



Effects of putative metformin targets on phenotypic age and leukocyte telomere length: a mendelian randomisation study using data from the UK Biobank

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Summary

Background Metformin, a first-line medication for type 2 diabetes, might also have a protective effect against ageing-related diseases, but so far little experimental evidence is available. We sought to assess the target-specific effect of metformin on biomarkers of ageing in the UK Biobank.

Methods In this drug target mendelian randomisation study, we assessed the target-specific effect of four putative targets of metformin (AMPK, ETV6, GPD1, and PEN2), involving ten genes. Genetic variants with evidence of causation of gene expression, glycated haemoglobin A_{1c} (HbA_{1c}), and colocalisation were used as instruments mimicking the target-specific effect of metformin via HbA_{1c} lowering. The biomarkers of ageing considered were phenotypic age (PhenoAge) and leukocyte telomere length. To triangulate the evidence, we also assessed the effect of HbA_{1c} on the outcomes using a polygenic mendelian randomisation design and assessed the effect of metformin use on these outcomes using a cross-sectional observational design.

Findings GPD1-induced HbA_{1c} lowering was associated with younger PhenoAge (β -5.26, 95% CI -6.69 to -3.83) and longer leukocyte telomere length (β 0.28, 0.03 to 0.53), and AMPK γ 2 (*PRKAG2*)-induced HbA_{1c} lowering was associated with younger PhenoAge (β -4.88, -7.14 to -2.62) but not with longer leukocyte telomere length. Genetically predicted HbA_{1c} lowering was associated with younger PhenoAge (β -0.96 per SD lowering of HbA_{1c}, 95% CI -1.19 to -0.74) but not associated with leukocyte telomere length. In the propensity score matched analysis, metformin use was associated with younger PhenoAge (β -0.36, 95% CI -0.59 to -0.13) but not with leukocyte telomere length.

Interpretation This study provides genetic validation evidence that metformin might promote healthy ageing via targets GPD1 and AMPK γ 2 (*PRKAG2*), and the effect could be in part due to its glycaemic property. Our findings support further clinical research into metformin and longevity.

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Introduction

Metformin, a first-line medication for type 2 diabetes in the general population, is increasingly recognised as having a potential protective effect against age-related diseases, and it has been proposed that metformin could promote healthy ageing. Observationally, metformin use is associated with a lower risk of mortality in different patient populations, although whether these associations are driven by confounding and immortal time bias remains unclear.¹ A small randomised controlled trial (MILES, n=14) provided preliminary evidence that metformin can lead to transcriptomic changes in pathways affecting ageing.² A larger 6-year trial, Targeting Aging with Metformin (TAME, n=3000), to explore the role of metformin in longevity is still in its preparatory stage.³

In the absence of data from randomised controlled trials and given the limitations of conventional observational studies, the use of genetics could provide

another strand of evidence on the use of metformin, and might increase the chance of successful drug development.⁴ Mendelian randomisation studies utilise genetic variants randomly allocated at conception and hence this study design is less susceptible to confounding than other observational study designs.⁵ Previous mendelian randomisation studies indicated potentially protective effects of metformin, via AMP-activated protein kinase (AMPK)-dependent and AMPK-independent pathways, against ageing-related diseases (ie, cardiovascular diseases, cancer, Alzheimer's disease, and osteoarthritis).⁶⁻⁸ To explore whether metformin can be repurposed for the promotion of healthy ageing while circumventing the limitations of conventional approaches such as residual confounding, we used a drug target mendelian randomisation study to decipher the target-specific effect of metformin on biomarkers of ageing in the UK Biobank as a form of

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Research in context

Evidence before this study

We searched PubMed for publications from database inception to March 31, 2023, with no language restrictions, using the terms “metformin” AND (“Mendelian randomization” OR “Mendelian randomisation”), and identified 20 studies. Results from these studies suggest potential protective effects of metformin, via AMP-activated protein kinase (AMPK)-dependent and AMPK-independent pathways, against ageing-related diseases (ie, cardiovascular diseases, cancer, Alzheimer’s disease, and osteoarthritis). To our knowledge, no drug target mendelian randomisation studies have yet assessed the role of metformin on the biomarkers of ageing.

Added value of this study

As a form of genetic validation, the drug target mendelian randomisation study uses randomly allocated variants in the protein-encoding genes to infer mechanism-based efficacy and safety outcomes from the pharmacological perturbation of a

drug target. We considered four putative metformin targets (AMPK, ETFDH, GPD1, and PEN2) and their corresponding ten encoding genes. We used variants with evidence of causation of gene expression, glycated haemoglobin A_{1c} (HbA_{1c}), and approximated colocalisation as instruments to mimic the effect of individual putative targets of metformin. HbA_{1c} lowering induced by the metformin targets GPD1 and AMPKγ2 (*PRKAG2*) was associated with younger phenotypic age. The putative effects might be in part due to the glycaemic property of metformin.

Implications of all the available evidence

Metformin is a highly affordable medicine with a known safety profile and has long been on the WHO Model List of Essential Medicines. Our study serves as a proof of concept that metformin could have potential benefits for healthy ageing and might foreshadow results from the Targeting Aging with Metformin (TAME) trial, a large trial evaluating the role of metformin in longevity that is in the preparatory stages.

genetic validation. To triangulate the evidence,⁹ we further explored the effect of glycated haemoglobin A_{1c} (HbA_{1c}) on the outcomes using a polygenic mendelian randomisation design and assessed the association of metformin use on these outcomes using a cross-sectional observational design in the UK Biobank.

Methods

Study design

We conducted a mendelian randomisation study to assess the target-specific effect of metformin on biomarkers of ageing in the UK Biobank. In the context of a drug target mendelian randomisation study, for a valid causal inference, genetic variants within or near the protein-coding gene should be: associated with the known downstream biomarker (ie, the exposure; a concept known as relevance); should share no unmeasured common cause with the outcome (independence); and should not affect the outcome except through the exposure (exclusion restriction).¹⁰ The latter two of these assumptions are not empirically verifiable. The directed acyclic graph of the study design is shown in the appendix (pp 7–8). This study is reported according to the STROBE-MR statement.

The relevance assumption was validated by selecting variants with evidence of corresponding gene expression (cis-acting expression quantitative trait loci [cis-eQTL]) and HbA_{1c} using mendelian randomisation, and colocalisation.¹¹ In addition, we assessed the instrument strength (F statistic) using an approximation method ($\beta^2/\text{standard error}^2$), where a higher F statistic (>10) suggests weak instrument bias is less likely. For the independence assumption, confounding by population

stratification is possible and hence we restricted our analysis to white British participants and adjusted for the top 20 principal components of ancestry. For the exclusion restriction assumption, mendelian randomisation methods with varying assumptions for valid estimation (eg, weighted median and mendelian randomisation-Egger regression [MR-Egger]) were used as sensitivity analyses when conducting mendelian randomisation with variants from multiple gene regions (polygenic mendelian randomisation). These methods cannot be used when conducting mendelian randomisation with variants from a single gene region (cis-mendelian randomisation); therefore, we used colocalisation to assess potential biases from linkage disequilibrium.¹¹

Participants

UK Biobank recruited around 500 000 participants (aged 37–73 years) from 2006 to 2010 across 22 assessment centres in England, Scotland, and Wales, where they completed a series of physical assessments and questionnaires on topics such as sociodemographic characteristics, lifestyle, and self-reported health conditions.¹² Participants also provided biological samples at baseline for biochemical assays and genotyping.¹² HbA_{1c} was measured in mmol/mol using high-performance liquid chromatography (Bio-Rad Variant II Turbo analysers, Bio-Rad Laboratories, Berkeley, CA, USA) and was converted to percentage according to the National Glycohemoglobin Standardization Program. Genotyping was performed using the Affymetrix UK BiLEVE Axiom array and Affymetrix UK Biobank Axiom array (Affymetrix Research Services Laboratory, Santa Clara, CA, USA) and imputation using a reference panel combining the UK10K haplotype and the Haplotype

See Online for appendix
For the STROBE-MR statement
see <https://www.strobe-mr.org>

Reference Consortium panels.¹² To reduce confounding by population stratification, the analyses were restricted to participants who were of self-reported and genetically verified white British ancestry. We also excluded participants who had withdrawn (February, 2022 update), who had sex chromosome aneuploidy, whose self-reported sex differed from genetic sex, who had excess relatedness (more than ten putative third-degree relatives), who had genotyping missingness of 1·5% or higher (which indicates low quality of genotyping) and who had missing data on blood chemistry biomarkers used to derive ageing metrics of interest.

The UK Biobank received ethical approval from the North-West Multi-center Research Ethics Committee (11/NW/0382), and all participants provided written informed consent.

Putative targets of metformin

The mechanism by which metformin acts is not clearly understood.¹³ In addition to the AMPK-dependent pathways via hepatic gluconeogenesis, and targeting of the lysosomal AMPK pathway through PEN2 by low-dose metformin,¹⁴ AMPK-independent mechanisms also exist, such as inhibition of mitochondrial glycerophosphate dehydrogenase.¹⁵ We systematically searched the literature and DrugBank, a curated pharmaceutical knowledge database from inception to March 1, 2023, using the term “metformin” and identified four putative protein targets of metformin: AMPK, ETFDH, GPD1, and PEN2 (appendix p 17). In mammals, AMPK is a heterotrimeric complex which is coded by different gene regions, including catalytic α subunits ($\alpha 1$ and $\alpha 2$ isoforms are encoded by *PRKAA1* and *PRKAA2*, respectively), regulatory β subunits ($\beta 1$ and $\beta 2$ isoforms by *PRKAB1* and *PRKAB2*, respectively), and γ subunits ($\gamma 1$, $\gamma 2$, and $\gamma 3$ isoforms by *PRKAG1*, *PRKAG2*, and *PRKAG3*, respectively).

To select valid instruments to proxy the target-specific effect of metformin, we first assessed the association of tissue-specific gene expression level with HbA_{1c}, followed by approximated colocalisation.^{7,16} Specifically, for the protein-encoding genes (*PRKAA1*, *PRKAA2*, *PRKAB1*, *PRKAB2*, *PRKAG1*, *PRKAG2*, *PRKAG3*, *ETFDH*, *GPD1*, and *PSENE1*), we obtained tissue-specific cis-eQTL from GTEx v8. We selected independent cis-eQTL using the panel of European ancestry with clumping r^2 of less than 0·001 and clumping window of 10 000 kb. We obtained the genetic associations of HbA_{1c} from the UK Biobank for 344 182 participants of European ancestry. Secondly, for variants showing putative causal associations of cis-eQTL and HbA_{1c} ($p < 0\cdot05$), we used an approximated colocalisation approach to differentiate between associations driven by a common causal variant or confounded by linkage disequilibrium. Only those variants with evidence of association and colocalisation were retained to proxy the target-specific effect of metformin.

Outcomes

No gold standard biomarker of ageing exists, although a variety of ageing metrics have been proposed in the geroscience framework.¹⁷ In this study, we considered phenotypic age (PhenoAge) as the primary outcome and leukocyte telomere length as the secondary outcome. Leukocyte telomere length does not correlate as strongly with longevity as PhenoAge,¹⁸ and some studies have suggested that leukocyte telomere length is a highly heritable trait that is largely determined at birth.¹⁹

PhenoAge is a measure based on chronological age and nine multisystem clinical chemistry biomarkers, which were selected using a Cox proportional hazard elastic net model for mortality using data from a US cohort (the third National Health and Nutrition Examination Survey [NHANES III]) with over 23 years of mortality follow-up.²⁰ PhenoAge is a predictor of mortality risk and is robust to population characteristics.²¹ In accordance with the established algorithm (appendix p 4), we computed PhenoAge (years) assuming generalisability of NHANES III training results to the UK Biobank.

Leukocyte telomere length at baseline was measured from DNA from peripheral blood leukocytes using a multiplex quantitative PCR assay, as detailed elsewhere.²² Leukocyte telomere length is expressed as the ratio between telomere repeat copy number (T) and a reference single copy gene, *HBB* (S).²² T and S were calculated relative to a calibrator sample (pooled DNA from 20 individuals).²² In this study, we used UK Biobank data field 22192, in which leukocyte telomere length was log-transformed to obtain a normal distribution, then Z-standardised using the distribution of all individuals with a leukocyte telomere length measurement.

Mendelian randomisation

Genetic associations with the outcomes were generated using a linear regression model assuming an additive effect, adjusted for age at recruitment, sex, genotyping array, and the top 20 principal components of genetic ancestry. For drug target mendelian randomisation analysis, we obtained variant-specific Wald estimates (genetic association on the outcomes divided by the genetic association on metformin target-induced HbA_{1c} lowering). To orientate the direction of the effects of instruments, we applied Steiger filtering, which examines whether the variance explained between each variant–exposure is larger than the variance explained between each variant–outcome effect, and therefore whether the instrument primarily affects the outcome through the exposure (rather than vice versa).

To further identify whether the putative target-specific effect of metformin on the biomarkers of ageing is explained by the glycaemic-dependent pathway, we performed a polygenic mendelian randomisation study and a cross-sectional study. For the polygenic mendelian randomisation study of HbA_{1c} on biomarkers of ageing,

For the metformin entry on DrugBank see <https://go.drugbank.com/drugs/DB00331>

For the GTEx database see <https://www.gtexportal.org/home>

we obtained uncorrelated variants (clumping $r^2 < 0.001$ and clumping window of 10 000 kb) that were associated with HbA_{1c} ($p \leq 5 \times 10^{-8}$) from 344 182 participants of European ancestry in the UK Biobank.

The variant-specific Wald estimates were combined using inverse variance weighting with a multiplicative random-effects model, as the main analysis. To assess the robustness of findings, we also performed sensitivity analyses using methods that relied on different assumptions, including the weighted median, MR-Egger, MR-Robust Adjusted Profile Score and MR-Pleiotropy Residual Sum and Outlier. To orientate the direction of the effects of instruments, Steiger filtering was applied. For the cross-sectional analysis, we assessed the association of HbA_{1c} (%) with the biomarkers of ageing using multivariable linear regression, adjusted for

potential confounders, including age at recruitment, sex, BMI, educational attainment, Townsend deprivation index, self-reported smoking status and alcohol drinking status, and white blood cell (leukocyte) count (for leukocyte telomere length only), as per previous studies (appendix p 9).²³

To triangulate the putative findings from drug target mendelian randomisation, we assessed the biomarkers of ageing comparing users of metformin only with users of other antidiabetic drugs via propensity score matching. This cross-sectional observational analysis was restricted to 21 056 participants with type 2 diabetes at baseline. Baseline characteristics, including potential confounders of metformin use status and biomarkers of ageing, were summarised by the mean and SD for continuous variables and proportions for categorical variables. The baseline characteristics that were measured are described in the appendix (p 5). In addition, the standardised mean difference (SMD) between treatment groups was reported. To control for confounding as much as possible, we used propensity score matching with 1:1 nearest neighbour propensity score matching without replacement, with a caliper of 0.01 of the SD. The propensity score was estimated using logistic regression of the treatment on the potential confounders. To estimate the average treatment effect and its standard error, we fitted a linear regression model with PhenoAge and leukocyte telomere length as the outcomes, and the treatment groups and potential confounders as predictors, and included the matching weights in the estimation.

A two-sided p value of less than 0.05 was considered to indicate statistical significance. All analyses were performed using R version 4.2.2.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

321 412 participants (mean age 56.9 years [SD 8.0]) were included in the analyses, of whom 149 028 (46.4%) were men and 172 384 (53.6%) were women (table). In general, the women had a better health profile than the men, and they had younger PhenoAge and longer leukocyte telomere length. PhenoAge increased with chronological age, whereas leukocyte telomere length decreased with chronological age (p value for trend < 0.0001 ; appendix pp 10, 18). The correlation matrix of these ageing metrics is shown in the appendix (p 11).

Ten protein-encoding genes from four putative targets of metformin were considered (appendix p 17), among which seven genes (*ETFDH*, *GPDI*, *PSENE1*, *PRKAA1*, *PRKAG1*, *PRKAG2*, and *PRKAG3*) had evidence of associations of tissue-specific cis-eQTL on HbA_{1c} ($p < 0.049$). Nine of 65 associations from targets *GPDI*, *AMPKα1* (*PRKAA1*), and *AMPKγ2* (*PRKAG2*) had

	Overall (n=321 412)	Female participants (n=172 384)	Male participants (n=149 028)
Age, years	56.92 (8.00)	56.73 (7.92)	57.14 (8.08)
BMI, kg/m ²	27.43 (4.75)	27.06 (5.14)	27.85 (4.22)
Systolic blood pressure, mm Hg	138.33 (18.62)	135.67 (19.19)	141.40 (17.45)
Diastolic blood pressure, mm Hg	82.31 (10.11)	80.67 (9.93)	84.20 (9.99)
Townsend deprivation index	-1.56 (2.93)	-1.59 (2.89)	-1.52 (2.99)
HbA _{1c} , %	5.44 (0.58)	5.41 (0.52)	5.47 (0.65)
HbA _{1c} , mmol/mol	35.96 (6.37)	35.67 (5.65)	36.29 (7.10)
Random glucose, mmol/L	5.12 (1.21)	5.07 (1.05)	5.18 (1.37)
Total cholesterol, mmol/L	5.71 (1.14)	5.90 (1.13)	5.50 (1.13)
LDL cholesterol, mmol/L	3.57 (0.87)	3.65 (0.87)	3.49 (0.86)
HDL cholesterol, mmol/L	1.45 (0.38)	1.60 (0.38)	1.28 (0.31)
Triglycerides, mmol/L	1.76 (1.02)	1.56 (0.86)	1.98 (1.13)
eGFR, mL/min per 1.73 m ²	90.53 (13.19)	90.83 (13.20)	90.19 (13.18)
C-reactive protein, mg/L	2.60 (4.35)	2.71 (4.34)	2.47 (4.35)
Phenotypic age, years	52.38 (9.92)	51.33 (9.40)	53.60 (10.35)
Z-standardised leukocyte telomere length	-0.03 (0.99)	0.05 (0.99)	-0.13 (0.99)
Type 2 diabetes	23 656 (7.4%)	9210 (5.3%)	14 446 (9.7%)
Cardiovascular disease	170 021 (53%)	82 713 (48%)	87 308 (59%)
Chronic kidney disease	12 432 (3.9%)	5875 (3.4%)	6557 (4.4%)
Smoking status*			
Never	174 535 (54%)	101 937 (59%)	72 598 (49%)
Previous	113 126 (35%)	54 837 (32%)	58 289 (39%)
Current	32 651 (10%)	15 028 (8.7%)	17 623 (12%)
Drinking status*			
Never	10 081 (3.1%)	7549 (4.4%)	2532 (1.7%)
Previous	11 019 (3.4%)	6218 (3.6%)	4801 (3.2%)
Current	300 038 (93%)	158 468 (92%)	141 570 (95%)
Education			
Degree	146 605 (46%)	76 935 (45%)	69 670 (47%)
Non-degree†	115 045 (36%)	63 278 (37%)	51 767 (35%)
None of the above	57 018 (18%)	30 731 (18%)	26 287 (18%)

Data are presented as mean (SD) or n (%). HbA_{1c}=glycated haemoglobin A_{1c}. eGFR=estimated glomerular filtration rate. *Smoking and drinking status were self-reported. †Non-degree education includes A-Level, General Certificate of Secondary Education, National Vocational Qualification, Higher National Diploma, Higher National Certificate, or equivalents of any of these.

Table: Baseline characteristics of participants from the UK Biobank

evidence of approximated colocalisation ($r^2 > 0.80$). We excluded three instruments from the AMPK α 1 (*PRKAA1*) with F statistics of less than 10, indicating the presence of weak instrument bias. Validation of the instruments for each protein-encoding gene of metformin is described in the appendix (pp 19–28). After clumping, rs12581493 from *GPD1* (F statistic, 68) and rs3793342 from AMPK γ 2 (*PRKAG2*; F statistic, 27) were selected to mimic the target-specific effects of metformin via HbA_{1c} lowering (appendix p 29). GPD1-induced HbA_{1c} lowering was associated with younger PhenoAge (β -5.26, 95% CI -6.69 to -3.83) and longer leukocyte telomere length (0.28, 0.03 to 0.53). In addition, AMPK γ 2 (*PRKAG2*)-induced HbA_{1c} lowering was also associated with younger PhenoAge (β -4.88, 95% CI -7.14 to -2.62) but not with leukocyte telomere length (-0.05, -0.45 to 0.34; figure 1). Steiger directionality tests suggested that the variants primarily affected the biomarkers of ageing through metformin target-induced HbA_{1c} lowering (appendix p 30).

273 uncorrelated variants were strongly associated with HbA_{1c} ($p < 5 \times 10^{-8}$) from the UK Biobank; the F statistic for each variant was greater than 10, indicating that weak instrument bias was unlikely (appendix pp 31–34). Using inverse variance weighting, genetically predicted HbA_{1c} lowering was associated with PhenoAge (β -0.96 per SD lowering of HbA_{1c}, 95% CI -1.19 to -0.74) but not associated with leukocyte telomere length (0.014,

-0.007 to 0.035). Sensitivity analyses using methods that relied on different assumptions showed similar results, and Steiger direction tests suggested that the variants primarily affected the biomarkers of ageing through HbA_{1c} (appendix p 35). In the cross-sectional analysis, HbA_{1c} was associated with younger PhenoAge (β -3.31 per percentage point lowering of HbA_{1c}, 95% CI -3.35 to -3.28) but was not associated with leukocyte telomere length (0.004, -0.003 to 0.01; figure 2).

Among 21056 participants with type 2 diabetes at baseline, 3075 (14.6%) were classified as users of metformin and 17981 (85.4%) as users of other antidiabetic drugs. Users of metformin were more likely to have hypercholesterolaemia and hypertension, to be taking cholesterol-lowering agents and antihypertensives, to have worse profiles for glycaemia, and to have better profiles for blood pressure and lipids (appendix p 36). Nearest neighbour propensity score 1:1 matching yielded 3054 pairs of participants (6108 participants). The baseline characteristics of metformin users versus other antidiabetic drug users were well balanced after propensity score matching (all SMD < 0.1; appendix pp 12–16, 36). In the propensity score matched analysis, metformin use was associated with younger PhenoAge (β -0.36, 95% CI -0.59 to -0.13), but not with leukocyte telomere length (-0.04, -0.09 to 0.01).

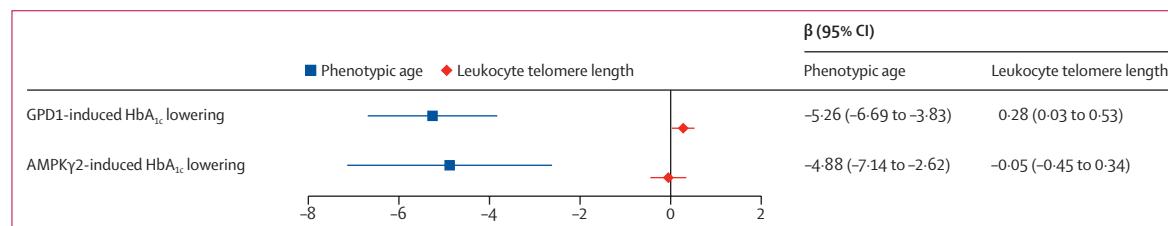


Figure 1: Target-specific effect of metformin on phenotypic age and leukocyte telomere length using drug-target mendelian randomisation
HbA_{1c}=glycated haemoglobin A_{1c}.

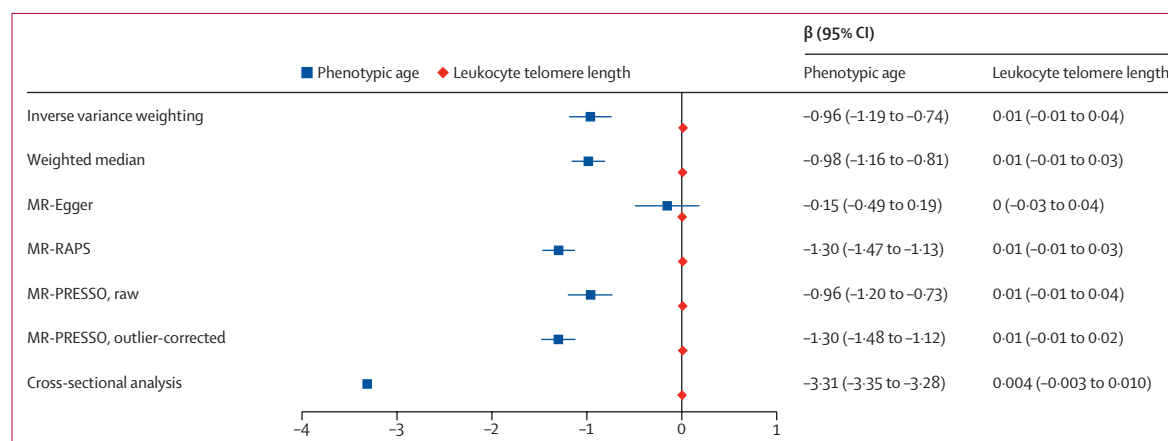


Figure 2: Effect of HbA_{1c} lowering on phenotypic age and leukocyte telomere length using polygenic mendelian randomisation analysis and cross-sectional analysis
HbA_{1c}=glycated haemoglobin A_{1c}. MR=mendelian randomisation. PRESSO=Pleiotropy Residual Sum and Outlier. RAPS=Robust Adjusted Profile Score.

Discussion

This is one of the first drug target mendelian randomisation studies to comprehensively explore the target-specific effect of metformin on biomarkers of ageing. The metformin targets GPD1 and AMPK γ 2 (*PRKAG2*) were associated with younger PhenoAge, which might be in part due to the reduction in HbA_{1c}, and hence reflected glycaemic effects of metformin. Using propensity score matching analysis to explore the association of metformin use with biomarkers of ageing, users of metformin had younger PhenoAge than did users of other antidiabetic drugs.

Animal studies have previously shown that metformin improves health and longevity in other species, such as *Caenorhabditis elegans* and mice, with a number of potential mechanisms considered. Our findings are consistent with observational studies in which metformin use was associated with reduced all-cause mortality risk among different patient populations with and without type 2 diabetes.²⁴ Our drug target mendelian randomisation study adds to the evidence by showing that these findings were unlikely to be due to confounding by indication, which is a common limitation of pharmaco-epidemiological studies. More importantly, our study extends the evidence that the geroprotective effects of metformin probably apply to the general population, given that the UK Biobank is a population-based cohort study.

AMPK activation due to complex I inhibition is one of the most frequently proposed mechanistic pathways by which metformin operates. Among the seven genomic regions determining the structure of mammalian AMPK, our study showed that AMPK γ 2 (*PRKAG2*)-induced HbA_{1c} lowering was associated with younger PhenoAge, which is consistent with the clinical evidence from patients with *PRKAG2* syndrome, which is caused by *PRKAG2* mutation and results in cardiac glycogenosis and lower longevity.²⁵ Activation of AMPK regulates autophagy via signalling of mTOR and ULK1, improves cellular stress resistance, and reduces inflammatory responses.²⁶ This proposed mechanism is also consistent with studies showing reduced AMPK activity with ageing,²⁷ and is related to other factors that are potentially beneficial for healthy ageing, such as intermittent fasting and calorie restriction, which trigger AMPK activity.²⁸

A more recently proposed mechanism of action for metformin is an increased cytosolic redox state due to inhibition of mitochondrial GPD activity, which results in the suppression of gluconeogenic reactions.¹⁵ Our findings showed that GPD1, one of three isoenzymes of human GPD, might play a role in the potential biological ageing-related benefit of metformin, consistent with previous studies using yeast models that showed GPD1 was highly expressed in long-lived models and the deletion of GPD1 reduces replicative lifespan.²⁹ GPD1 overexpression also seemed to enhance the

anticancer property of metformin *in vitro*.³⁰ Most previous studies concerning GPD1 have been conducted in non-humans; our study suggests that additional studies in humans are warranted to explore the impact of metformin on GPD1 activity, given its role in the glycerophosphate shuttle and downstream glucolipid metabolism, which might identify non-glycaemic mechanisms of the beneficial effects of metformin on ageing.

This study has some limitations. First, triangulating the evidence from different designs,⁹ we included conventional observational designs; the consistent findings across study designs improve the validity of evidence. However, these results should be interpreted with caution, given the likelihood of residual confounding which cannot be removed completely, as well as the possibility of immortal-time bias.¹ Nevertheless, these findings were generally consistent with our results from the drug target mendelian randomisation study, which is more resistant to residual confounding. Second, the targets and mechanisms of metformin are still under investigation,¹³ and hence we only reported target-specific effects via HbA_{1c} lowering induced by metformin to inform future target-specific drug trials and experiments. We could not derive the overall effect of metformin given that the contribution of metformin in healthy ageing via different mechanisms is not well studied. Third, we used HbA_{1c} to select related genetic variants from each protein encoding gene of putative targets of metformin because HbA_{1c} reduction is the best known downstream effect of metformin. Intestinal metformin action has been linked to weight loss and reduced appetite due to GDF15 secretion,³¹ but the relative importance of these effects is unknown. Future studies using more direct phenotypes for metformin action, such as instrumenting target functions using cis-acting protein quantitative trait loci for each putative protein target, and AMPK catalytic activity, would improve overall inference concerning the effect of metformin. Fourth, our mendelian randomisation analyses did not consider time-varying effect of HbA_{1c}, which could be explored in future studies using structural mean models. Fifth, the mendelian randomisation estimates might be different from those in clinical trials because of differences in dose and timing of metformin administration. Sixth, PhenoAge provides a snapshot of an individual's biological age (ie, physiological dysfunction) at a specific point in time and does not necessarily represent all domains of ageing. Furthermore, the calculation of PhenoAge included glucose, which can be modified by metformin, and hence might reflect the effect of metformin on glycaemia rather than biological ageing. Future studies using ageing clocks that incorporated multi-omics or are tailored to model specific functions (eg, BrainAge and DNA methylation PhenoAge)²⁰ will help verify the findings in this study. Seventh, we used standardised leukocyte telomere length as per the recommendation by

UK Biobank. Although this does not impact inference within this study, it might complicate the comparison of effect sizes across populations with varying SD of leukocyte telomere length, with corresponding implications for trial designs in different populations. Eighth, selection bias could be an issue because participants in the UK Biobank are, on average, healthier than the general population, possibly leading to an overestimation of the beneficial effect of metformin observed in this study. Lastly, the analyses were restricted to only white British participants to reduce confounding by population stratification; however, this might limit their application to other ethnic groups.

Metformin is a highly affordable medicine with a known safety profile and has long been on the WHO Model List of Essential Medicines. Our study serves as a proof of concept that metformin could have potential in targeting the biology of ageing and might foreshadow results from the proposed TAME trial. The beneficial effects of metformin on ageing could involve HbA_{1c} lowering via GPD1 and AMPK γ 2 (*PRKAG2*) targets, indicating that the glycaemic property of metformin could be one of its mechanistic pathways. Future studies of specific downstream targets and signatures (eg, proteomics and lipidomics) would improve understanding of the underlying mechanisms by which metformin could affect longevity.

Contributors

SL contributed to funding acquisition, investigation, methodology, formal analysis, validation, visualisation, and writing of the manuscript. ICKW contributed to funding acquisition and supervision. CSLC, JZ, and YH contributed to methodology. CMS contributed to funding acquisition and supervision. SLAY contributed to conceptualisation, data curation, funding acquisition, investigation, methodology, project administration, resources, and supervision. All authors contributed to reviewing and editing the manuscript, and approved the final version to be published. SL and SLAY accessed and verified the data reported in the study. SL is the guarantor of the findings.

Declaration of interests

ICKW has received research funding outside the submitted work from Amgen, Bristol-Myers Squibb, Pfizer, Janssen, Bayer, GSK, Novartis, the Hong Kong Research Grant Council, the Hong Kong Health and Medical Research Fund, the UK National Institute for Health Research, the European Commission, and the Australian National Health and Medical Research Council, and also received speaker fees from Janssen and Medice in the previous 3 years. CSLC has received grants outside the submitted work from the Food and Health Bureau of the Hong Kong Government, Hong Kong Research Grant Council, Hong Kong Innovation and Technology Commission, Pfizer, IQVIA, MSD, and Amgen; and personal fees from PrimeVigilance. All other authors declare no competing interests.

Data sharing

Researchers registered with UK Biobank can apply for access to the UK Biobank Resource. The summary data for all tables and figures are available in the appendix.

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