Review

A Clinical Perspective of the Multifaceted Mechanism of Metformin in Diabetes, Infections, Cognitive Dysfunction, and Cancer

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Abstract: In type 2 diabetes, ecological and lifecourse factors may interact with the host microbiota to influence expression of his/her genomes causing perturbation of interconnecting biological pathways with diverse clinical courses. Metformin is a plant-based or plant-derived medicinal product used for the treatment of type 2 diabetes for over 60 years and is an essential drug listed by the World Health Organization. By reducing mitochondrial oxidative phosphorylation and adenosine triphosphate (ATP) production, metformin increased AMP (adenosine monophosphate)-activated protein kinase (AMPK) activity and altered cellular redox state with reduced glucagon activity, endogenous glucose production, lipogenesis, and protein synthesis. Metformin modulated immune response by directly reducing neutrophil to lymphocyte ratio and improving the phagocytic function of immune cells. By increasing the relative abundance of mucin-producing and short-chain-fatty-acid-producing gut microbes, metformin further improved the host inflammatory and metabolic milieu. Experimentally, metformin promoted apoptosis and reduced proliferation of cancer cells by reducing their oxygen consumption and modulating the microenvironment. Both clinical and mechanistic studies support the pluripotent effects of metformin on reducing cardiovascular–renal events, infection, cancer, cognitive dysfunction, and all-cause death in type 2 diabetes, making this low-cost medication a fundamental therapy for individualization of other glucose-lowering drugs in type 2 diabetes. Further research into the effects of metformin on cognitive function, infection and cancer, especially in people without diabetes, will provide new insights into the therapeutic value of metformin in our pursuit of prevention and treatment of ageing-related as well as acute and chronic diseases beyond diabetes.

Keywords: metformin; diabetes; mechanisms; anticancer action; infections; cognition; cardioprotection

1. Introduction

In 2021, an estimated 537 million people or 10.5% of the world’s population were affected by diabetes, the majority having type 2 diabetes (T2D) with major healthcare and socioeconomic implications [1]. Pharmacological treatment plays an important role in the prevention and treatment of T2D. Understanding the physiology of glucose homeostasis as elegantly defined by Gerich JE is critical to understanding the mechanisms of these glucose-lowering drugs (GLDs) [2]. Glucose and free fatty acids are the main energy substrates essential for survival with excess energy stored as glycogen in the liver and muscle, and triglycerides in adipose tissues. Under metabolic stress with low oxygen and glucose supply, lactate and ketones are alternative fuels. Chronic exposure to high glucose can lead to glucotoxicity causing dysregulation of metabolic, vascular, inflammatory, and cell signaling pathways resulting in widespread organ damage [3].
For survival purpose, the human body possesses a set of mechanisms to maintain blood glucose within a narrow range of 4–8 mmol/L, irrespective of energy intake or expenditure. Type 2 diabetes is characterized by chronic hyperglycemia due to non-suppression of glucagon and reduced post-prandial insulin secretion, often worsened by obesity-associated insulin resistance. During fasting, glycogen and triglyceride are broken down to release glucose and free fatty acids, which are interchangeable through the Randle cycle as energy source. Glucose can also be generated through gluconeogenesis where protein can be broken down by counter-regulatory hormones, such as cortisol, growth hormone, and catecholamines into amino acids which are then converted to glucose to maintain energy balance. In a prandial state, insulin is released to promote glycogen and fat storage while excess glucose is excreted through the kidney, albeit with reabsorption. To date, all GLDs utilize some of these mechanisms to regulate blood glucose levels by reducing energy intake, suppressing endogenous glucose production, reducing glucose reabsorption from gut or kidney, and/or redistributing energy storage [4].

2. Metformin Pharmacology and Mechanisms of Action

2.1. Metformin Pharmacology

Metformin is currently recommended as the first-line GLD in patients with T2D [5]. This plant-derived medicinal product has been used in the treatment of T2D for over 60 years [6]. Galegine or guanidine is a chemical extracted from the herbal plant, Galega officinalis [7]. Metformin is a synthetic guanidine with two coupled molecules (biguanide) and additional chemical substitutions. Metformin is transported into the cell via organic transporter-3 (OCT-3) and OCT-1. It is mainly absorbed from the upper small intestine with an absolute bioavailability of 50–60%. The half-life of plasma level of metformin ranges from 0.9–2.6 h although the latter may vary with different formulations with reports of prolonged half-life due to accumulation in other tissues such as red blood cells [8]. Metformin is excreted unchanged in the urine. Using C11 positron emission tomography, orally administered metformin is mainly concentrated in the liver, kidneys, and bladder with the highest concentrations detected in the liver [9] and jejunal sites [10].

Apart from its low oral bioavailability and short half-life, gastro-intestinal side effects are not uncommon with metformin therapy. Drug delivery systems have been designed to overcome these limitations associated with conventional dosage forms of metformin. Development of novel formulation (e.g., microparticles, and nanoparticles) may improve its bioavailability, reduce the dosing frequency and decrease gastrointestinal side effects with improved effectiveness in the treatment of diabetes and, possibly, cancer [11].

In the last decade or so, there have been a large number of publications on the clinical effects and molecular mechanisms of metformin, with the latter being elegantly summarized in the latest review by LaMoia et al. [12]. In the present article, we aim to interpret these molecular studies in the lens of practicing physicians and highlight the knowledge gap in translating this evidence to clinical practice especially in areas of unmet needs, such as cancer, infection, and cognitive dysfunction in people with or without diabetes.

2.2. Inhibition of Mitochondrial Metabolism and Endogenous Glucose Production

The primary action of metformin is mediated through its effects on mitochondrial metabolism. Metformin with positive charges tends to accumulate within the mitochondria due to the action potentials across its inner membranes. Within the mitochondria, metformin inhibits Complex I of the electron transport chain leading to reduced oxidative phosphorylation with decreased amount of adenosine triphosphate (ATP) as an energy unit. The increased adenosine monophosphate (AMP) to ATP ratio activates the AMP-activated protein kinase (AMPK) with reduced energy storage. The cellular increase in AMP inhibits adenylyl cyclase activity with reduced glucagon signaling needed for glycogenolysis to release glucose [13]. Metformin also reduced mitochondrial glycero phosphate dehydrogenase (mGPD). This leads to an altered cellular redox state with reduced endogenous glucose production including reduced conversion of lactate and glycerol to glucose and hepatic
activated protein kinase (AMPK) with reduced energy storage. At the same time, AMPK activation increases the activity of insulin receptors and translocation of glucose transporters (GLUT) including ubiquitously expressed GLUT-1 and GLUT-4 expressed in muscle to promote glucose uptake in the peripheral tissues [15].

**Figure 1.** Mechanisms of metformin. The multifaceted nature of the mechanisms of metformin targeting different organs, including liver, muscle, and gastrointestinal tract, including the microbiota, results in glucose-lowering, anti-inflammatory, and anti-cancer effects through AMPK and non-AMPK dependent pathways (adapted from references [16–19]).

Due to its inhibition of mitochondrial respiratory chain complex 1 which favours anaerobic respiration, metformin may increase accumulation of lactic acid with increased risk of lactic acidosis. However, metformin-associated lactic acidosis usually occurs within a setting of increased production due to hypoxia with acute cardiopulmonary events or sepsis and renal dysfunction with reduced clearance. In a non-stress situation, the production of lactate by metformin may improve the efficiency of energy metabolism. After donating its proton, lactic acid becomes lactate which is a more efficient cell-to-cell shuttle for delivery of oxidative and gluconeogenic substrates. Through direct uptake and oxidation of lactate produced elsewhere, metabolically active organs (such as cardiomyocytes, liver, and renal cells) can utilize lactate for immediate use without relying on glycolysis and endogenous glucose production [20].

Among metformin users, the incidence of lactic acidosis had been estimated to range from 2 to 9 cases per 100,000 person-years [21] with most of these events occurring within a setting of multi-organ dysfunction. A large body of real-world evidence supported the safety and efficacy of metformin with increasing prescription during the past two decades [22–24]. Apart from its benefits in glucose metabolism, there is a growing body of evidence from clinical trials and observational studies indicating that metformin might prevent or alleviate complications and co-morbidities of T2D such as cardiovascular diseases (CVD), chronic kidney disease (CKD), obesity, cancer, and infections including pneumonia, tuberculosis, and, more recently, coronavirus disease (COVID-19) [25], mediated by both AMPK-dependent and AMPK-independent mechanisms [26].
2.3. Metformin and Hepatic Gluconeogenesis

There are different mechanisms through which metformin can regulate hepatic gluconeogenesis. In terms of transcription alteration, metformin-activated AMPK directly downregulates expression of gluconeogenic genes. The accumulation of AMP inhibits adenylate cyclase and reduces cyclic AMP (cAMP) level which prevents transcription of gluconeogenic genes mediated by the cAMP-response element-binding protein (CREB) [12]. Besides, metformin inhibits mitochondrial glycerol-3-phosphate dehydrogenase-2 (GPD2) which converts glycerol to dihydroxyacetone phosphate [12]. By inhibiting GPD2, metformin increases glycerol and glycerol 3-phosphate levels with reduced gluconeogenesis. GPD2 inhibition is a redox-dependent enzyme and is part of the α-glycerophosphate shuttle. The latter maintains the NADH/NAD+ (nicotinamide adenine dinucleotide) ratio. By inhibiting GPD2, metformin alters the NADH/NAD+ ratio and cytosol redox state and inhibits gluconeogenesis from lactate and glycerol [12].

2.4. Modulation of Gut Microbiota and Inflammation

Metformin efficacy and tolerance are closely linked with the gastrointestinal physiology with accumulating evidence supporting the role of gut microbiota in glucose metabolism [27]. There are approximately 100 trillion micro-organisms, including bacteria, viruses, fungi, and protozoa, in the gastrointestinal tract of a typical person. While the human genome consists of about 23,000 genes, the collective genomes of this microbiota encode over three million genes producing thousands of metabolites which can influence the health and phenotypes of the host. Both animal and clinical studies indicated that different abundance of microbiota was associated with changes in inflammatory microenvironment and production of bile acids and short-chain fatty acids (SCFA) which could contribute to onset and progression in T2D [28].

In clinical trials, metformin-treated patients showed increased abundance of beneficial bacteria such as Akkermansia muciniphila which was negatively associated with the risk of T2D. In a 4-month, double-blind, placebo-controlled study involving treatment-naive patients with T2D [28], metformin treatment increased Akkermansia muciniphila, Bifidobacterium adolescentis and Lactobacillus fermentum, and decreased Intestinibacter bartlettii and Clostridium spp. Amongst these species, changes in Bifidobacterium adolescentis were directly related to the dosage of metformin. In this short-term study, there was no difference in body weight, body fat, or fasting plasma insulin between placebo and metformin although glycated haemoglobin (HbA1c) and fasting plasma glucose were reduced in the metformin group [29]. Two other clinical trials reported similar findings where metformin treatment increased mucin-producing Akkermansia muciniphila, and SCFA-producing microbes [30] including Butyribrio, Bifidobacterium bifidum, Megasphaera, and Prevotella [31]. These microbes utilized different dietary substrates to produce an array of metabolites which can influence the host microenvironment with beneficial metabolic effects. Whilst Bifidobacterium species had been shown to induce gene expression involved in carbohydrate metabolism [32], Prevotella species could degrade starch [31] and metabolize fructose to produce medium-chain carboxylic acids with improved fuel transport [33]. In a study involving newly diagnosed patients with T2D, 3-day treatment with metformin reduced the genus Bacteroides fragilis and increased the bile acid, glycoursodeoxycholic acid, which might contribute to the anti-inflammatory and metabolic effects of metformin [34]. Intriguingly, metformin had been shown to lengthen lifespan of the nematode worm, Caenorhabditis elegans, via changes in microbial folate and methionine metabolism [35].

3. Clinical Evidence for Pleiotropic Effects of Metformin

The multi-targeted actions of metformin are mediated both by the AMPK pathway ubiquitous in all cells for energy metabolism and non-AMPK mechanisms [36]. The clinical benefits of metformin have been reported in liver, pancreas, lungs, and gastrointestinal tract as well as cardiovascular–renal and nervous systems (Figure 2).
In this section, we reviewed the potential molecular mechanisms and clinical evidence regarding the effects of metformin in closely related conditions, including cardiovascular-renal disease, infection, cancer, non-alcoholic fatty liver disease (NAFLD), and cognitive dysfunction. Figure 3 summarizes the clinical effects of metformin in different disease conditions.

Figure 2. Clinical benefits of metformin in multiple systems. The multi-targeted actions of metformin are mediated both by the adenosine monophosphate activated protein kinase (AMPK) pathway and non-AMPK pathways. In the liver, metformin reduces glycogenolysis, hepatic glucose production, and gluconeogenesis [37]. In the lung, metformin modulates the tumor necrosis factor (TNF)-α/NF-kB/mammalian target of rapamycin (mTOR) pathways and expression of pro-inflammatory cytokines. In the intestines, metformin modifies gut microbiome and promotes incretin (e.g., glucagon-like peptide 1, GLP-1) secretion with increased glucose utilization. In the nervous system, metformin reduces amyloid plaque formation and decline of cognitive function. In the circulatory systems, metformin improves dyslipidemia and endothelial dysfunction with reduced cardiovascular–renal events. Metformin reduces site-specific cancer events, including prostate and liver, in part due to amelioration of insulin resistance with reduced activation of insulin/insulin-like growth factor (IGF-1). Metformin is eliminated by the kidney. Metformin alleviates podocyte loss, mesangial cells apoptosis, and tubular cells senescence through AMPK-mediated signaling pathways. In chronic kidney disease, renal fibrosis is ameliorated by metformin, mainly via AMPK activation. Reduced glomerular filtration and tubular secretion may lead to accumulation of metformin and increased risk of lactic acidosis, especially in stress situations [17] (adapted from reference [13]).

3.1. Putative Mechanisms of Metformin on Cardiovascular Systems

Diabetes is a major risk factor for CVD and CKD. In the 10-year follow-up analysis of the United Kingdom Prospective Diabetes Study (UKPDS), treatment with metformin was associated with reduced cardiovascular events and all-cause mortality [38]. The Diabetes Prevention Program conducted in the United States of America (USA) was the largest and longest clinical trial assessing the effects of metformin in people with impaired glucose tolerance (IGT). In this study, metformin was confirmed to prevent T2D which was translated to reduction in cardiovascular events in the post-trial period [39]. Apart from these two large randomised controlled trials (RCTs), most of the evidence in support of
the cardiovascular–renal effects of metformin were inferred from systematic reviews and meta-analysis of observational studies (Table 1).

Figure 3. Summary of clinical effects of metformin in different disease conditions. NAFLD, Non-alcoholic fatty liver disease (NAFLD); NASH, non-alcoholic steatohepatitis; CKD, chronic kidney disease; and UKPDS, United Kingdom Prospective Diabetes Study.

In a meta-analysis of RCTs including 2079 patients with T2D [40], metformin use was associated with reduced risk of cardiovascular death, myocardial infarction, and peripheral vascular disease compared with non-use of metformin. The most consistent benefits were observed for all-cause mortality with up to 16% risk reduction, albeit with an increased risk of stroke by 48% [39]. In a more recent meta-analysis involving 1,160,254 patients with T2D, metformin use was associated with decreased cardiovascular mortality (relative risk, RR = 0.44 (95% CI: 0.34–0.57) and incidence of CVD (RR = 0.73, 95% CI: 0.59–0.90)) [41]. However, the risk association of metformin with myocardial infarction and heart failure amongst patients with T2D [42,43] was not always consistent [44], with many of these meta-analyses and systematic reviews having low or critically low quality [25,44,45]. The heterogeneous clinical profiles, study design, and settings contributed towards these controversies calling for more RCTs with better-defined settings, populations, and study design, preferably with comparative drugs [44].
Table 1. Studies on the association of metformin use with clinical events in patients with type 2 diabetes.

| Author/Year | Study Design | Region       | No. of Participants | No. of Cases | Follow-Up (Years) | Comparisons and Outcomes | Main Conclusion |
|-------------|--------------|--------------|---------------------|--------------|-------------------|--------------------------|-----------------|
| Raee, 2017  | Cohort       | Iran         | 717                 | 446          | 3.0               | Glyburide versus metformin All-cause mortality: HR = 0.27, 95% CI: 0.10–0.73 Cardiovascular mortality: HR = 0.12, 95% CI: 0.20–0.66 | Compared with metformin, glyburide was associated with increased all-cause and cardiovascular mortality in patients with diabetes. |
| Scheller, 2014 | Retrospective cohort | Denmark       | 84,756               | 83,528       | 5.0               | Sitagliptin versus metformin All-cause mortality: HR = 1.25, 95% CI: 0.92–1.71 Incidence of CVD: HR = 1.22, 95% CI: 0.96–1.61 | Compared with metformin monotherapy, sitagliptin monotherapy was not associated with increased risk of all-cause mortality or CVD. |
| Roumie, 2012 | Retrospective cohort | USA           | 253,690              | 155,025      | 5.5               | Sulfonylurea versus metformin CVD (acute myocardial infarction and stroke) or death: HR = 1.21, 95% CI: 1.13–1.30 | Compared with metformin, use of sulfonylureas was associated with an increased hazard of CVD events or death. |
| Roumie, 2017 | Retrospective cohort | USA           | 131,972              | 65,986       | 0.9–1.1           | Sulfonylurea versus metformin Heart failure and cardiovascular death: HR = 1.32, 95% CI: 1.21–1.43 | Compared with metformin, sulfonylurea had a higher risk of heart failure and cardiovascular death. |
| Johnson, 2002 | Cross-sectional | Canada        | 4183                 | 1150         | 5.1               | Metformin versus sulfonylurea All-cause mortality: OR = 0.60, 95% CI: 0.49–0.74 cardiovascular-related mortality: OR = 0.53, 95% CI: 0.41–0.68 | Metformin therapy, alone or in combination with sulfonylurea, was associated with reduced all-cause and cardiovascular mortality. |
| Ekstrom, 2012 | Register-based cohort | Sweden        | 32,152               | 14,696       | 3.9               | Other-GLDs versus metformin Incidence CVD: HR = 1.02, 95% CI: 0.93–1.12 All-cause mortality: HR = 1.13, 95% CI: 1.01–1.27 | Metformin showed lower risk than insulin for CVD and all-cause mortality and slightly lowered risk for all-cause mortality compared with other GLDs. |
| Pantalone, 2012 | Retrospective cohort | USA           | 23,915               | 12,774       | 2.2               | Glipizide, glyburide, glimepiride versus metformin All-cause mortality: - glipizide: HR = 1.64 (1.39–1.94) - glyburide: HR = 1.59 (1.35–1.88) - glimepiride: HR = 1.68 (1.37–2.06) | Glipizide, glyburide and glimepiride were associated with an increased risk of overall mortality versus metformin. |
### Table 1. Cont.

| Author/Year | Study Design | Region | No. of Participants | No. of Cases | Follow-Up (Years) | Comparisons and Outcomes | Main Conclusion |
|-------------|--------------|--------|---------------------|--------------|-------------------|---------------------------|-----------------|
| Charytan, 2019 [53] | Clinical trails | USA | 4038 | 591 | 4.0 | Metformin versus non-metformin  
All-cause mortality: HR = 0.49, 95% CI: 0.36–0.69  
Cardiovascular death: HR = 0.49, 95% CI: 0.32–0.74  
Cardiovascular composite: HR = 0.67, 95% CI: 0.51–0.88  
Kidney disease composite: HR = 0.77, 95% CI: 0.61–0.98  
ESKD (end stage kidney disease): HR = 1.01, 95% CI: 0.65–1.55 | Metformin might be safer for use in CKD than previously considered with reduced risk of death and cardiovascular events in individuals with stage 3 CKD. |
| Cheng, 2014 [54] | Retrospective cohort | Taiwan | 14,856 | 10,857 | 4.0 | Metformin versus non-metformin  
Incidence stroke: HR = 0.38, 95% CI: 0.35–0.42 | Compared with non-metformin use, metformin use was associated with lower risk of stroke especially in high-risk patients |
| Mogensen, 2015 [55] | Retrospective cohort | Danish | 28,236 | 16,910 | 13.0 | Sulfonylureas + metformin versus metformin/ metformin + insulin  
All-cause mortality: RR = 1.81, 95% CI: 1.63–2.01  
Cardiovascular death: RR = 1.35, 95% CI: 1.14–1.60  
Composite endpoint (myocardial infarction, stroke and cardiovascular death): RR = 1.25, 95% CI: 1.09–1.42 | In combination with insulin, the use of sulfonylureas was associated with increased mortality compared with metformin. |
| Evans, 2006 [56] | Retrospective cohort | UK | 5617 | 2286 | 8.0 | Sulfonylurea versus metformin  
All-cause mortality: HR = 1.43, 95% CI: 1.15–1.77  
Cardiovascular mortality: HR = 1.70, 95% CI: 1.18–2.45 | Patients treated with sulfonylureas only, or combinations of sulfonylureas and metformin, were at higher risk of adverse cardiovascular outcomes than those treated with metformin alone. |
| Sillars, 2010 [57] | Retrospective cohort | Australia | 1271 | 390 | 10.4 | Metformin–sulphonylurea versus diet and metformin monotherapy  
All-cause mortality: HR = 0.82, 95% CI: 0.58–1.23  
Cardiovascular mortality: HR = 0.82, 95% CI: 0.53–1.27 | Combination metformin–sulphonylurea appeared to be as safe as other blood glucose-lowering therapies used in type 2 diabetes. |
| Author/Year | Study Design       | Region   | No. of Participants | No. of Cases | Follow-Up (Years) | Comparisons and Outcomes                                                                 | Main Conclusion                                                                                                                                 |
|------------|--------------------|----------|--------------------|--------------|------------------|------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| Morgan, 2014 [58] | Retrospective cohort | UK       | 80,999            | 68,139       | 2.9–3.1          | Sulfonylurea versus metformin                                                              | All-cause mortality: HR = 1.27, 95% CI: 1.02–1.58 MACE (adverse cardiovascular events): HR = 0.81, 95% CI: 0.57–1.15 All-cause mortality was increased in patients prescribed with sulphonylureas compared with metformin monotherapy. |
| Breunig, 2014 [59] | Retrospective cohort | USA      | 6271              | 5548         | 1.6              | Rosiglitazone, pioglitazone versus metformin                                              | Incidence of heart failure: Rosiglitazone: HR = 1.57, 95% CI: 1.15–2.15 Compared with metformin, there appeared to be higher risk of heart failure in patients started on rosiglitazone but not pioglitazone. |
| Fung, 2015 [60]  | Retrospective cohort | Hong Kong | 11,293            | 7493         | 5.0              | Metformin versus non–metformin                                                              | Incidence of coronary heart disease: HR = 0.87, 95% CI: 0.52–0.86 Incidence of stroke: HR = 0.75, 95% CI: 0.57–0.98 Incidence of CKD (eGFR < 30): HR = 1.08, 95% CI: 0.84–1.38 Patients who were started on metformin monotherapy showed improvement in many of the clinical parameters and a reduction in all-cause mortality and CVD events than lifestyle modifications alone. |

Note: CVD, cardiovascular disease; HR, hazard ratio; CI, confidence interval; RR, relative risk; GLDs, glucose-lowering drugs; CKD, chronic kidney disease; and eGFR, estimated glomerular filtration rate.
3.1.1. Metformin and Endothelial Dysfunction, Inflammation and Oxidative Stress

Pending definitive evidence on clinical outcomes, metformin has been shown to improve surrogate markers of CVD, including endothelial dysfunction, dyslipidemia, and systemic inflammation [38,61,62]. Metformin improves endothelial dysfunction by increasing nitric oxide synthase (eNOS) with increased generation of nitric oxide (NO), a potent vasodilator. Other mechanisms include suppression of mitochondrial complex 1, stimulation of AMPK and inhibition of apoptosis [63]. In experimental studies, AMPK activation by therapeutically relevant concentrations of metformin (50–500 µM) increased NO via increasing eNOS phosphorylation and eNOS interaction with heat shock protein 90 (HSP90) [64]. Metformin restores the impaired eNOS-HSP90 interaction in high-glucose exposed endothelial cells [64]. The eNOS activating effect of metformin (250 mg/kg/d) has also been demonstrated in endothelial progenitor cells from streptozotocin (STZ)-induced diabetic mice [65]. Metformin further attenuates glucose-induced endothelial dysfunction through enhancing guanosine 5′triphosphate cyclohydrolase 1 (GTPCH1) mediated eNOS recoupling and NADPH oxidase inhibition [66]. Metformin raises the tissue concentration of hydrogen sulfide (H2S), which is a major endothelium-derived hyperpolarising factor (EDHF) that causes vascular endothelial and smooth muscle cell hyperpolarization and vasorelaxation by activating the ATP-sensitive potassium channels through cysteine S-sulfhydration [67].

Metformin may exert anti-inflammatory effects and reduce oxidative stress via multiple pathways. This can occur via an AMPK-dependent inhibition of the inhibitory-kB kinase (IKK)/IkBalpha/NF-kB [67]. Metformin also inhibits tumor necrosis factor (TNF)-α induced gene expression of cell adhesion molecules that contribute to monocyte adhesion which promotes atherogenesis. Acting via AMPK, metformin also exhibits epigenetic effects and phosphorylates multiple substrates, including histone acetyltransferases class II histone deacetylases (HDACs) and DNA/histone methyltransferase [63]. For example, metformin may increase Sirtuin 1 (SIRT1) activity and protect against hyperglycemia-induced metabolic memory resulting in endothelial dysfunction [68].

3.1.2. Metformin on Blood Flow and Haemostasis

Several studies have shown favourable effects of metformin on blood flow. Metformin increased haemodynamic responses to L-arginine, the precursor of vasodilatory NO [69]. Metformin also lowered levels of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase (NOS) in T2D. Metformin reduced platelet activity and haemostasis with reduced clot formation [70]. In clinical studies, metformin reduced plasminogen activator inhibitor-1 (PAI-1) with increased fibrinolysis [71], although results are not always consistent [70]. In in vitro studies, metformin had been shown to reduce platelet activation by reducing extracellular mitochondrial DNA (mtDNA) release [72].

3.1.3. Metformin and Kidney Disease

The occurrence of CKD in patients with T2D markedly amplified the risk of CVD [73,74]. In patients with mild to moderate CKD (stages 3a and 3b with respective estimated glomerular filtration rate [eGFR] 45–59 and 30–44 mL/min/1.73 m²), the incidence of death due to CVD was considerably higher than that due to kidney failure, with these patients having the double burden of CVD and end-stage kidney disease (ESKD) [75]. Metformin reduces blood glucose without causing weight gain and hypoglycemia [76] which are conducive to the prevention of CVD. This low risk of hypoglycemia is particularly relevant to patients with CKD who are at high risk for both CVD and hypoglycemia which are closely associated. Despite the lack of definitive evidence from RCTs, cohort analyses, and real-world evidence suggested neutral or beneficial cardiovascular–renal effects of metformin in patients with T2D at different stages of CKD (Table 2). These clinical findings are supported by experimental findings where metformin use in rats with CKD prevented progression of renal dysfunction, reduced vascular calcification, and inhibited high bone turnover with reduced renal expression of cellular infiltration, fibrosis, and inflammation [77].
Table 2. Cohort studies on the association of metformin use with cardiovascular–renal outcomes at different CKD stages.

| Author/Year | Study Design                  | Sample Size          | Comparison                                                                 | Duration/Dose | Outcomes, Hazard Ratio (95% CI) | Main Conclusion                                                                 |
|-------------|-------------------------------|----------------------|-----------------------------------------------------------------------------|---------------|--------------------------------|--------------------------------------------------------------------------------|
| Whitlock, 2020 [78] | Retrospective Cohort (2006–2017) | 21,996 (metformin: 19,990) | metformin vs. sulfonylurea among patients with T2D (age > 18 years) | NA            | All-cause mortality: Overall: 0.48 (0.40–0.58) eGFR ≥90: 0.38 (0.27–0.53) eGFR 60–89: 0.42 (0.31–0.56) eGFR 45–59: 0.92 (0.53–1.61) eGFR 30–44: 0.85 (0.46–1.57) eGFR <30: 1.51 (0.58–3.95) CVD: Overall: 0.64 (0.41–1.00) eGFR ≥90: 0.78 (0.52–1.2) eGFR 60–89: 0.86 (0.45–1.64) eGFR 45–59: 0.62 (0.3–1.29) eGFR 30–44: 0.85 (0.46–1.37) eGFR <30: 0.56 (0.18–1.69) | Metformin use was associated with lower risk for all-cause mortality, cardiovascular events, and major hypoglycemic episodes when compared with sulfonylureas. CKD was a significant effect modifier for all-cause mortality, but not for cardiovascular events or major hypoglycemic episodes. |
| Kwon, 2020 [79] | Retrospective Cohort (2001–2016) | 10,426 | metformin vs. non-metformin among patients with type 2 diabetes kidney disease | Duration and dose | All-cause mortality: Overall: 0.48 (0.40–0.58) eGFR ≥45: 0.38 (0.27–0.53) eGFR 45–30: 0.42 (0.31–0.56) eGFR <30: 0.35 (0.37–0.81) ESKD: Overall: 0.67 (0.58–0.77) eGFR ≥45: 0.62 (0.51–0.76) eGFR 45–30: 0.73 (0.54–0.99) eGFR <30: 0.87 (0.67–1.12) | Metformin usage in advanced CKD patients, especially those with CKD 3b, was associated with reduced risk of all-cause mortality and incident ESKD. Metformin did not increase the risk of lactic acidosis. |
| Charytan, 2019 [53] | Retrospective analysis in trials | 4038 (591) | metformin vs. non-metformin among patients with diabetes and chronic kidney disease | NA            | All-cause mortality: Overall: 0.49 (0.36–0.69) CKD S1–3: 0.61 (0.44–0.82) CKD S4–5: 0.83 (0.54–1.27) CV-death: Overall: 0.49 (0.32–0.74) CKD S1–3: 0.59 (0.38–0.9) CKD S4–5: 0.80 (0.46–1.39) ESKD: Overall: 1.01 (0.65–1.55) CKD S1–3: 0.70 (0.53–0.92) CKD S4–5: 0.95 (0.7–1.29) | Metformin might be safer for use in CKD than previously considered with reduced risk of death and cardiovascular events in individuals with stage 3 CKD. |
| Author/Year | Study Design | Sample Size | Comparison | Duration/Dose | Outcomes, Hazard Ratio (95% CI) | Main Conclusion |
|------------|--------------|-------------|------------|--------------|---------------------------------|----------------|
| Bergmark, 2019 [80] | Retrospective analysis in trials (2010–2013) FU: 2.1 years | 12,156 (8971) | metformin vs. non-metformin among patients with diabetes and high CV risk | NA | All-cause mortality: 0.75 (0.59–0.95) \( \text{CV-death: 0.68 (0.51–0.91)} \) \( \text{MI: 1.08 (0.83–1.41)} \) \( \text{Stroke: 1.07 (0.77–1.48)} \) \( \text{Hear failure: 1.23 (0.94–1.6)} \) | Metformin use was associated with reduced risk of all-cause mortality, including after adjustment for clinical variables and biomarkers, but not lower rates of the composite end point of cardiovascular death, myocardial infarction, or ischemic stroke. |
| Roumie, 2019 [81] | Retrospective Cohort (2001–2016) FU: 1.1 year | 174,882 metformin and sulfonylureas users | metformin vs. sulfonylureas | NA | MACE: Overall: 0.80 (0.75–0.86) | Among patients with diabetes and reduced kidney function persisting with monotherapy, treatment with metformin, compared with a sulfonylurea, was associated with a lower risk of MACE. |
| Hung, 2015 [82] | Retrospective Cohort (2000–2009) FU: 2.1 years | 3252 (metformin 813) | metformin vs. non-metformin among patients with type 2 diabetes and stage 5 chronic kidney disease | Daily dose | All-cause mortality: 1.35 (1.2–1.51) | Use of metformin in people with type 2 diabetes and a serum creatinine concentration greater than 530 µmol/L was associated with an increased risk of all-cause mortality compared with non-users. Metformin use should not be encouraged in this patient group. |
| Ekstrom, 2012 [51] | Retrospective analysis in Swedish register (2004–2007) FU: 3.9 years | 51,675 patients with type 2 diabetes | Metformin monotherapy vs. other GLDs | NA | All-cause mortality: Overall: 1.13 (1.01–1.27) \( \text{Fatal/non-fatal CVD: Overall: 1.02 (0.93–1.12)} \) | Metformin showed lower risk vs. insulin for CVD and all-cause mortality, and lower risk for all-cause mortality vs. other GLDs |

Note: FU, follow-up; GLDs, glucose-lowering drugs; MACE, major adverse cardiovascular events; and ESKD, end-stage kidney disease.
The latest Kidney Disease Improving Global Outcomes (KDIGO) practice guideline recommended continuing use of metformin in CKD Stage 3b (eGFR: 30–44 mL/min/1.73 m$^2$) with dose adjustments and increasing frequency of eGFR monitoring [83]. In both the USA and European Union (EU), according to the label, metformin can be used in CKD stage 3 (eGFR: 30–60 mL/min/1.73 m$^2$), but not in stage 4 or stage 5. In the USA, metformin should not be initiated in CKD stage 3b, but may be continued in patients already treated with metformin [84]. Given the global burden of ESKD and the low cost of metformin, the safety and efficacy of metformin use in patients with CKD stage 3b and stage 4 should be further explored, preferably using RCT design due to a paucity of real-world data in these patients.

Metformin may protect the kidney via multiple mechanisms, such as reducing podocyte loss, tubulointerstitial injury, and mesangial cell dysfunction. Podocyte loss is the initiating event in the development of glomerular sclerosis in diabetic kidney disease (DKD). In animal models of T2D, metformin prevented podocyte loss via oxidative stress inhibition. In cultured podocytes, metformin reduced apoptosis via AMPK activation and inhibition of mTOR (mammalian target of rapamycin) activity [85]. In in vitro models, metformin attenuated palmitate-mediated mesangial apoptosis, ameliorated oxidative stress, and promoted autophagy. In high glucose-stimulated rat mesangial cells, metformin inhibited abnormal cell proliferation via the AMPK/SIRT1/forkhead box protein O1 (FOXO1) pathway [86]. Other studies demonstrated metformin in attenuating renal fibrosis in mice model of DKD by altering miR-192 expression [87]. Metformin also protected human epithelial cells against glucose-induced apoptosis by normalizing parkin protein expression and inducing mitophagy via repressing NF-KB expression [88].

More recently, beneficial effects of metformin have been shown in renal conditions other than DKD. Metformin was shown to inhibit cyst growth in patients with polycystic kidney disease (PKD) due to PKD1 mutation. In a zebrafish model, metformin inhibited cyst formation via activation of the AMPK pathway and modulated cellular events, such as autophagy, cellular proliferation, and inflammation [89].

3.2. Metformin and Infection

Metformin was originally introduced as an anti-influenza drug and had been proposed as an adjunct treatment in infective diseases [7]. During the current pandemic of COVID-19, there has been renewed interest in repurposing metformin as a host-directed adjunctive therapy to treat infections by altering the immune responses [37,90–92]. In this regard, metformin use was associated with a lower risk of death in patients with T2D affected by COVID-19 than their counterparts using other GLDs, especially among women with obesity [93,94]. Other studies indicated that metformin users who developed COVID-19 infection had lower levels of interleukin-6 (IL-6) [95] and other inflammatory markers than non-users [96]. Despite these observational data, definitive evidence from RCTs is lacking [92].

Patients with T2D are at high risk of pneumonia and other respiratory infections including chronic obstructive pulmonary diseases (COPD). In patients with community-acquired pneumonia, increased neutrophil-to-lymphocyte ratios indicating a heightened pro-inflammatory state had been associated with poor outcomes [13,97]. In a cohort of 3537 patients with T2D, long-term treatment with metformin was associated with reduced neutrophil-to-lymphocyte ratios, compared with sulfonylurea [98]. Previous studies reported a protective effect of metformin on pneumonia-related hospitalizations and pneumonia-related mortality among patients with T2D [99,100]. In an observational study of 36,990 patients aged >65 years with diabetes who were hospitalized with pneumonia, metformin users had a lower 30-day pneumonia-related mortality (odds ratio, OR = 0.80, 95% CI: 0.72–0.88) than non-users [100]. In a prospective diabetes register involving 15,784 patients with T2D in Hong Kong, metformin use was independently associated with lower incidence of pneumonia-related hospitalisation with a hazard ratio (HR) of 0.63 (95% CI: 0.52–0.77) and related-mortality (HR = 0.49, 95% CI: 0.33–0.73) adjusted for multiple confounders [101]. In a RCT comparing metformin vs. placebo for reducing adverse metabolic
effects of glucocorticoids, in the analysis of the adverse events, metformin-treated patients had a lower incidence of pneumonia than the placebo group accompanied by lower levels of pro-inflammatory cytokines [102].

Several mechanisms have been proposed for the protective effects of metformin on pulmonary infections. In animal models of hyperoxia-induced lung injury, metformin reduced inflammatory cytokines such as IL-6 and TNF-α [103]. In other animal studies, metformin reduced the excessive release of neutrophil extracellular traps (NETs). The latter are extracellular DNA with anti-microbial actions although its overproduction may cause excessive inflammatory responses with deleterious consequences [104]. Metformin had been advocated as adjunctive therapy to improve outcomes in patients with sepsis [105]. In patients with tuberculosis, metformin had been shown to improve T-cell immunity and phagocytosis [106,107].

3.3. Metformin and Cancer

Diabetes, obesity, and cancer frequently coexist in part due to insulin resistance where excessive stimulation of the insulin/insulin-like growth factor (IGF-1) pathway might cause abnormal cell signaling and cancer growth [108]. Other epidemiological studies suggested additive risk associations of glycemic variability and burden with all-site cancer and cancer-related death in T2D [109,110]. In breast cancer cells, metformin exerted anticancer effects by changing the metabolic milieu and reducing the circulating insulin levels by improving insulin resistance with reduced insulin/IGF-I receptor-mediated phosphoinositide 3-kinases (PI3K) signaling [111].

Metformin also inhibited mTOR pathway in cancer cells by activating AMPK and liver kinase B1 (LKB1) with reduced protein synthesis and cell growth [112]. In a systematic review, the Signal transducer and activator of transcription 3 (STAT3) was activated through the LKB1 and AMPK pathway which induced apoptosis in triple-negative breast cancer cells. Metformin had also been shown to influence the “sphingolipid rheostat”, shifting the balance away from Sphingosine-1-Phosphate towards ceramides with inhibition of cell growth and proliferation as demonstrated in an ovarian cancer cell line. Other anti-cancer mechanisms of metformin included increased fatty acids oxidation and reduced expression of transcription factors, such as specificity protein (Sp)1, Sp3, and Sp4 implicated in cancer growth [111]. Figure 4 summarizes the main mechanisms for the action of metformin and cancer.

**Figure 4.** The action of metformin on cancer. Metformin activates adenosine monophosphate activated protein kinase (AMPK), an immediate downstream effector of the tumor suppressor liver kinase B1 (LKB1), resulting in inhibition of tumor growth. The various downstream effects of
metformin-mediated AMPK activation in tumor growth inhibition include: (1) activation of the tuberous sclerosis complex (TSC) with inhibition of mammalian target of rapamycin (mTOR) activity, resulting in inhibition of protein synthesis and cell growth; (2) activation of p53 and p21 along with inhibition of cyclins, resulting in cell-cycle arrest; (3) inhibition of lipid and sterol biosynthetic pathways; (4) inhibition of sterol regulatory element-binding protein-1c (SREBP-1) by regulating its expression and phosphorylation, leading to down-regulation of fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC); (5) direct phosphorylation and inhibition of ACC; and (6) systemic effects on multiple organs such as reducing diabetes-associated cancers by improving glucose balance with reduced levels of growth factors such as insulin, insulin-like growth factor 1 (IGF-1) and leptin which can initiate and promote cancer growth with progression. Metformin had also been shown to reduce cancer events via AMPK-independent mechanisms [113,114] (adapted from reference [115,116]).

In experimental studies, metformin induced programmed cell death of cancer cells with inhibition of cell signals, such as vascular endothelial growth factor A (VEGFA), with reduced vascularization of tumor cells [36]. Metformin also modulated the immune response by activating anti-tumor T-cell activity [117]. By reducing oxygen and glucose consumption by tumor cells and increasing the intratumor oxygen levels, metformin sensitized patients’ responses to programmed death-ligand 1 (PD-L1) chemotherapy [118]. By altering the epigenetic signature of tumor cells, metformin also interfered with the signaling pathways that conferred chemoresistance of endometrial cancer cells and improved treatment responses to chemotherapy [119].

A large number of observational studies suggested an association of metformin use with reduced incidence of cancers [120]. In the Taiwan National Health Insurance Data Survey (2000–2007) including 12,005 metformin-users and 4597 non-metformin users, metformin use was associated with reduced risk of total, colorectal, liver and pancreatic cancer by up to 88% [121]. In a UK retrospective cohort of 62,809 patients, metformin monotherapy was associated with the lowest cancer risk, compared with insulin or sulfonylureas. Compared with metformin, sulfonylurea monotherapy was associated with a HR of 1.36 (95%CI: 1.19–1.54) for solid tumors (breast, colon, pancreas, and prostate cancer). The corresponding HR for combination therapy of metformin and sulfonylurea was 1.08 (95%CI: 0.96–1.21) [122]. Other observational studies also reported low incidence of breast cancer among long-term metformin users vs. non-users [123]. In a database of health records from Tayside of Scotland, a comparative analysis between new metformin users and users of other medications showed consistently lower hazard for diagnosed cancer amongst metformin users [124].

In the Hong Kong Diabetes Register of 2658 patients with T2D free from cancer at enrollment [125], metformin use was associated with reduced risk of cancer in a dose-dependent manner. After adjusting for covariates, metformin non-users with high-density lipoprotein (HDL)-cholesterol <1.0 mmol/L had 5.8-fold increased hazards of cancer compared with metformin users with HDL-cholesterol ≥1.0 mmol/L [125]. In a post-hoc analysis of rosiglitazone-based RCTs, including the ADOPT (A Diabetes Outcome Progression Trial) and RECORD (Rosiglitazone Evaluated for Cardiovascular Outcomes and Regulation of Glycaemia in Diabetes) Trials [126], there was no difference in the incidence of cancers between patients treated with metformin vs. rosiglitazone. Apart from short duration of follow up, these studies were not powered to evaluate cancer incidence as a predefined endpoint [127]. Of note, these retrospective studies are subject to time-related biases, including immortal time bias and time-window bias, which might inflate the association between metformin and reduced risk of cancer [128]. Besides, it remained plausible that the risk differential might be due to increased cancer risk in the comparator group [129].

In a systematic review of 11 studies with accrual of 4042 cancer events and 529 cancer deaths, the researchers reported a 31% reduction in all-cancer risk amongst metformin-users compared with users of other GLDs (RR = 0.69, 95% CI: 0.61–0.79) [129]. The negative association was significant for pancreatic and hepatocellular cancer, and nonsignificant for colon, breast, and prostate cancer. On the other hand, in a pooled analysis of 9 RCTs
including 821 patients with advanced or metastatic cancers from lung, breast, or pancreas as primary cancers, metformin did not improve tumor-related outcomes with a pooled OR of 1.23 (95% CI: 0.89–1.71) [130]. Similarly, metformin added to anticancer agents did not prolong progression-free survival (HR = 0.95, 95% CI: 0.75–1.21) or actual survival (HR = 0.97, 95% CI: 0.80–1.16) [130].

There are few RCTs that evaluated metformin with cancer as a predefined outcome measure. In a multi-center, double-blind, placebo-controlled phase 3 trial, nondiabetic adults who had resection of single or multiple colorectal adenomas or polyps were randomised to receive metformin 250 mg daily or placebo for 1 year. After 1 year, the total counts of polyps on colonoscopy was lower in the metformin group than the placebo group (relative risk (RR) = 0.67, 95% CI: 0.47–0.97). The corresponding RR for adenomas was 0.60 (95% CI: 0.39–0.92) [131].

In a phase 2 randomised trial, 40 women with metastatic breast cancer positive for estrogen receptor (ER)/progesterone receptor receiving chemotherapy were randomised to receive metformin (n = 22, mean age, 55 years) or placebo (n = 18, mean age, 57 years) for a mean of 151 days. The progression-free survival was 5.4 months in the metformin group vs. 6.3 months in the placebo group with a HR of 1.2 (95% CI: 0.63–2.31). The corresponding mean survival time were 20.2 and 24.2 months with a HR of 1.68 (95% CI: 0.79–3.55) suggesting that metformin did not confer survival benefits in patients with advanced disease although larger samples size and longer follow up period are needed to confirm these observations [132].

Obesity and hormonal dysregulation play important roles in the initiation and progression of some cancer events. Despite plausible mechanisms, the effects of metformin on hormone-responsive cancers, such as breast and endometrial cancers, remain to be clarified [133]. To this end, the NCIC Clinical Trials Group MA.32 had initiated a 5-year phase 3 RCT including 3649 women with early-stage breast cancer randomised to receive metformin 500 mg twice daily or placebo [134]. In an interim analysis at 6 months, metformin was associated with a tendency of increased insulin sensitivity, lower body mass index (BMI) [134], and reduced estradiol hormones vs. placebo [135].

### 3.4. Metformin and NAFLD

Metformin regulates cellular lipid and glucose metabolism by reducing mitochondrial oxidative processes resulting in activation of AMPK within the liver. This is accompanied by inhibition of de novo synthesis of fatty acids and increased β-oxidation of fatty acids leading to reduced liver steatosis. Metformin also reduced lipid accumulation by inhibiting differentiation of adipocytes with reduced production of adipokines [136]. In transgenic obese (ob/ob) mice with NAFLD, metformin reduced hepatic fat accumulation and liver steatosis and reversed hepatomegaly and abnormalities in liver enzymes [137]. In the high-fat-diet-induced NAFLD model of nondiabetic mice, metformin prevented and reversed liver steatosis and inflammation [138].

In the first open-label, pilot study, 20 non-diabetic patients with NAFLD were given metformin 500 mg thrice daily for 4 months but 6 patients did not adhere to treatment and were considered as control subjects. Insulin resistance was quantified using the euglycemic clamp technique and liver volume measurement by ultrasound scan. Metformin reduced the liver volume, moderately improved insulin sensitivity and normalized aminotransferase levels in 50% of the patients. Withdrawal of metformin was accompanied by a return of aminotransferase levels to the pre-treatment values. No changes in any of these parameters were observed in the control patients [139].

In a subsequent clinical trial using open-label, quasiexperimental design involving 28 overweight or obese patients with non-alcoholic steatohepatitis (NASH) treated with metformin 2000 mg daily for 12 months, the researchers reported improvement in insulin resistance, alanine transaminase (ALT), and histology [140]. In another comparative trial involving 34 patients with NASH treated with metformin 850 mg twice daily plus diet
(n = 17) vs. diet alone (n = 17) for six months, the metformin group had reduction in ALT but no effects on histology [141].

Other researchers compared metformin vs. therapies, such as thiazolidinedione (TZDs) or vitamin E, in patients with NAFLD. In an open-label, randomised study, 35 non-diabetic patients with NAFLD were assigned to 12-month treatment with metformin 2000 mg daily (n = 55), vitamin E 800 IU daily (n = 28) or weight-reducing diet (n = 27). Liver enzymes and weight loss improved in all groups with the metformin group showing the highest odds of normalization of liver enzymes and metabolic profile. In a subgroup of 17 metformin users with 14 being non-responders, metformin use was associated with reduction in liver fat, necro-inflammation, and fibrosis although these results were limited by the small sample size [142]. In another open-label RCT comparing metformin, rosiglitazone, and combination therapy with both drugs, changes in liver enzymes and histology were observed only in patients treated with rosiglitazone or rosiglitazone plus metformin group but not in metformin alone group [143].

Following these initial encouraging results, subsequent double-blind placebo-controlled trials tended to report lack of benefits of metformin in patients with NAFLD. In one such trial, 48 patients with biopsy-proven NAFLD were randomised to receive either metformin or placebo for 6 months followed by repeat liver biopsy. Despite the positive effects on body weight, lipids, and glycaemic control, metformin treatment did not improve NAFLD score based on liver transaminases. No differences were observed in parameters of liver steatosis, assessed either histologically or by imaging [144]. Similarly, in two RCTs evaluating metformin (1000 to 1500 mg per day) vs. placebo in children with obesity and NAFLD, metformin did not improve liver histology, ALT and aspartate transaminase (AST) levels, BMI or insulin resistance [145]. In the Diabetes Prevention Program, participants with IGT randomised to metformin had lower ALT levels which was rendered non-significant once adjusted for weight loss [146]. Based on the latest systematic review of available data, there is insufficient evidence to support the use of metformin for relieving NAFLD or NASH [147].

3.5. Metformin and Cognitive Function

Ageing, T2D, and cognitive dysfunction frequently coexist. Apart from metabolic and vascular causes, Alzheimer’s disease (AD) characterized by deposition of amyloid-β (Aβ) plaques, neuroinflammation, neurofibrillary tangles, and neuronal loss is an important cause of dementia in patients with or without T2D. Experimental studies suggested that metformin might prevent amyloid plaque formation via AMPK-dependent pathways [148]. In part due to its anti-inflammatory effects, metformin improved microenvironment to promote neuro-glial cell survival and differentiation [149]. Additionally, metformin might directly influence the functional phenotype of microglia favoring a M2 phenotype which facilitated neural tissue repair following infarction [150]. In cultured astrocytes, metformin increased cellular consumption of oxygen and glucose with suppressed intermediates of amino acid and fatty acid metabolites but increased lactate production due to predominant anaerobic glycolysis. However, the clinical significance of these findings remained uncertain [151].

In animal studies, male Wistar rats treated with metformin at a dose of 100 mg/kg/day exhibited a reversal of scopolamine-induced cognitive impairment [152]. However, other studies using the same rat models reported no effect of metformin-fortified diet on cognitive function, despite improving insulin sensitivity [153]. In ischemic models, metformin improved neuron survival in dentate gyrus of diabetic mice [154]. In a rat model of forebrain ischaemia, metformin treatment for 7 days restored regulation of the AMPK/brain derived neurotrophic factor (BDNF)/70-kDa ribosomal protein S6 kinase (p70S6K) pathway with enhanced learning and memory [155].

Several observational studies investigated the effects of metformin on cognitive function [156] although many of these studies were limited by small sample size with incomplete documentation or adjustment for confounders, such as diabetes duration, glycaemic con-
control, duration of metformin use, and comorbidities. One of the major confounders is the inhibitory effect of metformin on intestinal absorption of vitamin B12, especially with prolonged use in high dose where subclinical vitamin B12 deficiency had been associated with cognitive decline [157,158].

In a cross-sectional study of 4160 adults with normoglycemia (n = 1856), either with (n = 318) or without (n = 1986) metformin treatment, metformin use was associated with higher risk of vitamin B12 deficiency (odds ratio (OR) = 1.45, 95% CI: 1.03–2.02) and 1.3 times higher risk of cognitive impairment [159]. In a cross-sectional study conducted in Australia involving patients with AD and mild cognitive impairment [158], amongst patients with diabetes (n = 126), worse cognitive performance was associated with metformin use (OR = 2.23, 95% CI: 1.05–4.75), which was attenuated after adjustment for vitamin B12 levels (OR = 1.75, 95% CI: 0.81–3.78). On the other hand, in the Singapore Longitudinal Aging Study [160], the authors compared metformin use (n = 204) vs. non-use (n = 161) in diabetes patients with cognitive impairment (Mini-Mental State Exam ≤ 23). Metformin use was inversely associated with cognitive impairment prospectively (OR = 0.49, 95% CI: 0.25–0.95) with the lowest risk in those treated with metformin for 6 years or more [160].

Recently, in the prospective Sydney Memory and Ageing study including 1037 community-dwelling adults without dementia aged 70–90 years [161], participants underwent comprehensive neuropsychological testing every 2 years including executive function, visuospatial testing, and magnetic resonance imaging of brain to assess hippocampal volumes. Amongst 123 patients with T2D, metformin users had slower decline in global cognition and executive function. After adjustment for age, sex, BMI, smoking, blood pressure, and apolipoprotein E (APOE) genotypes, metformin use was associated with 81% lower risk of incident dementia than non-users [161].

There are few interventional studies of metformin on cognitive function and dementia. In a pilot, cross-over study including nondiabetic adults, a daily dose of 2000 mg metformin for 8 weeks was associated with favorable effects on executive function and measures related to learning, memory, and attention capacity [162]. In another placebo-controlled RCT involving 52 patients with T2D and depression, 6-month treatment with metformin was associated with improvements in cognitive function [163]. In the Diabetes Prevention Program, 2280 participants with IGT were randomised to receive metformin, placebo or lifestyle intervention for a mean of 2.3 years. In the 12–14 years post-randomisation period, metformin was associated with reduced risk of diabetes and lower plasma glucose with a neutral effect on cognitive outcomes [164]. Taken together, these results suggested that the effects of metformin on cognitive function depend on the dose and duration of metformin treatment. Although the overall evidence supports the favourable effects of metformin on cognitive function, this will need to be confirmed in larger and long-term interventional trials [165].

4. Conclusions

Type 2 diabetes is a global health challenge associated with multiple morbidities beyond cardiovascular–renal disease. The current COVID-19 pandemic highlighted the vulnerability of people with diabetes during acute infection due to their abnormal metabolic and proinflammatory milieu. With better control of cardiovascular risk factors, notably blood pressure and lipids, persistent hyperglycemia and obesity continue to give rise to other comorbidities including NAFLD, CKD, cancer, and cognitive dysfunction. Optimal energy metabolism is critical in maintaining cellular structure and function in any organism. Metformin inhibits mitochondrial respiratory chain, reduces ATP supply, and activates the AMP kinase to reduce excessive endogenous glucose production and energy storage in the form of protein and fat. These metabolic changes can improve insulin resistance, an important feature in type 2 diabetes. By restoring the energy balance, metformin can improve insulin action, glucose metabolism, and energy utilization at cellular levels. With better understanding on the pathogenetic roles of gut microbiota, there are emerging evidence supporting interactions between unabsorbed metformin and gut microbiota in
reducing oxidative stress and inflammation which can trigger abnormal cell cycles in the host. These non-AMPK dependent mechanisms may contribute to the mitigating effects of metformin on cancer, infections, and age-related conditions, although further investigations are needed to confirm these hypotheses. Taken together, in patients with T2D, metformin forms a safe and efficacious low-cost base therapy for individualization of other GLDs. In patients without diabetes, the paucity of data especially RCTs limits the indications for use of metformin despite their potential benefits. Given the already extensive use of metformin in patients with T2D, large-scale RCTs in people without diabetes evaluating the effects of metformin on infection, cancer, and cognitive function will provide significant insights in our pursuit of reducing the burden of noncommunicable disease and aging-related co-morbidities.

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