REVIEW

Metformin as an anti-inflammatory agent: a short review

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Abstract

Metformin is a biguanide drug widely used as the initial treatment of type 2 diabetes. Despite its widespread use, its precise mechanisms of action remain incompletely characterised. Its effect in lowering blood glucose is largely related to the suppression of gluconeogenesis in the liver, which is probably accomplished by partial inhibition of the mitochondrial respiratory chain complex 1 with a subsequent increase in intracellular AMP levels and activation of AMP kinase. Several local and systemic anti-inflammatory effects of metformin have been described. Many of these effects seem to be mediated by AMP kinase activation and downstream effects inhibiting mTOR and NF- κ B pro-inflammatory signalling cascades. However, there are also studies describing actions independent of AMP kinase action. In this review, we summarise the currently known mechanisms of metformin on inflammatory pathways and the clinical evidence underpinning the use of metformin as a potential anti-inflammatory drug.

Key Words

- ▶ immune system
- ▶ inflammatory diseases
- ▶ diabetes
- ▶ glucose metabolism

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Introduction

Metformin is a biguanide drug that is widely prescribed as an oral antihyperglycaemic agent internationally and is recommended as the initial drug of choice for type 2 diabetes (T2D) according to recent joint European–American clinical guidelines (Buse *et al.* 2020). Metformin has been used in the treatment of type 2 diabetes since 1958 when it was first introduced to clinical practice in Europe (Bailey 2017). Two other biguanide compounds, phenformin and buformin, were, however, used more widely due to their more potent antihyperglycaemic effects until they were withdrawn in most countries by the late 1970s due to an increased risk of severe lactic acidosis (Stumvoll *et al.* 2007). All the biguanides are ultimately derived from guanidine, galegine and other related compounds extracted from the plant *Galega officinalis* (also known as goat's rue, French lilac, Italian fitch, Spanish sainfoin and professor's weed). This plant may have already been used in medieval times

as a folk medicine for diabetes-related symptoms such as excessive thirst and urination (Bailey & Day 2004). As research on the molecular mechanisms of metformin progressed over the years, proposed applications of metformin widened to diverse conditions such as cancer, non-alcoholic fatty liver disease and polycystic ovary disease, although clinical evidence supporting the use of metformin in these diseases is scant (Marshall 2017). It has also been proposed as a general 'anti-ageing' drug based on observations of increased longevity in some, but not all, animals treated with it (Glossmann & Lutz 2019).

One of the most widely studied aspects of the non-glycaemic effects of metformin is its effects on immune cells and inflammatory processes. This review aims to provide an overview of the preclinical and clinical evidence supporting such immune-modulating and anti-inflammatory effects.

Clinical use and precautions

The daily dose of metformin used for the treatment of T2D is typically 1–3 g divided in two to three doses taken orally with major meals. Absorption primarily occurs in the small intestine and the drug is then widely distributed in the body to tissues such as the liver and kidney where uptake is mediated largely by various organic cation transporters (Gong *et al.* 2012). The drug is excreted unchanged in the urine with an elimination half-life of around 5 h in subjects with normal renal function (Graham *et al.* 2011). Although the risk is much smaller than for other biguanides, the accumulation of metformin in the body carries a risk of lactic acidosis. Metformin is, therefore, considered to be contraindicated with a glomerular filtration rate (GFR) of <30 mL/min/1.73 m² and dose reduction is needed at GFR levels of 30–60 mL/min/1.73 m² (Imam 2017). In addition, severe impairment of hepatic function is also a contraindication for metformin as it can also increase the risk of lactic acidosis. The chemical structures of metformin and the other biguanide compounds are shown below in Fig. 1.

Glucose-lowering effect

The effect of metformin to lower blood glucose is primarily mediated by the suppression of hepatic glucose production. Metformin appears to accumulate in mitochondria, preferentially in hepatocytes, and it partially inhibits the mitochondrial respiratory chain complex 1. This causes intracellular AMP accumulation which in turn activates AMP-activated kinase (AMPK) and leads to inhibition of gluconeogenesis through several downstream pathways (Zhou *et al.* 2001, Foretz *et al.* 2019). Other suggested

mechanisms include direct inhibition of mitochondrial glycerol 3-phosphate dehydrogenase leading to an increase of cytosolic NAD hydrogen (NADH) levels and altered redox state in hepatocytes. This may also contribute to increased glucagon-like peptide-1 secretion and decreased gluconeogenesis in intestinal cells (He 2020). There are also results suggesting an increase in local glucose utilisation of the gut (Koffert *et al.* 2017) as well as potentiation of insulin-mediated glucose uptake in skeletal muscle (Kristensen *et al.* 2014). Recent research has also postulated a link between metformin treatment and a change in gut microbiota, effects possibly mediated through increased intestinal levels of the bile acid glyoursodeoxycholic acid and antagonism of the farnesoid X receptor (Sun *et al.* 2018). The quantitative contributions of the several known and potential mechanisms to the overall antihyperglycaemic effect of metformin remain a matter of ongoing research and debate.

Anti-inflammatory effects

The following sections will provide an overview of the effects of metformin on systemic and local inflammatory responses. We provide an overview of the most important molecular mechanisms of metformin, grouping them into AMPK-dependent and AMPK-independent effects (Fig. 2).

Adipose tissue

Obesity has been linked to chronic low-grade inflammation in adipose tissue contributing to insulin resistance and ultimately the development of T2D (Wu & Ballantyne 2020). Targeting this low-grade inflammation may represent an effective way of treating these comorbid

Figure 1
Chemical structures of biguanide and its derivatives metformin, buformin and phenformin.

Figure 2

AMPK-dependent and AMPK-independent cellular effects of metformin. Metformin enters the cell via organic cation transporters and inhibits the respiratory chain complex 1 within the mitochondria leading to an increased intracellular AMP, ATP ratio. This activates AMP kinase and leads to several downstream anti-inflammatory effects, notably including inhibition of the TNF- α /NF- κ B and mTOR signalling pathways. Apart from AMPK-mediated effects, several effects have been described that appear to be independent of AMPK action. This includes activation of Dicer, iPKF2 and miRNA leading to lower expression of several pro-inflammatory cytokines such as TNF- α and IL-6. AMPK, AMP kinase; ATF3, activating transcription factor 3; ChREBP, carbohydrate-responsive element-binding protein; COX2, cyclooxygenase 2; Drp-1, dynamin-related protein 1; Erk, extracellular signal-regulated kinase; FOXO1, forkhead box protein O1; FOXP3, forkhead box P3; HDAC5, histone deacetylase 5; HIF-1 α , hypoxia-inducible factor-1 α ; HMGB1, high mobility group box 1; HSP60, heat shock protein 60; IFN- β , interferon- β ; IL-1 β , interleukin-1 β ; IL-6, interleukin-6; iPKF2, inducible phosphofructokinase 2; IRAK1, interleukin-1 receptor-associated kinase 1; JAK2, Janus kinase 2; JNK, c-Jun N-terminal kinase; mTOR, mechanistic target of rapamycin; NF- κ B, nuclear factor-kappa B; NLRP3, NLR family pyrin domain containing 3; NOX2, NADPH oxidase 2; PPA2, pyrophosphatase 2; PTEN, phosphatase and tensin homologue; TLR4, toll-like receptor 4; TRAF3IP2, TRAF 3 interacting protein 2; TRAF6, TNF receptor-associated factor 6.

conditions. Several models to study adipose inflammation have been used to address metformin effects. Many of these effects appear to be mediated through the activation of AMPK. A study performed in a mouse model of systemic lupus erythematosus (SLE) demonstrated that metformin could enhance immunoregulatory functions of adipose-derived mesenchymal stem cells. AMPK activation by metformin led to the inhibition of mechanistic target of rapamycin (mTOR) and activation of signal transducer and activator of transcription 1 (STAT1). Features of lupus nephritis in the animals, such as proteinuria and anti-dsDNA IgG production, were significantly ameliorated (Jang *et al.* 2020). In parapancreatic adipose tissue of rats, metformin-mediated AMPK activation inhibited mTOR signalling, which led to a decrease in the levels of the inflammatory cytokines interleukin (IL)-1 β and IL17-A. Metformin also induced expression of forkhead box P3 (FOXP3), a master regulator of regulatory T cells (Treg), which can modulate inflammatory responses and has

been shown to ameliorate the severity of autoimmune diseases in animal models (Putlin *et al.* 2020). In a mouse model, metformin increased AMPK activity in perivascular adipose tissue, leading to increased expression of sirtuin 1, which is associated with improved insulin sensitivity. It also decreased nuclear factor-kappa B (NF- κ B) p65 phosphorylation, leading to lower levels of inflammatory cytokines such as tumor necrosis factor alpha (TNF- α), IL-6 and C-reactive protein (CRP). In addition, levels of the anti-inflammatory adipocyte-derived hormone adiponectin increased and signs of endothelial dysfunction improved (Sun *et al.* 2014). Another AMPK-mediated effect shown in a study of mice was the inhibition of dynamin-related protein 1 (Drp1)-mediated mitochondrial fission, which prevented stress-induced activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome. NLRP3 is linked to the initiation and maintenance of inflammation by downstream activation of the important inflammatory cytokine IL-1 β (Li *et al.* 2016a).

Other studies have identified effects that appear to be independent of AMPK. In rats treated with metformin and in adipose tissue incubated with metformin, levels of hypoxia-inducible factor 1 α (HIF-1 α) and fibrosis markers were reduced (Li *et al.* 2016b). Metformin did not lower intracellular oxygen tension and was shown to reduce ATP production. This was not seen with another powerful AMPK activator (AICAR), thus suggesting this effect to be AMPK-independent. In experiments with adipocytes in cell culture, metformin was shown to prevent phosphorylation of c-Jun N-terminal kinase (JNK) p46 and lipopolysaccharide-induced gene expression of IL-1 β and TNF- α (Qi *et al.* 2017). Interestingly, these anti-inflammatory effects seemed to be mediated by inducible 6-phosphofructokinase-2 (iPFK2) as they were not seen in iPFK2-knockdown adipocytes and there was no increase in AMPK activation. Anti-inflammatory effects were also seen in a mouse model of olanzapine-induced insulin resistance in which metformin not only reduced common inflammatory cytokines such as TNF- α , IL-1 β and IL-6 but also counteracted macrophage infiltration and M1 polarisation (Guo *et al.* 2021a). Similar effects have been shown in obese high fat-fed mice with a clear shift in polarisation from M1 to M2 in those animals that received metformin (Jing *et al.* 2018).

Heart

Both acute myocardial ischaemia and the development of chronic heart failure are associated with maladaptive inflammatory responses (Adamo *et al.* 2020) and targeting the IL-1 β pathway with the MAB canakinumab has been shown to reduce the risk of cardiovascular events in patients with ischaemic heart disease (Ridker *et al.* 2017). Metformin has exhibited several anti-inflammatory effects in animal and cell models. In mice, metformin was shown to inhibit aldosterone-induced cardiac fibroblast migration *in vitro* and to reduce cardiac fibrosis *in vivo*. This effect was mediated through AMPK activation and inhibition of TRAF 3 interacting protein 2 (TRAF3IP2), an important oxidative stress-responsive adapter molecule that can induce inflammatory cytokines such as IL-17, IL-18 and IL-6 (Mummidi *et al.* 2016). In mouse studies of heart ischaemia-reperfusion injury, metformin activates AMPK and suppresses NLRP3 inflammasome activity in macrophages which enhances autophagy. This seems to be linked to a protective effect on heart muscle cells from being damaged and dying (Fei *et al.* 2020, Zhang *et al.* 2020). However, there is also contradicting evidence that rather implicates a downregulation of autophagy through

activation of the Akt signalling pathway as protective metformin effects; different results may be related to differences in methodology, for example, administering metformin prior to ischaemia or during reperfusion (Huang *et al.* 2020). Another key result of AMPK activation by metformin seems to be inhibition of toll-like receptor 4 (TLR4), leading to significantly lower levels of pro-inflammatory cytokines and attenuation of left ventricular dysfunction in mouse models of myocardial infarction (Soraya *et al.* 2012, 2014, Vaez *et al.* 2016). In accordance with these findings, metformin similarly decreased levels of pro-inflammatory cytokines in a model of endotoxin-induced myocarditis (which would act partially through TLR signalling) (Liu *et al.* 2017). Other studies have shown that metformin may inhibit cardiomyocyte apoptosis by activating the protective, anti-inflammatory Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) signalling pathway (Gao *et al.* 2019) and by activating the mitochondrial pyrophosphatase PPA2 independently of the glycogen synthase kinase 3 beta/myeloid cell leukemia 1 (GSK3 β /MCL1) pathway (Cheng & Li 2020). Interestingly, metformin also acts on the epicardial adipose tissue by reducing inflammation and regulating adiponectin signalling which may prevent remodelling and development of atrial fibrillation (Li *et al.* 2020).

Blood vessels

Atherosclerosis is considered a chronic inflammatory disease, with accumulation of low-density lipoprotein (LDL) cholesterol in the vessel wall triggering a plethora of immune responses and the formation of atherosclerotic plaques (Wolf & Ley 2019). The effects of metformin on cells of blood vessels have been extensively researched. An important anti-inflammatory effect is the inhibition of TNF- α /NF- κ B signalling. In human umbilical vein endothelial cells, the activation of AMPK by metformin led to inhibition of TNF- α and subsequent NF- κ B activation (Hattori *et al.* 2006, Huang *et al.* 2009). AMPK activation in endothelial cells may furthermore be enhanced by the activation of phosphatidylinositol 3-kinase (PI3K) (Huang *et al.* 2009), an effect not seen in vascular smooth muscle cells (Isoda *et al.* 2006). In a sepsis model of endotoxin-induced endothelial inflammation, AMPK activation by metformin led to histone deacetylase 5 (HDAC5) phosphorylation and subsequent upregulation of Krüppel-like factor 2 (KLF2), which inhibited TNF- α (Tian *et al.* 2019). Another effect of metformin-induced AMPK activity appears to be inhibition of the nuclear factors carbohydrate

response element-binding protein (ChREBP) and forkhead box protein O1 (FOXO1), leading to decreased expression of thioredoxin-interacting protein (TXNIP), which in turn leads to reduced cellular oxidative stress and so could exert a protective effect on vascular endothelium (Li *et al.* 2015). Recent evidence also points to the role of metformin and AMPK in promoting autophagic flux to counteract intracellular lipid accumulation and thereby to reduce pro-inflammatory responses (Kim *et al.* 2020).

In vascular smooth muscle cells, metformin inhibited IL-1 β -induced activation of NF- κ B, the MAP kinases Akt, p38 and Erk, but not, as mentioned previously, activity of PI3K (Isoda *et al.* 2006). However, a later study was able to show that metformin-induced AMPK action resulted in the activation of phosphatase and tensin homologue (PTEN), a known negative PI3K regulator, and this suppressed the TNF- α /NF- κ B pro-inflammatory signalling cascade (Kim & Choi 2012).

Several animal models have similarly shown beneficial effects of metformin on vascular inflammation associated with the process of atherosclerosis. For instance, in atherosclerotic mice treated with metformin, AMPK activation reduced the expression of Drp-1 which in turn suppressed mitochondrial fission, attenuated oxidative stress, ameliorated endothelial dysfunction and inhibited vascular inflammation (Wang *et al.* 2017). In hypertensive rats, metformin exhibited anti-inflammatory effects by reducing plasma TNF- α levels and tissue NADPH oxidase 2 (NOX2) as well as cyclooxygenase 2 (COX2) expression while also improving parameters of cardiac autonomic dysfunction (Oliveira *et al.* 2020). Interestingly, in a study of rats fed a high-fat diet, it appears that metformin could exert anti-inflammatory effects on the endothelium by increasing levels of the micro-RNAs miR-146a and miR155, in turn decreasing expression of IL-1 receptor-associated kinase1 (IRAK1), TNF receptor-associated factor 6 (TRAF6) and NF- κ B p65. These molecules are crucial components of the pro-inflammatory NF- κ B pathway (Gou *et al.* 2020). Furthermore, in atherosclerotic rabbits, metformin treatment decreased plasma inflammatory cytokine levels and reduced atherosclerotic lesion burden. Analysis of atherosclerotic plaques revealed significantly reduced macrophage content in the metformin-treated animals and attenuation of the pro-inflammatory cytokines monocyte chemoattractant protein 1 (MCP1), IL-6 and TNF- α (Yang *et al.* 2018). Another study in mice showed that metformin-induced AMPK activation negatively regulates STAT3 activity, which inhibited both inflammation and monocyte-to-macrophage differentiation. Similarly, plaque formation in the metformin-treated animals

was attenuated, with reduced monocyte infiltration (Vasamsetti *et al.* 2015).

Macrophages/monocytes

Macrophages and monocytes are cells of the innate immune system that play a crucial role in the host defence against pathogens. They have also been implicated in the pathogenesis of inflammatory disorders such as atherosclerosis and obesity. Several effects of metformin on these cells have been described. As for other mechanisms detailed above, AMPK seems to be an important mediator. In a mouse macrophage study, metformin treatment of lipopolysaccharide-stimulated macrophages induced AMPK activation, suppressed the NF- κ B pathway and reduced expression of the chemokines MCP1, C-X-C motif chemokine ligand 10 (CXCL10) and CXCL11 (Ye *et al.* 2018). Suppression of NF- κ B signalling in macrophages was also shown in other studies, along with inhibition of the mitogen-activated protein (MAP) kinases such as Erk and JNK (Buřdak *et al.* 2016, Wang *et al.* 2020). AMPK activation by metformin has been shown to counteract the effect of advanced glycation end products (AGE) in promoting inflammation through the receptor for advanced glycation end products (RAGE)/NF- κ B pathway and also to induce M2 polarisation in murine macrophages. M2 macrophages help resolve inflammation and promote immune tolerance (Zhou *et al.* 2016). Further experiments have also shown the role of AMPK in increasing the expression of activated transcription factor-3 (ATF-3) which in turn could reduce IL-6 and TNF- α expression and so reduce inflammation by attenuating macrophage activation (Kim *et al.* 2014). In PTEN-knockdown murine macrophages, metformin not only blocked reactive oxygen species (ROS) generation and Akt activation but also led to significant apoptosis causing growth inhibition (Lin *et al.* 2013).

Interestingly, the effects of metformin to inhibit the inducible nitric oxide synthase (iNOS) and thereby reducing ROS formation appear to be independent of AMPK but rather mediated through inhibition of interferon- β in the myeloid differentiation primary response 88 (MyD88)-independent signalling pathway (Kato *et al.* 2010). Another AMPK-independent effect of metformin is the direct binding to and inhibition of the alarmin high mobility group box 1 (HMGB1), which induces inflammation by stimulating various receptors such as TLR4 and RAGE (Horiuchi *et al.* 2017). Studies in endotoxaemic mice have indicated that metformin can also inhibit the release of HMGB1, which, however, is mediated by AMPK (Tsoyi *et al.* 2011). Furthermore, it has been shown that metformin can

suppress IL-6 and TNF- α by inducing Dicer, a key miRNA enzyme, and thereby increasing levels of miR-34a-5p and miR-125b-5p (Luo *et al.* 2020). NF- κ B pathway inhibition may be further mediated by the suppression of scavenger receptors in macrophages such as cluster of differentiation 36 (CD36) and scavenger receptor A (SR-A) (Hyun *et al.* 2013). Finally, in human monocyte cells, metformin has exhibited strong stimulatory effects on the expression of the mitochondrial chaperone protein heat shock protein 60 (HSP60) (Tsuei & Martinus 2012). In macrophages, downregulation of HSP60 has been associated with an increased oxidised LDL accumulation and M1 polarisation, which could substantially contribute to the atherosclerotic process in vessel walls (Shirsath *et al.* 2021).

T cells/B cells/APCs

T cells, B cells and antigen-presenting cells (APC) play a central role in adaptive immunity. Dysfunctional responses by these cells have been implicated in a wide array of autoimmune diseases. In mouse T cells, metformin exhibited antioxidant effects by reducing intracellular lipid peroxidation and increasing glutathione levels, which resulted in inhibition of T-cell proliferation (Solano *et al.* 2008). Metformin has furthermore been shown to suppress mechanistic target of rapamycin complex 1 (mTORC1) signalling in mouse CD8⁺ T lymphocytes in an AMPK-independent manner, a key pathway involved in the growth and proliferation of antigen-activated T cells (Zarrouk *et al.* 2014). In CD4⁺ T cells from both human healthy controls and patients with SLE, metformin inhibited transcription of interferon-stimulated genes by inhibiting phosphorylation of pSTAT1 and its binding to interferon-stimulated response elements. These effects were independent of AMPK activation or mTORC1 inhibition but rather appeared to be mediated through inhibition of mitochondrial respiratory chain complexes (Titov *et al.* 2019).

Antigen-presenting cells such as dendritic cells play an important role in T-cell activation and primary T-cell responses. Metformin has, in mouse dendritic cells, been shown to decrease MHC class I- and class II-restricted ovalbumin presentation while also suppressing the expression of MHC molecules and co-stimulatory factors such as CD54, CD80 and CD86 (Shin *et al.* 2013).

Much research has focused on the effects of metformin in animals models of various autoimmune diseases. Interestingly, a study in mice with autoimmune insulinitis, a model of type 1 diabetes, showed that metformin reduced the severity of insulinitis by

suppressing the proliferation of pro-inflammatory Th1 and Th17 cells while promoting the development of Tregs. This was mediated through activation of AMPK and subsequent inhibition of mTOR/HIF-1 α signalling (Duan *et al.* 2019). Similar findings have been demonstrated in mice with the multiple sclerosis model autoimmune encephalomyelitis (Nath *et al.* 2009, Sun *et al.* 2016). In autoimmune arthritis, metformin not only favourably affects the Th17/Treg balance but also promotes brown adipose tissue differentiation, induces fibroblast growth factor 21 (FGF21) expression, suppresses osteoclastogenesis, and corrects impaired autophagic flux (Kang *et al.* 2013, Son *et al.* 2014, Yan *et al.* 2015, Kim *et al.* 2018).

In a murine SLE model, apart from affecting T cells, metformin could inhibit B cell differentiation into plasma cells, germinal centre formation and decrease autoantibody levels through AMPK activation and inhibition of the mTOR-STAT3 pathway (Lee *et al.* 2017). This has similarly been shown in a model of Sjögren's syndrome (Kim *et al.* 2019).

Clinical studies

T2D and obesity

In the United Kingdom Prospective Diabetes Study (UKPDS) trial, metformin treatment led to a significantly lower risk of diabetes-related death and adverse diabetes-related outcomes in overweight T2D patients as compared to diet intervention or treatment with sulphonylurea or insulin (UKPDS Group 1998). The decrease in diabetes-related endpoints included events of atherosclerotic cardiovascular disease, where chronic, low-grade inflammation is an important pathogenic factor. Thus, it was later hypothesised that the anti-inflammatory effects of metformin may be partly responsible for its beneficial effect. In the Hyperinsulinaemia: the Outcome of its Metabolic Effect (HOME) trial, 390 insulin-treated T2D patients were randomised to either metformin or placebo. Sixteen weeks of metformin treatment led to improvement in several blood plasma markers of endothelial function, but no decrease in the inflammatory parameters CRP and soluble intercellular adhesion molecule-1 (sICAM1) (De Jager *et al.* 2005). It is noteworthy that the long-term follow-up of this trial showed a significant decrease in the secondary composite macrovascular endpoint in the metformin-treated group (Kooy *et al.* 2009). Conversely, the A Diabetes Outcome Progression Trial (ADOPT) study showed that glyburide treatment led to fewer serious

cardiovascular events as compared to either metformin or rosiglitazone treatment, although the trial was not powered to detect a significant difference in cardiovascular events (Kahn *et al.* 2006). In a study of T2D patients, 4 weeks of metformin treatment led to a decrease in plasminogen activator inhibitor-1 (PAI-1) and leptin levels, but not in CRP, indicating effects on adipose tissue (Eriksson *et al.* 2007). Another study involving non-obese patients with T2D utilised a crossover design to compare the effects of metformin and the insulin secretagogue repaglinide. With glycaemic levels being similar in both groups, metformin led to significantly lower levels of TNF- α and sICAM-1, but not CRP or IL-6 (Lund *et al.* 2008). The effect of metformin was further compared with the thiazolidinedione drug rosiglitazone in a randomised trial. At the end of 12 weeks, serum levels of IL-6 and TNF- α had decreased significantly in both groups compared to baseline, but there was no difference between the groups (Fidan *et al.* 2011). Another trial compared the effects on inflammatory parameters after 1 year of treatment with the α -glucosidase inhibitor acarbose and metformin. There were no significant differences between the groups although in both, compared to baseline, there were significant decreases in TNF- α and IL-6, but not in IL-2 and IL-1 β (Mo *et al.* 2019).

In an open-label study of overweight individuals with impaired glucose tolerance, half of the participants received the β -hydroxy β -methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor simvastatin and the other half received metformin. After 16 weeks, both groups exhibited significant decreases in CRP and IL-6 as compared to baseline, with no change in TNF- α and no significant differences between the groups (Bulcão *et al.* 2007). Further placebo-controlled studies in patients with prediabetes and lipid-lowering drugs showed anti-inflammatory effects. Persons with impaired fasting glucose treated with simvastatin were randomised to either placebo or metformin 3 g daily for 90 days, and metformin treatment resulted in reduced plasma levels of CRP and sICAM1 while also inhibiting lymphocyte release of IL-2, IFN- γ and TNF- α (Krysiak & Okopien 2012). A similar trial in patients with impaired glucose tolerance treated with fenofibrate showed that metformin reduced plasma CRP levels and also suppressed lymphocyte release of IFN- γ and TNF- α , although sICAM1 levels and IL-2 release were not significantly affected (Krysiak *et al.* 2013). A study of T2D patients demonstrated significantly lower serum CRP levels compared to placebo, along with reduced oxidative and nitrosative stress (Chakraborty *et al.* 2011). Conversely, an earlier study involving obese T2D patients did not show any changes in serum levels

of CRP and IL-6 after 12 weeks of metformin treatment (Ersoy *et al.* 2008). Interestingly, however, there is also some evidence that increased metformin dosages may exert greater anti-inflammatory effects. In a cross-sectional study of metformin-treated T2D patients, those taking 3000 mg/day had significantly lower serum levels of TNF- α than those treated with 1000 or 2000 mg/day (Amoani *et al.* 2021).

In another T2D study, treatment with 500–2000 mg/day metformin led to significantly lower levels of NF- κ B, IL-1 β and the nicotinic acid receptor GPR109A in peripheral blood leukocytes (Xu *et al.* 2017). In patients with carotid artery atherosclerosis, most of whom were free from diabetes, metformin at a dose of 1000 mg/day led to a significant decrease in plasma levels of hsCRP, IL-6 and TNF- α . In peripheral blood mononuclear cells, this effect appeared to be mediated by induction of sirtuin 1, leading to reduced p65 acetylation and inhibition of NF- κ B activation (Xu *et al.* 2015).

Inflammatory and autoimmune diseases

Metformin has also been proposed as an adjunct treatment in classical inflammatory and autoimmune diseases. In a trial of patients receiving glucocorticoid treatment for various chronic inflammatory diseases, 12 weeks of treatment with metformin 2550 mg/day resulted in lower serum levels of high-sensitive CRP as compared to placebo. Furthermore, carbohydrate-challenged TNF- α levels increased significantly in the placebo-treated group but not in the metformin-treated group (Pernicova *et al.* 2020). However, a larger trial involving SLE patients did not show a significant decrease in the incidence of lupus flares in the metformin-treated group (Sun *et al.* 2020). In a translational gout study, it was shown that metformin could inhibit mTOR signalling in monocytes exposed to monosodium urate crystals and metformin use was associated with a lower flare frequency in a retrospective analysis of 42 gout patients (Vazirpanah *et al.* 2019). Metformin has also been tried as an adjunct therapy for patients with chronic obstructive pulmonary disease (COPD). In a trial of 52 patients hospitalised for severe COPD exacerbations, 1 month of metformin therapy did, however, not result in any reduction of CRP or improvement in clinical outcomes (Hitchings *et al.* 2016). Other inflammatory conditions where metformin use has been proposed include asthma (Guo *et al.* 2021b), non-alcoholic steatohepatitis (Li *et al.* 2019) and inflammatory bowel disease (Lee *et al.* 2015). However, prospective clinical trials in these disorders are lacking.

Table 1 Summary of clinical studies of metformin effects on inflammatory markers in humans.

Reference	Subjects	Intervention	Effects
(De Jager <i>et al.</i> 2005, Kooy <i>et al.</i> 2009)	390 insulin-treated T2D patients	Metformin vs placebo	No effect on CRP, sICAM1 at 16 weeks; lower risk of secondary composite macrovascular endpoint at 4.3 years of follow-up
(Eriksson <i>et al.</i> 2007)	21 T2D patients	Metformin vs placebo	↓ PAI-1, leptin, no effect CRP
(Lund <i>et al.</i> 2008)	96 non-obese T2D patients	Metformin vs repaglinide	↓ TNF- α , sICAM-1, no effect CRP, IL-6
(Fidan <i>et al.</i> 2011)	40 T2D patients	Metformin vs rosiglitazone	↓ IL-6, TNF- α compared to baseline; no difference between groups
(Mo <i>et al.</i> 2019)	70 T2D patients	Metformin vs acarbose	↓ TNF- α and IL-6, no effect IL-2 and IL-1 β compared to baseline; no difference between groups
(Bulcão <i>et al.</i> 2007)	41 overweight individuals with impaired glucose tolerance	Metformin vs simvastatin	↓ CRP and IL-6, no effect TNF- α compared to baseline; no difference between groups
(Krysiak & Okopien 2012)	62 simvastatin-treated patients with impaired fasting glucose	Metformin vs placebo	↓ CRP, sICAM1
(Krysiak <i>et al.</i> 2013)	80 fenofibrate-treated patients with impaired glucose tolerance	Metformin vs placebo	↓ lymphocyte release of IL-2, IFN- γ , TNF- α
(Chakraborty <i>et al.</i> 2011)	208 T2D patients	Metformin vs placebo	↓ CRP
(Ersoy <i>et al.</i> 2008)	24 obese T2D patients	No control group	↓ oxidative and nitrosative stress
(Amoani <i>et al.</i> 2021)	209 metformin-treated T2D patients	Cross-sectional study	No effect CRP, IL-6
(Xu <i>et al.</i> 2017)	117 T2D patients	Metformin vs placebo	↓ TNF- α in 3000 mg/day group compared to 1000–2000 mg/day
(Xu <i>et al.</i> 2015)	42 patients with carotid artery atherosclerosis	Metformin vs placebo	↓ NF- κ B, IL-1 β , GPR109A in peripheral leukocytes
(Pernicova <i>et al.</i> 2020)	53 patients receiving glucocorticoid treatment for chronic inflammatory diseases	Metformin vs placebo	↓ hsCRP, IL-6, TNF- α
(Sun <i>et al.</i> 2020)	140 SLE patients	Metformin vs placebo	↓ CRP, no increase in carbohydrate-challenged TNF- α
(Vazirpanah <i>et al.</i> 2019)	42 gout patients	Cross-sectional study	No effect on SLE flare frequency
(Hitchings <i>et al.</i> 2016)	52 hospitalised COPD patients	Metformin vs placebo	↓ gout flares with metformin use
			No effect on CRP levels or clinical outcomes

The findings of the clinical studies are summarised in Table 1.

Several randomised controlled trials are currently being conducted to examine this further (Samuel *et al.* 2021).

Antimicrobial effects

An intriguing aspect of metformin is its antimicrobial effects shown in several preclinical models, including against *Mycobacterium tuberculosis* (Rodriguez-Carlos *et al.* 2020), *Staphylococcus aureus* (Garnett *et al.* 2013, Kalsi *et al.* 2019), Zika and dengue virus (Farfan-Morales *et al.* 2021). In the currently ongoing coronavirus pandemic, COVID-19, metformin has been suggested as an adjunct therapy regardless of diabetes status. This is based on its known anti-inflammatory properties (Samuel *et al.* 2021). While there are some observational data suggesting a lower risk of death in COVID-19 among metformin-treated women with obesity or T2D (Bramante *et al.* 2021), this has not yet been shown in any prospective clinical studies.

Conclusions

Metformin is a compound that has been used for several decades in the treatment of T2D, but its molecular mechanisms of action have been explored only in the last few decades. At the cellular level, it consistently exhibits anti-inflammatory actions largely due to its effects to modulate mitochondrial function and thus increasing intracellular AMP levels and thereby activating AMPK. However, several effects that are independent of AMPK have also been described. Apart from its established indication as a drug for type 2 diabetes, it may hold promise as part of adjunct therapy for several classical inflammatory diseases. Additional prospective, randomised clinical trials in larger

cohorts are required to establish the clinical efficacy of metformin in these disorders.

Declaration of interest

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