Is the classical oral antidiabetic metformin the novel miracle drug to combat aging?

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Metformin is first or second choice for oral treatment of type 2 diabetes and is licensed for its prevention. Numerous articles propagate metformin as a drug that combats diseases and lowers the risks that are associated with advanced chronological age, such as cancer, diabetes, frailty, coronary heart disease and, most recently, even COVID-19. However, no clinical studies yet exist which prove that metformin delays the onset of such age-related diseases in elderly healthy individuals. Metformin – contrary to expectations – interferes with the beneficial effects of endurance or resistance training, is suspected to rapidly lead to mild anaemia and proven to cause vitamin B12 deficiency.

Metformin, its discovery and modes of action

A brief history of metformin’s discovery is presented in Box 1 (online supplementary figure 1) and Figure 1. Intracellular targets are presented in Figure 2.

Treatment of type 2 diabetes mellitus with metformin

Treatment follows guidelines, which now differ between, e.g., the USA and Europe. Until recently, almost all of the guidelines recommended metformin (after failure of diet and lifestyle changes) as the first drug to use. Metformin, the ‘golden standard’, lowers HbA1c significantly the higher the initial value is and does not cause hypoglycaemia. It is now regarded safe even in patients with moderate kidney dysfunction and can lead to weight loss, especially in obese patients. The weight loss (which may affect both lean and fat mass) is due to decreased caloric intake and increased satiation. Weight loss per se in obese persons (especially loss of visceral and liver fat) may be beneficial for cardiovascular and other health outcomes, as is also suggested by calorie restriction interventions. For reasons yet unknown, up to one-third of freshly diagnosed T2D are metformin ‘non-responders’. Gastrointestinal disturbance (diarrhoea, pain) is relatively common and intolerability may lead to cessation of the drug. A serious adverse effect is undisputed: vitamin B12 deficiency can occur by inhibition of intestinal uptake with the consequence of peripheral neuropathy. This neuropathy is irreversible in contrast to other consequences of a vitamin B12 deficit.
Another, so far completely overlooked adverse effect, discovered by post hoc analysis, is mild anaemia, most likely not due to a vitamin B12 deficit. Lactic acidosis (in contrast to phenformin and buformin) is extremely rare.

### Metformin for prediabetes

In addition to treatment of T2D, metformin is approved for prevention of diabetes in individuals with prediabetes. Diagnosis and thereby prevalence of prediabetes depend on definition. Prevalence may be 4.3 or 43% in the adult US population, depending on which parameters are employed. Approval of the slow-release form of metformin for prediabetes is based on the Diabetes Prevention Program (DPP, NCT00004992) and its long-term follow-up, Diabetes Prevention Program Outcomes Study (DPPOS, NCT00038727). Compared to placebo, intensive lifestyle intervention (ILI) reduced the risk for developing diabetes by 58% and metformin by 31%. Post hoc analysis revealed that metformin has very little effect in participants older than 60 years or in females without a known risk factor for diabetes (gestational diabetes mellitus). When participants of DPP and DPPOS were screened for frailty (see Section ‘Measuring aging in humans and the metformin biomarker’), approximately 12 and 14 years after randomization, metformin was not better than placebo. In contrast, there was a ‘legacy effect’ of ILI and frailty was significantly less compared to the other two groups.

### Metformin’s organ targets: liver, intestine, skeletal muscle

A ‘hepato-centric’ view, supported by preclinical experiments, postulates that metformin acutely inhibits T2D-increased endogenous glucose production (EGP) in the liver, either by stimulating AMPK (directly or indirectly), directly blocking an enzyme involved in shuttling NADH from glycolysis to mitochondria.
Diabetes

Metformin does not inhibit EGP in healthy humans

Jean Sterne reported more than seven decades ago that metformin does not lower fasting blood glucose in healthy persons as is expected by EGP inhibition. His observation has been reproduced numerous times, including fasting by healthy human subjects for 42 hours when most of the glycogen reserves (mainly in the liver) are depleted. Instead, it is a common finding that upon acute or short-term metformin treatment, EGP is increased. Plasma levels of cortisol (a stress hormone), glucagon, lactate and sometimes adrenaline are elevated (proven wrong) or increasing AMP and thereby changing the balance between glycolysis and gluconeogenesis.

Figure 2. Shown are a mitochondrion (symbolic) and Complex I of the electron transport chain, which transfers electrons from NADH+H+ to Coenzyme Q, mechanistic target of rapamycin 1 (mTORC1), AMP-dependent protein kinase (AMPK) and a lysosome (symbolic). Mitochondrial hypothesis for AMPK activation: to reach its mitochondrial target Complex I, metformin, for which no carrier or transporter across the inner mitochondrial is yet known, is postulated to be driven into the matrix by the strong negative potential and inhibits NADH+H+ oxidation. As a result, ATP synthesis is inhibited, membrane potential lowered and NADH/NAD status altered. The cytosolic raise in the AMP/ATP ratio leads to activation (phosphorylation of the α subunit in T172) of AMPK. A consistent observation with cellular systems is that, despite entry into the cytosol, half-maximal concentrations of metformin to activate AMPK are millimolar for short incubation times but may decrease to micromolar after 24 or more hours. Other antidiabetics are much more powerful to inhibit Complex I. The SGLT2 inhibitor canagliflozin has an IC50 value of 18 µM, which is three orders of magnitude lower compared to metformin and acts within 20 minutes to stimulate AMPK four- to fivefold. Activated AMPK can inhibit mTORC1 via phosphorylation but mTORC1 may also inhibit AMPK. Lysosomal (damage) hypothesis: AMPK is located in at least two cellular compartments, namely in the cytosol or via an attached lipid embedded in the lysosomal membrane. There is (mainly) in vitro evidence that metformin at 200–300 µM concentrations can activate AMPK and inhibit mTORC1 via a ‘lysosomal pathway’. In contrast to the mitochondrial hypothesis, no molecular target for the drug within the extremely complex lysosomal system has yet been identified nor uptake into the intra-lysosomal space investigated. Two antimalarials, chloroquine and hydroxychloroquine, are disease-modifying antirheumatic drugs (DMARDs). DMARDS, by their ability to enter the lysosome and being trapped as uncharged molecules (lysosomotropic agents), are reported to activate AMPK and inhibit mTORC1 in cellular systems. Similar to the development of metformin from an active plant molecule, the two antimalarials had an ancestor in the active ingredient of the cinchona bark. Use of hydroxychloroquine in patients is associated with cardiovascular protection and less cancer. It is approved since 2014 as an antidiabetic in India and speculated to fight COVID-19, similar to metformin. (The Complex I illustration has been derived from the RCSB PDB [Molecule of the Month, David S. Goodsell], the AMPK illustration has been derived from the RCSB PDB [depositing authors Ngoei K. et al., DOI: 10.2210/pdb6B1U/pdb] and the mTORC1 illustration has been derived from the RCSB PDB [depositing authors Yang H. et al., DOI: 10.2210/pdb5H64/pdb]; all have been licensed under CC BY-SA; the illustration of the lysosome is the authors’ own work.)
Diabetes in healthy subjects or in prediabetics. An example is shown in Figure 3. Raised glucagon and adrenaline are signals for the liver to increase gluconeogenesis and glycogenolysis – contrary to the proposed EGP inhibition.

Metformin increases glucose uptake from blood into the intestine which switches to more glycolysis and delivers more lactate to the liver

Increased disposal of glucose upon metformin has puzzled clinicians for decades. Skeletal muscle was once suggested as a novel consumer but, as is exemplified for healthy volunteers in Figure 4, the intestine is responsible. In a placebo-controlled trial with T2D, the small intestine increased its glucose import twofold and the colon threefold after metformin (see Box 2, Supplementary Information). The metformin-induced increase in consumption of glucose of the intestine is counterbalanced in healthy subjects by endogenous insulin-antagonists: plasma glucose remains in the normal range.

A 50-year-old report (Figure 5) exemplifies acute actions of metformin when a high glucose load is presented to the upper part of the intestine (see Box 2, Supplementary Information). The human intestine and the liver in T2D and many prediabetes adapt to chronically given metformin as such counterregulatory responses in contrast to short-term drugged healthy individuals are not observed. A new equilibrium resulting in lowered blood glucose, is apparently reached when initial glucose levels are higher compared to healthy subjects who are able to defend their normally set value. Adaptation of metabolism is reflected by constantly raised plasma levels of alanine, about 10% higher compared to placebo or basal values, in the long-term CAMERA trial (NCT00723307). It is suggested that the source may be excess intestinal lactate converted back to pyruvate and transamination by the liver.

Metformin stimulates release of GLP-1 and PYY even in the fasted state

One feature of T2D is a blunted secretion of incretin hormones upon a glucose load such as an oral glucose tolerance test (OGTT). This phenomenon is reversible, e.g., with a carbohydrate-restricted diet, suggesting that impairment is secondary to high glucose-induced glucotoxicity. Metformin increases secretion of the incretin hormones glucagon-like peptide 1 (GLP-1) and peptide tyrosine tyrosine (PYY) in healthy subjects and T2D. This is a direct effect of the drug. Human mucosa, obtained by biopsies, when exposed to micromolar concentrations of metformin, responds within minutes. Release of the hormones can be blocked by metformin uptake inhibitors. GLP-1 and PYY are produced and stored in enteroendocrine (L) cells. Secretion is normally stimulated by nutrients. Release is to neighbouring cells,
especially to excite vagal afferents located just below the mucosa (paracrine action). Afferent signalling to the brain (gut-brain-axis) ends in the nucleus tractus solitarius, one of the two brain regions where GDF15 acts. Spillover to the portal vein occurs. PYY and GLP-1 and fragments thereof enter the general circulation (endocrine action) as a fraction of what acted locally. GLP-1 sensitizes β-cells of the pancreas to respond to glucose and inhibits glucagon secretion. Metformin elevates GLP-1 for T2D in the fasting state, mimicking presence of nutrients. Most of the glucose-lowering effects of metformin in T2D can be reversed by infusion of a GLP-1 antagonist.

**Figure 4.** Right side: Three compounds are shown as examples for non-invasive assessment of metabolism and changes upon metformin treatment or for comparison of healthy and T2D subjects. 2-Deoxy-2\[^{18}\text{F}\]-fluoro-D-glucose (\[^{18}\text{F}\]-FDG) is an ‘irreversible’ tracer entering cells in competition to glucose via different glucose carriers, termed GLUTs. After phosphorylation the label is trapped as the 6-phosphate and cannot be further metabolized. Tracer uptake measures both entry and the first step in glucose metabolism. Sodium-dependent glucose co-transporters (SGLT-1) do not recognize the analogue which favours rapid excretion of the label by the kidney. 1-\[^{13}\text{C}\]-Pyruvate was used with hyperpolarized \(^{13}\text{C}\) MRI to investigate effects of acute (infusion) or chronic application of metformin on heart or liver of rats and was translated recently to investigate cardiac metabolism in T2D. Interestingly, the human intestine has not yet been studied. 4-\[^{18}\text{F}\]-Fluorobenzyltriphenylphosphonium and related tracers, already tested in human pilot trials, are employed to quantitate the mitochondrial (or cellular) membrane potential (MP). The postulated decrease of MP after inhibition of oxidative phosphorylation (see Figure 4.) has been demonstrated for phenformin and metformin in tumour-bearing mice but not yet in humans. **Left side:** Example of a healthy volunteer before and after 7 days of 1 g metformin in two divided doses. A massive increase of \[^{18}\text{F}\]-FDG accumulation in the colon is observed with PET/CT. For eight lean subjects, uptake into the sigmoid was increased on average 3.6-fold, for the total colon 2.7-fold. A better structural resolution, distinguishing lumen and wall of the intestine, is achieved by PET/MRI (magnetic resonance imaging). (The images have been taken from Bahler et al. PLOS ONE 12 (5) e0176242 and have been licensed under CC BY-SA.)
Diabetes

Skeletal muscle – a matter of concern

Skeletal muscle contraction (exercise) promotes glucose uptake by increasing the density/activity of GLUT4 in sarcolemma and transverse tubules. The chain of events leading to this insulin-independent effect is still under investigation and may include increased production of reactive oxygen species. As a bout of exercise also leads to transient AMPK activation, it was once speculated that AMPK was responsible. In 2002 a small (eight subjects) T2D trial with metformin indeed reported that after 10 weeks the ATP content decreased by 24% and phosphocreatine by 34% whereas AMPK activity and phosphorylation (inactivation) of one key enzyme in fatty acid metabolism (acetyl-CoA carboxylase, ACC) were increased in skeletal muscle biopsies. AMPK activation has been reproduced for healthy, rather than slim aging women and men who participated in a placebo-controlled, double-blind, multicentre trial with progressive resistance exercise (MASTERS, NCT02308228). There are several smaller clinical trials before MASTERS with healthy or prediabetic subjects which reported either attenuation of effects of exercise on cardiovascular risk factors, decreased peak aerobic capacity or decreased mitochondrial function, and no increase of insulin sensitivity upon endurance training. MASTERS confirmed the benefits of exercise plus placebo for muscle growth, strength and change of muscle fibre types. To the dismay of the trialists, the metformin-treated subjects demonstrated blunting of all the benefits of exercise on skeletal muscle: hypertrophy, strength and normal muscle density gains.

Measuring aging in humans and the metformin biomarker

In 2001 the geriatric syndrome ‘frailty’ was described which was highly predictive for poor health outcomes. Five Fried criteria, with standardized cut-off points, define frailty: weak hand grip strength, slow walking speed, low physical activity, unintentional weight loss in the last year and self-reported exhaustion. Another conventional and easy approach to measure biological age is the use of clinical chemistry biomarkers, such as albumin, glucose, C-reactive protein, white blood cell counts, etc. When these data are used in a formula that also has chronological age as a variable, phenotypic age is associated with all-cause mortality. Next to telomere length or DNA-methylation status, plasma proteomic (and other omics) age clocks are constructed for healthy aging in humans and the metformin biomarker). Weight loss and rise of GDF15 levels in the metformin-treated arm are correlated. There is little doubt that the origin of this unexpected ‘metformin-gut-brain axis’ via GDF15 stems mainly from the drug-loaded intestine. The cellular mechanism for this up-regulation (transcription and translation of the GDF15 gene) is the ‘Integrated Stress Response’. Mitochondrial pathways, linking elevated NADH/NAD ratios or perturbations of the mitochondrial membrane potential, may be linked to metformin’s postulated activities (Figure 2). They can increase four- to fivefold after a marathon run, which supports a speculative role of metformin as an ‘exerkine’ or ‘exercise mimetic’.

Figure 5. Relative changes of glucose, insulin and lactate concentrations after 50 g glucose infused into the duodenum. Control data (100%) were obtained on a different day and the procedure repeated 2 hours after 1 g oral metformin. Non-diabetic subjects (n = 4) had an implanted portal vein as well as peripheral vein catheter. The observation time was from 30 to 120 minutes. Much less glucose enters the portal vein and into the general circulation after metformin. Lactate is increased dramatically in the portal vein but blood levels are only marginally raised. Detailed investigations with glucose tracers in T2D demonstrate that whole body uptake of a glucose load, which takes up to 300 minutes, may be identical for placebo- and metformin-treated patients. Increased glycolysis and stimulation of whole-body glucose disposal from peripheral blood are consistently reported. (The data has been derived from Berger, W. and Künzli, H. Diabetologia (1970) 6:37.)
Diabetes

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Metformin: evidence for benefits in T2D beyond lowering elevated glucose

The anti-aging metformin hypothesis posits that the drug mitigates an underlying universal process of aging and thereby delays the onset of numerous diseases associated with advancing age. An eye- catching title of a 2014 publication was: ‘Can people with type two diabetes live longer than those without?’ It was suggested, based on mortality data, that metformin therapy of T2D patients may prolong life compared to non-diabetic controls. For lower order species, consult Box 3 (Supplementary Information). At the first glance the evidence obtained for T2D seems convincing.

The 1998 UKPDS-34 (United Kingdom Prospective Diabetes Study 34) is termed a landmark trial which influenced therapy of T2D worldwide up to today. In UKPDS patients with overweight, freshly diagnosed T2D without pre-existing cardiovascular disease were randomized to either diet or metformin. The trial was not blinded and did not have a placebo control. UKPDS-34 concluded that 10-year total mortality and myocardial infarction were significantly reduced in the metformin cohort. The remarkable metformin benefits in UKPDS-34 – compared to diet – have been cited many thousand times. To cite Richard Feynman: “Extraordinary findings require extraordinary evidence”. Perhaps, to the reader’s surprise, findings have not been replicated in a properly designed randomized, controlled clinical trial (RCT) for macrovascular or microvascular complications (all-cause mortality, myocardial infarction, stroke, kidney failure, blindness, and neuropathy) of diabetes. The recent 2020 Cochrane Database of Systematic Reviews meta-analysis of RCTs (excluding UKPDS-34) comparing metformin monotherapy to all other T2D treatments found no clear evidence for the claim that metformin is better than even diet.

On the other hand, most (but not all) reviews come to the opposite conclusion, namely that metformin lowers total mortality, offers cardiovascular protection, prevents numerous cancers, may protect against tuberculosis or COVID-19 and delays dementia. Critics argue that immortal time bias and confounding by indication may have contributed to the claimed benefits. Insulin and/or sulfonylureas are taken by non-metformin users because of more progressive T2D.

Newer antidiabetics such as inhibitors of the sodium-glucose co-transporter 2 (SGLT-2), GLP-1 receptor agonists or dipeptidyl peptidase-4 (DPP-4) inhibitors are tested in megatrails (mainly in diabetic patients with already existing cardiovascular complications) with several thousand participants. Retrospective analysis of the megatrails also concludes that patients with background metformin have better outcome (less all-cause mortality, but surprisingly missing cardiovascular benefit) compared to non-users.

It seems that many of the criteria, defined by Sir Bradford-Hill for causality in observational studies, are fulfilled and that the case is settled. A powerful method to probe for causality is Mendelian randomization (MR). AMPK activation is one of the proposed mechanisms of action of metformin. One MR has reported that participants of a large UK Biobase with a higher AMPK score (i.e., variants with predicted higher enzymatic activity) were associated with lower HbA1c, less T2D, overall cancer and coronary artery disease, providing support for metformin. Experts suggest that AMPK activation can inhibit cancer development but at a later stage may worsen outcome.

Metformin trials in elderly people

Not unlike metformin, oestrogen replacement therapy or vitamin D once enjoyed a high-profile publicity for
some placebo-controlled, double-blind studies investigate if metformin can improve outcome of patients with chronic kidney disease (Reno MET, NCT03831464) or decrease hospitalizations in patients >60 years or <60 years with comorbidities (diabetics are excluded) undergoing elective surgery (UPMC REMAP, SPRY, NCT03861767). Results of the latter trial will be interesting as preclinical studies offer opposing views, namely that GDF15 either sensitizes for or protects against sepsis.

Conclusions

The hypothesis that metformin is an ‘anti-aging drug’ is based on its multiple ‘beneficial’ effects in cellular systems, preclinical models or gene expression changes as well as extrapolation from clinical trials or observational studies with T2D. There is no doubt that metformin, in addition to its decade-long overlooked role as acting as an antidiabetic mainly via the gut, may change mitochondrial status in all cells outside of the intestine, where uptake is occurring. No clinical studies yet exist which prove that metformin thereby delays the onset of age-related diseases in elderly healthy, non-obese people, who follow the (hopefully outdoors) ‘10,000 steps/day’ recommendation and eat a balanced diet. Please note that most of the on-going or planned trials mentioned in Section ‘Metformin trials in elderly people’ recruit individuals who are not ‘healthy’. Adverse effects of metformin on gaining more muscle mass or strength upon resistance training or improved metabolic health and increased muscle mitochondrial capacity by endurance training are a clear warning signal that harms may outweigh benefits. Metformin has discrete effects on circulating white blood cells with unknown consequences, is suspected to rapidly cause mild anaemia and has proven to lower the vitamin B12 status.

For some time, the drug will be first or second choice for oral treatment of T2D or its prevention but there is no clinical evidence that it changes the fundamental processes of aging.
Diabetes

Further Reading

- Boussageon, R., Gueyffier, F. and Cornu, C. (2016) Metformin as firstline treatment for type 2 diabetes: are we sure? BMJ. 352, h6748. DOI: 10.1136/bmj.h6748

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