obes Metab*. 2018;20(10):2494-2499. doi:10.1111/dom.13399
33. Johnson JA, Bowker SL. Intensive glycaemic control and cancer risk in type 2 diabetes: A meta-analysis of major trials. *Diabetologia*. 2011;54(1):25-31. <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed10&AN=2011193622>.
34. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet*. 1998;352(9131):837-853. [http://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(98\)07019-6/abstract](http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(98)07019-6/abstract).
35. Power ML, Schulkin J. Sex differences in fat storage, fat metabolism, and the health risks from obesity: possible evolutionary origins. *Br J Nutr*. 2008;99(5):931-940. doi:10.1017/S0007114507853347
36. Wannamethee SG, Papacosta O, Lawlor DA, et al. Do women exhibit greater differences in established and novel risk factors between diabetes and non-diabetes than men? The British Regional Heart Study and British Women's Heart Health Study. *Diabetologia*. 2012;55(1):80-87. doi:10.1007/s00125-011-2284-4
37. Wallace IR, McKinley MC, Bell PM, Hunter SJ. Sex hormone binding globulin and insulin resistance. *Clin Endocrinol (Oxf)*. 2013;78(3):321-329. doi:10.1111/cen.12086

38. Le TN, Nestler JE, Strauss JF, Wickham EP. Sex hormone-binding globulin and type 2 diabetes mellitus. *Trends Endocrinol Metab.* 2012;23(1):32-40. doi:10.1016/j.tem.2011.09.005
39. Matsumoto M, Yamaguchi Y, Seino Y, et al. Estrogen signaling ability in human endometrial cancer through the cancer-stromal interaction. *Endocr Relat Cancer.* 2008;15(2):451-463. doi:10.1677/ERC-07-0227
40. Howe LR, Subbaramaiah K, Hudis CA, Dannenberg AJ. Molecular pathways: adipose inflammation as a mediator of obesity-associated cancer. *Clin Cancer Res.* 2013;19(22):6074-6083. doi:10.1158/1078-0432.CCR-12-2603
41. Adams TD, Stroup AM, Gress RE, et al. Cancer incidence and mortality after gastric bypass surgery. *Obesity (Silver Spring).* 2009;17(4):796-802. doi:10.1038/oby.2008.610
42. Lega IC, Wilton AS, Austin PC, Fischer HD, Johnson JA, Lipscombe LL. The temporal relationship between diabetes and cancer: A population-based study. *Cancer.* 2016:1-8. doi:10.1002/cncr.30095
43. Singh AK, Singh R. Is gliclazide a sulfonylurea with difference? A review in 2016. *Expert Rev Clin Pharmacol.* 2016;9(6):839-851. doi:10.1586/17512433.2016.1159512
44. Johnson JA, Carstensen B, Witte D, et al. Diabetes and cancer (1): Evaluating the temporal relationship between type 2 diabetes and cancer incidence. *Diabetologia.* 2012;55(6):1607-1618. doi:10.1007/s00125-012-2525-1
45. Ray WA. Evaluating Medication Effects Outside of Clinical Trials: New-User Designs. *Am J Epidemiol.* 2003;158(9):915-920. doi:10.1093/aje/kwg231
46. Suissa S. Immortal time bias in observational studies of drug effects. *Pharmacoepidemiol Drug Saf.* 2007;16(3):241-249. doi:10.1002/pds.1357
47. Lévesque LE, Hanley JA, Kezouh A, Suissa S. Problem of immortal time bias in cohort studies: example using statins for preventing progression of diabetes. *BMJ.* 2010;340:b5087. doi:10.1136/BMJ.B5087
48. Suissa S, Azoulay L. Metformin and the risk of cancer: time-related biases in observational studies. *Diabetes Care.* 2012;35(12):2665-2673. doi:10.2337/dc12-0788
49. Kowall B, Rathmann W, Kostev K. Are Sulfonylurea and Insulin Therapies Associated With a Larger Risk of Cancer Than Metformin Therapy? A Retrospective Database Analysis. *Diabetes Care.* 2014. doi:10.2337/dc14-0977
50. Nijenhuis-Rosien L, Hendriks SH, Kleefstra N, Bilo HJG, Landman GWD. Nationwide diabetes-related lower extremity amputation rates in secondary care treated patients with diabetes in the Netherlands (DUDE-7). *J Diabetes Complications.* 2017;31(4):675-678. doi:10.1016/j.jdiacomp.2017.01.015
51. Rutten G, De Grauw WJC, Nijpels G, et al. NHG-Standaard Diabetes mellitus type 2 (Tweede herziening). *Huisarts Wet.* 2006;49:137-152.
52. Rutten G, De Grauw WJC, Nijpels G, et al. NHG-Standaard Diabetes mellitus type 2 (derde herziening). *Huisarts Wet.* 2013;56(10):512-525.
53. Ubink-Veltmaat LJ, Bilo HJ, Groenier KH, Rischen RO, Meyboom-de Jong B. Shared care with task delegation to nurses for type 2 diabetes: prospective observational study. *Neth J Med.* 2005;63(3):103-110. <http://www.ncbi.nlm.nih.gov/pubmed/15813422>.
54. Schrijnders D, Wever R, Kleefstra N, et al. Addition of sulphonylurea to metformin does not relevantly change bodyweight: A prospective observational cohort study (ZODIAC-39). *Diabetes, Obes Metab.* June 2016. doi:10.1111/dom.12700
55. Bryere J, Dejardin O, Launay L, et al. Socioeconomic status and site-specific cancer incidence, a Bayesian approach in a French Cancer Registries Network study. *Eur J Cancer Prev.* 2018;27(4):391-398. doi:10.1097/CEJ.0000000000000326
56. Zhu S, Hu J, McCoy TP, et al. Socioeconomic Status and the Prevalence of Type 2 Diabetes Among Adults in Northwest China. *Diabetes Educ.* 2015;41(5):599-608. doi:10.1177/0145721715598382
57. Volaco A, Cavalcanti AM, Filho RP, Precoma DB. Socioeconomic Status: The Missing Link Between Obesity and Diabetes Mellitus? *Curr Diabetes Rev.* 2018;14(4):321-326. doi:10.2174/1573399813666170621123227

58. Onitilo AA, Engel JM, Glurich I, Stankowski R V, Williams GM, Doi SA. Diabetes and cancer II: role of diabetes medications and influence of shared risk factors. *Cancer Causes Control*. 2012;23(7):991-1008. doi:10.1007/s10552-012-9971-4
59. Walker JJ, Johnson JA, Wild SH. Diabetes treatments and cancer risk: The importance of considering aspects of drug exposure. *Lancet Diabetes Endocrinol*. 2013;1(2):132-139. doi:10.1016/S2213-8587(13)70028-7
60. Oosterom N, Gant CM, Ruiterkamp N, et al. Physical Activity in Patients With Type 2 Diabetes: The Case for Objective Measurement in Routine Clinical Care. *Diabetes Care*. 2018;41(4):e50-e51. doi:10.2337/dc17-2041

9

Nederlandse Samenvatting

INTRODUCTIE

Type 2 diabetes (T2D) is een aandoening die wereldwijd veel mensen treft. In Nederland hebben zijn meer dan 1 miljoen mensen bekend met T2D. Het is de verwachting dat het aantal patiënten bekend met T2D de komende jaren verder toe zal nemen. Dit heeft voor een groot deel te maken met toename in obesitas en verminderde lichamelijke activiteit. Het risico op het ontwikkelen van T2D neemt daarnaast ook toe met de leeftijd. Het is bekend dat T2D kan leiden tot problemen aan kleine en grote bloedvaten (zogenoemde micro- en macrovasculaire problemen). Microvasculaire problemen kunnen leiden tot zenuw schade, oogschade en nierfunctiestoornissen. Macrovasculaire problemen kunnen leiden tot onder andere hart- en herseninfarcten en amputaties van de onderste extremiteiten. Naast diabetes komt kanker ook in Nederland in toenemende mate voor, o.a. in samenhang met de veroudering. In de afgelopen jaren zijn er steeds meer aanwijzingen dat bepaalde vormen van kanker vaker gezien worden bij patiënten met obesitas en ook bij patiënten met T2D.

Overgewicht is een factor die zowel bij T2D als bij een aantal typen kanker een rol speelt bij het ontwikkelen van de ziekte. Daarnaast wordt er gesuggereerd dat er ook nog een relatie zou kunnen zijn met de medicatie die gebruikt wordt bij het behandelen van T2D. In recente studies wordt daarom ook gesuggereerd dat een deel van de verhoogde kans op kanker mogelijk gerelateerd is aan verstorende factoren, waarmee tot nu toe onvoldoende rekening is gehouden. Dit wordt “bias” genoemd. Hierbij kan bijvoorbeeld worden gedacht aan lichaamsgewicht en blootstelling aan bepaalde medicatie. Maar ook bijvoorbeeld dat een patiënt bekend met diabetes vaker contact heeft met een dokter en er dus mogelijk ook een hogere kans is dat een bepaalde ziekte eerder opgemerkt wordt.

Daarnaast speelt mogelijk het chronisch gebruik van sommige vormen van medicatie een rol. Sulfonylureumderivaten (SU-derivaten) (een bepaalde groep van glucose verlagende medicatie waar glibenclamide, tolbutamide, glimepiride en gliclazide onder vallen) worden vaak als 2^{de} stap na metformine ingezet in de behandeling van T2D. Er wordt gesuggereerd dat gebruik van deze medicatie mogelijk gepaard gaat met een hoger risico op kanker. Echter, de studies waarin dit onderzocht en geconcludeerd is, lijken onvoldoende van kwaliteit.

Daarnaast komt als resultaat uit deze studies ook dat de middelen binnen deze groep mogelijk een verschillend risico op kanker hebben met een mogelijk lager kankerrisico bij patiënten die gliclazide gebruiken. Gliclazide is op dit moment in Nederland het medicijn van voorkeur wanneer er een SU-derivaat wordt voorgeschreven.

Het doel van dit proefschrift was het onderzoeken van kankerrisico in een grote groep patiënten met T2D, die bij de huisarts behandeld werden, waarbij met name werd onderzocht of het gebruik van de verschillende SU-derivaten gepaard ging met een verschil in kanker risico. De studies in dit proefschrift zijn onder andere gebaseerd op data verzameld binnen de ZODIAC-studie. Dit is een groot cohort onderzoek met patiënten die voor hun T2D in de eerstelijns worden behandeld. Dit cohort is in 1998 opgezet in de regio Zwolle, en daarna in 2006, 2009 en 2012 uitgebreid naar andere regio's. Jaarlijks wordt er door de aangesloten huisartsen data met betrekking tot patiënten met T2D verzonden naar het diabetes kenniscentrum in Zwolle. In deze data zit onder andere patiënt specifieke data als leeftijd, geslacht en gewicht en diabetes specifieke data als datum van diagnose, HbA1c en medicatiegebruik. HbA1c is een maat voor de ernst van de T2D. Met name het jaarlijks verzamelen van gewicht en HbA1c is uniek in dit type cohort. Dit cohort is gekoppeld met gegevens over diezelfde patiënten, als die aanwezig waren binnen de Nederlandse Kankerregistratie om zo het gecombineerde ZODIAC-NKR cohort te verkrijgen. In het ZODIAC-NKR cohort daardoor is naast data over de behandeling van T2D ook data beschikbaar over het optreden verschillende vormen van kanker, wanneer de kanker is ontstaan en hoe deze is behandeld.

RESULTATEN

In **hoofdstuk 2** is er middels een systematische review onderzocht wat er in de klinische en preklinische literatuur al bekend is over of de verschillende soorten SU-derivaten onderling verschillen qua kankerrisico. Klinische studies worden verricht met patiënten. Preklinische studies bestaan over het algemeen uit onderzoeken op cellen in petrischalen. Uit de *preklinische* studies komen een heel aantal potentiële mechanismen naar voren hoe SU-derivaten van invloed zouden kunnen zijn op (de snelheid van) kankercelgroei, DNA-schade, bloedvatontwikkeling en metastasen (uitzaaiingen). De meeste studies onderzochten de effecten van één specifieke SU-derivaat, glibenclamide, en laten in preklinische studies een lager kankerrisico zien. Er waren maar weinig preklinische studies met onderzoek naar gliclazide.

De resultaten van de *klinische* studies laten zien dat gebruikers van gliclazide een lagere kans op kanker lijken te hebben in vergelijking met gebruikers van de andere SU-derivaten. Daarnaast laten de resultaten zien dat gebruikers van glibenclamide een hoger risico op kanker lijken te hebben ten opzichte van gebruikers van tolbutamide en gliclazide. Omdat de methodologie van de verschillende studies onderling te veel ver-

schilde konden de resultaten niet goed worden vergeleken. De anti-kankereffecten van glibenclamide gevonden in de *preklinische* studies konden dus niet worden bevestigd in de *klinische* studies. Het merendeel van de klinische studies lieten juist een hogere risico op kanker zien, daar waar in de preklinische studies juist een beschermend effect zichtbaar lijkt te zijn. Voor de overige SU-derivaten is er op dit moment onvoldoende bewijs dat het kanker risico door het gebruik van die middelen wordt beïnvloed. Concluderend kan er gesteld worden dat er op basis van de preklinische studies mogelijk interessante en plausibele anti-kankereffecten zijn gevonden. Echter, de klinische studies met patiënten zijn van een dermate matige kwaliteit dat er een grote kans is dat versturende factoren de gevonden relatie verklaren, en er geen concreet advies voor patiënten op kan worden gebaseerd.

In **hoofdstuk 3** werd de tijd tussen start van SU-derivaat en de behoefte aan intensivering van de behandeling met insuline onderzocht. T2D is vaak een progressief verlopende ziekte. Op het moment dat de glucose controle verslechtert en de behandeling moet worden geïntensiveerd, kan de toevoeging van eenmaal daags insuline worden beschouwd als een aanwijzing voor ziekteprogressie. Uiteindelijk kan insuline worden toegevoegd nadat de maximaal toegestane dagelijkse dosis van metformine en het SU-derivaat is bereikt. In deze studie werd de initiatie van insuline gedeeltelijk beschouwd als het surrogaat-eindpunt voor gebruik (op dat moment) van de maximale (getolereerde) SU-dosis. Dit zou zich kunnen vertalen in een lagere cumulatieve blootstelling aan een SU-derivaat; de exacte gevolgen van een dergelijke bevinding zijn onduidelijk. Bovendien zal een insulinebehandeling (in het algemeen) leiden tot hogere insulineconcentraties in vergelijking met een orale behandeling. Hogere insulineconcentraties worden geassocieerd met een hoger risico op kanker. Daarnaast zal, zodra een SU-derivaat niet meer voldoende werkzaam is, ook er eerder blootstelling aan hogere HbA1c-niveaus zijn. Hogere HbA1cs zijn ook geassocieerd met een verhoogd risico op kanker.

De resultaten suggereren dat de duur van blootstelling aan SU-derivaat tot het moment dat insuline moet worden gestart niet significant verschilt tussen de verschillende SU-derivaten. Dat betekent dus, dat de duur van de blootstelling niet verschilt tussen de verschillende SU-derivaten, wat dan weer een aanwijzing is, dat de duur van blootstelling geen rol lijkt te spelen als het gaat om risico in een mogelijk verschil in kankerrisico tussen de verschillende SU-derivaten

In **hoofdstuk 4** werd onderzocht of na starten van verschillende SU-derivaten er daadwerkelijk gewichtstoename optreedt en of dit voor de verschillende medicamenten verschillend is.

Een van de doelen van dit proefschrift was om versturende effecten op de relatie tussen medicatie en kanker te onderzoeken. Aangezien overgewicht in verband is gebracht met de ontwikkeling van bepaalde specifieke kankertypen en er wordt aangenomen dat het gebruik van SU-derivaten leidt tot gewichtstoename, is het belangrijk om te weten of er een verschil in gewichtsstijging is tussen de verschillende SU-derivaten. Verschillen tussen de individuele SU-derivaten in gewichtsverandering na het starten van een SU-derivaat kunnen dan immers een mogelijke relatie tussen gebruik van SU-derivaat en kanker vertekenen.

Kankers van de slokdarm, maag, darm, lever, galblaas, alvleesklier en nier zijn in verband gebracht met obesitas bij mannen en vrouwen in de algemene bevolking. De resultaten lieten zien dat er geen verschil is tussen de verschillende SU-derivaten maar ook dat de mensen die starten met een SU-derivaat überhaupt geen significante gewichtstoename hebben. Hiermee wordt veel gehoorde stelling in de spreekkamer ontkracht.

In de studie in **hoofdstuk 5** werd de relatie tussen BMI en obesitas-gerelateerde kanker bij mannen en vrouwen onderzocht. In deze studie werd BMI geassocieerd met obesitas-gerelateerde kankers bij mannen met T2D, met uitzondering van gevorderde prostaatkanker. Bij vrouwen met T2D werd weliswaar een associatie gevonden tussen BMI en obesitas-gerelateerde kankers, maar bij uitsplitsen tussen algemene en geslacht specifieke obesitas gerelateerd kankers werd uiteindelijk alleen een duidelijke relatie gevonden met geslacht specifieke obesitas-gerelateerde kankers (ovarium-, endometrium- en postmenopauzale borstkanker). Bij vrouwen werd de groep niet-geslachtsgebonden obesitas gerelateerde kankers (maag, colon, lever, galblaas, alvleesklier en nierkanker en adenocarcinoom van de slokdarm) echter niet geassocieerd met BMI. Dit zou kunnen betekenen dat bij vrouwen met T2D, BMI geen risicofactor is voor deze groep kankers, in tegenstelling tot bij mannen met T2D. Deze studie bevestigt eerdere bevindingen in andere studies met betrekking tot de relatie van verschillende kankers met BMI. Daarom moet er dan ook rekening mee worden gehouden dat het verhoogde voorkomen van kanker bij patiënten met T2D in hoge mate te wijten kan zijn aan de hogere BMI's bij patiënten met T2D. Verder laten de resultaten zien dat de aanname in de literatuur dat gevorderde prostaatkanker gerelateerd is aan obesitas niet in ons cohort werd gevonden en mogelijk heroverwogen moet worden. Het laat ook zien dat afzonderlijke kankersoorten gepaard gaan met verschillende risico's voor kanker en ook afzonderlijk moeten worden geanalyseerd.

Eerdere studies hebben gesuggereerd dat de incidentie van kanker rond de diagnose van diabetes hoger is in vergelijking met de incidentie van kanker bij de rest van de populatie. In **hoofdstuk 6** laten de resultaten zien dat zowel bij mannen als bij vrouwen een

piek bestaat in de incidentie van obesitas-gerelateerde kankers in de jaren rondom het stellen van de diabetes diagnose. Vrouwen, in tegenstelling tot mannen, hadden al vijf jaar vóór de diabetes diagnose een toegenomen incidentie van obesitas-gerelateerde kanker. Na de diabetes diagnose blijven vrouwen een verhoogde incidentie van obesitas-gerelateerde kanker behouden. Bij mannen daalt de incidentie van obesitas-gerelateerde kanker na diagnose van diabetes tot een niet-significante verhoogde incidentie in vergelijking met de algemene populatie. Deze resultaten kunnen impliceren dat ten minste een deel van het relatieve voordeel van vrouwen in de algemene bevolking met betrekking tot het risico op kanker blijvend verloren gaat bij vrouwen met T2D; een vergelijkbaar fenomeen is waargenomen voor hart- en vaatziekten.

CONCLUSIE EN AANBEVELINGEN VOOR DAGELIJKSE PRAKTIJK.

De resultaten van dit proefschrift laten zien dat in patiënten met overgewicht en obesitas, gecombineerd met diabetes, de aandacht niet alleen uit moet gaan naar het voorkomen van micro- en macrovasculaire complicaties, maar ook naar de relatie tussen overgewicht en kanker. Dit zou ook moeten worden besproken met patiënten door hun hulpverleners. Een ander belangrijk resultaat is dat in een goed ingestelde populatie, het toevoegen van een SU-derivaat aan metformine niet leidt tot gewichtstoename. Voor toekomstig onderzoek is het belangrijk BMI dan wel BMI verandering als een verstorende factor te zien. Deze verstorende factoren zouden ook tijdsafhankelijk meegenomen moeten worden in het statistische model. Hoewel het op dit moment gezien de huidige privacywetgeving extreem lastig is, zou het verder koppelen van verschillende databases meer details aan de informatie kunnen toevoegen, waardoor het ook mogelijk zou zijn tot meer relevante bevindingen en conclusies te komen. Hierbij valt te denken aan het daadwerkelijk medicatiegebruik en de dosis. Hierdoor zouden betere analyses gemaakt kunnen worden. Om beter inzicht in de leefstijl van patiënten te krijgen zou het op de hoogte zijn van dieetgewoonten en de hoeveelheid beweging goed zijn. Dit zijn echter gegevens die nu vaak niet in andere grote cohorten beschikbaar zijn. Eerder onderzoek heeft laten zien dat patiënten met een lage socio-economische status (SES) hebben een hogere kans op het ontwikkelen van diabetes en ook een lagere levensverwachting. Informatie over socio-economische status (SES) van patiënten zou daarom een waardevolle toevoeging zijn.

D

Dankwoord

DANKWOORD

Eindelijk, na ruim 3 jaar fulltime en daarna een kleine 2 jaar nog schrijven naast mijn klinische werkzaamheden is het toch gelukt om de promotie af te ronden. Ik ben zelf trots op het resultaat maar had dat natuurlijk nooit alleen kunnen doen. Ik wil graag een aantal mensen in het bijzonder bedanken.

Alle mensen die hebben meegewerkt aan het opstellen van de ZODIAC-database. Dat is toch de basis geweest van het grootste deel van de stukken in dit proefschrift. Zonder hen was dat nooit mogelijk geweest.

Henk Bilo. Beste Henk, de afgelopen jaren zijn niet makkelijk geweest voor jou. Nu pas achteraf realiseer ik mij dat de donkere storm die het Kenniscentrum zou raken bij mijn aanvang al voor de kust lag. Aan het begin van mijn promotietraject had ik te weinig contact met jou. Ik kwam er pas na 2 jaar achter welke enorm waardevolle toevoegingen jij kunt doen. Altijd betrokken bij zowel het werk als ook privé, originele inbreng, leerzame casussen uit jouw eigen praktijk. Altijd te bereiken en bereid advies te geven. Wat zonde dat ik dat mij niet eerder gerealiseerd heb. Bedankt voor jouw bevlogen en enthousiaste begeleiding.

Truuske de Bock. Beste Truuske, jij was niet gelijk vanaf het begin aan betrokken bij mijn promotietraject maar wat ben ik blij dat je er uiteindelijk bij bent gekomen. Natuurlijk vanwege je bijdragende en opbouwende opmerkingen bij de stukken in dit proefschrift, maar vooral ook vanwege de korte wekelijkse telefoongesprekjes in de laatste twee jaar. Dat heeft voor mij ook als stok achter de deur gewerkt. Jouw tips en suggesties over hoe bepaalde zaken aan te vliegen hebben zeker geholpen. Bedankt dat je jouw duidelijke mening wou geven als onafhankelijk 'scheidsrechter' als er discussie in het promotieteam bestond.

Gijs Landman. Beste Gijs, wat kon ik toch af en toe weer balen als ik een stuk weer helemaal rood van je terugkreeg. En af en toe gefrustreerd zijn als ik jouw opmerkingen dan verwerkte en het vervolgens weer terug kreeg met jouw nieuwe suggestie die in mijn ogen toch weer dicht bij het origineel lag. Dat is zeker geen diskwalificatie, want ondanks mijn frustraties, maar vooral dankzij jouw opmerkingen zijn de stukken in dit proefschrift zeker beter geworden dan ze waren. Ik heb geleerd kritisch naar studies van anderen te kijken, maar vooral ook kritisch te zijn op de stukken van mijzelf. Bedankt voor je tijd, energie en brainstorms tijdens ons overleg op de dinsdagen.

Nanno Kleefstra. Beste Nanno, jouw originele en out-of-the-box denken verraste mij toch elke keer weer, en bracht mij ook tot nieuwe inzichten. Je probeert altijd het beste in de promovendi naar boven te halen. Ook voor jou zijn de afgelopen jaren niet makkelijk geweest, en daarbij was je heel teleurgesteld dat ik in januari 2018 ervoor koos terug de kliniek in te gaan. Ik wil je bedanken voor jouw prettige kritische blik en de levendige discussies bij het koffieapparaat. Beiden hebben het proefschrift naar een hoger niveau getild.

Klaas Groenier. Beste Klaas, hartelijk dank voor jouw statistische input. Geen vraag was je te gek. Ondanks dat ik veel statistiek zelf heb uitgevoerd had ik dat nooit kunnen doen zonder jouw hulp en tips. De soms onnavolgbare e-mails die meerdere keren gelezen moeten worden om ze volledig te begrijpen hebben zeker bijgedragen aan wat dit proefschrift nu is.

Hans van Hateren. Beste Hans, dank voor jouw behulpzaamheid en tijd om mij zaken uit te leggen en kritisch naar manuscripten of onderzoeksvoorstellen te kijken.

Prof. dr. P. Denig, prof. dr. B.H.R. Wolffenbuttel en prof. dr. S. Siesling wil ik bedanken voor hun bereidwilligheid om zitting te nemen in de leescommissie en voor het goedkeuren van mijn proefschrift.

Alle co-auteurs van de hoofdstukken van dit proefschrift. Ik wil jullie allen hartelijk danken voor jullie kritische blik, feedback en fijne samenwerking.

Anne Hendriks en Hilde Jalving. Beste Anne en Hilde, het heeft even geduurd maar uiteindelijk hebben we samen denk ik een heel mooi review neergezet. Bedankt voor de vele nuttige (Skype) overleggen, e-mails en suggesties.

Beste collega-onderzoekers van het Diabetes kenniscentrum. Steven, Laura, Ilse, Angelien en Leonie, wat was het fijn om met jullie een kamer te delen. Vaak allemaal keihard aan het werk, maar soms ook supergezellig. Steven, we hebben samen twee prachtige stukken geschreven. En fijn dat jij altijd bereid was mij iets over het ZODIAC uit te leggen. Laura, jouw pragmatische instelling en werkt tempo werkte aanstekelijk (ondanks dat het mij alsnog 5 jaar heeft gekost). Ilse, bedankt voor de gezellige dagen. Angelien en Leonie, uiteindelijk waren wij nog maar met zijn drieën over. Bedankt voor de fijne gesprekken die gelukkig niet altijd over onderzoek gingen.

Integraal Kankercentrum Nederland. Bedankt voor de mogelijkheid onze ZODIAC database te koppelen aan jullie dataset. Pauline Vissers en Sabine Siesling, bedankt voor jullie hulp.

Lieve Pap en Mam, jullie steunen mij altijd onvoorwaardelijk en staan altijd voor mij, Mira en Elena klaar. Of het nou verhuizen, schilderen of oppassen is, jullie zijn er met raad en daad. Zonder jullie zou ik niet zijn wie ik nu ben. Dank jullie wel.

Lieve Mira, wat hou ik toch ontzettend veel van jou. En wat hebben we veel meegemaakt de afgelopen jaren. We zijn getrouwd, tweemaal verhuisd en hebben we een pracht van een dochter gekregen. Zonder jou had ik het proefschrift nooit af gekregen. We hebben het pad echt samen gelopen. Een luisterend oor als ik weer eens vast zat of juist een doorbraak had. De tijd om te schrijven in het weekend. Gelukkig is het nu klaar, en hebben we tijd voor onze verwachte zoon.

Lieve Elena. Wat een lieve en makkelijke meid ben jij. Ik hou onvoorwaardelijk van jou. Door jouw vrolijke humeur en begroeting kom ik altijd graag weer thuis. De toekomst is van jou.

C

Curriculum Vitae

CURRICULUM VITAE

Dennis Schrijnders, geboren op 18 oktober 1985 in Uitgeest behaalde zijn VWO diploma aan het Bonhoeffer College in Castricum en begon in 2005 met de opleiding Medische Informatiekunde na te zijn uitgeloot voor de opleiding Geneeskunde. In 2007 werd hij toch decentraal geplaatst voor Geneeskunde aan de Universiteit van Amsterdam. Tijdens zijn studie Geneeskunde deed hij tijdens zijn wetenschappelijk stage aan het Westmead Millenium Institute te Sydney onderzoek naar adiponectine niveau's in niet alcoholisch lever steatose. Na zijn afstuderen in 2014 kreeg hij de gelegenheid om te starten met een promotieonderzoek naar de relatie tussen sulfonylureumderivaten en kanker bij type 2 diabetes patiënten. Naast zijn promotieonderzoek was hij vanaf januari 2018 ook werkzaam als ANIOS interne geneeskunde in het Meander Medisch Centrum. In maart 2019 is hij gestart met de opleiding tot huisarts.

Dennis is getrouwd met Mira Pfeufer en zij hebben samen een dochter en in verwachting van een zoon.

P

List of publications

LIST OF PUBLICATIONS

Hendriks AM, Schrijnders D, Kleefstra N, de Vries EGE, Bilo HJG, Jalving M, Landman GWD. "Sulfonylurea derivatives and cancer, friend or foe?". *Eur J Pharmacol.* 2019 Oct 15;861:172598. Doi: 10.1016/j.ejphar.2019.172598

Dennis Schrijnders , Steven H. Hendriks, Nanne Kleefstra, Pauline A. J. Vissers, Jeffrey A. Johnson, Geertruida H. de Bock, Henk J. G. Bilo, Gijs W. D. Landman. "Sex differences in obesity related cancer incidence in relation to type 2 diabetes diagnosis (ZODIAC-49)" *PLoS One.* 2018 Jan 25;13(1):e0190870. doi: 10.1371/journal.pone.0190870

Hendriks SH, Schrijnders D, van Hateren KJ, et al "Association between body mass index and obesity-related cancer risk in men and women with type 2 diabetes in primary care in the Netherlands: a cohort study (ZODIAC-56)" *BMJ Open* 2018;8:e018859. doi: 10.1136/bmjopen-2017-018859

Schrijnders D, de Bock G, Houweling S, van Hateren K, Groenier K, Johnson J, Bilo H, Kleefstra N, Landman G. "Within-class differences in cancer risk for sulfonylurea treatments in patients with type 2 diabetes (ZODIAC-55) - a study protocol" *BMC Cancer.* 2017 Jun 23;17(1):444. doi: 10.1186/s12885-017-3433-z.

Hartog LC, Schrijnders D, Landman GW, Groenier K, Kleefstra N, Bilo HJ, van Hateren KJ. "Is orthostatic hypotension related to falling? A meta-analysis of individual patient data of prospective observational studies." *Age Ageing.* 2017 Feb 25;1-8. doi: 10.1093/ageing/afx024.

Dennis Schrijnders, Raiza Wever, Nanne Kleefstra, Sebastiaan T. Houweling, Kornelis J J van Hateren, Geertruida H. de Bock, Henk J G Bilo, Klaas H. Groenier, and Gijs W D Landman. 2016. "Addition of Sulphonylurea to Metformin Does Not Relevantly Change Body Weight: A Prospective Observational Cohort Study (ZODIAC-39)." *Diabetes Obes Metab.* 2016 Oct;18(10):973-9. doi: 10.1111/dom.12700..

Dennis Schrijnders, Laura C. Hartog, Nanne Kleefstra, Klaas H. Groenier, Gijs W D Landman, and Henk J G Bilo. 2016. "Within-Sulfonylurea-Class Evaluation of Time to Intensification with Insulin (ZODIAC-43)." *PLoS One.* 2016 Jun 21;11(6):e0157668. doi: 10.1371/journal.pone.0157668.

Dennis Schrijnders, Sebastiaan T. Houweling, Gijs W.D. Landman. 2015. "Comment on Genuth. Should Sulfonylureas Remain an Acceptable First-Line Add-on to Metformin Therapy in Patients With Type 2 Diabetes? No, It's Time to Move On!" *Diabetes Care*. 2015 Jun;38(6):e94. doi: 10.2337/dc15-0293

Dennis Schrijnders, Nanne Kleefstra, Gijs W D Landman. 2015. "Within-Class Differences of the Sulfonylureas Should Be Accounted For." *Diabetologia*. 2015 Jun;58(6):1374-5. doi: 10.1007/s00125-015-3556-1.

F. G. I. van Vilsteren, L. Alvarez Herrero, R. E. Pouw, D. Schrijnders, C. M. T. Sondermeijer, R. Bisschops, et al. "Predictive factors for initial treatment response after circumferential radiofrequency ablation for Barrett's esophagus with early neoplasia: a prospective multicenter study" *Endoscopy* 2013; 45(07): 516-525. DOI: 10.1055/s-0032-1326423

Van der Poorten, David, Caroline F. Samer, Mehdi Ramezani-Moghadam, Sally Coulter, Marina Kacevska, Dennis Schrijnders, Lindsay E. Wu, et al. "Hepatic Fat Loss in Advanced Nonalcoholic Steatohepatitis: Are Alterations in Serum Adiponectin the Cause?" *Hepatology*. 2013 Jun;57(6):2180-8. doi: 10.1002/hep.26072.

D

Previous Dissertations

PREVIOUS DISSERTATIONS

“St. Langerhans Onderzoeksinstituut”

This thesis is published within the Langerhans Medical Research Group (St. Langerhans Onderzoeksinstituut).

Previous dissertations at / in cooperation with the Langerhans Medical Research Group:
E.A.J.M. Spaans-Hummelink. (2019). *Scope of epidemiology and daily practice in children with type 1 diabetes in the Netherlands*. Promotores: Prof. dr. H.J.G. Bilo, Prof. dr. P.L.P. Brand. Copromotor: Dr. N. Kleefstra.

L.C. Hartog. (2017). *Orthostatic hypotension in elderly patients*. Promotor: Prof. dr. H.J.G. Bilo. Copromotores: Dr. K.J.J. van Hateren en dr. N. Kleefstra.

S.H. Hendriks. (2017). *Sex and gender differences in diabetes care*. Promotores: Prof. dr. H.J.G. Bilo en prof. dr. A.H.E.M. Maas. Copromotores: Dr. K.J.J. van Hateren en dr. N. Kleefstra.

Van Dijk P.R. (2015). *Continuous intraperitoneal insulin infusion in the treatment of type 1 diabetes mellitus*. Promotores: prof. dr. H.J.G. Bilo, prof. dr. J.F.M. Wetzels. Copromotorer: dr. N. Kleefstra, dr. S.J.J. Logtenberg.

Drion I. (2014). *Renal function estimation: the implications for clinical practice*. Promotores: prof. dr. H.J.G. Bilo, prof. dr. J.F.M. Wetzels. Copromotor: dr. N. Kleefstra.

Hortensius H. (2013). *Self-monitoring of blood glucose in insulin-treated patients with diabetes*. Promotores: Prof. dr. H.J.G. Bilo, Prof. dr. R.O.B. Gans. Copromotores: Dr. J.J. van der Bijl, Dr. N. Kleefstra.

Alkhalaf A. (2013). *Novel Approaches in diabetic nephropathy*. Promotores: Prof. Dr. G.J. Navis, Prof. dr. H.J.G. Bilo. Copromotores: Dr. S.J.L. Bakker, Dr. N. Kleefstra.

Van Hateren K.J.J. (2013). *Diabetes care in old age*. Promotores: Prof. dr. H.J.G. Bilo, Prof. dr. K. van der Meer. Copromotores: Dr. N. Kleefstra, Dr. S.T. Houweling.

Landman G.W.D. (2012). *Mortality predictors in patients with type 2 diabetes*. Promotores: Prof. dr. H.J.G. Bilo, Prof. dr. R.O.B. Gans. Copromotores: Dr. N. Kleefstra, Dr. K.H. Groenier.

Kleefstra N. (2010). *Self-care interventions in type 2 diabetes*. Promotores: Prof. dr. H.J.G. Bilo, Prof. dr. R.O.B. Gans. Copromotores: Dr. S.T. Houweling, Dr. K.H. Groenier.