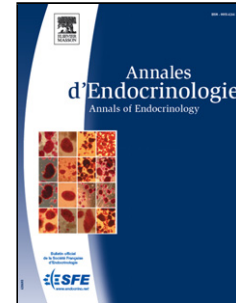# Journal Pre-proof

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Metabolic Complications of Glucocorticoids – Prevention by Metformin

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Abstract

Glucocorticoid treatment is prescribed in 2-3% of the population for various diseases. Chronic exposure to excess glucocorticoid can lead to iatrogenic Cushing's syndrome, which is associated with increased morbidity, especially from cardiovascular diseases and infections. While several 'steroid-sparing' drugs have been introduced, glucocorticoid treatment is still applied in a large number of patients. We have previously showed that the enzyme AMPK plays a key role in mediating the metabolic effects of glucocorticoids.

While metformin is the most widely used drug for treatment of diabetes mellitus, its mechanism of effect is still debated. Among several effects, it stimulates AMPK in peripheral tissue, affects the mitochondrial electron chain, influences gut bacteria and stimulates GDF15. We have hypothesised that metformin will counteract the metabolic effects of glucocorticoids, even in patients without diabetes. We have conducted two double-blind placebo-controlled randomised clinical studies: in the first, we studied glucocorticoid-naive patients and started metformin treatment early together with the glucocorticoid treatment. While in placebo group glycaemic indices worsened, these sequelae were prevented in the metformin group, suggesting a beneficial effect of metformin on glycaemic control in non-diabetic patients receiving glucocorticoid treatment. In the second study, we treated patients already on established glucocorticoid therapy for a longer period with metformin or placebo. In addition to the beneficial effects on glucose metabolism, we observed significant improvement in lipid, liver, fibrinolysis, bone and inflammatory parameters, as well as fat tissue and carotid intima media thickness. Moreover, patients had a lower risk of developing pneumonia and a reduced number of admissions to hospital, representing financial advantage for the health service. We believe that the routine use of metformin for patients on glucocorticoid treatment would represent a key advantage in the care for this patient population.

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Introduction

Glucocorticoids (GCs) are potent immunosuppressive and anti-inflammatory agents prescribed for various conditions. Apart from being used as replacement therapy in patients with adrenal insufficiency, GCs are more frequently used for immunomodulatory purposes. Their benefits are derived from several underlying mechanisms. However, unawareness of the adverse effects after commencing GCs therapy may lead to hyperglycaemic sequelae including prediabetes, worsening hyperglycaemia in patients with diabetes mellitus, unmasked undiagnosed diabetes mellitus, or corticosteroid-induced hyperglycaemia (1). In turn, high blood glucose levels can increase the risk of infection, which can deleteriously impact on the prognosis of the primary illness with increased morbidity and mortality.

Metformin has been widely used as a glucose-lowering agent for more than six decades. Since GCs and metformin work similarly on numerous pathways, but in opposing directions (Figure 1), there is growing evidence in the potential ability of improving glycaemic complications and other metabolic effects resulted from GCs treatment.

Glucocorticoids and its metabolic complications

GCs, lipophilic substances, primarily function by binding to the cytoplasmic glucocorticoid receptor and can affect several steps involved in the production and utilisation of glucose. In hepatocytes, GCs directly increase endogenous glucose production via activating a number of genes involved in the hepatic metabolism of carbohydrates leading to enhanced gluconeogenesis. Glyceroneogenesis, a metabolic pathway involving fatty acid and glycerol 3-phosphate (G3P), is regulated by enzyme phosphoenolpyruvate carboxykinase (PEPCK). GCs enhance PEPCK expression resulting in increased glycerol production (2). In addition, GCs also promote glucose 6-phosphatase (G6Pase) mRNA expression, catalysing the terminal step in gluconeogenesis and glycogenolysis, and raising free glucose levels in the circulation (3,4). Moreover, increased fatty acids disrupt glucose utilisation and induce insulin resistance.

Regarding high saturated fatty acids occurring after steroid exposure, extensive evidence suggests that these fatty acids activate Toll-like receptor 4 (TLR4), a member of the pathogen recognition receptor families, which can trigger inflammatory cascades and also inhibit phosphorylation of insulin-mediated insulin receptor substrate 1 (IRS1) (5). Furthermore, this leads to a lack of protein kinase B/Akt (PKB/Akt) activation, resulting in inhibiting the translocation of GLUT4 to the cell membrane. Hence, muscle and adipose tissues require more insulin to import glucose, which suggests insulin resistance (5). Fatty acids in the liver increase ceramide synthesis, which can further inhibit PKB/Akt, leading to a hindering of liver-specific GLUT2 translocation. It is evident that GCs can promote hepatic ceramide synthesis resulting in high fatty acids and ceramide levels, which can trigger the TLR4 and nuclear factor kappa B (NF-kB) pathway and further repress insulin signalling (6,5).

In adipose tissue, GCs locally activate 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), a key enzyme responsible for the intracellular conversion of inactive cortisone to physiologically active cortisol (7). GCs exposure attenuates the expression of lipogenic genes in mature adipocytes including PPARG2, SREBP1C, CEBPA, and FABP4, leading to reduced lipogenesis (8). However, it is known that long-term GCs use has both lipolytic and lipogenic actions, leading to the expansion of the visceral adipose tissue and a reduction in subcutaneous adipose tissue since glucocorticoid receptors are more expressed in visceral adipose tissue than

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subcutaneous adipose tissue (9,10). Lipolysis in peripheral fat then enhances the release of free fatty acids and glycerol, further accelerating insulin resistance. GCs also alter genes involving lipolysis in white adipose tissue, including adipose triglyceride lipase (ATGL) and hormone sensitive lipase, resulting in increased lipolysis (11).

In skeletal muscles, GCs enhance protein breakdown and reduce protein synthesis, leading to high levels of circulating amino-acids and muscle wasting (12). Degradative pathways involving protein catabolism include ubiquitin-proteosome system and the autophagy-lysosome system (13). The stimulation of these two systems is mediated through an increase in expression of several atrogenes, which are genes altered in muscle atrophy, such as FOXO, ATROGIN-1 and MURF1, as well as several autophagy genes including LC3, BNIP3, and GABARAPL1 (14-18). On the other hand, mechanistic target of rapamycin (mTOR) is an essential component of the anabolic pathway for protein synthesis. GCs affect this synthetic pathway by blunting amino-acid-induces phosphorylation of 4E-binding protein 1 (4E-BP1) and ribosomal protein S6 kinase β-1 (S6K1), which are essential components in the IGF-1/PKB/Akt/mTOR pathway (19).

In the pancreas, the direct relationship between GCs and pancreatic beta-cell dysfunction remains a controversial area of research. The effects of GCs on various steps of insulin signalling vary depending on the times of drug exposure. In animal models, short-term GCs exposure causes an increase in beta-cell compensatory phenomena including up-regulating beta-cell mass, leading to increased insulin synthesis and secretion as a result of beta-cell hypertrophy and hyperplasia (20,21). Nevertheless, it should be noted that the acute beta-cell secretory response may vary among different individuals, as can be observed in the report of Wajngot *et al.*, who explored the effects of the oral administration of dexamethasone 15 mg over 48 hours on glucose and insulin levels in high-insulin responders and low-insulin responders. Among the participants, high-insulin responders experienced significantly lower fasting glucose levels and higher incremental insulin levels, comparing to low-insulin responders. In addition, a hyperglycaemic clamp study revealed a significant increase in insulin response in the high-insulin responder group after acute exposure of dexamethasone (22). As GCs can lower GLUT2 expression and numbers at the cell membrane, decreased glucose transportation into cells leads to lowering of intracellular ATP/ADP ratios, which directly allow K+ efflux and further indirectly lower Ca influx. Moreover, GCs can indirectly affect Ca influx and also inhibit protein kinase A/ protein kinase C (PKA/PKC) activity. Furthermore, serum- and glucocorticoid-inducible kinase 1 (SGK1), one of key regulators of voltage-gated K_V channels, was found to be upregulated in transcription and expression in insulin-secreting cells, resulting in lowered Ca^{2+} influx and suppressed insulin release (23). In addition, GCs trigger an unfolding protein response in the endoplasmic reticulum (ER) following ER stress, resulting in impaired ER homeostasis, which may lead to apoptotic beta-cell death (24). Also, beta-cell survival is hypothesised to be involved with thioredoxin-interacting protein (TXNIP), a negative regulator of the antioxidant thioredoxin. TXNIP in beta-cells is strongly induced by GCs via the glucocorticoid receptor and its induction is dependent on p38 mitogen-activated protein kinase (MAPK) activation. Both human and mouse models revealed that TXNIP potentiated apoptosis, whereas thioredoxin attenuated beta-cell death. On the contrary, downregulation of endogenous TXNIP expression reduces the sensitivity to GC-mediated apoptosis (25). Additionally, as tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) and the TRAIL

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death receptor (DR5) pathway appear to be crucial for proliferation and survival in various cell types, it is noteworthy that GCs can induce apoptosis of beta-cells via upregulation of the TRAIL/DR5 pathway. Interestingly, this effect was attenuated by the glucocorticoid receptor inhibitor, mifepristone (26).

In terms of glucagon responses, a study from Wise *et al.* examining the influence of 3-day dexamethasone treatment on the pancreatic alpha-cell response, found that GCs increased plasma glucagon concentrations in obese individuals in both the basal state and after protein ingestion or aminogenic stimulation, when compared to the non-obese group. Further, this effect of GCs was also reported in patients with Cushing's syndrome, representative of chronic hypercortisolism (27). This finding was supported by a mouse model illustrating that glucagon receptor blockade could successfully prevent GC-induced hyperglucagonaemia (28). However, the exact mechanism behind the elevated glucagon secretion remains unclear.

Metformin and its protective effects on metabolic and non-metabolic complications

Metformin has been used for treatment of diabetes mellitus since 1957 in Europe and 1995 in the USA (29). Although precise mechanisms are still incompletely understood, one of its primary mechanisms of action is activation of 5'-AMP-activated protein kinase (AMPK), a multi subunit enzyme which is a master cellular regulator of lipid and glucose metabolism. However, it is evident that metformin acts on both AMPK-dependent and AMPK-independent pathways.

In the liver, uptake of metformin into hepatocytes occurs via the organic cation transporter 1 (OCT1). It accumulates at high concentration within mitochondria and inhibits complex I of the mitochondrial transport chain, leading to suppression of ATP production while concurrently raising intracellular ADP and AMP levels, and finally activates AMPK via a lysosomal pathway (30). AMPK switches on catabolic pathway generating ATP, and also switches off anabolic pathway consuming ATP (31) including gluconeogenesis. AMPK activation results in reduced levels of PEPCK and G6Pase, which are key enzymes in hepatic glucose production, via the phosphorylation and cytoplasmic sequestration of transcriptional cofactor cAMP-response element-binding protein (CREB)-regulated transcription coactivator-2 (CRTC2) (32,33). In addition, metformin-induced AMP accumulation inhibits glucagon-induced activation of adenylate cyclase, resulting in lowered catalysis of ATP into cAMP. Lower levels of intracellular cAMP lead to a decreased protein kinase A (PKA) activity and decreased phosphorylation of key regulators in gluconeogenesis including 6-phosphofructo-2-kinase/fructose-2,6-biphosphate 1, inositol triphosphate receptor and CREB1, resulting in decreased glucagon-stimulated glucose production (33). In addition, metformin inhibits fructose-1,6-bisphosphatase (FBP1) by inducing an elevated AMP:ATP ratio, leading to a lowering of blood glucose. Regarding its effects on lipids, chronic metformin-induced AMPK activation inhibits acetyl-CoA carboxylase, which in turn lowers carnitine palmitoyltransferase (CPT1) and improves hepatic steatosis (33). Another essential model regarding AMPK-independent pathway involves a direct inhibition the enzymatic activity of mitochondrial glycerol-3-phosphate dehydrogenase (mG3PDH), which raises NADH levels while lowering NAD+ levels (34). This increase in cytosolic redox potential (NADH:NAD$^+$) hinders lactate from being converted to pyruvate, which in turn results in a decrease in hepatic glucose production.

It is well-established that impaired GLUT4 mediated glucose uptake is the primary underlying mechanism of insulin resistance, and the severity of insulin resistance correlates

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with GLUT4 expression (35,36). Improvement in adipose tissue GLUT4 mRNA expression after metformin therapy has been seen in human studies (37,38). Nevertheless, the actual molecular mechanisms of this action remain elusive. Theoretical mechanisms include insulin signalling involving phosphatidylinositol 3-kinase (PI3K) and Akt activation which are necessary for insulin-stimulated GLUT4 translocation (39), as well as AMPK activity which is related to rate of glucose uptake and GLUT4 translocation (40). In skeletal muscle, metformin can stimulate AMPK alpha-2 activity leading to enhancing peripheral glucose disposal and increasing muscular glycogen concentration in type 2 diabetes mellitus (41).

In the pancreas, high glucose levels are known to cause the opening of permeability transition pore (PTP), a Ca^{2+}-sensitive mitochondrial inner membrane channel, which results in cell death. Metformin can prevent PTP opening in permeabilised and intact INS-1 cells, leading to preserving beta-cell viability under a glucolipotoxicity condition (42). Metformin also reduces ER stress via AMPK and PI3K activation, resulting in restored insulin secretion and reduced ER stress-induced apoptosis in a mouse pancreatic cell line (43). However, these protective effects are not related to the unfolded protein response (43). In addition, metformin has effects on transcriptional regulation in pancreatic beta-cell line Min6, including stimulation of insulin promoter factor (IPF1) (44), as well as upregulation of glucagon-like peptide-1 receptor (GLP1R), glucose-dependent insulinotropic polypeptide receptor (GIPR), and peroxisome-proliferator activated receptor-α (PPARα) expression (45).

In the intestine, metformin is known to inhibit glucose absorption in the proximal intestine, which is considered to be associated with increased glucose utilisation by the enterocytes(29). Proposed mechanisms involving metformin-induced intestinal glucose utilisation include GLUT2 redistribution at apical membranes (46), as well as increased upper small intestinal microbiota which leads to increased sodium-glucose cotransporter-1 (SGLT1) expression (47).

Regarding its suppressive effect on appetite, metformin was reported in a preclinical model that the stress response mitokine with respect to inducing expression and secretion of growth differentiating factor 15 (GDF15), which in turn can reduce food intake resulting in lower body weight (48,49).

To date, the advantages of metformin from an anti-inflammatory standpoint are becoming more obvious. Metformin's effect on leucocyte mitochondrial was previously explored in polycystic ovarian syndrome (PCOS) patients with insulin resistance. The lower mitochondial oxygen consumption, membrane potential, mitochondrial mass and gluthathione levels were restored after metformin administration; whereas reactive oxygen species (ROS), IL-6, and TNF-α were decreased (50). ROS plays an important role in the clearance of bacterial pathogens. By enhancing mitochondrial ROS and AMPK phosphorylation, metformin also demonstrated evidence of suppressed growth of *L. pneumophila* in a time- and concentration-dependent manner in macrophages, both in vitro and in vivo model (51). Regulatory functions of macrophages are indisputably critical in atherosclerotic process, and numerous preclinical and clinical studies have demonstrated a preventive role of metformin in atherosclerotic cardiovascular disease through various steps of involvement, irrespective of diabetes status (52). Metformin can hinder NF-κB via blocking the PI3K-Akt pathway, which exerts a direct vascular anti-inflammatory effect (53), as well as reducing monocyte adhesion to endothelial cells and decreasing the inflammatory response of macrophage (54).

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Cholesterol biosynthesis in macrophages can also be inhibited, which is related to metformin-induced ROS in macrophages (55). In addition, this drug reduces lipid accumulation in macrophages via reducing forkhead box transcription factor O1 (FOXO1)-mediated fatty acid binding protein 4 (FABP4) transcription (56). Furthermore, metformin has the ability to decrease foam cell formation, a crucial step in plaque progression, by lowering oxidised low-density lipoprotein (LDL)-induced cholesterol accumulation and increasing cholesterol efflux to high-density lipoprotein, which is associated with an upregulation of ATP binding cassette (ABC) cholesterol transporter (57). Lastly, it has been reported that metformin attenuates lipid accumulation in macrophages by preventing oxidised LDL-induced macrophage apoptosis and inhibiting lipid uptake of macrophages (58).

Apart from the primary effect on glycaemic control, metformin can improve bone heath by directly affecting bone metabolism. AMPK activation by metformin promotes progenitor mesenchymal stem cells (MSC) differentiation into osteoblasts via the upregulation of the runt-related transcription factor 2 (RUNX2), an osteoblast-specific transcription factor, as well as decreasing adipogenesis by the suppression of PPARγ (59-61). Additionally, PPARγ establishes osteoclastogenesis and bone resorption by increasing the expression of receptor activator for nuclear factor- κB (RANKL) signalling (62). Activation of the Akt/AMPK pathway can also suppress the nuclear factor of activated T-cells cytoplasmic 1 (NFATC1), as well as promoting Sirtuin 6 (SIRT6) and NF-κB expression, contributing to the suppression in osteoclastogenesis via RANKL signalling (63,64). Moreover, the activation of AMPK promotes RUNX2 expression via AMPK/USF1-SHP axis and the mTORC1-Notch axis, and suppresses RUNX2 expression through the AMPK-SMURF1 axis (65)(Figure 2).

Patients taking metformin may experience weight loss, which can be attributed to both direct and indirect effects on appetite suppression, These include the suppression of orexigenic hypothalamic agouti-related peptide (AgRP) neurons via AMPK signalling (66), an increase in hypothalamic leptin sensitivity (67), as well as stimulation of the release of anorectic hormone glucagon-like peptide-1 (68,69) and peptide YY (69) from intestinal L-cells. Additionally, lactate accumulation from the inhibition of the mitochondrial electron transport system by metformin may contribute to appetite suppression (70,71). Recent evidence also reported that metformin can induce expression and secretion of GDF15, a cytokine with anti-inflammatory effect and insulin sensitising property, resulting in the suppression of appetite and promotion of weight loss (72,73).

Metformin and the clinical reversal of metabolic complications of glucocorticoids

Our team have conducted two randomised controlled trials in order to explore the protective effects on metabolic complications during GCs usage in non-diabetic patients. The first trial performed in patients already on GCs treatment, either prednisone, prednisolone, or methylprednisolone for at least 4 weeks (average prednisolone doses were 35 mg/day and 30 mg/day in metformin and placebo, respectively), found that prescription of metformin was able to stabilise plasma glucose levels (74). After a 4-week intervention period, effects on glycaemic control were clearly seen in fasting glucose levels and 2-hour area under curve (AUC) glucose, Homeostatic Model Assessment for Insulin (HOMA) index, fasting glucose, and fasting insulin. Our findings corresponded with those of a previous non-randomised, open-label study in non-

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diabetic patients on long-term (15-20 years), low-dose prednisone administration (<0.3 mg/kg/day), which revealed a reduction in HOMA-IR and the insulin-to-glucose ratio, as well as an improvement in the quantitative insulin sensitivity check index (QUICKI) following metformin treatment (75). It should be noted that HOMA-IR and HOMA%B can be used to evaluate longitudinal change of insulin resistance and beta-cell function, respectively, in order to explore the natural history of glycaemic status, as well as the effect of treatment (76). Hence, these findings indicate a preservation of glucose tolerance and improvement in insulin resistance.

Our second study was conducted in patients continuously receiving 20 mg daily or more of prednisolone or its cumulative dose equivalent for 4 weeks or longer, and subsequently remaining on at least 10 mg daily, or more, of prednisolone equivalent for 12 weeks. Average cumulative exposure of GCs was similar between groups, which was 1860 mg, and 1770 mg prednisolone-equivalent, in metformin and placebo groups, respectively (77). After the 12-week study period, truncal subcutaneous fat contributing to the deleterious cardiometabolic risk profile and associated with central adiposity, was significantly lower in the metformin group. It should be noted that visceral adipose tissue and subcutaneous adipose tissue have a deleterious impact on insulin sensitivity and secretion, with greater visceral adipose tissue being a stronger predictor of insulin resistance (78). Also, metformin was able to improve glucose and A1C, as well as lowering the number of patients with dysglycaemia. Regarding effects on insulin resistance and beta-cell function, metformin hindered worsening HOMA-IR and improved HOMA2%B/HOMA2IR disposition index, referring to a compensatory phenomenon in enhanced insulin secretion. We also found total cholesterol and LDL-cholesterol concentrations were improved in the metformin group. Liver profile, including AST-to-ALT ratio and gamma glutamyl transferase, was also improved in the treatment group. It is worth noting that this improvement in hepatic markers is associated with a decrease in insulin resistance, which is considered a trigger of non-alcoholic fatty liver disease by the "two-hit" theory. As previously mentioned, AMPK activation by metformin leads to suppression of acetyl-CoA carboxylase activity resulting in inhibition of lipogenesis and a stimulation of fatty acid oxidation, as well as inhibition of transcription factor SREBP1c, which consequently decreases fatty acid synthesis. It was also hypothesised that metformin could trigger fibroblast growth factor 21 expression, which in turn prevents fatty liver disease in animal model (79). Moreover, progression of carotid intimal media thickness (CIMT) was significantly lower in the metformin group. This finding is consistent with studies including the *Reducing with MetfOrmin Vascular Adverse Lesions* (REMOVAL) trial in type 1 diabetes mellitus (80), type 2 diabetes mellitus (81), obese children (82) and PCOS (83). This effect is supported by a recent systematic review regarding the administration of metformin being associated with a significant reduction of CIMT, especially for a duration longer than 12 months at dose of 1500 mg daily or less (84). However, it is important to note that these beneficial effects of metformin regarding CIMT and carotid plaque score were rare in the *Carotid Atherosclerosis: Metformin for insulin ResistAnce* (CAMERA) study, which was conducted in non-diabetic patients with a high cardiovascular risk, taking statins (85).

In terms of anorectic effects, metformin was associated with suppressed appetite and sugar craving, even in patients using GCs, which are known to increase appetite. This effect is hypothesised to be influenced by prevention of the GCs-induced effects on AMPK in

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hypothalamic neurons as previously mentioned (86). The beneficial effect of metformin on weight has been previously shown in various non-diabetic populations (87-90).

With respect to fibrin clot lysis time, an assay assessing fibrinolytic potential was reduced in the metformin group. This effect is apparently crucial in this population, as poorly treated inflammatory disease and overexposure to GCs both contribute to prothrombotic events, which are linked to impaired fibrinolysis via increasing levels of plasminogen activator inhibitor-1 (PAI-1), a major inhibitor of endogenous thrombolysis, contributing to a risk factor for thrombosis and atherosclerosis (91,92). This effect was hypothesised to be a contributing factor to the reduced cardiovascular risk in metformin-treated individuals in the *UK Prospective Diabetes Study* (UKPDS) (93). An analysis of the *Biguanides and the Prevention of the Risk of Obesity* (BIGPRO) 1 trial in non-diabetic obese patients revealed a greater decrease in PAI-1 antigen and activity in the metformin group, and this favourable outcome was observed in participants who experienced weight loss (94), which is consistent with the previous finding regarding the effect of metformin on improving the hypofibrinolytic status via reducing adipose tissue expression of PAI-1 (95). It is interesting to note that βCTX, a bone resorption marker, was decreased in the metformin group, with the overall bone turnover consistent with an increase in bone mass, as can be seen from the fact that bone mineral density at the hip area increased in the metformin group. Notably, this effect remained regardless of the body composition, vitamin D status, or bisphosphonate therapy. Metformin was histomorphometrically demonstrated to prevent GCs-induced bone loss in animal model by suppressing bone resorption and stimulating bone formation in trabecular bone, in contrast to the mechanism of action of bisphosphonate (96). Advantageous effects of metformin on fracture risk or bone mass were also noted in patients with polycystic ovarian syndrome (97) and type 2 diabetes mellitus (98).

The prophylactic effectiveness of metformin to prevent prednisolone-induced hyperglycaemia has also demonstrated in haematological cancer patients (99). Co-administration of metformin with antipsychotic medications to reduce metabolic side-effects of these drugs has also been explored (100-106). Additionally, there are mounting evidence regarding the study on benefits of metformin in non-diabetic patients as illustrated in Table 1. Here we provide a brief summary of studies concentrating on anthropometric and metabolic profiles, cardiovascular outcomes, nonalcoholic fatty liver disease/ steatohepatitis, inflammation, bone, polycystic ovary syndrome (except fertility-related studies), pregnancy and elderly.

Conclusion

The multifaceted benefits of metformin beyond glucose lowering are evident. Taken together, we suggest the routine prescription of metformin for individuals receiving GCs, unless there are specific contraindications.

Acknowledgement

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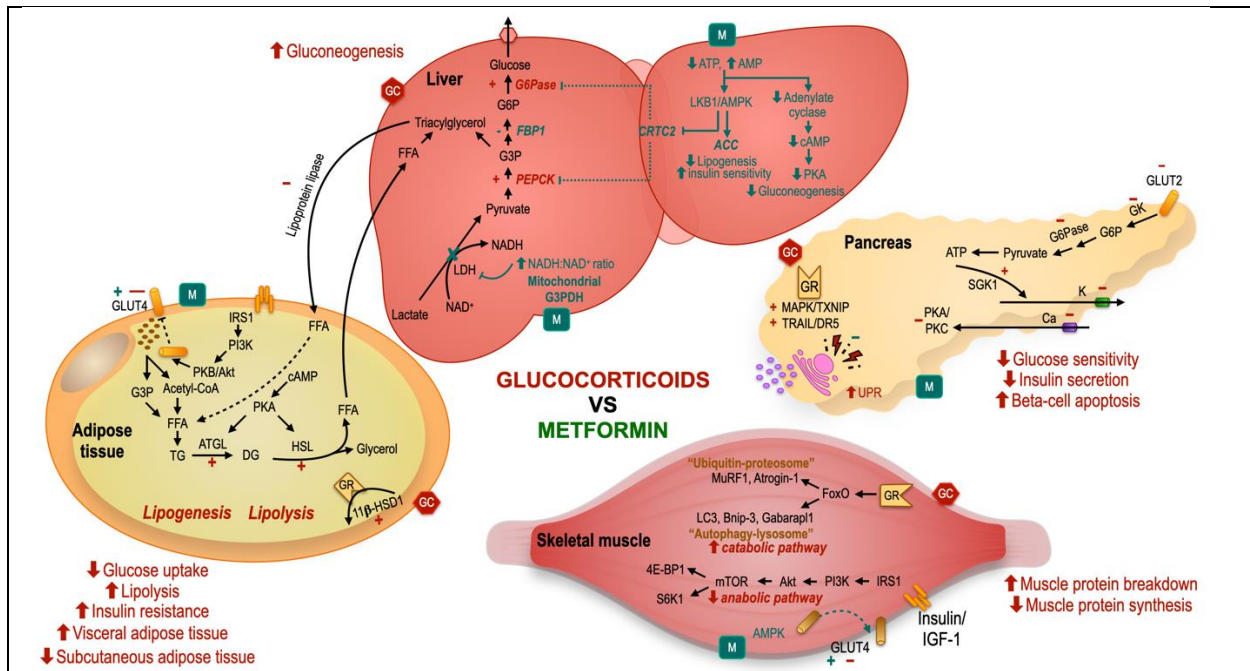
Figure 1 Integrated effects of glucocorticoids and metformin on main organs involvement. GCs act on the liver, enhancing hepatic glucose production by activation of PEPCK and G6Pase. In adipose tissue, GCs are activated by 11**β**-HSD1, then binds to their receptors, causing a decrease in glucose uptake by affecting glucose transporter, increase in lipolysis by enhanced ATGL and HSE activity, and increase in insulin resistance by hindering phosphorylation of IRS1. In skeletal muscle, GCs enhance protein catabolism by stimulation of ubiquitin-proteosome system and autophagy-lysosome system, as well as reduce protein synthesis by affecting IGF-1/Akt/mTOR pathway. In the pancreas, GCs have an action on beta cells, leading to impaired response to glucose, decreased insulin secretion, and beta cell death. Contrary, metformin acts on the liver, reducing PEPVK and G6Pase activity by AMPK activation. In addition to inhibiting FBP1, high AMP levels also have an impact on LKB1/AMPK signalling and cAMP synthesis, contributing to a decrease in lipogenesis and hepatic glucose production, respectively. Direct inhibition of mitochondrial G3PDH by metformin has been suggested as an alternative model inhibiting hepatic gluconeogenesis. In adipose tissue, improvement in GLUT4 mRNA expression by metformin has been hypothesized. In skeletal muscle, metformin enhances AMPK activity, which leads to a switching on catabolic pathway and generating ATP, as well as switching off anabolic pathway, consuming ATP. In the pancreas, metformin reduces ER stress via AMPK and PI3K activation which results in restoration of impaired insulin secretion and reduction in ER stress-induced apoptosis in a mouse pancreatic cell line, Moreover, metformin prevents Ca^{2+}-induced PTP opening, resulting in preserving beta cell viability under glucolipotoxicity condition.

4E-BP1, 4E-binding protein 1; 11**β**-HSD1, 11beta hydroxydehydrogenase type 1; Akt, protein kinase B; AMPK, AMP-activated protein kinase; AR, adrenergic receptor; ATGL, adipose triglyceride lipase; Bnip-3, Bcl-2/adenovirus E1B 19-kDa-interacting protein 3; DG, diglyceride; DR5, TRAIL death receptor; FoxO, forehead box O; Gabarapl1, GABA A receptor-associated protein-like 1; G3P, glycerol 3-phosphate; G3PDH, glycerol-3-phosphate dehydrogenase; G6P, glucose 6-phosphate; G6Pase, glucose 6-phosphatase; GK, glucokinase; GLUT, glucose transporter; GR, glucocorticoid receptor; HSL, hormone sensitive lipase; IGF-1, insulin growth factor-1; IRS1, insulin receptor

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substrate 1; LC3, light chain 3; LKB1, liver kinase B1; LPL, lipoprotein lipase; mTOR, mammalian target of rapamycin; MAFbx, muscle atrophy F-box; MuRF1, muscle RING finger-1; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKC, protein kinase C; SGK1, serum and glucocorticoid inducible kinase-1; S6K1, S6 kinase beta-1; TG, triacylglycerol; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand; TXNIP, thioredoxin-interacting protein; UPR, unfolded protein response

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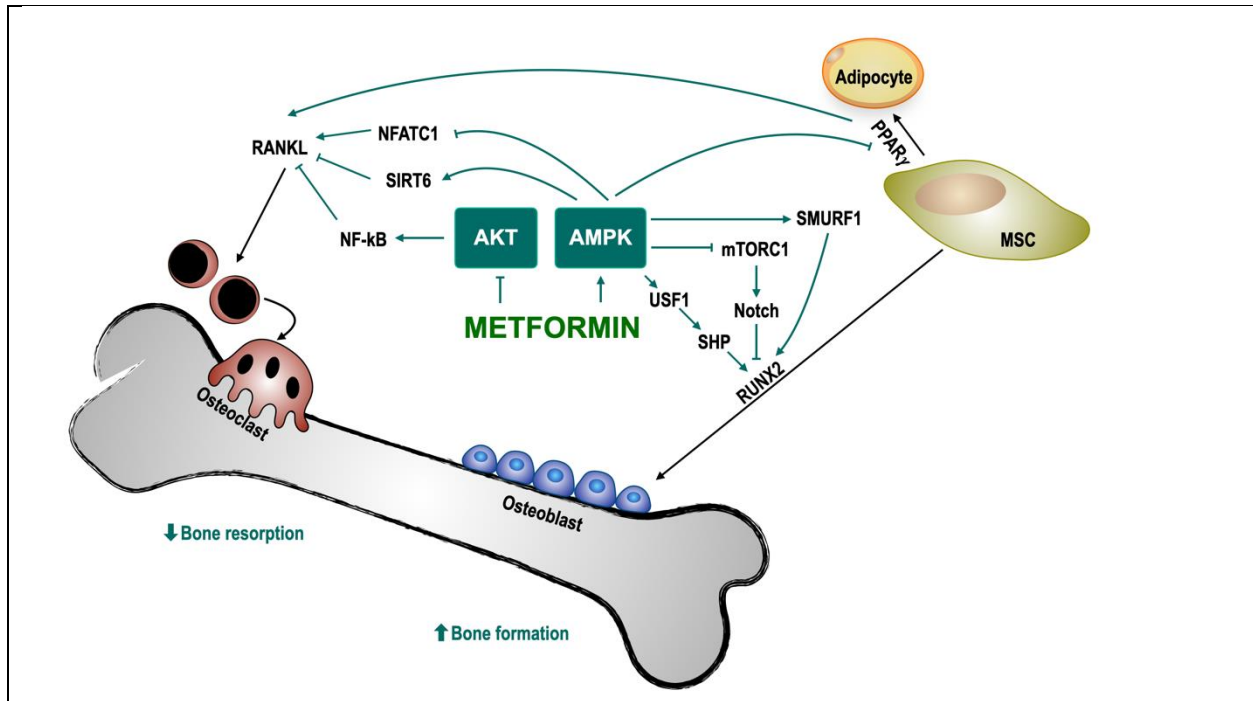
Figure 2 Metformin and its effects on bone homeostasis. AMPK activation by metformin affects bone formation via RUNX2 activation, as well as suppresses bone resorption via inhibition of PPARγ, NFATC1, NF- κB, resulting in RNKL suppression, which in turn can inhibit osteoclastogenesis.

Akt, protein kinase B; AMPK, AMP-activated protein kinase; mTORC1, mammalian target of rapamycin complex 1; NFATC1, nuclear factor of activated T-cell, cytoplasmic 1; NF- κB, nuclear factor-kB; peroxisome-proliferator activated receptor-γ (PPARγ); RANKL, receptor activator for nuclear factor-kB; RUNX2, runt-related transcription factor 2; SHP, small heterodimer partner; SIRT6, sirtuin 6; SMURF1, Smad ubiquitin regulatory factor 1; USF1, upstream transcription factor 1

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Table 1 Selected human studies on benefits of metformin in non-diabetic patients

Reference	Participants	Outcome variables	Results/ Conclusion
Anthropometric / Metabolic profiles			
Ochola *et al.,* 2020 (99)	24 haematological cancer patients currently or newly commencing high-dose prednisolone-based chemotherapy, 7 participants received metformin	-Fasting capillary blood glucose -2-hour postprandial capillary blood glucose	-After 2 weeks, the metformin group had lesser proportion of newly diagnosed prediabetes -Significant differences in mean 2-hour postprandial glucose were seen after 2nd week
Seifarth *et al.,* 2013 (90)	199 adults with BMI at least 27 kg/m2, 154 patients received metformin	-Weight change -HOMA and Matsuda index	-After 6 months, weight loss was 5.8 ± 7.0 kg in the metformin group, compared to 0.8 ± 3.5 kg in the control group -Patients with severe insulin resistance significantly lost more weight as compared to insulin sensitive patients
Fontbonne *et al.,* 2009 (107)	457 upper-body obese participants without cardiovascular disease, 85 participants received metformin	-Anthropometric measurement -Blood pressure -Fasting plasma glucose, insulin levels -2-hour post-load glucose and insulin levels -Serum lipids -Fibrinolytic parameters: PAI-1 activity, PAI-1 antigen, t-PA antigen levels	-After 1 year, the metformin group in IFG/IGT subset showed significantly improved in systolic blood pressure, fasting glucose, total cholesterol, and LDL cholesterol levels -Weight, waist-hip ratio, 2-hour post-load glucose and insulin, HDL cholesterol, TG, and fibrinolytics markers were comparable between groups -Metformin showed benefits in subjects at high risk of developing diabetes
Rodriguez *et al.,* 2004 (108)	21 obese insulin resistant individuals, 10 participants received metformin	-Anthropometric measurement -Body composition -Serum lipids and fatty acid composition levels -Plasma glucose and insulin levels -Mean OGTT glucose and insulin levels -HOMA insulin secretion and insulin sensitivity -Insulin sensitivity index	-After 20 weeks, there were significant decreases in body weight, BMI, percentage body fat, the sum of saturated fatty acids in serum phospholipids and increase in insulin sensitivity index in both groups, when compared to baseline -However, these changes were not significantly different between groups
Charles *et al.,* 2000 (109)	168 men with hypertension, hypertriglyceridaemia, and central obesity, 83 patients received metformin	-Fasting plasma glucose, insulin levels -Serum lipids, Apo AI and Apo B	-After 3 months, fasting plasma insulin, total cholesterol, and Apo B levels significantly declined in the metformin group

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		-Haemostatic parameters: PAI-1 activity, PAI-1 antigen, t-PA antigen, vWF, fibrinogen levels	-t-PA antigen levels significantly decreased only in the metformin group but this change was not significantly different from the placebo group -Blood pressure, plasma TG, and PAI-1 levels were comparable between groups
Paolisso *et al.,* 1998 (88)	30 obese, normotensive patients, 15 participants received metformin	-Anthropometric measurement -Food intake -Fasting plasma leptin levels	-After 15 days, metformin significantly lowered body weight, body fat, plasma leptin levels, and food intake -These changes in food intake correlated with plasma leptin levels regardless of sex or change in body fat
Carlsen *et al.,* 1996 (110)	60 men previously treated with coronary artery bypass surgery or angioplasty with high total cholesterol and/or low HDL cholesterol levels	-Fasting serum lipids and glucose -Side effects	-Metformin lowered LDL/HDL cholesterol ratio at week 4 and 12, as well as reduced body weight at week 12 -These effects were also observed in patients whose body weight and fasting glucose were not alter by metformin
Fendri *et al.,* 1993 (111)	13 obese individuals received metformin	-Glucose disposal using euglycaemic clamp technique	-After 1 month, 6 participants experienced weight loss -Glucose disposal significantly affected in only the weight loss group -Metformin does not appear to influence peripheral insulin-mediated glucose metabolism unless there is weight loss
Landin *et al.,* 1991 (112)	9 non-obese, non-smoking, untreated hypertension patients, all participants received metformin	-Anthropometric measurement -Plasma insulin, C-peptide levels -Glucose disposal using euglycaemic clamp technique -Serum lipids -t-PA antigen, PAI-1, and fibrinogen levels	-After 6 weeks, metformin significantly decreased total cholesterol, LDL cholesterol, TG, insulin, and C-peptide levels; whereas increased glucose disposal, when compared to baseline -t-PA antigen levels were significantly decreased; while PAI-1 and fibrinogen levels were unaffected by metformin -Withdrawal of metformin was associated with the return of both blood pressure and metabolism to their baseline levels
Pentikainen *et al.,* 1990 (113)	24 type IIB hyperlipidaemia, all participants received metformin and placebo in a crossover fashion	-Anthropometric measurement -Blood glucose, plasma insulin -Serum lipoproteins -Blood lactate -Platelet function -Urine prostanoids	-After 9 weeks, there were a greater fall in total cholesterol and LDL cholesterol in the higher dose of metformin group -Body weight, blood glucose, plasma insulin, blood lactate, platelet function, and urinary excretion of prostanoids remained unchanged

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Munro *et al.,* 1969 (87)	90 refractory obese women with normal OGTT, 26 participants received metformin, 24 participants received phenformin	-Body weight -Side effects -Blood glucose and plasma bicarbonate levels	-After 16 weeks, there was a significant difference between the mean weight change of the treated and the control groups -However, there was no difference between those treated by metformin or phenformin
Cardiovascular outcomes			
Ladeiras-Lopes *et al.*, 2021 (114)	54 adults with metabolic syndrome and diastolic dysfunction, 27 patients received metformin on top of lifestyle counselling	-Change in mean e' velocity -HOMA-IR -Functional capacity -QoL (SF-36 questionnaires)	-After 24-month follow-up, the metformin group showed a significant improvement in diastolic function and HOMA-IR when compared to the control group -The functional capacity and QoL were comparable between groups
Brittain *et al.,* 2020 (115)	20 patients with idiopathic or heritable pulmonary arterial hypertension, all participants received metformin	-RV function -Plasma metabolomic analysis -RV TG content -Safety: lactic acidosis, study withdrawal, plasma oxidant stress markers	-After 8 weeks, metformin significantly improved RV fractional area and RV TG content. -Exploratory analysis showed the metabolomic correlates in the peripheral blood of subjects who achieved at least 50% reduction in RV TG content -There was no clinically significant lactic acidosis or change in oxidant stress markers
Larsen 2020 *et al.,* 2020 (116)	36 HFrEF patients, 19 participants received metformin on top of standard heart failure therapy	-Body composition -Myocardial efficiency expressed as work metabolic index at resting and exercise -6MWD -Hand grip strength -Heart failure QoL evaluation	-After 3 months, metformin significantly increased work metabolic index and reduced myocardial oxygen consumption -Changes in resting and exercise ejection fraction, global longitudinal strain, and exercise capacity were comparable between groups
Mohan *et al.,* 2019 (117)	68 patients having coronary artery disease with insulin resistance and/or prediabetes, 34 participants received metformin	-Body weight -LVM, LVMI change -Subcutaneous adipose tissue -Marker for oxidative stress: thiobarbituric acid reactive substance	-After 12 months, metformin significantly reduced LVM, LVMI, body weight, subcutaneous adipose tissue, systolic blood pressure, and oxidative stress when compared to the placebo group
Bassols *et al.,* 2019 (82)	18 pre-pubertal and early pubertal children with obesity and risks for metabolic syndrome, 9 participants received metformin	-Anthropometric measurement -Insulin level -Serum lipids, leptin -hs-CRP levels -Body composition -CIMT	-After 12 months, the metformin group showed a reduction in weight, BMI, leptin, leptin-to-high-molecular-weight adiponectin ratio, hs-CRP, CIMT, fat mass, and liver fat, and these effects maintained after completing 24-month treatment

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Sardu et al., 2019 (118)	258 propensity score-matched patients with stable angina and nonobstructive coronary stenosis, 86 patients with normoglycaemia, 86 patients with prediabetes, and 86 patients with prediabetes received metformin	-Endothelial function -MACE	-After 24 months, prediabetic patients receiving metformin showed a significantly lower percentage of endothelial LAD dysfunction and a lower rate of MACE, compared to the prediabetes group -Treatment with metformin may reduce the risk of cardiovascular events in prediabetes
Liao et al., 2018 (119)	93 patients with pulmonary arterial hypertension associated with congenital heart defects, 48 participants received bosentan, and 43 participants received bosentan/metformin	-WHO functional class -6MWD -NT-proBNP -Right heart haemodynamic parameters	-After 3 months, the combination group revealed the significant improvement in 6MWD and pulmonary vascular resistance index, as well as a significant decrease in pulmonary endothelin 1, a strong vasoconstrictor and proliferative cytokine -Both groups showed the significant improvement in WHO functional class, 6MWD, NT-proBNP, and right heart haemodynamic parameters.
Hartman et al., 2017 (120)	379 STEMI patients undergoing PCI, 191 participants received 4-month metformin	-MACE	-After 2-year follow-up, incidence of MACE and individual components of MACE were comparable between both groups
Eppinga et al., 2016 (121)	371 STEMI patients undergoing PCI, 191 participants received 4-month metformin	-Serum lipids and lipoprotein subfraction particle levels -Cardiac MRI	-After 4 months, metformin significantly decreased LDL cholesterol levels, as well as large LDL particles and LDL size -After adjustment for covariates, high level of small-sized HDL particles and medium-sized VLDL particles at 24 hours after STEMI predicted higher LVEF and smaller infarct size, respectively
Lexis et al., 2014 (122)	379 STEMI patients undergoing PCI, 191 participants received 4-month metformin	-LVEF -NT-proBNP -MACE	-After 4-month follow-up, LVEF, NT-proBNP, and MACE were comparable between groups
Li et al., 2014 (123)	152 metabolic syndrome patients following PCI	-Markers for myocardial injury: CK-MB, troponin I levels -Clinical outcomes	-Post-PCI myocardial injury markers were significantly lower in the metformin group -After 1-year follow-up, the metformin group showed the significant lower proportion of the composite endpoint of death from any cause, post PCI MI, MI after PCI hospitalisation or ischaemia-driven target lesion revascularisation

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Preiss *et al.*, 2014 (85)	173 patients with coronary heart disease and large waist circumference, 86 participants received metformin	-CIMT -A1C, fasting glucose, insulin, HOMA-IR -Serum lipids -hs-CRP -t-PA	-After 18 months, there was no significant difference in CIMT progression, change of carotid plaque score, or hs-CRP between groups
Wong *et al.*, 2012 (124)	62 congestive heart failure patients with insulin resistance	-Cardiopulmonary exercise testing -Fasting insulin resistance index	-After 4 months, metformin significantly decreased insulin resistance index and body weight when compared to the placebo group -Peak oxygen uptake was comparable between groups but metformin significantly improved the slope of the ratio of minute ventilation to carbon dioxide production
Meaney *et al.*, 2008 (125)	60 patients with metabolic syndrome without structural cardiac disease, 30 patients received metformin	-Anthropometric measurement -Fasting glucose -Serum lipids -Nitroxidant metabolites, nitric oxide, carotid vascular stiffness, CIMT, CRP	-After 1 year, the metformin group showed reductions in total cholesterol and CIMT -There were better endothelial function profile and lower CRP levels in the metformin group -Metformin has a beneficial effect on nitroxidation, endothelial function and CIMT in patients with metabolic syndrome
Jadhav *et al.*, 2006 (126)	33 women with a history of normal coronary angiography but two consecutive positive exercise tolerance tests, 16 participants received metformin	-Microvascular function using laser Doppler imaging combined with iontophoresis -HOMA-IR	-Endothelium-dependent microvascular responses significantly improved with metformin -The metformin group revealed significant reductions in weight and HOMA-IR
Vague *et al.*, 1987 (127)	18 obese women were compared to age-matched control	-Plasma insulin, TG levels -PAI capacity -Euglobulin fibrinolytic activity	-Obese participants had higher levels of PAI capacity, lower euglobulin fibrinolytic activity, higher plasma insulin, and TG levels when compared to the control group -After 15 days, metformin significantly decreased in PAI capacity, plasma insulin, and TG levels, as well as increased euglobulin fibrinolytic activity when compared to the baseline
Nonalcoholic fatty liver disease/ steatohepatitis			
Green *et al.*, 2022 (128)	10 insulin-resistant, overweight/obese NAFLD patients, all participants received metformin	-Anthropometric measurement -Intrahepatic TG -Hepatic de novo lipogenesis -Fatty acid oxidation	-After 12 weeks, metformin showed a significant reduction in body weight and improvement in insulin sensitivity; whereas intrahepatic TG and fatty acid oxidation remained unchanged

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			-Metformin significantly decreased VLDL-TG levels; while increased the relative contribution of de novo lipogenesis-derived fatty acids to VLDL-TG
Anushiravani et al., 2019 (129)	150 NAFLD patients were assigned to 5 groups including lifestyle, metformin, silymarin, pioglitazone, and vitamin E	-Anthropometric measurement -Liver chemistry: ALT, AST	-After 3 months, all groups except the control group experienced a significant decline in ALT and AST levels
Handzlik et al., 2019 (130)	42 NAFLD patients, 21 participants received metformin on top of dietary advice	-Anthropometric measurement -Liver chemistry -Liver ultrasonography	-Metformin combined with dietary treatment significantly decreased controlled attenuation parameter values at 3 and 5 months, as well as reduced liver stiffness value at 5 months
Corey et al., 2015 (131)	173 NAFLD children were assigned to placebo, metformin, and vitamin E groups	-Serum lipids -Liver histology	-After 96 weeks, children with NASH resolution had significant decreases in cholesterol and non-HDL cholesterol compared with those without NASH resolution -TG, HDL level, TG/HDL ratio remained unchanged in either group
Sofer et al., 2015 (132-134)	63 patients with NAFLD, 32 participants received metformin	-Fasting glucose, insulin levels -Serum lipids -HOMA-IR -Circulating osteoprotegerin -CRP -Fibrinogen -Liver chemistry -Pulse wave velocity, augmentation index -P1NP	-After 4 months, fasting glucose, TG, alkaline phosphatase levels were significantly decreased in the metformin group; whereas a significant increase in HDL cholesterol level was observed -Metformin significantly decreased osteoprotegerin levels and a regression analysis revealed that metformin treatment was the only significant independent predictor of endpoint and delta osteoprotegerin -Pulse wave velocity and augmentation index were significantly decreased in the metformin group -P1NP was significantly lower in metformin-treated patients and the effect on P1NP was independent of glucose lowering effect.
Zhuang et al., 2015 (135)	45 participants (23 NAFLD and 22 control subjects), 23 NAFLD patients received metformin	-Anthropometric measurement -HOMA-IR -Liver chemistry -Chemerin level	-HOMA-IR, TG, ALT, AST, and chemerin levels in the NAFLD group were significantly higher than in the control group -After 24 weeks, metformin significantly reduced the levels of HOMA-IR, TG, ALT, AST, and chemerin

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Kazemi *et al.,* 2012 (136)	33 biopsy-proven NASH patients, 15 participants received metformin	-Anthropometric measurement -Liver chemistry -Liver histology -Adverse effects	-After 6 months, both groups experienced significant decreases in liver enzymes when compared to baseline; however, there was no significant difference between groups -Subjects with higher initial ALT or AST levels experienced greater improvement after intervention
Shargorodsky *et al.,* 2012 (137)	63 NAFLD patients, 27 participants received metformin	-Fasting glucose, insulin levels -Serum lipids -HOMA-IR -Liver chemistry -hs-CRP -Central aortic augmentation index -Plasma adiponectin level	-In metformin group, augmentation index significantly decreased during the study -ALT and ALP decreased during initial 4-month period, then raised to the pretreatment values after 12 months -Regression analysis revealed that the independent predictors of improvement in arterial stiffness were metformin treatment and increase in circulating adiponectin levels
Akcam *et al.,* 2011 (138)	67 obese adolescents with liver steatosis were assigned to placebo, metformin, and vitamin E groups	-Anthropometric measurement -Fasting glucose, insulin levels -HOMA-IR	-After 6 months, all groups demonstrated significant decreases in BMI, fasting insulin, and HOMA-IR when compared to baseline -Among the groups, the metformin group showed significant improvement in metabolic control and HOMA -Change of adiponectin levels were comparable among the groups
Lavine *et al.,* 2011 (139)	173 children and adolescents with biopsy-confirmed NAFLD were assigned to placebo, metformin, and vitamin E groups	-Anthropometric measurement -Body composition -Fasting glucose, insulin, C-peptide levels -HOMA-IR -Serum lipids -Leptin -CRP -Liver chemistry -Liver histology -Paediatric QoL	-After 96 weeks, sustained reduction in ALT level was observed and comparable between groups -44% of patients treated with metformin had significant improvement in hepatocellular ballooning compared with the placebo group -No other significant improvement was found in those treated with metformin compared with placebo in terms of steatosis, inflammation, change in NAFLD activity score, or resolution of NASH
Garinis *et al.,* 2010 (140)	50 overweight/obese patients with ultrasonographic diagnosis of hepatic steatosis, 25 participants received	-Anthropometric measurement -Fasting glucose, insulin levels -Serum lipids -HOMA-IR -Serum adiponectin level	-After 6 months, echographic evidence of fatty liver was significantly improved in both groups -In the metformin group fasting glucose, insulin resistance, and serum adiponectin significantly decreased, as well as the proportion of IFG and

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	metformin on top of dietary advice	-Liver chemistry -Liver ultrasonography	metabolic syndrome which were significantly declined, when compared to baseline
Tock *et al.,* 2010 (141)	35 postpubertal obese male adolescents with NAFLD, 21 participants received metformin on top of lifestyle intervention	-Anthropometric measurement -Fasting glucose, insulin levels -HOMA-IR -Liver chemistry -Ultrasonic measurements of hepatic steatosis, visceral and subcutaneous adipose tissue	-After 12 months, metformin significantly improved body weight, BMI, insulin, HOMA-IR, and visceral fat -There was a positive correlation between the degree of ultrasonic liver steatosis with insulin levels and HOMA-IR
Haukeland *et al.,* 2009 (142)	48 patients with biopsy-proven NAFLD, 24 participants received metformin	-Anthropometric measurement -Liver chemistry -Liver steatosis by CT and histology	-After 6 months, no significant differences in changes in liver steatosis, NAFLD activity score, ALT, markers of insulin resistance or inflammation were observed between groups
Loomba *et al.,* 2009 (143)	28 patients with biopsy-proven NASH	-Anthropometric measurement -Body fat composition -Hepatic fat content and liver using MRI liver -Visceral and subcutaneous abdominal fat using CT abdomen -Liver chemistry: ALT, AST -Liver histology -HOMA-IR	-After 48 weeks, most patients lost weight and histologic response was achieved 30% -There was a significant association between weight loss and improvement in NASH activity index and ALT levels
Nadeau *et al.,* 2009 (144)	50 obese, insulin-resistant adolescents, 37 participants received metformin	-Anthropometric measurement -Fasting glucose, insulin levels -2-hour postload glucose and insulin levels -Serum lipids -Liver chemistry -Liver ultrasonography	-Prevalence of fatty liver was 74% and more common in male and Hispanic subjects -After 6 months, fatty liver prevalence and severity, as well as fasting insulin level significantly improved in the metformin group
Idilman *et al.,* 2008 (145)	74 newly diagnosed NASH patients were assigned to conventional diet and insulin-sensitiser group (24 and 25 participants received metformin and rosiglitazone, respectively)	-Anthropometric measurement -Body fat content -Liver chemistry -Liver histology -Fasting glucose, insulin levels -HOMA-IR	-After 48 weeks, insulin sensitisers significantly improved ALT, AST, HOMA-IR, CRP, and histologic features, while diet and exercise improved ALT and AST, when compared to baseline

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Nobili *et al.,* 2008 (146)	60 overweight/obese children with biopsy-proven NASH, all participants received metformin; whereas a control group (n=30) was selected from another parallel study	-Anthropometric measurement -Liver chemistry -Liver histology -HOMA-IR	-After 24 months, body weight, ALT, HOMA-IR, histologic steatosis, NAFLD activity score were improved in both metformin and control groups
Duseja *et al.,* 2007(147)	25 NAFLD patients who did not achieve ALT normalisation after 6-month lifestyle intervention and UDCA, all participants received metformin; whereas a control group (n=25) was selected from the same cohort treated only with lifestyle interventions	-Anthropometric measurement -Liver chemistry -Liver histology -Plasma glucose, insulin, C-peptide levels -Serum lipids -HOMA-IR -TNF-α	-After 6 months, all patients treated with metformin had partial biochemical response and 56% of them achieved ALT normalisation
Bugianesi *et al.,* 2005 (148)	110 NAFLD patients were assigned to weight-reducing diet, metformin, and vitamin E groups	-Anthropometric measurement -Liver chemistry -Liver histology -HOMA-IR	-After 12 months, ALT improved in all groups, especially in the metformin group -Multivariate analysis revealed that metformin treatment was associated with higher rates of ALT normalisation -A control biopsy in 17 metformin-treated cases (14 nonresponders) showed a significant decrease in liver fat, necroinflammation, and fibrosis
Schwimmer *et al.,* 2005 (149)	10 obese children with biopsy-proven NASH, all participants received metformin	-Liver chemistry: ALT, AST -Liver fat using MR spectroscopy -Markers for insulin sensitivity: QUICKI -Health-related QoL	-After 24 weeks, mean ALT, mean AST, liver fat, as well as insulin sensitivity and QoL showed significant improvement from baseline -Overall, normalized ALT and AST occurred 40% and 50% respectively
Uygun *et al.,* 2004 (150)	36 NASH patients, 17 participants received metformin on top of dietary treatment	-Liver chemistry: ALT, AST -Liver ultrasonography -Liver histology -Plasma glucose, insulin, C-peptide levels -Serum lipids -HOMA-IR	-After 6 months, metformin significantly improved ALT, AST, insulin, C-peptide levels, and insulin resistance, when compared to baseline, as well as when compared to the control group -Metformin showed a trend of improvement in the necro-inflammatory activity
Inflammation			

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Krysiak *et al.*, 2022 (151)	147 men with BMI at least 24 kg/m^2 and IGT and FPG between 95-125 mg/dL from prospective case-control study (early-onset androgenic alopecia versus normal hair growth), all received metformin	-A1C, glucose, lipids, indices of insulin sensitivity and resistance -Sex hormone -hs-CRP -25-hydroxyvitamin D	-After 12 months, fat content, waist circumference, glycaemic control, and TG levels improved in both groups. -Metformin decreased hs-CRP and bioavailable testosterone levels in the control group -Treatment-induced changes in glucose homeostasis parameters correlated with impact of metformin on hs-CRP and 25-hydroxyvitamin D levels.
Padmapriydarsini *et al.*, 2022 (152)	322 adults with pulmonary tuberculosis in a randomised open-label trial, 160 patients received metformin	-Time to sputum conversion -Chest X-ray finding -Plasma acute phase proteins: alpha-2 macroglobulin, CRP, haptoglobin, serum amyloid P -Inflammatory markers: IFN-γ, TNF-α, IL-17A, IL-1β	-The median time to sputum culture conversion were similar between standard ATT and the intervention group. -The inflammatory markers and extent of cavitary lesions were significant lower in the intervention group.
Polverino *et al.*, 2021 (153)	3804 participants in The COPDGene study, 115 received metformin	Index of emphysema progression	-Over 5 years of follow-up, a slower progression of emphysema and a slower decrease of adjusted lung density were illustrated in the metformin group.
Planas *et al.*, 2021 (154)	22 ART-treated, virologically suppressed PLWH with CD4$^+$/CD8$^+$ T-cell ratios < 0.8, all participants received metformin	-CD4$^+$ T-cell counts, CD4$^+$/CD8$^+$ T-cell ratios, plasma markers of inflammation/gut damage -Cell-associated integrated HIV-DNA and HIV-RNA levels -Sigmoid colon biopsy	-After 12 weeks, metformin significantly decreased CD4$^+$ T-cell infiltration in the colon and mTOR activation/phosphorylation, particularly in CD4$^+$ T-cell expressing the Th17 marker CCR6 CD4$^+$ T-cells -Metformin decreased the HIV-RNA/HIV-DNA ratios in colon-infiltrating CD4$^+$ T-cells -Metformin reduced residual HIV transcription in the colon CD4$^+$ T-cells
Cameron *et al.*, 2016 (155)	1. Population cohort study: 9295 type 2 diabetes mellitus patients received metformin 2. Randomised, double-blind, placebo-controlled study: 33 non-diabetes mellitus with insulin resistance and congestive heart failure received metformin	-Neutrophil to lymphocyte ratio -Fasting insulin resistance index -Cytokines	-Metformin has a stronger effect on neutrophil to lymphocyte ratio. -Metformin significantly improved fasting insulin resistance index. -Anti-inflammatory properties of metformin are exerted irrespective of diabetes mellitus status.
Hitchings *et al.*, 2016 (156)	52 hospitalised COPD patients with exacerbation taking oral	-In-hospital blood glucose levels -Fructosamine levels	-Mean in-patient capillary blood glucose levels were comparable between groups

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	prednisolone 30 mg/day for at least 7 days, 34 participants received metformin	-CRP -Scores on COPD assessment	-After 1 month intervention, there no significant between-group differences
Xu *et al.,* 2015 (157)	42 patients with coronary artery atherosclerosis, 21 received metformin	-hs-CRP -IL-6 -TNF-α	-After 12 weeks, hs-CRP, IL-6, TNF-α levels were significantly decreased in the metformin group. -Metformin also ameliorated the inflammatory response via SIRT1 induction, p65 acetylation reduction, NF- κB inactivation, and inflammatory inhibition in peripheral blood mononuclear cells.
Goldberg *et al.,* 2014 (158)	3234 participants in the DPP, 1073 participants received metformin	-Fasting insulin -hs-CRP -t-PA -fibrinogen level	-CRP and t-PA levels fell in the lifestyle and metformin groups at 1 year and remained lower at end of study (3.4 years) but remained unchanged in the placebo group.
Carlsen *et al.,* 1998 (159,160)	60 men with coronary heart disease treating with diet, lifestyle advice, and lovastatin 40 mg/day, 29 participants received metformin	-Fasting plasma glucose and glucose area under OGTT -Marker for insulin resistance: insulin area/glucose area -TNF- α and soluble TNF receptor levels	-After 12 weeks, metformin significantly increased TNF-α in non-obese subgroup; whereas soluble TNF receptors remained unchanged in both groups -Metformin does not improve insulin sensitivity by lowering circulating TNF- α levels -Fasting plasma glucose, glucose area during OGTT were comparable between groups -Metformin significantly decreased insulin resistance and this effect was mainly found in obese subgroup
Bone			
Schwartz *et al.,* 2021 (161)	3234 participants in the DPP, 2775 continued in DPPOS, 369 participants received metformin	BMD from DXA scan	-At DPPOS year 12, femoral neck BMD and prevalence of osteoporosis were similar in the lifestyle intervention and the metformin groups.
Lingaiah *et al.,* 2019 (162)	Post-hoc study of a prospective, placebo-controlled, randomised study: 40 non-obese and 17 obese PCOS received metformin	-Bone formation marker: P1NP -Bone resorption marker: CTX	-After 3 months, P1NP and CTX levels were significantly reduced in both obese and non-obese in the metformin group.
De Zegher *et al.,* 2018 (163)	34 low-birth weight girls with precocious puberty, 17 participants received metformin	-Body composition from DXA -Hepatic, abdominally subcutaneous, and visceral fat by MRI	-Metformin-treated girls had normal tempo of bone aging, gained more height per bone-age year, and had less visceral and hepatic fat, comparing to the untreated group.
Polycystic ovary syndrome			

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Gangale *et al.,* 2011 (164)	140 hyperinsulinaemic overweight PCOS patients, all participants received metformin	-Liver chemistry -Fasting glucose, insulin levels -Serum lipids -hormonal study: cortisol, testosterone, androsteinedione, estradiol, FSH, LH, DHEAS, 17-OH-P, SHBG, free androgen index, prolactin levels	-At baseline, NAFLD was diagnosed in 57.85% of PCOS patients, and metabolic syndrome was present only in the NAFLD patients -After 12 months, metformin significantly reduced the prevalence of metabolic syndrome, as well as improved hepatic parameters and decreased oligomenorrhea
Begum *et al.,* 2009 (165)	59 PCOS patients, 29 participants continued metformin throughout pregnancy	-Anthropometric measurement -FSH, LH, testosterone, and 36DHEAS levels -Fasting glucose and insulin levels	-Rate of gestational diabetes mellitus was lower in the metformin group -All baby born in the metformin group had average birthweight; whereas LGA occurred in the control group
Preiss *et al.,* 2008 (166)	82 obese PCOS patients	-Anthropometric measurement -Liver chemistry -Serum glucose, insulin, leptin levels -CRP levels	-After 8 months, mean weight, ALT, GGT significantly decreased from baseline -There was a highly significant association between weight loss and decreases in serum ALT and GGT that were independent of changes in HOMA-IR
Khattab *et al.,* 2006 (167)	360 PCOS patients, 200 participants continued taking metformin throughout pregnancy	-Incidence of gestational diabetes mellitus diagnosed by 75g OGTT -Incidence of pre-eclampsia	-Metformin significantly reduced the incidence of gestational diabetes mellitus and pre-eclampsia
Khattab *et al.,* 2006 (168)	200 PCOS patients, 120 participants continued taking metformin throughout pregnancy	-Anthropometric measurement -FSH, LH, estradiol, and 36DHEAS levels -Rate of pregnancy loss	-Rate of early pregnancy loss was significantly lower in the metformin group
Ortega-Gonzalez *et al.,* 2005 (169)	57 obese hyperinsulinaemic, insulin resistant PCOS patients, 17 women received metformin, 17 women received pioglitazone, others were lost to follow-up, non-compliant, or became pregnant	-Anthropometric measurement -Ferriman-Gallwey hirsutism score -2-hour OGTT for glucose and insulin measurement -2-hour metoclopramide test for prolactin measurement -HOMA-IR -QUICKI, fasting glucose-insulin ratio	-After 24 weeks, hirsutism score, fasting insulin levels, and AUC-insulin significantly decreased in both groups -AUC-prolactin significantly increased at the end of study in both groups -Either metformin or pioglitazone was associated with an improvement in the endogenous hypothalamic dopaminergic tone and an amelioration of the insulin resistance
Chou *et al.,* 2003 (170)	30 obese PCOS patients	-Anthropometric measurement -FSH, LH, testosterone, and SHBG levels -Fasting lipids, glucose and insulin levels	-After 90 days, metformin significantly reduced total testosterone and total cholesterol levels

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		-Menstrual cycle	
Baysal *et al.,* 2001 (89)	15 obese PCOS patients, all participants received metformin	-Anthropometric measurement -Fasting serum insulin and testosterone levels -Menstrual cycle	-After 12 months, mean BMI significantly decreased, as well as menstrual patterns were improved -Fasting insulin and testosterone levels remained unchanged
Pregnancy			
Nascimento *et al.,* 2020 (171)	357 obese pregnant women with gestational age ≤ 20 weeks, 171 participants received metformin	-Delivery route -LGA newborn	-Rates of caesarean delivery were significantly lower in the metformin group
Elderly			
Laksmi *et al.,* 2017 (172)	120 elderly aged ≥ 60 years with pre-frail status, 43 participants received metformin	-Handgrip strength -Gait speed -Serum mystatin level -Health-related QoL	-After 16-weeks intervention, metformin statistically improved the mean gait speed -Hand grip strength, serum myostatin level, and QoL were comparable between groups

The Medline and Embase databases (accessed on 11 April 2023) were searched for relevant articles published in any language involving keywords synonymous to "metformin", "non-diabetic", "fatty liver disease", and "polycystic ovary syndrome". Papers were excluded which concentrated on metformin effects on cancer, gut microbiota, antipsychotics and infertility/assisted pregnancy - related PCOS.

17-OH-P, 17-hydroxyprogesterone; 6MWD, 6-min walking distance; ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; ATT, anti-tuberculosis treatment; AUC, area under curve; BMD, bone mineral density; CIMT, carotid intima media thickness; CK-MB, creatine kinase-MB; COPD, chronic obstructive pulmonary disease; CTX, carboxy-terminal cross-linking telopeptide of type 1 collagen; DEXA, dual-energy X-ray absorptiometry; DHEAS, dehydroepiandrosteronesulfate; DPP, Diabetes Prevention Program; DPPOS, Diabetes Prevention Program Outcome Study; FSH, follicle-stimulating hormone; HFrEF, heart failure and reduced ejection fraction; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; hs-CRP, high-sensitivity C-reactive protein; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IL-6, interleukin-6; LAD, left anterior descending coronary; LGA, large-for-gestational age; LH, luteinising hormone; LVEF, left ventricular ejection fraction; LVM, left ventricular mass; LVMI, left ventricular mass index; MACE, major adverse cardiac events; MI, myocardial infarction; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NF-κB, nuclear factor-kB; NT-proBNP, N-terminal pro-brain natriuretic peptide; PAI, plasminogen activator inhibitor; P1NP, procollagen type 1 amino-terminal propeptide; PCI, percutaneous coronary intervention; PCOS, polycystic ovary syndrome; PLWH, people living with HIV; QoL, quality of life; RV, right ventricle; SHBG, sex hormone binding globulin; STEMI, ST-segment elevation myocardial infarction; TG, triglyceride; TNF-α, tumor necrosis factor α; t-PA, tissue plasminogen activator; UDCA, ursodeoxycholic acid; VLDL, very low-density lipoprotein; vWF, von Willebrand factor; WHO, World Health Organization

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