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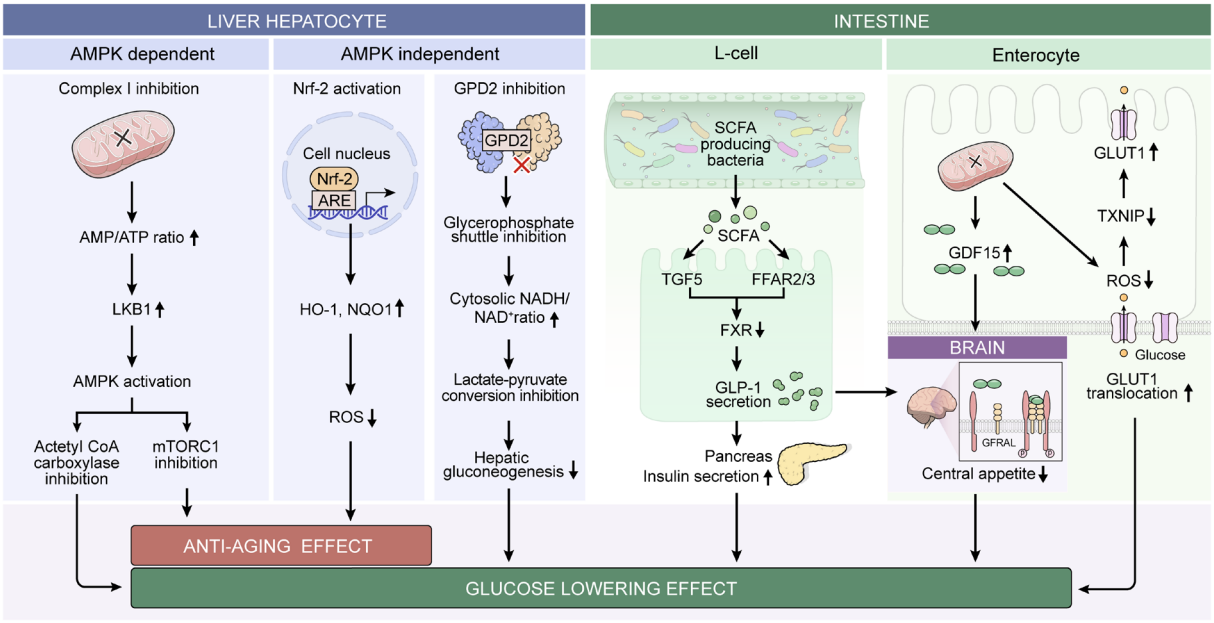

Highlights

- **Metformin is a pleiotropic metabolic modulator beyond glucose lowering.**
- **Coordinates mitochondrial and redox reprogramming with the gut–brain–liver axis.**
- **Engages intestinal glucotonic effect alongside AMPK signaling.**
- **Targets hallmarks of aging with geroprotective potential.**
- **Promising for precision medicine; awaits large, long-term RCT validation.**

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Metformin beyond Glycemic Control: New Mechanistic Insights and Expanding Therapeutic Horizons

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Metformin, while central to diabetes management, functions as a highly pleiotropic agent with mechanisms that extend far beyond simple glycemic control. In age-related degenerative diseases, including neurodegenerative disorders, it may modulate mitochondrial function, reduce oxidative stress, and influence longevity-related pathways, suggesting possible anti-aging effects. Emerging evidence also points to anticancer activity, with studies reporting reduced incidence and improved outcomes across several malignancies, potentially through mammalian target of rapamycin (mTOR) inhibition, metabolic reprogramming, and suppression of inflammatory signaling. Furthermore, the 'intestinal glucotonic effect' has been proposed to involve glucose excretion from the circulation into the gut lumen through reactive oxygen species-dependent upregulation and membrane localization of glucose transporter type 1 (GLUT1), an adenosine monophosphate-activated protein kinase (AMPK)-independent process that may contribute to the reprogramming of systemic glucose flux and provides metabolic substrates for the microbiota. Metformin also alters the gut microbiome by increasing the abundance of multiple short-chain fatty acid-producing bacteria and enhancing intestinal barrier function, which may contribute to systemic metabolic and immunologic benefits. Collectively, metformin is a pleiotropic agent with broad effects on aging biology, cancer pathophysiology, host–microbiome interactions, and immunometabolic regulation. Despite decades of clinical use, important gaps remain in understanding how these mechanisms converge to influence outcomes in individuals with diabetes and beyond.

Keywords: Aging; Metformin; Microbiota; Neoplasms

INTRODUCTION

Although metformin was originally synthesized in 1922 from French lilac, its metabolic potential was largely overlooked following the advent of insulin until its re-evaluation in the 1950s. After its therapeutic introduction in France in 1957, it received regulatory approval in the United States in 1995 and subsequently became an established agent in metabolic pharmacology.

Since its clinical emergence, metformin has served for decades as a first-line therapy for type 2 diabetes mellitus (T2DM) and remains the world's most widely prescribed glucose-lowering agent [1-3]. Although its ability to improve glycemic control and reduce diabetes-related complications is well established [4,5], its precise mechanisms of action remain incompletely understood. Traditionally, its effects were attributed primarily to reduced hepatic gluconeogenesis [6]. More recent studies, however, suggest a far more complex narrative. Metformin appears to act through multiple pathways, including

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adenosine monophosphate-activated protein kinase (AMPK) activation [7], mitochondrial modulation [8], alterations in cellular redox balance [9], and gut-centered mechanisms [10,11], highlighting a degree of biological pleiotropy that far exceeds its classical description.

Over the past decade, interest in metformin has expanded rapidly as evidence has accumulated for benefits beyond T2DM management. Emerging studies suggest that metformin may influence aging biology and age-related disorders [12,13]. Improvements in mitochondrial efficiency, reductions in oxidative stress, and favorable effects on longevity-associated signaling pathways have raised the possibility of geroprotective properties [14,15], prompting further investigation into its potential to delay age-related decline.

Epidemiologic and experimental studies have also increasingly pointed to potential anticancer properties [16-19]. Another emerging interest involves the gut microbiome, as metformin shifts microbial composition to enhance short-chain fatty acid (SCFA) production and improve intestinal barrier function [20,21]. These microbiome-related effects may contribute to its broader systemic actions in addition to its metabolic benefits [22].

Collectively, these findings portray metformin as a drug with a remarkably wide spectrum of biological effects, extending far beyond its historical role as a glucose-lowering therapy. However, substantial mechanistic gaps remain, particularly in understanding how these distinct pathways interact to influence clinical outcomes. In this review, we summarize current knowledge on the expanding roles of metformin and discuss recent mechanistic insights.

PHARMACOKINETIC ARCHITECTURE: THE FOUNDATION OF METFORMIN'S MULTI-ORGAN PLEIOTROPY

Metformin exhibits a distinctive pharmacokinetic profile characterized by incomplete absorption, extensive intestinal sequestration, and transporter-dependent tissue distribution. Following oral administration, its bioavailability is approximately 50% to 60%, with a considerable proportion of the administered dose remaining within the lumen and mucosal layer of the small intestine [7,23,24]. This markedly elevated intraluminal concentration, which exceeds plasma levels by several orders of magnitude [24], has reshaped current understanding of metformin's primary sites of action. Rather than acting predominantly through liver-directed mechanisms, metformin exerts potent gut-mediated metabolic and endocrine effects, including modulation of glucose utilization, lactate production, bile acid flux, and entero-endocrine signaling [11,25-27].

After absorption, metformin enters the portal circulation, where concentrations exceed those in the systemic circulation, facilitating preferential hepatic exposure [28]. Therapeutic dosing of 1 to 2 g/day typically results in plasma concentrations of 1 to 40 μM [7,23], whereas hepatic concentrations are estimated to be two- to three-fold higher, reflecting both first-pass exposure and high transporter expression [29,30]. Studies using radiolabeled metformin have demonstrated substantial drug accumulation in the liver, kidney, and small intestine, reinforcing the concept of organ-selective pharmacodynamics [23,28,29]. The elimination half-life ranges from 1.5 to 6.5 hours. As metformin undergoes negligible hepatic or biliary metabolism and is excreted unchanged, renal clearance represents the primary determinant of systemic exposure [23].

Due to its hydrophilic and cationic nature, metformin depends almost entirely on a repertoire of electrogenic transporters for cellular uptake and distribution. Intestinal absorption is primarily mediated by the plasma membrane monoamine transporter (PMAT, *SLC29A4*), localized on the luminal surface of enterocytes, where it facilitates uptake from the gut lumen [31,32]. Organic cation transporter 1 (OCT1) is expressed on the basolateral membrane of enterocytes and contributes to transport into the interstitial space and portal circulation [30]. In the liver, OCT1 (*SLC22A1*), and to a lesser extent OCT3 (*SLC22A3*), serve as the principal mediators of hepatic metformin uptake, supporting the high intrahepatic accumulation of metformin [32,33]. In the kidney, metformin handling is driven by OCT2 (*SLC22A2*) on the basolateral membrane of proximal tubular cells, which mediates uptake from the circulation, while luminal efflux into the urine is mediated by multidrug and toxin extrusion proteins (MATE1, *SLC47A1*) and MATE2-K (*SLC47A2*) [34,35]. Given the absence of clinically relevant metabolism, this OCT2-MATE transport axis serves as the primary determinant of systemic clearance (Fig. 1).

Importantly, genetic variation in key transporters, particularly OCT1, has been associated with inter-individual variability in glycemic response. Reduced-function variants of OCT1 may limit hepatic drug accumulation and attenuate metformin-mediated suppression of hepatic glucose production [30]. Emerging evidence also suggests that glucose transporter type

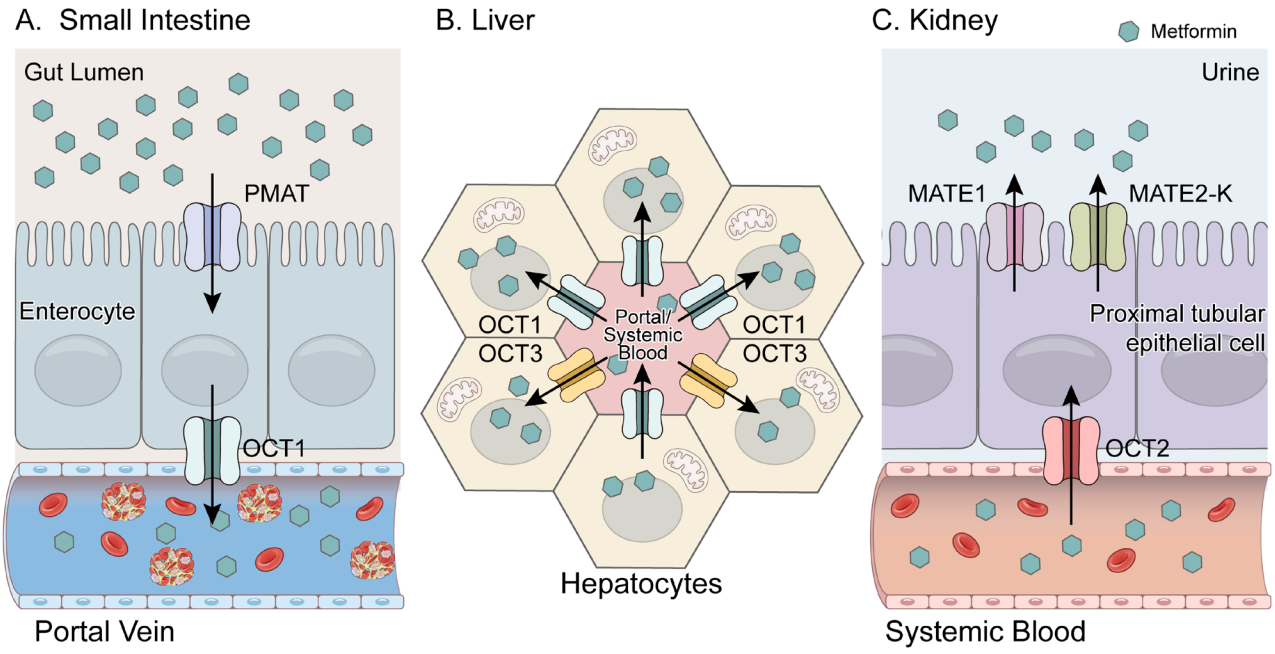

Fig. 1. Transporter-mediated pharmacokinetics of metformin. (A) Small Intestine: Following oral administration, metformin accumulates within the gut lumen and is absorbed into enterocytes via transporters, such as plasma membrane monoamine transporter (PMAT) and organic cation transporter 1 (OCT1), subsequently entering the portal vein. (B) Liver: Metformin is predominantly taken up by hepatocytes through OCT1 (and, to a lesser extent, OCT3). Intracellularly, it localizes to mitochondria, where it modulates cellular energy balance and suppresses gluconeogenesis. (C) Kidney: Metformin is excreted unchanged via active tubular secretion. OCT2 mediates uptake from the systemic circulation into proximal tubular epithelial cells, while multidrug and toxin extrusion protein 1 (MATE1) and MATE2-K facilitate apical efflux into the urinary lumen for elimination.

2 (GLUT2) may influence metformin responsiveness through modulation of hepatic glucose handling and intestinal glucose flux [36].

These pharmacokinetic principles underscore that the pleiotropic biological effects of metformin cannot be accounted for solely by circulating plasma concentrations. Instead, its therapeutic actions arise from spatially defined drug distribution shaped by saturable intestinal absorption, preferential hepatic exposure, and transporter-mediated cellular uptake. This framework provides a mechanistic basis for metformin's multiorgan activity and highlights the relevance of transporter biology in determining variability in drug response and informing precision dosing strategies.

MECHANISMS OF METFORMIN ACTION: CLASSICAL VERSUS EMERGING EVIDENCE

The classical model: AMPK activation pathway

The canonical framework for metformin's mechanism of action has traditionally centered on its capacity to regulate cellular energy homeostasis through activation of AMPK, a central sensor of nutrient availability and metabolic stress. Following OCT1-mediated uptake into hepatocytes, metformin reaches intracellular concentrations sufficient to influence mitochondrial energetics [30,33]. Within this paradigm, the primary initiating event involves the partial inhibition of mitochondrial respiratory-chain complex I, leading to reduced oxidative phosphorylation, modest depletion of adenosine triphosphate (ATP), and concomitant increases in intracellular adenosine monophosphate (AMP) and adenosine diphosphate (ADP) levels [37]. This perturbation of the adenylate charge, characterized by elevated AMP/ATP and ADP/ATP ratios, promotes allosteric binding of AMP and ADP to the regulatory γ-subunit of AMPK, thereby facilitating its phosphorylation by upstream kinases such as liver kinase B1 [38,39].

AMPK-mediated metabolic reprogramming is thought to suppress hepatic glucose production by limiting ATP availability for energetically demanding gluconeogenic processes and

by downregulating transcription of key gluconeogenic genes [40]. Furthermore, AMPK inhibits acetyl-coenzyme A carboxylase and mammalian target of rapamycin complex 1 (mTORC1), thereby reducing lipogenesis, enhancing fatty acid oxidation, and improving metabolic flexibility [41,42].

However, accumulating evidence has challenged the requirement of AMPK as the central mediator of metformin's glucose-lowering effects. Studies demonstrate that suppression of hepatic glucose output is preserved in AMPK-deficient models [8,9,43,44]. Although AMPK activation is observed at low metformin concentrations [45], it is increasingly conceptualized as a secondary adaptive response to metformin-induced metabolic stress rather than the primary driver of its hypoglycemic action [46]. Importantly, recent findings indicate that AMPK activation may occur in a compartment-specific and concentration-dependent manner, including lysosome-localized signaling pathways that operate independently of overt mitochondrial energy depletion [47].

Direct mitochondrial action: beyond canonical AMPK signaling

Extensive mechanistic evidence indicates that a significant proportion of metformin's metabolic effects arises from direct mitochondrial actions that are independent of AMPK activation. Experimental studies have demonstrated that metformin induces a modest but functionally meaningful inhibition of mitochondrial respiratory activity, thereby constraining oxidative metabolism and limiting gluconeogenic flux [8,37,48]. Specifically, metformin suppresses mitochondrial respiration and reverse electron transport through complex I, thereby attenuating mitochondrial reactive oxygen species (ROS) production [44,49]. This reduction in ROS is relevant because ROS-dependent signaling pathways regulate mitochondrial redox balance and tricarboxylic acid cycle-associated gluconeogenesis [9]. Notably, these effects persist in AMPK-deficient systems, supporting the view that mitochondrial reprogramming constitutes a fundamental component of metformin's mechanism of action [9,35,43,44]. Furthermore, recent evidence from primate models and human embryonic stem cell–derived systems indicates that metformin enhances cellular antioxidant capacity through activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway via a cell-autonomous mechanism [50]. By promoting nuclear accumulation of phosphorylated Nrf2, metformin upregulates the expression of cytoprotective genes, including heme oxygenase-1 (*HO-1*) and NAD(P)H quinone dehydrogenase 1 (*NQO-1*), thereby reinforcing redox homeostasis and limiting oxidative macromolecular damage.

Metformin also directly inhibits mitochondrial glycerophosphate dehydrogenase (mGPD; GPD2), a key enzyme that links glycerol metabolism to hepatic gluconeogenesis and mitochondrial redox homeostasis [7,8]. In hepatocytes, glycerol is phosphorylated to glycerol-3-phosphate and subsequently oxidized to dihydroxyacetone phosphate (DHAP) by mGPD. As DHAP is an essential intermediate in gluconeogenesis, mGPD activity facilitates the entry of glycerol into glucose production pathways. Beyond substrate conversion, mGPD is a redox-sensitive component of the glycerophosphate shuttle, one of the two major cellular nicotinamide adenine dinucleotide (NADH) shuttles, alongside the malate–aspartate shuttle, which together maintain cytosolic and mitochondrial NADH/NAD^+ balance. Metformin-mediated inhibition of mGPD disrupts this shuttle, elevating cytosolic NADH levels and selectively impairing redox-dependent gluconeogenesis from reduced substrates, such as lactate and glycerol [7,8]. Consequently, this redox-driven modulation provides a distinct AMPK-independent mechanism wherein metformin selectively suppresses hepatic glucose production.

Although discrepancies regarding the role of mGPD in gluconeogenic flux and hepatic lipid handling have been reported [51], these differences might reflect the physiological distinction between partial, context-dependent inhibition by metformin and the systemic compensatory responses to chronic genetic deficiency. Additionally, heterogeneity in experimental metformin concentrations and exposure regimens can determine which mitochondrial pathways predominate, thereby contributing to divergent mechanistic interpretations across studies [45].

Old and new: gut–brain signaling as a driver of metformin action

Beyond hepatocentric mechanisms, a substantial body of evidence indicates that metformin exerts potent metabolic effects through the intestinal microbiome. The brand name Glucophage, derived from 'gluco' (glucose or sugar) and 'phage' (eater), reflects its capacity to sequester and utilize glucose within the gut—a concept that aligns with accumulating evidence identifying the intestine as a major site of its metabolic activity [52]. As early as the 1960s, studies demonstrated that metformin enhances glucose utilization and lactate production in the

intestinal mucosa [53-55], suggesting the existence of a prehepatic mechanism of action. Given that metformin accumulates in the gut at concentrations substantially exceeding those in plasma, it reshapes microbial composition and function. An early mechanistic study in *Caenorhabditis elegans* demonstrated that metformin extends lifespan by altering microbial folate and methionine metabolism, highlighting the microbiota as a primary target of metformin [14]. Contemporary microbiome-based models thus extend, rather than replace, these earlier gut-centric observations.

Multiple studies have demonstrated that metformin increases the abundance of SCFA-producing bacteria, leading to enhanced intestinal production of butyrate and propionate [22]. Nevertheless, these effects appear to be taxon-specific rather than universal, as reductions in certain SCFA-producing taxa have also been reported following metformin exposure [21,56]. *Akkermansia muciniphila*, a mucin-degrading bacterium associated with host–microbe crosstalk and intestinal barrier integrity [57], is consistently enriched following metformin treatment in both human and experimental models [10,11,58]. In parallel, metformin-induced reductions in *Bacteroides fragilis* have been linked to increased levels of bile acids, such as glycoursodeoxycholic acid, which antagonize intestinal farnesoid X receptor (FXR) signaling and improve glucose tolerance. These findings support the involvement of a gut microbiome–bile acid–FXR axis as a key component of metformin action [11,25,59]. Additionally, metformin-driven microbial production of SCFAs can stimulate intestinal gluconeogenesis, which triggers portal glucose sensing and vagal afferent signaling to the brain [60]. This gut–brain–liver neurogenic pathway subsequently suppresses hepatic glucose output independently of insulin and AMPK [61].

Through coordinated alterations in SCFA and bile acid signaling, metformin stimulates enteroendocrine L-cells via free fatty acid receptors (FFAR2/3) and the bile acid receptor Takeda G protein-coupled receptor 5 (TGR5), promoting glucagon-like peptide-1 (GLP-1) secretion [62,63]. Enhanced GLP-1 release contributes to improved glucose homeostasis through integrated effects on pancreatic insulin secretion, central appetite regulation, and hepatic glucose production, largely independent of direct AMPK activation. In addition, microbiome-derived metabolites and bile acids delivered via the portal circulation provide a direct gut–liver axis through which hepatic glucose output can be rapidly modulated, complementing both endocrine and neural pathways (Fig. 1) [25].

More recently, growth differentiation factor 15 (GDF15) has emerged as a metformin-responsive hormone linking intestinal stress signaling to central regulation of energy balance [64,65]. Circulating GDF15 levels increase in a dose-dependent manner following metformin treatment, suggesting its utility as a novel biomarker of metformin action [64]. GDF15 suppresses appetite and promotes weight loss and metabolic improvement by acting on its receptor, glial cell line–derived neurotrophic factor family receptor alpha-like (GFRAL), which is expressed in the area postrema and the nucleus tractus solitarius of the brainstem [65]. These findings indicate that metformin coordinates systemic metabolism through integrated gut–brain signaling pathways, including endocrine, neural, and gut-derived signals such as GDF15, thereby integrating local intestinal signaling with central energy homeostasis.

Metformin efficacy varies across individuals and may be modulated by the gut microbiome. In a recent analysis of individuals with T2DM, clinical responsiveness to metformin was associated with enrichment of several taxa, including multiple members of the *Collinsella* genus, whereas non-response was associated with enrichment of oral-origin bacteria such as *Streptococcus mitis*, *Fusobacterium animalis*, and *Gemella sanguinis*. These findings suggest that colonization by translocated oral bacteria may attenuate metformin efficacy (manuscript in preparation).

Despite these promising findings, drawing definitive conclusions remains challenging due to the inherent heterogeneity of gut microbiome research. This inconsistency stems from both methodological disparities and profound inter-individual variability. Methodologically, variations in sample handling, sequencing platforms (e.g., 16S rRNA vs. shotgun metagenomics), and bioinformatic pipelines often lead to divergent results.

On a biological level, the microbiome is highly dynamic, shaped externally by diet, regional differences, and concurrent medications [66]. Intrinsically, recent large-scale cohorts reveal that disease risk and drug responses are deeply rooted in strain-level variations, such as within-species phylogenetic diversity and strain-specific gene carriage [67]. Acknowledging these external and intrinsic factors is essential for reconciling the often-conflicting reports in current microbiome literature.

The intestinal glucotonic effect: reprogramming glucose flux across the blood–lumen axis

Alterations in intestinal glucose metabolism associated with

metformin have been recognized for decades, particularly through ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (PET/CT) imaging, which has consistently demonstrated increased glucose uptake in the small intestine and colon of patients receiving metformin [68-71].

While evidence supporting this concept remains nascent, it is steadily accumulating. Human PET/CT studies have demonstrated a marked increase in intestinal glucose uptake following metformin administration, indicating a significant shift in glucose handling [72]. This is further corroborated by preclinical models showing the translocation of circulating glucose directly into the intestinal lumen. Notably, experimental modulation of this pathway has been linked to improved systemic glucose homeostasis, underscoring its viability as a novel therapeutic target [73]. Accumulating evidence indicates that the glucose-lowering effects of metformin involve intrinsic intestinal metabolic processes that extend beyond established gut–liver axis signaling [74] and microbiome-mediated mechanisms. Within this framework, glucose regulation is no longer viewed solely as absorption from the intestinal lumen into the circulation. Instead, it may also involve the 'intestinal glucotonic effect,' defined as the active uptake of glucose from the circulation into intestinal epithelial cells followed by its release into the intestinal lumen [75]. This concept represents an emerging paradigm that may extend beyond a passive luminal absorption to active glucose uptake from the circulation into intestinal epithelial cells and subsequent excretion into the lumen. This intestine-centered reprogramming of systemic glucose flux mirrors metabolic changes observed in other therapeutic contexts, such as bariatric surgery [73], suggesting that the intestine may function as an active regulator of glucose disposal rather than solely as an absorptive barrier, and given that the intestine represents a primary site of metformin action, these alterations in intestinal glucose flux been increasingly recognized as an important component in elucidating the mechanism of action of metformin.

Recent investigations have elucidated both the physiological impact and molecular drivers of this metformin-induced, intestinal lumen-directed flux. Sakaguchi et al. [76] demonstrated that the increased intestinal glucose signal observed on PET/CT following metformin administration may reflect substantial translocation of glucose from the circulation into the gut lumen, rather than simple intracellular sequestration. Quantitative analyses suggest that metformin induces a previously underrecognized pathway of glucose excretion from the bloodstream into the intestinal lumen. Importantly, this blood-derived glucose is proposed to serve as a metabolic substrate for the gut microbiota, driving SCFA production. Mechanistically, inhibition of mitochondrial complex I by metformin has been shown to induce antioxidant reprogramming in intestinal epithelial cells, where reduced ROS levels suppress thioredoxin-interacting protein (TXNIP) expression. Subsequently, this enhances GLUT1 translocation to the plasma membrane, facilitating glucose uptake from the circulation and its redistribution into the lumen [75]. Importantly, this ROS–TXNIP–GLUT1 axis has been suggested to operate independently of AMPK activation, providing an intestine-specific pathway for systemic glucose lowering.

Collectively, these findings support a model in which metformin reprograms glucose flux across the blood–intestine–lumen axis. The intestinal glucotonic effect may complement hepatocentric and microbiome-based mechanisms, providing an integrated framework for understanding the multifaceted glucose-lowering actions of metformin (Fig. 2).

EXPANDING THE THERAPEUTIC FRONTIER: AGING AND ONCOLOGY

Neuroprotective potential in Alzheimer's and Parkinson's diseases

Substantial epidemiological evidence indicates that metformin use is associated with a reduced risk of neurodegenerative disorders, particularly dementia. Large population-based studies have consistently demonstrated a lower incidence of all-cause dementia and Alzheimer's disease among metformin users than among individuals receiving alternative glucose-lowering therapies [77-80]. Conversely, discontinuation of metformin has been associated with an increased risk of dementia, independent of glycemic control [81]. Although findings for Parkinson's disease remain less consistent, several studies have also reported a potential protective association between metformin use and Parkinson's disease risk [82,83]. Collectively, these clinical data support the hypothesis that metformin exerts neuroprotective effects that extend beyond glycemic regulation.

Metformin's neuroprotective potential is fundamentally anchored in its ability to reshape mitochondrial bioenergetics and redox homeostasis. In neurodegenerative landscapes, mitochondrial failure and oxidative stress act as synergistic drivers of neuronal loss [84-87]. Metformin counteracts this by

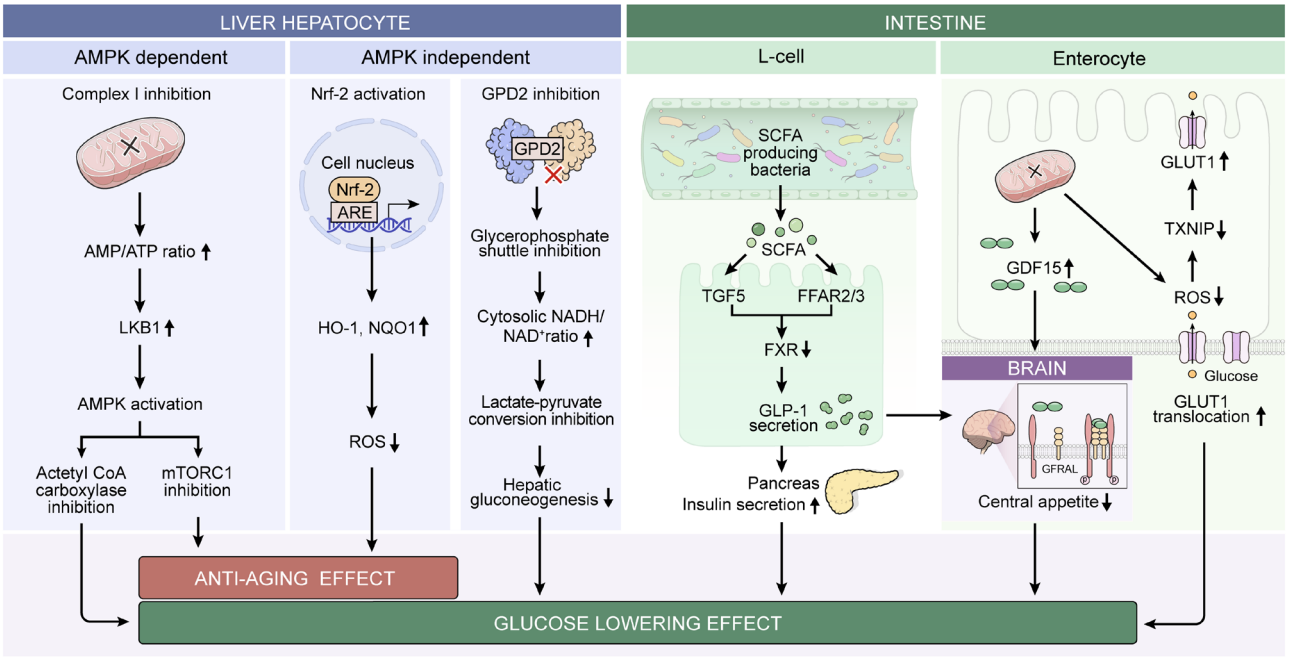

Fig. 2. Pleiotropic mechanisms of metformin action. Metformin improves systemic glucose tolerance through coordinated actions in the liver and intestine. In the liver, it inhibits mitochondrial complex I, triggering both adenosine monophosphate-activated protein kinase (AMPK)-dependent and -independent pathways. The resulting energy deficit (↓adenosine triphosphate [ATP], ↑adenosine monophosphate [AMP]) activates liver kinase B1 (LKB1) and AMPK, which subsequently inhibit mammalian target of rapamycin complex 1 (mTORC1) and acetyl-coenzyme A (CoA) carboxylase. Independently of AMPK, metformin suppresses hepatic gluconeogenesis by inhibiting glycerol-3-phosphate dehydrogenase 2 (GPD2), thereby disrupting the glycerophosphate shuttle and altering the cytosolic nicotinamide adenine dinucleotide (NADH)/NAD^+ ratio. Additionally, metformin activates nuclear factor erythroid 2-related factor 2 (Nrf2), which upregulates downstream antioxidant targets such as heme oxygenase-1 (HO-1) and NAD(P)H quinone dehydrogenase 1 (NQO1); this effectively reduces reactive oxygen species (ROS) levels, thereby exerting an anti-aging effect. In the intestine, metformin alters the luminal microbiome to increase short-chain fatty acid (SCFA) production. This change activates Takeda G protein-coupled receptor 5 (TGR5) and free fatty acid receptor 2/3 (FFAR2/3) while inhibiting farnesoid X receptor (FXR), stimulating glucagon-like peptide-1 (GLP-1) secretion. Within enterocytes, complex I inhibition reduces ROS and thioredoxin-interacting protein (TXNIP) levels, driving glucose transporter type 1 (GLUT1) membrane translocation. Furthermore, metformin stimulates the secretion of growth differentiation factor 15 (GDF15), which travels to the brain and binds to the GDNF family receptor alpha-like (GFRAL) in the hindbrain, resulting in the central suppression of appetite.

finely tuning mitochondrial respiratory activity, thereby dampening the excessive ROS production that typically overwhelms vulnerable populations like dopaminergic neurons [88].

Beyond redox control, metformin reinforces mitochondrial quality control—a critical defense against the impaired biogenesis and stalled mitophagy characteristic of Alzheimer's and Parkinson's disease [89-92]. By modulating the mTORC1-autophagy axis, it streamlines the clearance of damaged organelles alongside proteotoxic aggregates like amyloid-β, tau, and α-synuclein [93-96]. This 'cellular housekeeping' is further complemented by the induction of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), a master regulator of biogenesis that is typically suppressed in Parkinsonian models [97,98]. Through this dual promotion of mitochondrial clearance and renewal, metformin provides a multi-layered defense to preserve synaptic integrity in the aging brain [82,99].

Emerging evidence further suggests that gut microbiome-derived metabolites, including SCFAs, bile acids, and indole derivatives, can modulate mitochondrial homeostasis and influence neurodegenerative processes. Accordingly, the neuroprotective effects of metformin may, at least in part, be mediated through microbiome-dependent mechanisms that converge on mitochondrial function [100,101]. Despite promising mecha-

nistic insights and early observational data, evidence from randomized clinical trial are limited; currently, only a few pilot clinical studies have been conducted (Supplementary Table 1).

Metformin as a geroprotective agent: from cellular hallmarks to clinical translation

Metformin has evolved into a bona fide gerotherapeutic candidate by orchestrating several hallmarks of biological aging [13]. While its effects are traditionally linked to the AMPK–mTORC1 axis, its reach extends to critical AMPK-independent pathways—most notably direct sirtuin 1 (SIRT1) activation and nuclear factor-κB suppression [12,93,102]. This dual action allows metformin to simultaneously maintain cellular proteostasis and dampen 'inflammaging,' the chronic low-grade inflammation driving biological decline. This systemic defense is reinforced by the gut microbiome. Metformin-induced microbial shifts produce a distinct metabolic signature—rich in SCFAs, secondary bile acids, and indole derivatives—that converges upon these same longevity pathways [103].

Evidence from multiple experimental systems supports this translational framework. In *C. elegans* and murine models, metformin extends both lifespan and healthspan by reducing macromolecular damage and delaying cellular senescence [104-106]. Furthermore, a long-term primate study demonstrates that metformin slows biological aging across multiple tissues, reducing composite measures of biological age [50]. Notably, pronounced neuroprotective effects were observed, including an approximate six-year reversal in brain aging metrics, accompanied by reduced brain atrophy and improved cognitive function. A meta-analysis of clinical data corroborated these findings, reporting reductions in all-cause mortality and incidence of age-related cancers and cardiovascular events [107]. Human mechanistic insights were further confirmed by a randomized trial in older adults, where six weeks of metformin treatment induced tissue-specific transcriptomic reprogramming in skeletal muscle and subcutaneous adipose tissue—targeting biological aging pathways such as DNA repair, mitochondrial fatty acid oxidation, and extracellular matrix remodeling [108]. To further evaluate these effects in individuals without diabetes, the Targeting Aging with Metformin (TAME) trial has been initiated [109]. Unlike conventional disease-specific trials, TAME is designed to assess whether metformin can delay the onset of multiple age-related morbidities, including cardiovascular disease and cognitive decline.

Despite these promising findings, the geroprotective effects of metformin are highly context-dependent and may occasionally counteract positive physiological adaptations. In older adults, metformin has been shown to attenuate adaptive responses to aerobic exercise by impairing mitochondrial remodeling in skeletal muscle [110]. This complexity is also reflected in experimental aging models; although generally protective, late-life administration in *C. elegans* paradoxically shortens lifespan by exacerbating mitochondrial dysfunction because of an age-related loss of metabolic plasticity and fatal ATP exhaustion [111]. Similarly, clinical data on musculoskeletal health remain heterogeneous. While Mendelian randomization analyses suggest an association between metformin exposure and reduced risk of osteoporosis [112], its impact on sarcopenia remains unclear. One clinical trial reported improvements in biomarkers of muscle strength and physical performance [113], whereas another found no significant benefit in walking speed and highlighted poor tolerability among older participants [114]. Collectively, metformin represents a promising but complex gerotherapeutic candidate. Its non-uniform efficacy, context-dependent effects, and potential to interfere with physiological adaptations, such as exercise, highlight the need for careful patient stratification. These considerations underscore the importance of large-scale prospective clinical trials and precision medicine approaches to identify populations most likely to derive meaningful geroprotective benefit.

Targeting metabolic and immunometabolic vulnerabilities in cancer

Early observational studies derived from real-world evidence generated considerable interest in repurposing metformin for oncology, reporting reduced cancer incidence and improved clinical outcomes [18,19]. However, subsequent methodological re-evaluations have indicated that many of these associations were likely influenced by immortal time bias and may have been overstated [16,17,115,116]. Consequently, the current paradigm has shifted: rather than acting as a direct cytotoxic agent, metformin's potential may lie in its ability to modulate the cancer-associated immune and metabolic landscape.

Cancer cells undergo extensive metabolic reprogramming to sustain proliferation within nutrient- and oxygen-constrained tumor microenvironments, thereby generating metabolic liabilities that may be therapeutically exploitable [117,118]. At the cellular level, metformin-mediated inhibition of mitochondrial function imposes energetic stress, limiting ATP availabil-

ity and constraining anabolic processes required for tumor growth, in part through AMPK–mTORC1 signaling and redox modulation [96]. Importantly, these cell-intrinsic effects remain highly context-dependent and are most evident in tumors that retain a high degree of mitochondrial respiration [119].

Beyond these direct effects on tumor cell metabolism, metformin also exerts anticancer activity through remodeling of the tumor immune microenvironment (TIME) [120]. The TIME constitutes a dynamic and highly complex ecosystem in which malignant, stromal, and immune cell populations interact to regulate tumor progression and therapeutic response [121]. Immunosuppressive cell populations, including myeloid-derived suppressor cells and tumor-associated macrophages (TAMs), contribute to immune evasion, angiogenesis, and metastatic progression while impairing effective T-cell–mediated antitumor immunity [121]. Metformin counteracts these constraints by enhancing the effector function of $CD8^+$ T cells—central mediators of the cancer-immunity cycle—and may thereby augment responses to immune checkpoint blockade targeting the programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) axis and facilitate $CD8^+$ T-cell infiltration into the TIME [122,123].

In parallel, metformin enhances innate immunosurveillance, particularly natural killer (NK) cell cytotoxic activity. This effect is mediated, at least in part, through attenuation of CXC motif chemokine ligand 1 (CXCL1) signaling, which regulates signal transducer and activator of transcription 1 (STAT1) and STAT3 phosphorylation, particularly in hematologic malignancies [124-126], and upregulation of immune-regulatory microRNAs, such as miRNA-50 and miRNA-155 [127]. Through these coordinated effects, metformin alleviates metabolic and inflammatory constraints within the tumor microenvironment, functioning as an immunometabolic adjuvant that restores both innate and adaptive antitumor immunity. This immunomodulatory capacity complements its metabolic effects on tumor cells and establishes a mechanistic link between metabolic modulation and effective immune surveillance in cancer therapy. The clinical setbacks in certain cancers notwithstanding, the ability of metformin to alleviate immunosuppression within the TIME and inhibit TAM activity keeps the door open for its use as an immunometabolic adjuvant (Supplementary Table 1). We should await further evidence regarding its impact on the tumor immune landscape.

CONCLUSIONS

Metformin has evolved beyond its conventional role as a glucose-lowering agent and is now recognized as a multifaceted metabolic modulator with broad systemic effects. In addition to classical AMPK-dependent signaling, its clinical actions reflect the integration of mitochondrial reprogramming, redox regulation, and gut–brain–liver communication, complemented by microbiome-derived metabolites and the intestinal glucotonic effect. These interconnected pathways converge to enhance cellular stress adaptation, optimize bioenergetic efficiency, and constrain inflammatory signaling, providing a cohesive framework for its pleiotropic biological activity.

These mechanisms intersect with fundamental hallmarks of aging, supporting a potential geroprotective role through preservation of metabolic flexibility, maintenance of mitochondrial integrity, and modulation of immune–metabolic crosstalk. It must be acknowledged, however, that this review does not encompass the full breadth of the vast AMPK signaling network, including emerging research on tissue-specific isoforms and non-canonical regulatory pathways that fall outside our immediate scope. Furthermore, the context-dependent nature of metformin's effects—reflected in inter-individual variability in response and its capacity to attenuate beneficial physiological adaptations—underscores the need for a precision medicine approach. Future applications of metformin require stratification based on metabolic phenotypes, tissue-specific vulnerability, and microbiome composition. Within this framework, metformin may serve as a model for mechanism-informed, individualized therapeutic strategies in the management of chronic metabolic and age-related diseases.

SUPPLEMENTARY MATERIALS

Supplementary materials related to this article can be found online at https://doi.org/10.4093/dmj.2026.0248.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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Supplementary Table 1. Outcomes of metformin intervention for Alzheimer's disease and malignancies from randomized clinical trials

Target disease	Population	Intervention/duration	Measurement	Results	Reference
Alzheimer's disease	20 Nondiabetic patients with mild cognitive impairment and positive Alzheimer's disease biomarkers participants	8-week Metformin → 8-week placebo (crossover); 16 weeks total	The level of 7 proteins (AZU1, CASP-3, CCL11, CCL20, IL32, PRTN3, and REG1A) of plasma and CSF proteomics data	Correlation between changes in plasma and CSF levels of the 7 proteins after metformin use relative to baseline levels was high (r=0.98)	NCT01965756
	80 Overweight or obese individuals with amnestic mild cognitive impairment but without diabetes	Metformin 1,000 mg twice a day or matching placebo for 12 months	Total recall of the selective reminding test and the score of the Alzheimer's disease assessment scale-cognitive subscale	Changes in total recall of the selective reminding test favored the metformin group (9.7±8.5 vs. 5.3±8.5, P=0.02)	NCT00620191
Malignancies	3,649 Patients with breast cancer without diabetes	Metformin twice daily or placebo for 5 years	Invasive disease-free survival	The addition of metformin vs placebo to standard breast cancer treatment did not significantly improve invasive disease-free survival	NCT01101438
	24 Patients with treatment-refractory microsatellite stable metastatic colorectal cancer	Metformin twice daily during lead-in phase (day 1 to day 14) followed by novolumab IV every 4 weeks for 2 years	Overall response rate	Metformin alone failed to increase the infiltration of T-cell subsets in the tumor	NCT03800602
	Patients with esophageal adenocarcinomas	1,000 mg/day metformin during a 2-week period followed by neoadjuvant chemotherapy	Activation of the tumor immune microenvironment after 2-week metformin treatment	Recruiting	NCT06687876

AZU1, azurocidin 1; CASP-3, caspase-3; CCL11, CC motif chemokine ligand 11; CCL20, CC motif chemokine ligand 20; IL32, interleukin 32; PRTN3, proteinase 3; REG1A, regenerating family member 1 alpha; CSF, cerebrospinal fluid.