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Original article

Diabetes

Reassessing the evidence of a survival advantage in Type 2 diabetes treated with metformin compared with controls without diabetes: a retrospective cohort study

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Abstract

Background: Previous research has suggested that individuals with Type 2 diabetes and initiated on metformin monotherapy present with a survival advantage compared with the general population without diabetes. This finding has generated considerable interest in the prophylactic use of metformin against age-related morbidity.

Methods: Utilizing Danish National Health Registers, we assessed differences in survival associated with metformin monotherapy for Type 2 diabetes compared with no diagnosis of diabetes in both singleton and discordant twin populations between 1996 and 2012. Data were analysed in both nested case–control and matched cohort study designs, with incidence rate ratios (IRRs) and hazard ratios estimated using conditional logistic regression and Cox proportional hazards regression, respectively.

Results: In case–control pairs matched on birth year and sex or co-twin (sex, birth year and familial factors), incident Type 2 diabetes with treatment by metformin monotherapy initiation compared with no diagnosis of diabetes was associated with increased mortality in both singletons (IRR = 1.52, 95% CI: 1.37, 1.68) and discordant twin pairs (IRR = 1.90, 95% CI: 1.35, 2.67). After adjusting for co-morbidities and social indicators, these associations were attenuated to 1.32 (95% CI: 1.16, 1.50) and 1.64 (95% CI: 1.10, 2.46), respectively. Increased mortality was observed across all levels of cumulative use and invariant to a range of study designs and sensitivity analyses.

Conclusions: Treatment initiation by metformin monotherapy in Type 2 diabetes was not associated with survival equal or superior to that of the general population without

diabetes. Our contrasting findings compared with previous research are unlikely to be the result of differences in epidemiological or methodological parameters.

Key words: metformin, type 2 diabetes, epidemiology, mortality, survival, longevity, ageing, age-related disease

Key Messages

- Previous research has suggested that when individuals with incident Type 2 diabetes are initiated on metformin monotherapy, they present with better survival compared with the general population without diabetes, despite exhibiting substantially higher morbidity.
- This finding has generated considerable interest in the prophylactic use of metformin against age-related morbidity and contributed to the rationale of the forthcoming Targeting Ageing with Metformin trial.
- In our study reassessing these findings, no evidence of a survival advantage or equalization was observed in comparison with either the general population or those with more similar profiles of familial risk or co-morbidity outside of diabetes.
- Importantly, we show that our contrasting findings are unlikely to be the result of differences in epidemiological or methodological parameters.
- Further research is necessary before interpreting this line of research as supporting evidence for the anti-ageing or geroprotective potential of metformin.

Introduction

Metformin is the most commonly prescribed oral hypoglycaemic agent worldwide.[1] In the landmark UK Prospective Diabetes trial, treatment by intensive blood-glucose control with metformin was associated with reductions in diabetes-related and all-cause mortality of 42% and 36%, respectively, compared with conventional treatment.[2,3] In observational studies, metformin has also been associated with protective effects against both cancer and coronary heart disease, to an extent that may not be entirely explained by improvements in glycaemic control.[4,5] There is strong interest in repurposing metformin for these indications and numerous clinical trials are planned or ongoing to assess its chemopreventive, chemotherapeutic and cardioprotective potential in populations without diabetes.[6,7] On the basis of its established safety and evidence of improvements in healthspan and lifespan in several model organisms, metformin has more recently been considered a strong contender in the search for pharmacological interventions that may attenuate the ageing process in humans.[8]

One line of epidemiological evidence has been particularly influential in generating support for this hypothesis, originating from a 2014 study by Bannister *et al.* entitled 'Can people with type 2 diabetes live longer than those without? A comparison of mortality in people initiated with metformin or sulphonylurea monotherapy and matched, non-diabetic controls'.[9] Following the UK's General Practice Research Database population, this study demonstrated lower mortality in individuals with Type 2 diabetes initiated on metformin monotherapy than not only those on other first-line treatments, but also the general population without diabetes, despite exhibiting substantially higher morbidity.[9] Even with strong confounding by indication, metformin initiators presented with at least equal survival compared with age- and sex-matched controls without diabetes, and a 15% longer median survival time after further adjustment for baseline cardiovascular prophylaxis.[9]

This finding has been widely interpreted as supporting the idea that metformin may confer a large and generalized protective effect against age-related morbidity and contributed to the rationale of the forthcoming Targeting Ageing with Metformin (TAME) trial.[10–15] Moreover, the Bannister *et al.* (2014) study has received significant attention in the public sphere due to its dissemination through TED (Technology, Entertainment and Design) talks, popular podcasts featuring longevity researchers and digital communities interested in health and longevity.[16–19] However, its findings have yet to be replicated and warrant several methodological concerns, due to informative censoring of metformin users and a disadvantageous selection bias into the control group without diabetes.

Given the widespread attention this study has received, we set out to reassess its findings. We conducted a Danish register-based cohort study of survival in metformin-initiated

Type 2 diabetes compared with the general population, first using methods comparable to previous research. We then conducted a nested case–control study that permitted dose–response analyses of cumulative exposure to metformin. Lastly, we considered the analysis of twin pairs discordant on metformin and diabetes exposure, which may provide greater power to observe evidence of a survival advantage through adjustment for a wide range of familial confounders. We hypothesized that metformin initiators would exhibit at least equal survival compared with controls without diabetes from the general population and that this effect would increase in discordant twin pairs, at higher levels of use of metformin and after adjustment for socio-economic factors and co-morbidities.

Materials and methods

Study population

Singleton cohorts were derived from a 5% random sample of the Danish population recorded in the Civil Registration System (CRS) since 1968 and twin cohorts from all twin pairs recorded in the population-based Danish Twin Registry (DTR).[20,21] Our cohorts comprised all singletons and same-sex twin pairs who were alive, aged ≥18 years and residing in Denmark on 1 January 1996, 1 year after the initiation of the Danish National Prescription Register (DNPR).[22] Prevalent antidiabetic users, defined as those redeeming diabetes-related prescriptions prior to 1996, were excluded. Due to the nationwide scope of our study, loss to follow-up was only possible by emigration and could be tracked through the CRS. All eligible individuals were observed from cohort entry on 1 January 1996 until at the latest 31 December 2012, the end of our observed data. After cohort entry in 1996, the maximum duration of follow-up was 16 years.

Exposure

The exposure of interest in our study was incident Type 2 diabetes stratified by the initiating treatment class of oral hypoglycaemic agent compared with no use of any antidiabetic medications. Information on exposure status was derived from the DNPR, which contained individual-level data on all prescriptions redeemed at Danish pharmacies since 1995.[22] Anatomical Therapeutic Chemical (ATC) code group A10 was used to identify prescriptions for the treatment of diabetes. We stratified individuals with Type 2 diabetes according to their first-line therapy into groups of metformin monotherapy initiation, sulfonylurea monotherapy initiation and other.[9] We were primarily interested in individuals initiating treatment with metformin, whilst those initiating sulfonylureas were included for positive control comparisons with previous research. Incident exposure after cohort entry was based on observing a single prescription of diabetes medication on 1 January 1996 or later. Initiation of metformin or sulfonylurea monotherapies as a first-line therapy were assumed to be indicated for the treatment of Type 2 diabetes in all cases and the presence of no antidiabetic use was assumed to mean no diagnosis of diabetes. Extensive analyses assessing the impact of these assumptions were performed (see 'Sensitivity analyses' section).

Study design

For comparison with Bannister *et al.* (2014), we first employed a prevalent new-user matched cohort design, where individuals initiating metformin monotherapy were followed from the date of their first prescriptions and compared with those from the background population without diabetes.[23] Metformin monotherapy initiators were matched 1:1 to persons who were alive and free of diabetes at the date of exposure. For each exposed person, persons without diabetes were sampled without replacement from a risk set matched on birth year and sex, and then assigned the index date of their exposed match. This process ensured that immortal time prior to exposure was not differentially excluded or misclassified and that the distributions of index dates were identical across the matched metformin and comparison cohorts. Matched pairs were followed until whichever came first out of death, emigration, the end of the observed data, incident diabetes in the matched persons without diabetes at baseline, or the censor date of their respective match.

The study design employed in Bannister *et al.* (2014) had several characteristics that may have induced bias in favour of metformin initiators.[9] First, metformin initiators were censored if they added additional antidiabetic medications to their treatment regimen, which is likely informative due its association with disease progression and mortality. Second, the comparison group was based on never-exposed controls, where individuals were only eligible to be selected if they were free of diabetes throughout all of follow-up. In settings with high incidence rates of the exposure, this categorization induces a disadvantageous selection bias into the control group whereby they are more likely to experience the outcome event (i.e. death).[24] In order to avoid these biases in our main analyses whilst providing direct comparability with Bannister *et al.* (2014), we explored the impact of these design parameters as separate sensitivity analyses (see 'Sensitivity analyses' section).

Next, we employed a nested case–control study design within the fully enumerated cohorts to assess associations

between Type 2 diabetes and mortality at varying levels of cumulative metformin use following monotherapy initiation. Here, all person-time since cohort entry on 1 January 1996 was eligible for sampling. Cases were defined as those experiencing death prior to the end of follow-up. Controls (i.e. non-cases) were sampled by risk set sampling over calendar time, where for each case we first identified a risk set of individuals from the fully enumerated cohort still alive at the index date (death date) of the case. We then sampled 1:1 with replacement from this risk set matched on birth year and sex. Exposure was treated as a binary time-dependent variable relative to the index date of each case–control pair. Exposed individuals could serve as a control in either their unexposed or exposed person-time until they experienced an outcome or were censored. Cumulative use was assessed in categories of months since first prescription (<12, 12–48, >48), number of 3-month calendar periods with at least one metformin prescription (<4, 4–12, >12) and sums of all defined daily doses (DDDs) of metformin redeemed since the date of first prescription (1–100, 201–500, 501–1000 and ≥1000). Three-month calendar periods were chosen due to varying intended lengths of prescriptions issued in primary care. The defined daily dosage of metformin (2.0 grams) was defined in terms of the assumed average maintenance dose per day when used for Type 2 diabetes in adults, in accordance with the World Health Organization's ATC/DDD methodology. A limitation of this study design was its reduced scope for describing characteristics and outcomes at baseline and over follow-up due to its case–control presentation.

The analysis of discordant same-sex twin data with binary exposures and outcomes can be viewed as one-to-one matched cohort or case–control studies, where matching is performed on sex, age and a large number of unobservables relating to shared early-life environment and genetic risk factors.[25] For analyses in twins, we formulated study designs analogous to those described previously for singletons. Due to shared familial risk for increased diabetes risk and mortality, we hypothesized that a protective association of metformin exposure in discordant twins would be at least equal to or increased compared with an analysis of singletons from the general population and may be better powered to observe evidence of a survival advantage.[25–28]

Statistical analyses

We first assessed mortality over follow-up in the matched cohort design by comparing mortality rates and survival estimates across matched cohorts of metformin monotherapy initiators and those without diabetes in both singleton and discordant twin populations. Crude mortality rates were computed as the number of deaths divided by total cumulative person-time and expressed per 1000 person-years. Age-standardized mortality rates were computed with respect to the age distribution of the cohort of singleton metformin initiators stratified into 5-year age bins. Comparisons of age-standardized rates across singleton and twin metformin cohorts were performed to test for evidence of selection bias into improved survival within intact twin pairs.

Cox proportional hazards regression and conditional logistic regression stratified on matched pairs estimated hazard ratios (HRs) and incidence rate ratios (IRRs) with 95% CIs in our matched cohort and nested case–control designs, respectively. HRs and IRRs in our setting measured the association between mortality and developing Type 2 diabetes and initiating metformin monotherapy compared with no diagnosis of diabetes. When controls in a case–control analysis are sampled by risk set sampling, conditional logistic regression has been shown to yield an unbiased estimator of the IRR in the full cohort as opposed to an odds ratio of the outcome.[29] Under the definition of monotherapy initiation, all data were analysed as intention-to-treat and no informative censoring was applied upon deviation from initial treatment regimens (see 'Sensitivity analyses' section).

Unadjusted analyses in both study designs controlled for birth year and sex via exact matching, and in twins also unobservables relating to shared early-life environment and genetic risk.[25] Limiting matching to these variables in singletons allowed the comparison with discordant twins to isolate the effect of familial and genetic factors, and provided an interpretable baseline on which further adjustments could be made parametrically. In adjusted models, we included covariates measuring lipid-lowering, antiplatelet, antihypertensive, pulmonary, psychiatric and dementia medication use as indicators of morbidity, as these closely reflected diagnostic activity in general practice. We also included covariates measuring education level and marital status as social indicators. In the matched cohort design, covariates were measured at baseline (i.e. at the exposure index date of each matched pair), with medication use measured ≤2 years prior. In the nested case–control design, all covariates were treated as time-dependent relative to the index date (i.e. death date of the case) of each case–control pair. We reported analyses as unadjusted (matched on birth year and sex for singletons and twin pair for twins), partially adjusted (all covariates excluding education level) and fully adjusted (all covariates including education level). Partially adjusted models were included due to limited coverage of the education register and missing values for derived covariates within older strata of our cohorts.[30] Lastly, the assumption of proportional hazards

with Cox regression was assessed by inspection of trends in scaled Schoenfeld residuals.

All data processing and analysis were performed using R version 4.0.3 (2020–10-10).[31]

Sensitivity analyses

We conducted extensive sensitivity analyses to assess the robustness of our results to the definition of exposure, study design and statistical methods. In the matched cohort design, we assessed changes in estimates resulting from maximum durations of follow-up, informative censoring based on regimen change (i.e. medication switch or progression from monotherapy to combined therapy) and a never-exposed categorization of the matched cohort without diabetes, as in Bannister *et al.* (2014).[9] We also employed a validated register-based definition of Type 2 diabetes to identify individuals without diabetes, using a combination of data from hospitalizations, insurance claims relating to blood-glucose measurements and podiatric treatments, and prescriptions.[32]

For both study designs, we assessed the sensitivity of our results to imposing mandated durations of treatment before being considered exposed and procedures for excluding patients with first-line use of metformin that may not be indicated for Type 2 diabetes, specifically those with polycystic ovary syndrome or gestational diabetes.[33] We compared our conditional logistic regression results to those from the full cohort with estimation by time-dependent Cox proportional hazards regression on data in counting process form. To assess residual confounding in our models, we employed an additional measure of comorbidity status in the form of a register-based Charlson Comorbidity Index derived from hospital ICD-10 codes.[34] Lastly, we assessed differences in mortality between metformin and sulfonylurea initiators for comparison with meta-analyses of previous observational studies assessing such comparisons.[35–38] Detailed descriptions of these sensitivity analyses are contained in the Supplementary material (available as Supplementary data at *IJE* online).

Results

Study population

Our source populations comprised 445 662 singletons from a 5% random sample of the Danish population alive in or born since 1968 and all 151 091 individual twins recorded in the population-based DTR. After removing individuals who were <18 years old, had died or emigrated from Denmark or were prevalent users of antidiabetic drugs prior to cohort entry in 1996, the fully enumerated singleton cohort consisted of 216 916 eligible individuals present and unexposed to antidiabetic drugs in Denmark on 1 January 1996. After applying the above criteria pairwise to the twin population base, as well as excluding opposite-sex dizygotic twin pairs and higher-order births, the fully enumerated twin cohort consisted of 19 763 twin pairs intact, present and unexposed in Denmark on 1 January 1996. Supplementary Figures S2.1 and S2.2 (available as Supplementary data at *IJE* online) depict flow charts outlining this selection process and numbers lost for each criterion.

Matched cohorts

Table 1 describes the baseline characteristics of the singleton and twin matched cohorts stratified according to exposure status in the matched cohort study design. We identified 7842 singletons with incident Type 2 diabetes who were initiated on metformin monotherapy and 976 twin pairs discordant on diabetes where metformin monotherapy was the initiating treatment in the first exposed twin. Markedly increased use of cardiovascular medications, including lipid-modifying agents, antihypertensives and antiplatelet therapy, was observed in both singletons and twins with Type 2 diabetes compared with their matched cohorts without diabetes.

Table 2 describes characteristics of exposure and mortality over follow-up in the singleton and twin matched cohorts. Mean duration of follow-up was 4.2 and 4.1 years in singleton and twin matched cohorts, respectively, with mean durations of metformin monotherapy of 3.0 and 2.8 years, respectively. After adjusting for differential age distributions, no differences in mortality were observed between singleton and twin metformin initiators (24.9 vs 24.7, $P = 0.92$). Both singleton and twin cohorts of metformin initiators exhibited higher age-standardized mortality rates over follow-up than their matched cohorts without diabetes (singletons 24.9 vs 16.9, $P < 0.001$; twins 24.7 vs 12.9, $P < 0.001$).

For direct comparison with previous research, equivalent data are reported for initiators of sulfonylurea monotherapy in Supplementary Section 2 (available as Supplementary data at *IJE* online).

Cases and controls

Table 3 describes the characteristics of risk set case–control pairs selected from the fully enumerated singleton and twin cohorts in the nested case–control study design. Throughout follow-up, 44 629 individuals within the singleton cohort died and were matched 1:1 with eligible controls. In the twin cohort, 5744 individual twins died but

Table 1 Baseline characteristics of matched cohorts of metformin initiators and those without diabetes

Characteristic[a]	Singleton matched cohorts		Twin matched cohorts	
	Metformin[b]	Matched without diabetes[c]	Metformin[b]	Co-twin without diabetes[c]
Number of individuals (*n*)	7842	7842	976	976
Age at index date				
Mean (SD)	60.1 (13.4)	60.1 (13.4)	58.9 (12.2)	58.9 (12.2)
Median (IQR)	61.0 (18)	61.0 (18)	60.0 (15)	60.0 (15)
Men, *n* (%)	4130 (52.7%)	4130 (52.7%)	545 (55.8%)	545 (55.8%)
Index year, *n* (%)				
1996–2000	484 (6.17%)	484 (6.17%)	49 (5.02%)	49 (5.02%)
2001–2004	1229 (15.7%)	1229 (15.7%)	172 (17.6%)	172 (17.6%)
2005–2008	2244 (28.6%)	2244 (28.6%)	292 (29.9%)	292 (29.9%)
2009–2012	3885 (49.5%)	3885 (49.5%)	463 (47.4%)	463 (47.4%)
Medications, *n* (%)[d]				
Lipid-lowering	3576 (45.6%)	1206 (15.4%)	438 (44.9%)	182 (18.6%)
Antiplatelet	2403 (30.6%)	1155 (14.7%)	283 (29.0%)	155 (15.9%)
Antihypertensive	4980 (63.5%)	2437 (31.1%)	590 (60.5%)	336 (34.4%)
Pulmonary	1218 (15.5%)	804 (10.3%)	152 (15.6%)	107 (11.0%)
Psychiatric	2470 (31.5%)	1867 (23.8%)	296 (30.3%)	227 (23.3%)
Dementia	176 (2.24%)	122 (1.56%)	26 (2.66%)	17 (1.74%)
Highest education, *n* (%)[e]				
Missing or no education	499 (6.36%)	624 (7.96%)	46 (4.71%)	42 (4.30%)
Middle or high school	3453 (44.0%)	2904 (37.0%)	464 (47.5%)	422 (43.2%)
Vocational training	670 (8.54%)	1045 (13.3%)	78 (7.99%)	93 (9.53%)
Short or medium cycle	227 (2.89%)	252 (3.21%)	13 (1.33%)	30 (3.07%)
Candidate or PhD	2993 (38.2%)	3017 (38.5%)	375 (38.4%)	389 (39.9%)
Marital status, *n* (%)[f]				
Unmarried	1132 (14.4%)	970 (12.5%)	214 (21.9%)	173 (17.7%)
Married	4686 (59.8%)	4978 (64.1%)	523 (53.6%)	586 (60.0%)
Divorced	1101 (14.0%)	1010 (13.0%)	149 (15.3%)	122 (12.5%)
Widowed	918 (11.7%)	803 (10.3%)	90 (9.22%)	95 (9.73%)
Zygosity (same sex), *n* (%)				
Monozygotic	–	–	304 (31.1%)	304 (31.1%)
Dizygotic	–	–	587 (60.1%)	587 (60.1%)
Unspecified	–	–	85 (8.7%)	85 (8.7%)

[a]Unless otherwise stated, values reflect characteristics at the index (metformin exposure) date.
[b]Metformin monotherapy initiation for treatment of Type 2 diabetes, at least one observed prescription.
[c]Free of diabetes at index date; matched on birth year and gender in singletons, or twin pair in same-sex-twins.
[d]Characteristic in the 2-year period leading up to index date.
[e]Grouped short and medium cycles, and middle and high school for reporting per data usage agreement. Separated in analysis.
[f]Includes registered partnerships, terminated partnerships and longest-living of two partners.
IQR, interquartile range.

only 4264 had an eligible match at their index date due to their co-twin having previously died.

Metformin monotherapy initiation and mortality

Figure 1 displays Kaplan–Meier survival curves with 95% CIs over 8 years of follow-up in the singleton and twin matched cohorts. Estimates of cumulative survival with 95% CIs at Years 3, 5 and 8 are presented in Table 2. Singleton and twin cohorts with incident Type 2 diabetes and treatment initiation by metformin monotherapy exhibited lower rates of survival over follow-up compared with their matched cohorts without diabetes.

Table 4 describes estimates of the association at varying levels of adjustment from Cox proportional hazards and conditional logistic regression models. Compared with no diagnosis of diabetes, incident Type 2 diabetes with metformin monotherapy initiation in singletons was associated with an increased risk of mortality in both matched cohort (HR = 1.48, 95% CI: 1.32, 1.64) and nested case–control designs (IRR = 1.52, 95% CI: 1.37, 1.68). After adjustment for the full range of available covariates, these

Table 2 Follow-up in matched cohorts of metformin monotherapy initiators and those without diabetes

Outcome	Singleton matched cohort		Twin matched cohort	
	Metformin[a]	Matched without diabetes[b]	Metformin[a]	Co-twin without diabetes[b]
Duration of follow-up				
Mean (SD)	4.20 (3.47)	4.20 (3.47)	4.07 (3.28)	4.07 (3.28)
Median (IQR)	3.27 (4.64)	3.27 (4.64)	3.31 (4.46)	3.31 (4.46)
Duration of monotherapy				
Mean (SD)	2.97 (2.86)	–	2.84 (2.68)	–
Median (IQR)	2.13 (3.41)	–	2.08 (3.24)	–
Mortality Rate (95% CI)				
Crude/1000 person-years	24.93 (23.23, 26.64)	16.86 (15.46, 18.27)	21.68 (17.09, 26.26)	10.08 (6.96, 13.21)
Age-standardized[c]	24.93 (23.23, 26.64)	16.86 (15.46, 18.27)	24.73 (19.50, 29.96)	12.94 (8.93, 16.95)
Survival (95% CI)				
Year 3	0.93 (0.92, 0.93)	0.95 (0.95, 0.96)	0.94 (0.92, 0.95)	0.97 (0.96, 0.99)
Year 5	0.89 (0.88, 0.90)	0.92 (0.91, 0.93)	0.91 (0.89, 0.93)	0.95 (0.93, 0.97)
Year 8	0.83 (0.82, 0.84)	0.88 (0.87, 0.89)	0.83 (0.79, 0.87)	0.93 (0.90, 0.96)

[a]Metformin monotherapy initiation for treatment of Type 2 diabetes, at least one observed prescription.
[b]Free of diabetes at index; matched on birth year and gender in singletons, or twin pair in same-sex twins.
[c]Age-standardization performed with respect to the age distribution of singleton metformin initiators.
IQR, interquartile range.

estimates were attenuated in both matched cohort (HR = 1.33, 95% CI: 1.16, 1.54) and nested case–control designs (IRR = 1.32, 95% CI: 1.16, 1.50). In discordant twin pairs, the exposure was similarly associated with an increased risk of mortality (HR = 2.15, 95% CI: 1.48, 3.13; IRR = 1.90 95% CI: 1.35, 2.67), which was attenuated upon adjustment for the full range of available confounders (HR = 1.80, 95% CI: 1.11, 2.91; IRR = 1.64, 95% CI: 1.10, 2.46).

Table 5 describes estimates of a cumulative analysis within the nested case–control design, with metformin use grouped by time since first prescription and cumulative DDD categories. All associations compared cumulative measures in incident Type 2 diabetes with metformin monotherapy initiation to the reference group of no diabetes. Metformin monotherapy initiators exhibited increased mortality compared with those without diabetes across all treatment durations including <12, 12–48 and >48 months. Similar findings were reported when taking into account breaks in medication use through an analysis of cumulative 3-month calendar exposure periods. Moreover, increased mortality was observed regardless of the cumulative amount of metformin used, including categories defined by 1–200, 201–500, 501–1000 and >1000 DDDs. A dose–response effect was observed due to the known efficacy of intensive blood-glucose control with metformin reducing mortality in Type 2 diabetes.[2,3] However, no duration since treatment or level of metformin use in Type 2 diabetes was associated with survival superior or equal to that of the general population without diabetes.

The size of our twin cohort was insufficient to conduct subgroup analyses of cumulative metformin use; however, we included the results for this sample in the Supplementary Table S2.6 (available as Supplementary data at *IJE* online) for transparency.

Sensitivity analyses

Our findings were robust to imposing maximum durations of follow-up, informative censoring based on regimen changes, and a never-exposed categorization of the matched cohorts without diabetes in the matched cohort design as in Bannister *et al.* (2014).[9] Our findings were also unchanged when expanding our definition of diabetes to include data from podiatric visits, diabetes-related outpatient or inpatient hospitalizations and insurance claims related to blood-glucose tests. Moreover, our findings were robust to imposing mandated durations of exposure, excluding strata that were likely to have the highest rates of use of metformin for polycystic ovary syndrome or gestational diabetes and additional measures of co-morbidity based on hospital registers. Lastly, the results of direct comparisons between metformin and sulfonylurea initiators were consistent with previous meta-analyses of a large literature of observational studies, suggesting generalizability of our study cohorts.[35–38] All other sensitivity analyses were consistent with our main results and complete descriptions of these can be found in the Supplementary material (available as Supplementary data at *IJE* online).

Table 3 Characteristics of risk set sampled case–control pairs in the nested case–control design

Characteristic	Singleton case–control pairs		Twin case–control pairs	
	Cases[a]	Matched controls[a]	Cases[a]	Co-twin controls[a]
Number of individuals (*n*)	44 629	44 629	4264	4264
Age at index date (years)				
Mean (SD)	76.4 (13.8)	76.4 (13.8)	68.3 (14.2)	68.3 (14.2)
Median (IQR)	79 (19)	79 (19)	70 (20)	70 (20)
Men, *n* (%)	21 869 (49.0%)	21 869 (49.0%)	2161 (50.7%)	2160 (50.7%)
Exposure, *n* (%)[b]				
Metformin	942 (2.11%)	653 (1.46%)	105 (2.46%)	67 (1.57%)
Sulfonylurea	2002 (4.49%)	1201 (2.69%)	171 (4.01%)	103 (2.42%)
Other	382 (0.86%)	82 (0.18%)	55 (1.29%)	17 (0.40%)
No antidiabetic use	41 303 (92.5%)	42 693 (95.7%)	3933 (92.2%)	4077 (95.6%)
Medications, *n* (%)[c]				
Lipid-lowering	6084 (13.6%)	5970 (13.4%)	632 (14.8%)	656 (15.4%)
Antiplatelet	19 884 (44.6%)	15 407 (34.5%)	1481 (34.7%)	1132 (26.5%)
Antihypertensive	32 637 (73.1%)	25 308 (56.7%)	2759 (64.7%)	1985 (46.6%)
Pulmonary	13 287 (29.8%)	8890 (19.9%)	1260 (29.5%)	869 (20.4%)
Psychiatric	32 744 (73.4%)	22 645 (50.7%)	3047 (71.5%)	2052 (48.1%)
Dementia	2565 (5.75%)	1261 (2.83%)	289 (6.78%)	128 (3.00%)
Highest education, *n* (%)[d]				
Missing or no education	16 545 (37.1%)	16 294 (36.5%)	686 (16.1%)	653 (15.3%)
Elementary or high school	16 067 (36.0%)	14 069 (31.5%)	1972 (46.2%)	1930 (45.3%)
Vocational training	8637 (19.4%)	9341 (20.9%)	1163 (27.3%)	1196 (28.0%)
Short or medium cycles	2553 (5.72%)	3570 (8.00%)	355 (8.33%)	365 (8.56%)
Candidate or PhD	827 (1.85%)	1355 (3.04%)	88 (2.06%)	120 (2.81%)
Marital status, *n* (%)[e]				
Unmarried	5201 (11.7%)	3840 (8.60%)	828 (19.4%)	602 (14.1%)
Married	20 216 (45.3%)	22 723 (50.9%)	2329 (54.6%)	2639 (61.9%)
Divorced	3725 (8.35%)	2561 (5.74%)	382 (8.96%)	293 (6.87%)
Widowed	15 487 (34.7%)	15 505 (34.7%)	725 (17.0%)	730 (17.1%)
Zygosity (same sex), *n* (%)				
Monozygotic	–	–	1287 (30.2%)	1287 (30.2%)
Dizygotic	–	–	2627 (61.6%)	2627 (61.6%)
Unspecified	–	–	350 (8.21%)	350 (8.21%)

[a]Cases defined as having died, controls matched on birth year and gender in singletons, or twin pair in same-sex twins.
[b]Initiation of treatment for diabetes as monotherapy prior to index (case death) date.
[c]Characteristic measured at any point prior to index date.
[d]Grouped short and medium cycles, and middle and high school for reporting per data usage agreement. Separated in analysis.
[e]Includes registered partnerships, terminated partnerships and longest-living of two partners.
IQR, interquartile range.

Discussion

In this study we followed up on previous epidemiological findings within the UK General Practice Research Database suggesting that individuals with Type 2 diabetes who initiate metformin monotherapy as their first-line treatment exhibit lower mortality over follow-up compared with the general population without diabetes.[9] This surprising finding has been widely cited as providing strong support for the initial testing of metformin as a geroprotector and contributed to the rationale for the upcoming TAME trial.[10–15] The results of our study failed to establish support for this line of evidence. In the Danish population, individuals with incident Type 2 diabetes who were initiated on metformin monotherapy were placed at a strong disadvantage within the general population, exhibiting increased mortality compared with those without diabetes matched on sex and birth year. These associations persisted across all levels of cumulative use of metformin and diabetes duration, and after adjustment for a wide range of co-morbidities and other confounders, including

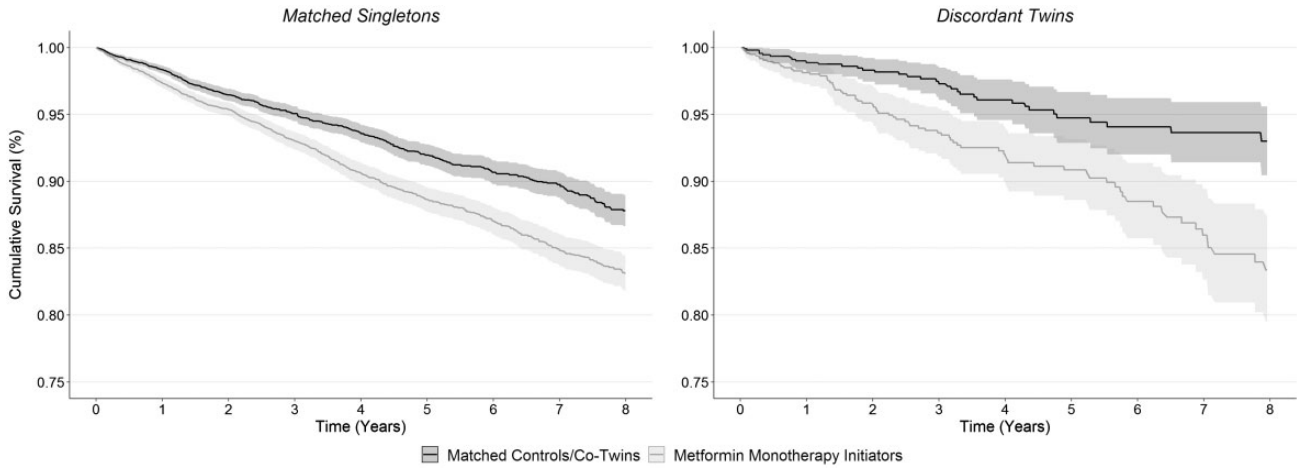

Figure 1 Kaplan–Meier cumulative survival estimates (with 95% CIs) in matched cohorts of metformin monotherapy initiators and those without diabetes over 8 years of follow-up

Table 4 Associations between metformin monotherapy initiation and mortality compared with no diagnosis of diabetes

Cohort	Adjustment	Matched cohort design		Nested case–control design	
		HR	95% CI	IRR	95% CI
Singletons	Unadjusted[a]	1.48	1.32, 1.64	1.52	1.37, 1.68
	Partially adjusted[b]	1.32	1.16, 1.50	1.35	1.21, 1.51
	Fully adjusted[c]	1.33	1.16, 1.54	1.32	1.16, 1.50
Twins	Unadjusted[a]	2.15	1.48, 3.13	1.90	1.35, 2.67
	Partially adjusted[b]	1.70	1.09, 2.64	1.68	1.14, 2.48
	Fully adjusted[c]	1.80	1.11, 2.91	1.64	1.10, 2.46

[a]Previously matched on birth year and gender in singletons, or twin pair in same-sex twins; no additional adjustment.
[b]Additional adjustment for cardiovascular, psychiatric, pulmonary and dementia medication use and marital status.
[c]Additional adjustment for highest attained education level as well as previous covariates.
HR, hazard ratio; IRR, incidence rate ratio.

unobservables relating to shared early-life environment and genetic risk in discordant twin pairs as well as social indicators. No evidence of a survival advantage or survival equalization was observed in comparison with either the general population or those with more similar profiles of familial risk or co-morbidity outside of diabetes.

Type 2 diabetes has been associated with reductions in life expectancy of ~6 years and increased risk for a wide range of vascular and non-vascular diseases.[39] Our data support this mainstream view that Type 2 diabetes is associated with substantial morbidity and mortality compared with those without the disease. However, recent research has suggested that large reductions in several specific risk factors after diagnosis may be associated with complete amelioration of excesses in several key outcomes.[40,41] Specifically, optimal control of glycated haemoglobin, systolic blood pressure, LDL cholesterol, albumin and smoking within specified ranges in those with Type 2 diabetes was shown to be associated with mortality equal to that of the background population without diabetes.[41] However, only 5% of patients achieved such risk profiles and all comparisons were adjusted for socio-economic indicators.[41] Therefore, the presence of a survival advantage or equalization in a population presenting with substantial co-morbidity and selected only for their first-line initiation of metformin monotherapy would be unlikely.

Various comparisons between our study and that of Bannister *et al.* (2014) suggest that our contrasting findings are unlikely to be the result of differences in methodological or epidemiological parameters.[9] First, descriptive parameters of the singleton metformin matched cohorts across studies were similar, including mean age at baseline (60.1 in this study vs 61.2 in Bannister *et al.*), duration of monotherapy (3.0 vs 3.0 years), proportion of men (53% vs 57%), prior lipid-lowering therapy (46% vs 50%), prior antihypertensive therapy (64% vs 66.0%) and prior antiplatelet therapy (31% vs 36%). Interestingly, crude mortality rates per 1000 person-years for the matched cohorts

Table 5 Associations between metformin monotherapy initiation and mortality at different levels of cumulative use in the nested case–control study design

Cohort[f]	Exposure	Unadjusted[a]		Partially adjusted[b]		Fully adjusted[c]	
		IRR	95% CI	IRR	95% CI	IRR	95% CI
Singletons	Time since first prescription						
	<12 months	1.70	1.35, 2.13	1.57	1.22, 2.01	1.58	1.19, 2.12
	12–48 months	1.50	1.28, 1.76	1.31	1.10, 1.56	1.24	1.02, 1.51
	>48 months	1.62	1.37, 1.91	1.44	1.21, 1.72	1.44	1.18, 1.75
	Cumulative exposed periods[d]						
	<4 calendar quarters	1.73	1.44, 2.07	1.58	1.29, 1.93	1.70	1.34, 2.14
	4–12 calendar quarters	1.43	1.21, 1.69	1.23	1.03, 1.48	1.16	0.94, 1.42
	>12 calendar quarters	1.45	1.22, 1.72	1.29	1.07, 1.56	1.23	0.99, 1.51
	Cumulative use (DDD)[e]						
	1–200	1.71	1.44, 2.03	1.57	1.30, 1.89	1.67	1.35, 2.08
	201–500	1.41	1.16, 1.71	1.26	1.02, 1.56	1.18	0.93, 1.50
	501–1000	1.57	1.24, 1.97	1.37	1.06, 1.76	1.26	0.95, 1.67
	1000+	1.56	1.23, 1.98	1.32	1.03, 1.71	1.27	0.96, 1.68

[a]Previously matched on birth year and gender. No additional adjustment.
[b]Additional adjustment for cardiovascular, psychiatric, pulmonary and dementia medication use and marital status.
[c]Additional adjustment for highest attained education level as well as previous covariates.
[d]Calendar quarters (January–March, April–June, July–September, October–December) where at least one metformin prescription was redeemed.
[e]WHO Index DDD for metformin is 2.0 grams.
[f]Twin sample underpowered for a cumulative analysis; results deferred to Supplementary material (available as Supplementary data at *IJE* online) for transparency.
DDD, defined daily dose; IRR, incidence rate ratio.

without diabetes were almost identical (metformin matched cohort without diabetes, 16.9 vs 15.2; sulfonylurea matched cohort without diabetes, 28.4 vs 28.7), as well as for sulfonylurea monotherapy initiators (49.0 vs 50.9). Differences in crude mortality rates were only observed in those initiating metformin monotherapy (24.9 in our study vs 14.4 in Bannister *et al.*). Lastly, various sensitivity analyses suggested that the potential biases present in Bannister *et al.* (2014), specifically due to informative censoring of metformin initiators and a disadvantageous selection bias into the control group without diabetes, likely had only marginal impact. Importantly, these differences did not explain the extent of the contrast observed in our study. Full presentation of our analyses of sulfonylurea monotherapy initiators and other sensitivity analyses can be found in Supplementary Sections 2 and 3 (available as Supplementary data at *IJE* online).

This study contributes to a growing literature reassessing early epidemiological findings that suggested considerable promise with metformin. Its use in Type 2 diabetes has been previously associated with protection against a wide range of conditions, including various forms of cancer, cardiovascular and cerebrovascular disease, age-related cognitive decline, Alzheimer's and other forms of dementia, age-related macular degeneration and, more recently, COVID-19.[42–44] Many of the reported associations have been in excess of what is likely to be explained by improvements in glycaemic control or residual confounding, thus suggesting a general protective effect. In the case of cancer, for example, early meta-analyses of observational studies reported overall reductions in incidence and mortality associated with metformin of ~30%.[45–47] However, pervasive methodological flaws underlying these findings have since been identified and more recent observational research and phase II clinical trials have failed to demonstrate evidence of benefit.[48–51] Positive findings for the remaining conditions have yet to be sufficiently replicated or receive comprehensive bias assessments and meta-analyses of appropriate quality.[52]

This study had several limitations. First, due to the register-based nature of our data, our exposure definition did not include a primary care diagnosis, but rather relied on prescriptions for first-line Type 2 diabetes medication. In the interpretation of our findings, we assumed that unexposed treatment status was not contaminated by lifestyle-treated or undiagnosed Type 2 diabetes and that all first-line treatment with metformin indicated an underlying diagnosis of Type 2 diabetes. However, various comparisons and sensitivity analyses suggested that related assumptions are not likely to affect our estimates or the

interpretation of our study. Moreover, in the case that our control group was contaminated by unidentified Type 2 diabetes, this would serve to increase mortality and to therefore bias towards improved survival in metformin initiators. Second, there are inherent limitations to the use of DDDs in pharmacoepidemiology that are unable to account for varying dosages used in the treatment of diabetes depending on severity.

Lastly, we aimed to assess whether the protective effect of metformin on mortality in individuals with Type 2 diabetes was, as suggested by previous research, sufficient to increase survival above that of the population without diabetes, despite exhibiting substantially higher morbidity. Although our findings do not suggest this to be the case, our study is unable to rule out a protective effect of smaller magnitude that may also extend to ageing cohorts without Type 2 diabetes or other interactions based on clinical measures of diabetes severity such as HbA1c. However, considering the ongoing reappraisal and scepticism of metformin's wide-ranging epidemiological associations, substantiating or refuting previous influential findings on survival may be important for the ensuing interpretation of ongoing clinical trials.

Conclusion

Treatment initiation by metformin monotherapy in Type 2 diabetes was not associated with survival equal or superior to that of the general population without diabetes. Our contrasting findings compared with previous research are unlikely to be the result of differences in epidemiological or methodological parameters. Further research is necessary before interpreting this line of research as supporting evidence for the anti-ageing or geroprotective potential of metformin.

Ethics approval

This project was registered at the Research & Innovation Organization at the University of Southern Denmark (registration number 10.632), who approves all scientific projects for the university according to the Data Protection Regulation. No informed consent or approval by a regional ethical committee is required in register-based studies.

Data availability

Data for this research were obtained on a per-project basis in liaison with a government agency in Denmark and there are strict restrictions on its use and sharing. The data cannot be deposited in a public database and exports of summary data are only allowed as material for direct use in a scientific publication.

Supplementary data

Supplementary data are available at *IJE* online.

Author contributions

K.C. and J.H. conceived the study idea. M.T.K., M.T., J.H., L.A.L., D.A.P. and K.C. designed the study. M.T.K., L.A.L. and D.A.P. obtained and pre-processed the study data. M.T.K., L.A.L. and M.T. contributed to the analysis of study data. M.T.K. performed the data analysis and takes full responsibility for the integrity of the results. M.T.K. and K.C. wrote the initial manuscript. All authors worked on subsequent iterations of the manuscript and contributed intellectual content. All authors approved the final manuscript. The manuscript's guarantor (M.T.K.) affirms that this manuscript is an honest, accurate and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned have been explained.

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Conflict of interest

None declared.

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