Chapter

Glycemic and Extraglycemic Effects of Metformin in Patients with Diabetes

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Abstract

For several decades, metformin has been the mainstay of treatment of type 2 diabetes (T2D), not only due to its remarkable efficacy in both monotherapy and combination therapy regimens, but also due to its favorable safety profile, weight neutrality, and low cost. Other advantages have been reported, including improvements in lipid profile and inflammatory markers and reports of cardioprotective effects, albeit with scant evidence. The modification of the cellular energy metabolism is the core of metformin's mode of action. Metformin works to lower serum glucose concentration by inhibiting hepatic gluconeogenesis and countering the action of glucagon. Secondarily, it enhances glucose uptake in peripheral tissues, predominantly in the muscles. Long-term and widespread use of metformin has shed light on its other potential uses mediated by its effects on deranged metabolic pathways. Moreover, metformin is gaining research interest by demonstrating its potential in the treatment of multiple disorders other than diabetes and has been proven to have anti-cancer, immunoregulatory, and anti-aging properties. As a result, metformin is currently being researched as a potential treatment option for various diseases.

Keywords: metformin, diabetes, extraglycemic effects, longevity, microbiome, anticancer

1. Introduction

Metformin is a widely utilized oral treatment for type 2 diabetes (T2D), FDA-approved since 1998, this guanidine derivative has been thoroughly researched in molecular, biochemical, animal, human, and epidemiological trials first for its glycemic effects, with additional effects noted later. Metformin has come under the spotlight for its pleiotropic effects, which include anti-inflammatory, immunomodulatory, antibacterial, antiviral, anticancer, anti-aging, hormone regulatory, cardioprotective, and anti-lipid effects [1, 2]. These vast effects have led to research showing shared molecular mechanisms and multiple effects on cellular, biochemical, and other signaling pathways in the body, resulting in complex positive effects, particularly during chronic use. Furthermore, these extra glycemic effects are potentiated by the treatment of T2D, a risk factor for many conditions, including cardiovascular disease, cancer, infections, and obesity. Its low side-effect risk, low cost, and widespread use

strengthen the interest in using metformin for treating conditions other than T2D, with some researchers labeling it "the Aspirin of the twenty-first century."

2. Glycemic effects of metformin

In use for over 30 years, metformin is the most commonly prescribed oral antihyperglycemic and is the first-line treatment for T2D. It acts primarily by decreasing hepatic glucose production, with additional effects by decreasing intestinal absorption of glucose and improving peripheral glucose uptake (**Figure 1**). Hypoglycemia is unlikely with metformin, making its side effects more favorable when compared to other, older oral antihyperglycemics such as sulfonylureas. In primary hepatocytes, metformin activates the AMP-activated protein kinase (AMPK) pathway, which results in the inhibition of glucose production [3, 4]. Additionally, recent advances have shown that metformin's effects on gluconeogenesis may be independent of AMPK activation. Metformin acts on the respiratory chain in mitochondria, changing the intracellular ATP levels, thereby impairing the supply of ATP required for gluconeogenesis [3–6]. Another recently reported potential target of metformin may be mitochondrial glycerophosphate dehydrogenase [7]. Other mechanisms of metformin on glycemia include potential improvements in homeostasis *via* actions on glucagon-like peptide 1 and antagonization of glucagon, further suppressing hepatic gluconeogenesis [8–10].

The effect of metformin on the intestines involves several mechanisms. Fundamentally, metformin decreases proximal intestinal glucose absorption, possibly by increasing enterocytic glucose utilization and increased lactate production [11].

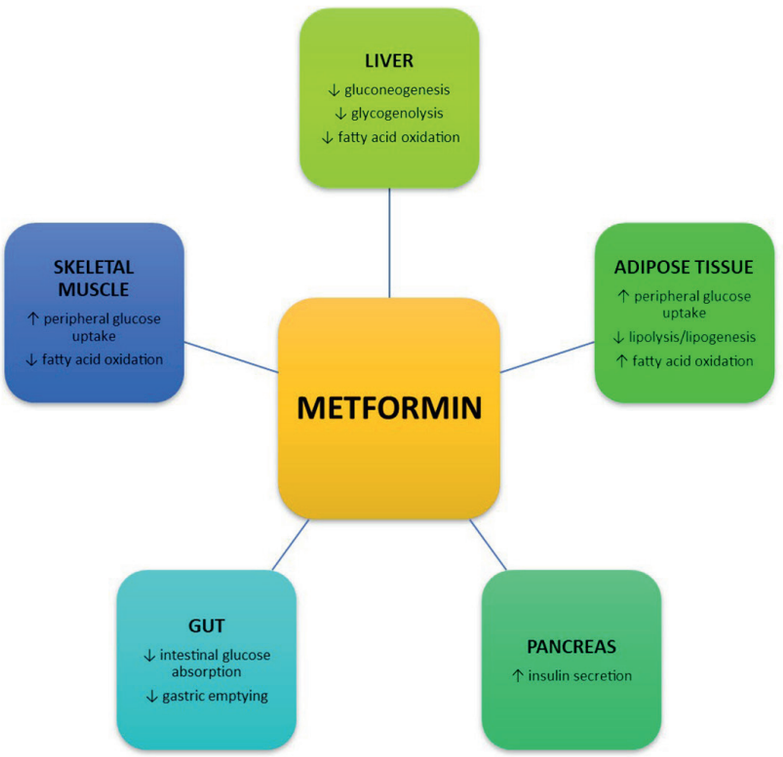

Figure 1.
Overview of metformin glycemic effects.

The complete mechanisms by which glucose utilization is increased are unclear; however, animal models indicate a role in increased GLUT2 expression on the enterocyte membrane. The other pathways of metformin's action on the gut involve its effects on the incretin system. Metformin increases GLP-1 secretion by enteroendocrine cells in the intestine, thereby enhancing glucose homeostasis. Mechanisms of this GLP-1 increase are under debate, and the currently prevailing opinion is that metformin acts by increasing GLP-1 production rather than by preventing its degradation by DPP-4 [12, 13]. Other glucoregulatory effects *via* the intestines include modulations of the gut-brain axis and its effects on the intestinal microbiome [14].

Metformin reduces gluconeogenesis and hepatic glucose production, increasing peripheral glucose uptake and improving insulin sensitivity.

3. Extraglycemic effects of metformin

3.1 Metformin and aging

Aging is an inevitable biological process occurring in all organisms and is defined by the accumulation of numerous detrimental alterations that are correlated with an increased risk for morbidity and mortality. Although aging itself cannot be called a disease, it is undeniable that age-related disorders are one of the major causes of mortality worldwide. Aging is regulated by numerous cellular signaling mechanisms, namely protein homeostasis, nutrient-sensing pathways, and ROS-mediated oxidative stress [15]. In recent years, there has been considerable interest in researching metformin as an anti-aging medicine that could not only improve health but also increase lifespan. Research showed that metformin can increase lifespan by modulating the generation of ROS *via* the SIRT-3, Nrf-2/GPx7, and PRDX-2/SKN-1 signaling pathways [16–18]. Furthermore, it was established that it promotes the autophagy-mediated clearance of decaying components while reducing mTOR-induced production of aging-related proteins (e.g., progerin). Several studies have already demonstrated metformin's positive effect on aging in humans. Metformin in longevity study (MILES) is a 3-year trial in which 14 senior individuals with impaired glucose tolerance were treated with metformin at 1700 mg/day. The findings of the study showed that metformin administration caused transcriptome alterations in aging-related pathways, such as mitochondrial pathways, adipose tissue, fatty acid metabolism, and DNA repair processes [19]. Campbell et al. conducted a systematic review and a meta-analysis of 53 studies that showed that metformin might enhance both health span and longevity irrespective of its antihyperglycemic effects, implying that metformin fits the criteria for the anti-aging medication [20]. Likewise, an analysis of over 180,000 T2D patients' medical records from the UK Clinical Practice Research Datalink indicated that, despite being more obese and having more comorbidities than their non-diabetic counterparts, metformin-treated diabetic patients had survival rates comparable to their matched nondiabetic control group [21]. On the other hand, some studies demonstrated that metformin is less effective than exercise and may negate some of the benefits of exercise [22, 23]. Despite mentioned positive findings, metformin should be used with caution beyond the treatment of T2D, particularly in older people who may be at risk of metformin toxicity due to their possible hepatic and renal impairment. Further research is needed to demonstrate metformin as potential prophylaxis for age-related complications.

3.2 Metformin and cancer

Diabetes types 1 and 2 are both associated with increased rates of developing certain types of cancer, a link noticed over 90 years ago [24]. Common risk factors for diabetes and certain cancer types deepen the correlation among these entities. Bearing this in mind, could treatment or prevention of diabetes play a role in cancer incidence? This large and productive study question was initiated by a 2005 epidemiological report describing a link between metformin use and decreased rates of cancer occurrence [25]. Similar observational studies of patients with diabetes type 2 with or without metformin report lower cancer incidence in patients on metformin [26]. This sparked numerous *in-vitro*, animal, retrospective, prospective, and randomized controlled trials (RCTs), with several hundred registered RCTs investigating the effects of metformin on different cancer types currently in progress. *In-vitro* and animal studies have shown elaborate evidence of the anticancer effects of metformin as well as its modes of action on various mechanisms in cancer cells. Metformin has been shown to disrupt cellular energy mechanisms by affecting AMPK and mTOR pathways and by decreasing available glucose, has further effects on inhibition of protein synthesis and cell growth, as well as anti-angiogenic and anti-inflammatory effects [2, 27]. An important barrier to translating the findings of *in-vitro* and animal studies is the concentration of metformin used to achieve these effects, which is frequently 10–1000x times higher than the usual human dose. However, after oral intake, metformin distribution is different across the body, and for certain targets, such as the gastrointestinal, hepatic, and urinary systems, reaching these concentrations is possible. In the gut, the concentration of metformin remains 30–300 times higher than in plasma. At the same time, imaging studies show metformin accumulates in the liver, kidneys, and urinary bladder at concentrations far higher than those in plasma [28–30]. Another important factor for the responsiveness of cells was the expression of organic cation transporters, whose increased expression enables a very high concentration of metformin in the endoplasmic reticulum and mitochondria [31]. A wide body of findings indicates a potentially high therapeutic potential of metformin in cancer, particularly in these organ systems. However, definitive evidence of its beneficial effects, particularly in human studies, is lacking and often contradicting. It is studied for various cancer types at various stages, such as prevention, chemotherapeutic and neoadjuvant treatment. Meta-analysis of observational studies showed a 31% overall relative risk reduction (CI 0.61–0.79) for overall cancer development in participants taking metformin compared with other antidiabetic drugs. Promising trends were noted for breast, colon, pancreatic, and hepatocellular cancer [32]. Epidemiological and observational studies show a risk reduction of colorectal cancer incidence in metformin users, particularly diabetics. However, no RCTs have identified any association, but a protective effect was noted when metformin was compared to other antihyperglycemics [33, 34]. Similarly, for pancreatic cancer, observational and epidemiological studies show a 44% risk reduction, while RCTs show no protective effects of metformin. A systematic review of an RCT and nine observational studies for liver cancer showed a significant protective effect (OR 0.34; CI 0.19–0.60). As for pancreatic and colorectal cancer, observational studies showed a protective effect on stomach cancer, however, when pooled with results from RCTs, the protective effect was lost. Melanoma, prostate, kidney, lung, ovarian, and uterine cancer all showed no beneficial effects from metformin in both observational studies and RCTs, albeit metformin had a marginally protective effect against lung cancer in observational studies [34]. Its lack of effect on advanced cancer independent of the site or type of cancer was demonstrated in a recent meta-analysis [35].

Overall, the vast heterogeneity of published data indicates the need for meticulous RCTs with long follow-ups and adequate confounder control to fully investigate metformin's anticancer effects in humans for prevention, chemotherapy, or neoadjuvant treatment. Several hundred registered ongoing RCTs promise to elucidate the potential of metformin in cancer treatment.

3.3 Metformin and gut microbiota

Numerous microorganisms have a critical role in physiologic and metabolic processes in our body. Their habitat is mainly in our gut and therefore are called "gut flora" or "gut microbiota." The composition of the microbiota is influenced by both internal and external factors, such as the type of delivery, nutrition, exposure to antibiotics, gut inflammation, stress, menopause, and toxins. The process of altering the predominant microbiota is known as dysbiosis, and it has been linked to the development of various illnesses. The internal gut medium can be aggrieved by changes in the microbiome in a variety of ways, including altered pancreatic enzyme function, biliary acid degradation, damage to the intestinal brush border, and the development of dysregulated immunological responses due to bacterial antigens. These changes, however, are reversible [36]. There is a strong correlation between the incidence of inflammatory diseases and disturbance in the microbiome composition. The gut microbiota interacts closely with the inflammatory, renal, cardiovascular, and endocrine systems *via* metabolic, humoral, and neural signaling pathways [37]. Several important molecular and pathophysiologic mechanisms linking the microbiome, obesity, and diabetes mellitus have been elucidated, most prominently low grade-inflammation, lipopolysaccharides, bile acids, short-chain fatty acids (SCFAs), and reduced intestinal permeability [38]. Of note, besides their direct effects on glucose homeostasis and insulin resistance, the effects of intestinal microbiota span to other factors in diabetes development, like body mass index (BMI) and low-grade inflammation, as well as the consequences of existing DM, including chronic kidney disease and diabetic retinopathy [37].

Novel studies have shown the effects of metformin on the composition of the gut microbiome, resulting in changes affecting several processes and diseases, including effects on diabetes mellitus, the cardiovascular system, and aging (**Figure 2**) [39]. The half-life of metformin in the blood is 3–4 hours, while its glucose-lowering effects are observed for much longer. Furthermore, glucose lowering was shown to be stronger after intraduodenal than intravenous administration [13, 39]. Metformin treatment has been shown to result in positive changes in the microbiome composition both in animal models and humans, namely, increased relative rates of *Akkermansia* and *Firmicutes*/*Bacteroides* ratio [13, 40]. Even short-term metformin treatment is associated with a lowered abundance of *Bacteroides fragilis* in the intestine, leading to improved glucose tolerance, which was reversed when *B. fragilis* was reintroduced to the gut [41]. A randomized double-blind trial investigated the treatment of naïve T2DM patients versus placebo and showed improved glucose tolerance in mice with fecal transplants from metformin-treated patients. This indicated that the glucose-lowering effect of metformin is at least partially mediated by its effect on the microbiome [42]. The study conducted by Zhang et al. concluded that metformin modulated gut microbiota and contributed to the increase of SCFA metabolizing bacteria in treated rats [43].

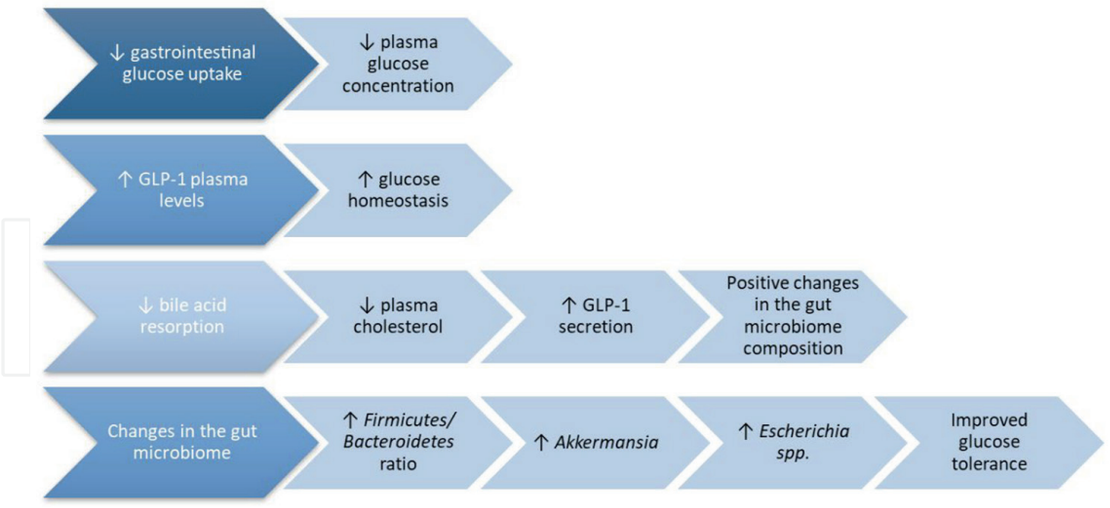

Figure 2.
Effects of metformin on diabetes via the gastrointestinal tract.

Metformin affects glycemia *via* several mechanisms resulting in a decrease of intestinal glucose absorption, positive changes in the gut microbiome leading to decreased "intestinal leak" and lower circulatory levels of proinflammatory cytokines resulting in improved glucose homeostasis. Decreased bile acid resorption has positive effects on the plasma lipid profile, as well as additional effects on glucose homeostasis *via* increased glucagon-like peptide 1 (GLP-1) secretion and changes associated with positive alterations of the intestinal microbiome.

3.4 Other extraglycemic effects of metformin

3.4.1 Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is an endocrine disorder marked by hyperandrogenism, polycystic ovaries, and disorders of ovulation, making it one of the most common causes of female infertility. It is a heterogeneous condition also associated with features of metabolic syndrome. Prevalence of PCOS, based on the Rotterdam consensus workshop, rates up to 15% [44]. Insulin resistance in women with PCOS was first described by Burghen et al. in 1980 [45]. Weight gain is associated with insulin resistance in both women with and without PCOS; however, PCOS is associated with insulin resistance even in 75% of lean women with PCOS, albeit less severe than in obese women with PCOS [46]. Another important component of metabolic derangements in PCOS is dyslipidemia, most commonly manifested as increased LDL and total cholesterol [47]. Interestingly, genes most commonly associated with PCOS are genes related to the insulin receptor, primarily insulin receptor substrate 1 and 2 (IRS-1 and 2), calpain 10, genes for the expression of androgen-producing enzymes, and polymorphisms peroxisome proliferator-activated receptor gamma (PPARγ) [48]. These findings represent the basis for the use of "insulin-sensitizers," such as metformin, for the treatment of PCOS. Studies show that the combination of metformin and lifestyle changes leads to more weight loss and improved menstrual cycle regularity when compared to lifestyle changes alone. The proposed benefits of metformin in PCOS stem from its effects on weight loss, lowering of serum testosterone, and beneficial effects on dyslipidemia and endothelial function [49]. Metformin

improves menstrual regularity, an effect more pronounced in lean patients, even those who are underweight [50]. Women (particularly obese) with PCOS are significantly more insulin resistant than their age- and BMI-matched female counterparts. Adipose tissue deposition in women with PCOS is more pronounced in the visceral and abdominal areas, termed android body fat distribution. Such fat deposition has adverse effects on hyperinsulinemia, causing consequent co-gonadotropic effects on the ovaries. Additionally, it endorses further android fat generation exacerbating weight gain [51]. Metformin does not primarily target fat tissue. Moreover, its effects on adipose tissue are unclear and many findings are debated. Many *in-vitro* and animal findings, such as increased glucose uptake and metabolism by adipocytes, effects on mitochondrial and peroxisomal fatty acid oxidation, lipolysis, and aerobic and anaerobic respiration, are yet unproven in human studies [49]. However, metformin may affect adipocytes through the activation of AMPK, which results in counteraction to the obesogenic effects of corticosteroids [52]. Metformin reduces hyperinsulinemia, effectively and safely ameliorating the reproductive, metabolic, and cardiovascular morbidity in PCOS. Metformin seems to have additional effects on the ovary itself. All current studies are *in-vitro* studies, and the exact mode of action is unclear. So far, it appears that metformin inhibits androgen production in human ovarian granulosa cells [53]. Metformin is a drug for all reasons. Other observed modes of action include AMPK-related pathways, similar to those in other tissues. In rat granulosa cells, metformin treatment resulted in AMPK activation and reduced steroidogenesis [54]. Studies evaluating pregnancy outcomes in women with PCOS remain inconclusive. In some patients, improved pregnancy outcomes could be a result of attenuation of hyperinsulinemia, reduced insulin resistance, and inhibition of the plasminogen activator, resulting in improved oocyte quality and folliculogenesis [55]. However, due to the non-significant improvement in outcomes, the use of metformin only in patients with impaired glucose tolerance [56].

3.4.2 Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is a term used for liver disorders often seen in obese individuals, particularly those with type 2 diabetes. These disorders include simple fatty liver disease, non-alcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). The prevalence of NAFLD in people with diabetes is between 50 and 90% [57]. Because of this high correlation, NAFLD is considered a novel T2D predictive indicator [58]. Initial treatment of NAFLD, as well as diabetes, includes weight loss and lifestyle changes to improve insulin sensitivity, reduce serum liver enzyme levels, and lower the degree of fatty change in the liver. Metformin has been shown to suppress hepatic gluconeogenesis, change hepatic fatty acid metabolism, increase fatty acid oxidation, suppress lipogenesis, and improve insulin sensitivity. Recently, it was revealed that metformin has beneficial effects on liver histology in patients with NAFLD/NASH [59]. Nonetheless, metformin is commonly prescribed off-label to patients with NAFLD since it is thought that activation of AMPK is associated with a myriad of positive benefits, such as reduction in oxidative stress and liver inflammation [60]. However, its long-term clinical effects on NASH patients, particularly in lowering the risk of HCC in NAFLD/NASH patients, are unknown. Patients with established T2D should be evaluated for NAFLD as it contributes to the progression of diabetes (**Figure 3**). Because viable noninvasive diagnostics for histological and biochemical indicators of NASH are unavailable, liver biopsy remains the current gold standard for diagnosis. The most practical approach

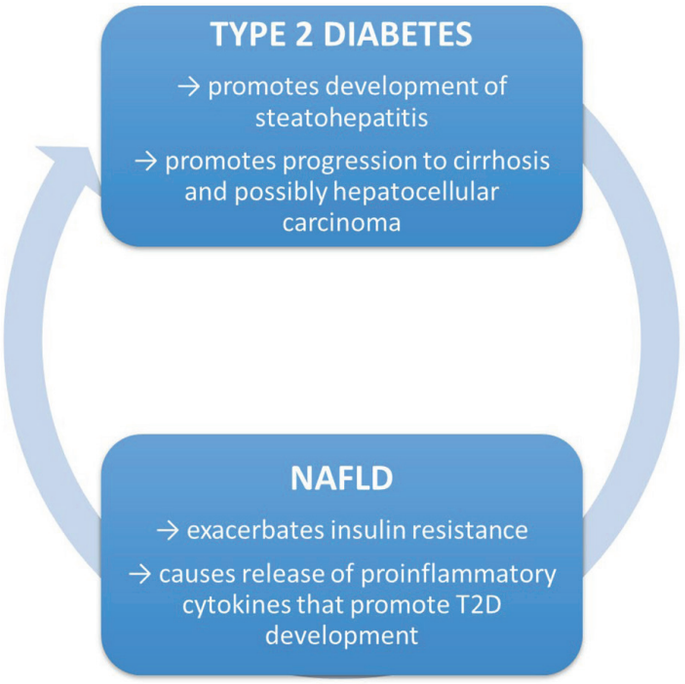

Figure 3.
Relationship between type 2 diabetes (T2D) and non-alcoholic fatty liver disease (NAFLD).

for detecting NAFLD is liver ultrasonography; however, it has yet to be proven for monitoring response to treatment. Therefore, metabolic markers remain viable indicators of therapeutic response.

In NAFLD the lipid accumulation within hepatocytes results in hepatic insulin resistance, hepatic insulin clearance is reduced and toxic cytokines are released which promotes the development of T2D. Type 2 diabetes is associated with higher rates of development of steatohepatitis, progression to liver cirrhosis, and possibly the development of hepatocellular carcinoma.

4. Future perspective

The described pleiotropic effects of metformin may be potentiated or affected when used in combination with other oral antihyperglycemic drugs. Frequently, the choice of second-agent drugs is dependent on the comorbidities, cost, and target goals individually tailored for each patient. The combined effects of other drugs, including GLP-1 receptor agonists, SGLT2 inhibitors, and DPP-4 are the focus of current research investigating their combined effects on glycemic control as well as influences outside regulation of plasma glucose concentration. In light of the new evidence and effects of other oral antihyperglycemics on the cardiovascular and renal systems, the Japanese guidelines are the first not to recommend metformin as a first-choice agent, but rather, one of the possible choices for first-line monotherapy. Other guidelines, including Korean and the American Diabetes Associations, recommend including different oral antihyperglycemics earlier than before [61]. Combination therapy of metformin with additional one or two drugs is currently being investigated for long-term safety and glycemic control. A recent study compared a metformin-DPP4 inhibitor combination compared to metformin, DPP-4, and an SGLT2 inhibitor, and the results showed similar long-term glycemic control, indicating better cost-effectiveness

of dual than triple therapy [62]. Of note, extraglycemic effects were more pronounced in patients on triple therapy; however, in selected populations, dual therapy may be similarly effective at a lower cost. Long-term efficacy studies and cost-effectiveness need to be studied for all possible combinations, and the results of these studies may guide physicians in selecting more appropriate treatments for their patients.

5. Conclusion

Metformin has been the first-line treatment for T2D for decades. The advantages of using metformin include its safety profile and low cost compared to newer medications such as GLP-1 receptor agonists and SGLT-2 inhibitors. Apart from its usage in the treatment of diabetes, growing evidence suggests that metformin may be beneficial in the treatment of cancer, PCOS, NAFLD, and a variety of other chronic diseases. By its effects on glucose homeostasis, the incretin axis, lipid metabolism, and the gut microbiome, it is reasonable to assume that metformin has a multitude of roles in disorders other than diabetes, and consequently, may increase healthspan and longevity.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Ursini F, Russo E, Pellino G, D'Angelo S, Chiaravalloti A, De Sarro G, et al. Metformin and autoimmunity: A "new deal" of an old drug. Frontiers in Immunology. 2018;**9**:1236

[2] Triggle CR, Mohammed I, Bshesh K, Marei I, Ye K, Ding H, et al. Metformin: Is it a drug for all reasons and diseases? Metabolism. 2022;**133**:155223

[3] Foretz M, Guigas B, Bertrand L, Pollak M, Viollet B. Metformin: From mechanisms of action to therapies. Cell Metabolism. 2014;**20**(6):953-966

[4] Miller RA, Chu Q, Xie J, Foretz M, Viollet B, Birnbaum MJ. Biguanides suppress hepatic glucagon signaling by decreasing the production of cyclic AMP. Nature. 2013;**494**(7436):256-260

[5] Hawley SA, Ross FA, Chevtzoff C, Green KA, Evans A, Fogarty S, et al. Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. Cell Metabolism. 2010;**11**(6):554-565

[6] Foretz M, Hébrard S, Leclerc J, Zarrinpashneh E, Soty M, Mithieux G, et al. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. The Journal of Clinical Investigation. 2010;**120**(7):2355-2369

[7] Madiraju AK, Erion DM, Rahimi Y, Zhang X-M, Braddock DT, Albright RA, et al. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. Nature. 2014;**510**(7506):542-546

[8] Maida A, Lamont BJ, Cao X, Drucker DJ. Metformin regulates the incretin receptor axis via a pathway dependent on peroxisome proliferator-activated receptor-α in mice. Diabetologia. 2011;**54**(2):339-349

[9] Mulherin AJ, Oh AH, Kim H, Grieco A, Lauffer LM, Brubaker PL. Mechanisms underlying metformin-induced secretion of glucagon-like peptide-1 from the intestinal L cell. Endocrinology. 2011;**152**(12):4610-4619

[10] Cuthbertson J, Patterson S, O'Harte FP, Bell PM. Addition of metformin to exogenous glucagon-like peptide-1 results in increased serum glucagon-like peptide-1 concentrations and greater glucose lowering in type 2 diabetes mellitus. Metabolism. 2011;**60**(1):52-56

[11] Bailey CJ, Mynett KJ, Page T. Importance of the intestine as a site of metformin-stimulated glucose utilization. British Journal of Pharmacology. 1994;**112**(2):671-675

[12] McCreight LJ, Bailey CJ, Pearson ER. Metformin and the gastrointestinal tract. Diabetologia. 2016;**59**(3):426-435

[13] Wu T, Horowitz M, Rayner CK. New insights into the anti-diabetic actions of metformin: From the liver to the gut. Expert Review of Gastroenterology & Hepatology. 2017;**11**(2):157-166

[14] Foretz M, Guigas B, Viollet B. Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. Nature Reviews. Endocrinology. 2019;**15**(10):569-589

[15] Hsu S-K, Cheng K-C, Mgbeahuruike MO, Lin Y-H, Wu C-Y, Wang H-MD, et al. New insight into the effects of metformin on

diabetic retinopathy, aging and cancer: Nonapoptotic cell death, immunosuppression, and effects beyond the AMPK pathway. International Journal of Molecular Sciences. 2021;**22**(17):9453. [Published: 31 August 2021]

[16] De Haes W, Frooninckx L, Van Assche R, Smolders A, Depuydt G, Billen J, et al. Metformin promotes lifespan through mitohormesis via the peroxiredoxin PRDX-2. Proceedings of the National Academy of Sciences of the United States of America. 2014;**111**(24):E2501-E2509

[17] Fang J, Yang J, Wu X, Zhang G, Li T, Wang X, et al. Metformin alleviates human cellular aging by upregulating the endoplasmic reticulum glutathione peroxidase 7. Aging Cell. 2018;**17**(4):e12765

[18] Karnewar S, Neeli PK, Panuganti D, Kotagiri S, Mallappa S, Jain N, et al. Metformin regulates mitochondrial biogenesis and senescence through AMPK mediated H3K79 methylation: Relevance in age-associated vascular dysfunction. Biochimica et Biophysica Acta - Molecular Basis of Disease. 2018;**1864**(4 Pt A):1115-1128

[19] Kulkarni AS, Brutsaert EF, Anghel V, Zhang K, Bloomgarden N, Pollak M, et al. Metformin regulates metabolic and nonmetabolic pathways in skeletal muscle and subcutaneous adipose tissues of older adults. Aging Cell. 2018;**17**(2):e12723

[20] Campbell JM, Bellman SM, Stephenson MD, Lisy K. Metformin reduces all-cause mortality and diseases of aging independent of its effect on diabetes control: A systematic review and meta-analysis. Ageing Research Reviews. 2017;**40**:31-44

[21] Bannister CA, Holden SE, Jenkins-Jones S, Morgan CL, Halcox JP, Schernthaner G, et al. Can people with type 2 diabetes live longer than those without? A comparison of mortality in people initiated with metformin or sulphonylurea monotherapy and matched non-diabetic controls. Diabetes, Obesity & Metabolism. 2014;**16**(11):1165-1173

[22] Malin SK, Braun B. Impact of metformin on exercise-induced metabolic adaptations to lower type 2 diabetes risk. Exercise and Sport Sciences Reviews. Jan 2016;**44**(1):4-11

[23] Konopka AR, Laurin JL, Schoenberg HM, Reid JJ, Castor WM, Wolff CA, et al. Metformin inhibits mitochondrial adaptations to aerobic exercise training in older adults. Aging Cell. 2019;**18**(1):e12880

[24] Wilson EB, Maher HC. Cancer and tuberculosis with some comments on cancer and other diseases. American Journal of Cancer Research. 1932;**16**(2):227-250

[25] Evans JMM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. BMJ. 2005;**330**(7503):1304-1305

[26] Kim HJ, Lee S, Chun KH, Jeon JY, Han SJ, Kim DJ, et al. Metformin reduces the risk of cancer in patients with type 2 diabetes: An analysis based on the Korean National Diabetes Program Cohort. Medicine (Baltimore). 2018;**97**(8):e0036

[27] Morale MG, Tamura RE, Rubio IGS. Metformin and cancer hallmarks: Molecular mechanisms in thyroid, prostate and head and neck cancer models. Biomolecules. 2022;**12**(3):357

[28] Bailey CJ, Wilcock C, Scarpello JHB. Metformin and the intestine. Diabetologia. 2008;**51**:1552-1553

[29] Gormsen LC, Sundelin EI, Jensen JB, Vendelbo MH, Jakobsen S, Munk OL, et al. In vivo imaging of human 11C-metformin in peripheral organs: Dosimetry, biodistribution, and kinetic analyses. Journal of Nuclear Medicine. 2016;**57**(12):1920-1926

[30] Marini C, Cossu V, Bauckneht M, Lanfranchi F, Raffa S, Orengo AM, et al. Metformin and cancer glucose metabolism: At the bench or at the bedside? Biomolecules. 2021;**11**(8):1231

[31] Chien H-C, Zur AA, Maurer TS, Yee SW, Tolsma J, Jasper P, et al. Rapid method to determine intracellular drug concentrations in cellular uptake assays: Application to metformin in organic cation transporter 1-transfected human embryonic kidney 293 cells. Drug Metabolism and Disposition. 2016;**44**(3):356-364

[32] DeCensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, Bonanni B, et al. Metformin and cancer risk in diabetic patients: A systematic review and meta-analysis. Cancer Prevention Research. 2010;**3**(11):1451-1461

[33] Lee M-S, Hsu C-C, Wahlqvist ML, Tsai H-N, Chang Y-H, Huang Y-C. Type 2 diabetes increases and metformin reduces total, colorectal, liver, and pancreatic cancer incidences in Taiwanese: A representative population prospective cohort study of 800,000 individuals. BMC Cancer. 2011;**11**:20

[34] Franciosi M, Lucisano G, Lapice E, Strippoli GFM, Pellegrini F, Nicolucci A. Metformin therapy and risk of cancer in patients with type 2 diabetes: Systematic review. PLoS One. 2013;**8**(8):e71583

[35] Gan X, Cao C, He Y, Hu X, Peng X, Su Y. Metformin has no significant anticancer effect on patients with advanced or unresectable cancer: A systematic review and meta-analysis. Current Pharmaceutical Design. 2022;**28**:1351-1358

[36] Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. Microbial Ecology in Health and Disease. 2015;**26**:26191

[37] Kanbay M, Onal EM, Afsar B, Dagel T, Yerlikaya A, Covic A, et al. The crosstalk of gut microbiota and chronic kidney disease: Role of inflammation, proteinuria, hypertension, and diabetes mellitus. International Urology and Nephrology. 2018;**50**(8):1453-1466

[38] Singer-Englar T, Barlow G, Mathur R. Obesity, diabetes, and the gut microbiome: An updated review. Expert Review of Gastroenterology & Hepatology. 2019;**13**(1):3-15

[39] Pascale A, Marchesi N, Govoni S, Coppola A, Gazzaruso C. The role of gut microbiota in obesity, diabetes mellitus, and effect of metformin: New insights into old diseases. Current Opinion in Pharmacology. 2019;**49**:1-5

[40] Yerevanian A, Soukas AA. Metformin: Mechanisms in human obesity and weight loss. Current Obesity Reports. 2019;**8**(2):156-164

[41] Sun L, Xie C, Wang G, Wu Y, Wu Q, Wang X, et al. Gut microbiota and intestinal FXR mediate the clinical benefits of metformin. Nature Medicine. 2018;**24**(12):1919-1929

[42] Wu H, Esteve E, Tremaroli V, Khan MT, Caesar R, Mannerås-Holm L, et al. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. Nature Medicine. 2017;**23**(7):850-858

[43] Zhang X, Zhao Y, Xu J, Xue Z, Zhang M, Pang X, et al. Modulation of gut microbiota by berberine and metformin during the treatment of high-fat diet-induced obesity in rats. Scientific Reports. 2015;**5**:14405

[44] Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertility and Sterility. 2004;**81**(1):19-25. DOI: 10.1016/j.fertnstert.2003.10.004

[45] Burghen GA, Givens JR, Kitabchi AE. Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. The Journal of Clinical Endocrinology and Metabolism. 1980;**50**(1):113-116

[46] Stepto NK, Cassar S, Joham AE, Hutchison SK, Harrison CL, Goldstein RF, et al. Women with polycystic ovary syndrome have intrinsic insulin resistance on the euglycaemic–hyperinsulinemic clamp. Human Reproduction. 2013;**28**(3):777-784. DOI: 10.1093/humrep/des463

[47] Toosy S, Sodi R, Pappachan JM. Lean polycystic ovary syndrome (PCOS): An evidence-based practical approach. Journal of Diabetes and Metabolic Disorders. 2018;**17**(2):277-285

[48] Hardy OT, Czech MP, Corvera S. What causes the insulin resistance underlying obesity? Current Opinion in Endocrinology, Diabetes, and Obesity. 2012;**19**(2):81-87

[49] Diamanti-KandarakisE,ChristakouCD, Kandaraki E, Economou FN. Metformin: An old medication of new fashion: Evolving new molecular mechanisms and clinical implications in polycystic ovary syndrome. European Journal of Endocrinology. 2010;**162**(2):193-212

[50] Anastasiou OE, Canbay A, Fuhrer D, Reger-Tan S. Metabolic and androgen profile in underweight women with polycystic ovary syndrome. Archives of Gynecology and Obstetrics. 2017;**296**(2):363-371

[51] Barber TM, McCarthy MI, Wass JAH, Franks S. Obesity and polycystic ovary syndrome. Clinical Endocrinology. 2006;**65**(2):137-145

[52] Christ-Crain M, Kola B, Lolli F, Fekete C, Seboek D, Wittmann G, et al. AMP-activated protein kinase mediates glucocorticoid-induced metabolic changes: A novel mechanism in Cushing's syndrome. The FASEB Journal. 2008;**22**(6):1672-1683. DOI: 10.1096/fj.07-094144

[53] Mansfield R, Galea R, Brincat M, Hole D, Mason H. Metformin has direct effects on human ovarian steroidogenesis. Fertility and Sterility. 2003;**79**(4):956-962

[54] Tosca L, Solnais P, Ferré P, Foufelle F, Dupont J. Metformin-induced stimulation of adenosine 5′ monophosphate-activated protein kinase (PRKA) impairs progesterone secretion in rat granulosa cells. Biology of Reproduction. 2006;**75**(3):342-351. DOI: 10.1095/biolreprod.106.050831

[55] Glueck CJ, Streicher P, Wang P. Treatment of polycystic ovary syndrome with insulin-lowering agents. Expert Opinion on Pharmacotherapy. 2002;**3**(8):1177-1189

[56] Messinis IE, Messini CI, Anifandis G, Dafopoulos K. Polycystic ovaries and obesity. Best Practice & Research. Clinical Obstetrics & Gynaecology. 2015;**29**(4):479-488

[57] Chan W-K, Tan AT-B, Vethakkan SR, Tah P-C, Vijayananthan A, Goh K-L.

Non-alcoholic fatty liver disease in diabetics—Prevalence and predictive factors in a multiracial hospital clinic population in Malaysia. Journal of Gastroenterology and Hepatology. 2013;**28**(8):1375-1383

[58] Fruci B, Giuliano S, Mazza A, Malaguarnera R, Belfiore A. Nonalcoholic fatty liver: A possible new target for type 2 diabetes prevention and treatment. International Journal of Molecular Sciences. 2013;**14**(11):22933-22966

[59] Said A, Akhter A. Meta-analysis of randomized controlled trials of pharmacologic agents in non-alcoholic steatohepatitis. Annals of Hepatology. 2017;**16**(4):538-547

[60] Smith BK, Marcinko K, Desjardins EM, Lally JS, Ford RJ, Steinberg GR. Treatment of nonalcoholic fatty liver disease: Role of AMPK. American Journal of Physiology. Endocrinology and Metabolism. 2016;**311**(4):E730-E740

[61] Yu J, Lee SH, Kim MK. Recent updates to clinical practice guidelines for diabetes mellitus. Endocrinology and Metabolism. 2022;**37**(1):26-37. DOI: 10.3803/EnM.2022.105

[62] Lin YY, Weng SF, Hsu CH, et al. Effect of metformin monotherapy and dual or triple concomitant therapy with metformin on glycemic control and lipid profile management of patients with type 2 diabetes mellitus. Frontiers in Medicine. 2022;**9**:995944. DOI: 10.3389/fmed.2022.995944 [Published: 14 October 2022]