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Metformin and Systemic Metabolism

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Abstract

Metformin can improve patients' hyperglycemia through significant suppression of hepatic glucose production. However, up to 300 times higher concentrations of metformin accumulate in the intestine than in the circulation, where it alters nutrient metabolism in intestinal epithelial cells and microbiome, leading to increased lactate production. Hepatocytes use lactate to make glucose at the cost of energy expenditure, creating a futile intestine-liver cycle. Furthermore, metformin reduces blood lipopolysaccharides and its initiated low-grade of inflammation and increased oxidative phosphorylation in liver and adipose tissues. These metformin effects result in the improvement of insulin sensitivity and glucose utilization in extrahepatic tissues. This review discusses the current understanding of metformin's impact on systemic metabolism and its molecular mechanisms of action in various tissues.

Keywords

Metformin; insulin resistance; nutrient metabolism; mitochondria

The Development and Use of Metformin

In medieval times, the herb *Gallega officinalis* was used to treat patients with diabetes in Europe. In the late 1800s, this plant was determined to be rich in guanidine [1]. In 1918, guanidine was found to lower the blood glucose levels in rabbits, but it was too toxic for clinical use [2]. Several biguanides, including metformin, were synthesized in 1929. Biguanides preserved the anti-diabetic effect of their parent compound with reduced toxicity, making them attractive treatment options. Jean Sterne, a physician scientist, conducted the first clinical trial on the use of metformin to treat diabetes and published his studies in 1957 [3]. After 20 years of use in Europe, the Food and Drug Administration approved metformin to treat type 2 diabetes (T2D) in the USA in 1995. The American Diabetes Association and the European Association for the Study of Diabetes officially recommended metformin as the initial drug for T2D in 2012 [4]. After over 60 years of clinical use, metformin has

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proven to be safe and affordable, making it the most commonly prescribed oral anti-diabetic agent worldwide, taken by over 150 million people annually [5]. In the USA, metformin was the fourth most prescribed drug in 2017 with 78.6 million prescriptions. Fascinatingly, patients treated with metformin have reduced cancer incidence [6, 7], and metformin increases the lifespan of *C. elegans*, mice, and diabetic patients [8–10].

Pharmacokinetics of Metformin

The maximum daily metformin dose prescribed to patients is 2.5 g. Following oral administration of a therapeutic dose, metformin is mostly distributed in the gastrointestinal tract, liver, and kidney, and metformin concentrations are around 40–70 μM in the portal and reaches peak levels in the liver in 60 min (Box 1) [11, 12]. Bidirectional transport leads to metformin uptake and elimination at similar rates in the liver [12–14]. The liver loses 80% of its metformin 4 h after the drug ingestion and eliminates 98% in 24 h [11]. In cultured Hepa1–6 cells, we found that metformin concentrations in the cytoplasm are similar to the concentrations in culture medium [15], suggesting that metformin in liver hepatocytes may be equilibrated with that in the portal vein. Circulating metformin concentrations are around 10–40 μM in animals and in humans [11, 16]. Metformin cannot be metabolized and is excreted unchanged in the kidney with a half-life of about 5 h. About 10 min after oral ingestion, metformin appears in the bladder, indicating rapid transport and early elimination [12].

Metformin Modulates Glucose Metabolism

In T2D, increased **hepatic glucose production (HGP)** (see Glossary) is the major cause of fasting hyperglycemia and metformin can alleviate hyperglycemia by inhibiting **gluconeogenesis** in the liver [5]. In cultured primary hepatocytes, we found that pharmacological metformin concentration inhibits cAMP-stimulated glucose production by 58% [15]. In animal models, metformin inhibited HGP by over 60% in a euglycemic-clamp study [27] and inhibited 50% of endogenous glucose production in high-fat-diet (HFD)-fed rats [28]. In human studies, metformin reduced gluconeogenesis in the liver by more than 33% [29, 30]. These data suggest that metformin can significantly inhibit gluconeogenesis in the liver.

The importance of AMPK in metformin inhibition of HGP

In 2001, Zhou et al. reported that metformin activates AMP-activated kinase (AMPK) [31]. Our study showed depletion of AMPKα1/2 subunits abolished the inhibition of glucose production by pharmacological metformin concentrations in primary hepatocytes [32]. Furthermore, our *in vivo* study showed that HFD-fed liver-specific AMPKα1/2 knockout mice treated with metformin for 12 weeks exhibited increased fasting blood glucose levels [15], and primary hepatocytes prepared from these AMPKα1/2 knockout mice displayed significantly increased glucose production and mRNA levels of *G6pc* and *Pck1*, the rate-limiting genes in the gluconeogenesis [15]. We also found that AMPKα1 or α2 plays a role in metformin inhibition of HGP and AMPKα1 is the principal AMPK catalytic subunit for metformin suppression of HGP [15].

In uncovering the molecular mechanism through which metformin activates AMPK, we found that metformin promotes the formation of the AMPKαβγ heterotrimeric complex in *in vitro* assembly assays and in liver of HFD-fed mice. Metformin-stimulated formation of the AMPKαβγ complex leads to a net increase in α subunit phosphorylation at T172 by augmenting phosphorylation by Liver kinase B1 (LKB1) and hindering dephosphorylation by protein phosphatase [33]. Our study showed that activation of AMPK by metformin increases CBP phosphorylation at S436, leading to the disassembly of the CREB-CBP/CRTC2 complex and inhibition of gluconeogenic gene expression and glucose production in the liver (Figure 1, lower) [34].

Metformin stimulates the relocation of AMPK and LKB1 to the lysosomes to activate the AMPK [35]. Metformin could not activate AMPK and had no effect on the alleviation of hyperglycemia in HFD-fed mice with liver-specific knockout of LKB1 [36], and mice with AMPK-targeted phosphorylation site mutations in ACC1/2 displayed insulin resistance and increased HGP [37]. These studies support a mechanism for metformin action through activation of LKB1-AMPK signaling. Furthermore, metformin can also inhibits HGP by inducing Fibroblast growth factor 21 (FGF21) through activated AMPK [38].

AMPK-independent pathways in metformin inhibition of HGP

In addition to activation of classical AMPK signaling, metformin can inhibit the activity of mG3PDH to increase cytoplasmic NADH levels and cytosolic redox state to mediate metformin's antihyperglycemic effect (Figure 1, lower)[39]. Since mitochondrial G3PDH is negatively regulated by AMPK, this metformin action could still be regulated by AMPK [5]. This phenomenon may be important for patients with high levels of serum **lactate** because elevated NADH levels inhibit the conversion of lactate to pyruvate. This action will halt the glucose production from lactate. Interestingly, pharmacological metformin concentrations reduced the ratio of NADH/NAD^+ in hepatocytes, the opposite of the proposed effect on hepatocellular redox state [40].

Metformin negatively modulates AMP deaminase to augment AMP levels [41] and AMP inhibits adenylate cyclase or FBP-1 to suppresses the HGP [42, 43], however, the metformin concentrations required to change AMP levels are ≥ 250 μM [32, 44], and these concentrations are unreachable in the portal vein under physiological conditions [5, 11]. Supra-pharmacological metformin concentrations drastically reduced ATP levels [15, 44] and the synthesis of glucose through gluconeogenesis is an energy-demanding process (Figure 1, lower); therefore, decreased cellular ATP should have a negative impact on HGP. Thus, the clinical relevance of AMP in the suppression of HGP is unclear, but it may have a role in suppressing gluconeogenesis in intestinal epithelial cells (see below).

Metformin affects glucose metabolism in the intestine

The intestine is an important organ for metformin action [45]. After oral ingestion, metformin concentrations in the intestinal mucosa are 30–300 times higher than in the circulation [16, 46]. Since supra-pharmacological metformin concentrations inhibit mitochondrial respiration in hepatocytes [15, 47, 48], this may occur in intestinal epithelial cells as well due to such high metformin concentrations. When mitochondrial oxidative

phosphorylation is inhibited, enterocytes may increase anaerobic glycolysis to maintain the cellular ATP levels. Indeed, metformin increases glucose utilization and lactate production in the intestine [49]. Subsequently, lactate is used to generate glucose in hepatocytes at the expense of energy expenditure, creating a futile intestinal-liver cycle [50] and contributing to metformin's glycemic control (Figure 1, upper) [45]. Metformin also significantly increases glucose uptake in the intestinal mucosa from either the circulation or the intestine lumen via GLUT2 relocation through metformin-mediated AMPK activation [51]. Since intestinal glucose production via gluconeogenesis contributes to 5–7% of systemic glucose production [52], high intestinal metformin concentrations may increase AMP by suppressing AMP deaminase and inhibition of adenylate cyclase and FBP-1 by AMP, leading to the reduction of gluconeogenesis in the intestine (Figure 1, upper)[42, 43].

Activation of AMPK by metformin increases GLP1 secretion from L cells and circulating GLP1 levels in T2D [53–55]. Importantly, low metformin concentration also increases *GLP1* expression in L cells through SGLT1 without AMPK activation (Figure 2, left)[56]. This mechanism may be important for metformin mediated GLP1 secretion in the colon because metformin concentrations decrease dramatically, and a large portion of L cells are found in the colon.

Metformin affects glucose metabolism in muscles and adipose tissues

Both myocytes and adipocytes have OCT3 for transporting metformin into cells [18]. In an animal study, chronic metformin treatment enhanced insulin-stimulated glucose uptake in muscle, and inactivation of AMPK blunted metformin's effect [57]; in patients, metformin increased glucose disposal and glycogen contents in skeletal muscle [58]. Metformin can increase glucose uptake in visceral adipose tissue of patients [59].

Metformin Modulates Lipid Metabolism

Metformin reduces lipid secretion from intestinal epithelial cells

Patients with T2D have abnormal lipid metabolism that contributes to markedly increased comorbidity of cardiovascular diseases and fatty liver diseases. Metformin has beneficial effects beyond glycemic control, including the improvement of lipid metabolism and reduction of up to 50% of **chylomicrons** in diabetic patients [60]. Patients with insulin resistance have increased levels of apoB-48 and apoA-IV, which are critical mediators of chylomicron synthesis and secretion. Metformin-mediated alleviation of insulin resistance, AMPK activation, and **Glucagon-like peptide- 1 (GLP-1**) induction downregulate the production of apoB-48 and apoA-IV and triglyceride synthesis, leading to the reduction of chylomicron packaging and secretion from enterocytes (Figure 2, left)[15, 53–55]. In addition, metformin reduces the reabsorption of bile acids in the intestine and increase the clearance of chylomicrons, thereby reducing circulating cholesterol [61].

Metformin alleviates liver steatosis

Over 70% of patients with obesity and T2D have nonalcoholic fatty liver disease (NAFLD) and most patients with NAFLD are also obese and/or have T2D. Currently, no approved pharmacological agent is available for the treatment of NAFLD. Metformin improves blood

lipid profile and lowers liver fat contents in patients and decreases liver fat contents in mice with diet-induced steatosis and genetically obese mouse models [62]. Our studies showed that pharmacological concentrations and clinically relevant doses of metformin activate AMPK [15, 32], and activated AMPK directly phosphorylates ACC1/2 to inhibit *de novo* lipogenesis. Indeed, mice with mutations in AMPK-targeted phosphorylation sites in ACC1/2 exhibited increased liver lipids contents [37]. Activated AMPK also phosphorylates SREBP1, the master regulator in lipogenesis, to inhibit its function [63]. Furthermore, inhibition of ACC1/2 by metformin reduces malonyl-CoA levels, leading to the disinhibition of CPT1 and an increase in fatty acid oxidation in the mitochondria (Figure 2, middle). Metformin can also reduce LDL-cholesterol through AMPK-dependent suppression of fatty acids desaturase expression along with increased recycling of LDL-cholesterol receptor [64]. These data suggest that metformin could be an effective treatment for NAFLD. However, in several human studies, metformin failed to improve liver steatosis [65]. These disconcerting data may result from different metformin treatment regimens, genetic variations in transporters and effectors, and methods used to measure liver lipids contents. The concept that metformin alleviates liver lipid metabolism deserves further study.

Metformin augments fatty acids oxidation in adipose tissues and muscle

In brown adipose tissues (BAT), metformin increases the uptake of fatty acids derived from VLDL-TG and drastically decreases lipid droplet content [66]. However, metformin can increase AMPKα phosphorylation at T172 without affecting the AMP/ATP ratio, suggesting that activation of AMPK was not due to the change in AMP/ATP ratio. Intriguingly, metformin significantly increases the proteins in the mitochondrial respiratory chain, which should increase oxidative phosphorylation and the utilization of fatty acids in mitochondria. Metformin can induce HSL expression to elevate lipolysis and in differentiated adipocytes pharmacological metformin concentrations increases the phosphorylation of AMPK, ACC and HSL, leading to elevated lipolysis (Figure 2, right)[66]. Metformin-induced FGF21 may be in part responsible for decreased adipose tissue mass by increasing fatty acids oxidation [67]. Increased fatty acid uptake and utilization in adipose tissues may explain the reduction in VLDL-triglyceride and loss of adipose tissue mass after metformin administration in patients and in HFD-fed mice [59, 68]. In skeletal muscle, metformin can inhibit fat accumulation by stimulating fatty acid oxidation as well [69].

Metformin Modulates Amino Acids and Protein Metabolism

Insulin has potent anti-proteolytic effects in muscles. Because muscle consumes ~ 80% of glucose and muscle mass is negatively associated with the development of T2D, maintaining or increasing muscle mass is an important avenue to reduce hyperglycemia in T2D. Diabetic patients exhibit significantly elevated amino acids in the blood; in particular, the branched chain amino acids, alanine, and aromatic amino acids [70]. These amino acids can be used to make glucose in the liver, and the blood amino acid profile has been suggested as a biomarker for predicting the risk of developing metabolic diseases including T2D. However, in diabetic patients, metformin administration leads to interesting changes in amino acid profiles. In one study, blood amino acids were measured after 6, 12, 18 months of metformin administration and metformin decreased blood levels of phenylalanine and tyrosine along

with increased branched chain amino acids, histidine and alanine levels [71]. In another human study, metformin administration significantly increased leucine, isoleucine, and tyrosine in the blood of diabetic patients with insulin resistance [72]. Since metformin significantly increased protein synthesis in muscle of patients with severe burns [73] and reduced the wasting effect induced by tumors in muscle through inhibition of proteolytic enzymes in an animal study [74], suggesting that metformin may improve net protein balance by increasing protein synthesis and preventing protein degradation. It remains mechanistically unexplored as to how metformin can augment protein contents in muscle, but metformin-mediated improvement of insulin signaling may have a role in augmenting protein synthesis (see section on how metformin improves insulin sensitivity).

Biphasic Effects of Metformin on Mitochondrial Respiration

Supra-pharmacological metformin concentrations inhibit mitochondrial respiration

While testing of the effects of guanidine on cellular respiration (Box 2), Hollinger used supra-pharmacological metformin (20 mM) and found that it could reduce oxygen uptake in mitochondria by 30% [76]. In 2000, two studies showed that metformin inhibited the activity of mitochondrial complex I, leading to a decrease in ATP production [47, 48]. It was thus proposed that the principal mechanism of metformin action is through inhibition of mitochondrial complex 1 activity [42, 44], however, metformin is a weak inhibitor of mitochondrial complex 1 with an IC_{50} of 19–66 mM [78, 79]. To support metformin inhibition of mitochondrial complex 1, it was proposed that mitochondrial membrane potential could drive the accumulation of positively charged metformin in the mitochondrial matrix [48]. However, metformin concentrations remained the same in mitochondria isolated from mouse livers between 30 min and 4 h after oral administration [46], which does not support metformin accumulation in hepatocyte mitochondria. In cultured hepatocytes, 1 mM of metformin drastically reduced mitochondrial oxygen consumption; unexpectedly, we found that metformin concentration in hepatocyte mitochondria was 64.5 μM, two orders of magnitude below the IC_{50} for mitochondrial complex 1 activity. This metformin concentration had no inhibitory effect on the activity of each mitochondrial complex [15].

In cultured hepatocytes, treatment with supra-pharmacological metformin concentrations increased mitochondrial membrane potential [15]. Membrane potential in mitochondria is used to generate ATP from ADP; if ADP is not available, the membrane potential cannot be utilized to make ATP, resulting in elevated mitochondrial membrane potential. This is exactly what we observed - treatment with supra-pharmacological metformin drastically reduced cellular ADP and the addition of exogenous ADP restored mitochondrial respiration inhibited by supra-pharmacological metformin concentrations [15].

Since metformin concentrations in the portal vein are around 40–70 μM [11], direct inhibition of mitochondrial complex 1 activity by metformin in hepatocytes is unlikely. However, metformin concentrations in the intestine are 30–300 times higher than that in the circulation [16, 46]. This high metformin concentration can inhibit mitochondrial respiration in intestinal epithelial cells, leading to increased glucose utilization through glycolysis to maintain cellular energy homeostasis along with the overproduction of lactate. This may be

a plausible explanation of metformin-mediated increased glucose utilization and lactate production in the intestine (Figure 1, upper) [49, 50].

Pharmacological metformin concentration increases cellular respiration in the liver

Patients with T2D have decreased mitochondrial numbers and respiratory activity in the liver and other metabolic tissues, and mitochondrial dysfunction is implicated in the development of T2D [80]. Human studies revealed that metformin activates mitochondrial respiratory chain activity in tissues and cells other than intestinal epithelial cells [81, 82] and we and others found that metformin significantly increased mitochondrial complex 1 activity in the liver of mice [9, 15]. Importantly, pharmacological metformin concentrations increased mitochondrial oxidative phosphorylation in the liver and primary hepatocytes [15, 40]. In HFD-fed mice, treatment with clinically relevant metformin (50 mg/kg/day) improved hyperglycemia and significantly increased mitochondrial numbers in the liver. Because the signaling pathway of PGC-1α-driven mitochondrial biogenesis was not affected by metformin, we examined metformin's effect on mitochondrial dynamics, and found that pharmacological metformin (75 μM) increased mitochondrial fission in primary hepatocytes. This was mediated by AMPK phosphorylation of mitochondrial fission factor (MFF) and dynamin-related protein 1 (DRP1) recruitment onto the mitochondrial membrane to initiate fission [15, 83].

We also determined the importance of mitochondrial fission in regulating mitochondrial respiration by blocking mitochondrial fission via DRP1 depletion. Primary hepatocytes prepared from liver-specific *Drp1* knockout mice displayed significantly decreased mitochondrial respiration, however, with increased fat accumulation [15]. Since mitochondrial fission is associated with oxidative phosphorylation, metformin-promoted mitochondrial fission increases nutrient oxidation in mitochondria (Figure 2, middle). In addition, the continual cycle of fusion and fission is important for quality control in mitochondria; metformin-promoted fission eliminates compromised mitochondria via mitophagy to maintain a healthy mitochondrial population.

Metformin increases cellular respiration in adipose tissues

In patients with T2D, metformin decreases adipose tissue mass [68]. In mice fed a Western diet equivalent, metformin increased uptake of VLDL-TG derived fatty acids in BAT, and significantly reduced the mass and lipid content in BAT without affecting the mRNA and protein levels of UCP1 [66]. Another animal study showed that metformin increased mitochondrial biogenesis and thermogenesis in BAT [59]. Both reports showed that metformin augmented PGC-1α expression and the contents of the mitochondrial respiratory chain in BAT, suggesting that metformin can increase mitochondrial biogenesis. Since metformin increased AMPKα phosphorylation at T172 in BAT [66], activated AMPK can drive mitochondrial fission and respiration chain activity [83], leading to increased utilization of fatty acids is likely (Figure 2, right).

Metformin Improves Systemic Insulin Sensitivity

Metformin improves insulin resistance and enhances insulin-mediated inhibition of HGP and insulin-stimulated glucose utilization in extrahepatic tissues [29, 30]. In human studies, metformin increases ~30% of insulin-stimulated glucose uptake and glycogen synthesis. In HFD-fed mice, metformin reduces plasma insulin levels and elevates biomarkers of insulin sensitivity [15]. These actions are also critical for improving the symptoms of T2D.

Metformin alters gut microbiota and maintains the integrity of the intestinal barrier

Patients with T2D have a different composition of intestinal microbiota compared to healthy individuals, and intestinal microbial dysbiosis is associated with obesity and T2D [84, 85]. HFD feeding leads to a two to three-fold increase in serum levels of lipopolysaccharides (LPS) that initiates LPS-induced inflammation and ER stress, causing insulin resistance [86]. But metformin can alter the composition of intestinal microbiota in HFD-fed and diabetic mice and reverse insulin resistance [87, 88]. In naïve patients with T2D, metformin quickly altered the composition and function of intestinal microbiota, and in an animal study, the transfer of metformin-altered microbiota to germ-free mice improved glucose tolerance [89]. This effect results in decreased serum LPS levels, reduced inflammation, and improved insulin sensitivity (Figure 3). Sequestration of bile acids in the intestine has been proposed as a therapy for T2D, metformin can reduce the resorption of bile acids, thereby increasing the amount of bile acids in the distal intestine, which indirectly affects the composition of intestinal microbiota [90].

HFD-fed mice and diabetic patients exhibit increased intestinal permeability. Short-chain fatty acids (SCFAs), such as lactate, butyrate, and propionate generated through glycolysis of glucose and metabolism of amino acids and organics, are important fuels for intestinal epithelial cells and strengthen the intestinal barrier to maintain intestinal integrity by activating AMPK [91]. Metformin increases intestinal levels of SCFAs and AMPK activation, strengthens of the intestinal barrier integrity and reduces LPS leakage from the intestine, resulting in the improvement of inflammation and insulin sensitivity in T2D (Figure 3)[88, 89].

Metformin alleviates low-grade of inflammation

Based on increased LPS levels observed in the liver of HFD-fed mice, we tested LPS's effect on insulin signaling and found that LPS treatment resulted in increased protein levels of acetyltransferase P300 and its abnormal appearance in hepatocyte's cytoplasm [92]. The abnormal cytoplasm appearing P300 disrupts insulin signaling by acetylating insulin receptor substrate 1/2 (IRS1/2), then blocking its association with insulin receptor. Because metformin augments IRS tyrosine phosphorylation in hepatocytes, metformin-mediated reduction of LPS improves insulin sensitivity through the blockade of LPS-induced acetyltransferase P300 in the liver (Figure 3, lower). LPS-induced activation of the NF-κB pathway and endoplasmic reticulum stress causes broad IRS serine/threonine phosphorylation by JNK and IKKβ to impair insulin signaling; however, metformin can improve the insulin signaling by activating AMPK to attenuate LPS-induced activation of the NF-κB pathway and endoplasmic reticulum stress in the liver (Figure 3, lower)[93].

Furthermore, metformin decreases the expression of inflammatory cytokines, such as TNFα and CRP, in circulating mononuclear cells in patients with T2D. Interestingly, supra-pharmacological metformin prevents monocyte-to-macrophage differentiation via AMPK activation to reduce the production of proinflammatory cytokines and promote the macrophage polarization to anti-inflammatory functional M2 phenotypes [94]. This effect may occur in the intestine, where metformin concentration is the highest. Metformin also increases oxidative phosphorylation with an increase in fatty acid oxidation and thus reduces fatty accumulation in the liver (Figure 2, middle)[15, 37, 95]. The reduction of fat accumulation in the liver and inflammatory cytokines in the circulation and liver leads to the improvement of insulin sensitivity.

Metformin affects insulin signaling in extrahepatic tissues

SHIP2 is a negative regulator of insulin signaling that dephosphorylates the 5'-position of PtdIns(3,4,5)P3 to generate PtdIns(3,4)P2. Metformin binds to SHIP2 to inhibit its enzymatic activity and increase glucose uptake by reducing GLUT4 endocytosis in muscle cells [96]. It was also reported that metformin improves insulin signaling in skeletal muscle by regulating the expression of miR-2 and TGFβ/smad7; moreover, metformin-mediated activation of AMPK and suppression of TGFβ/smad3 in adipose tissues improves systemic insulin sensitivity in obese models [97]. Additionally, AMPK activation by metformin suppresses PTEN and improves insulin signaling in pre-adipocyte 3T3 cells (Figure 3, lower) [98]. Of note, metformin has only a mild effect on β cell functions, such as insulin secretion, regulation of pancreatic islets, and cell viability in islets [99]. In human and animal studies, decreased food intake influenced by metformin reduces body weight, further contributing to the improvement of insulin sensitivity (Box 3). Collectively, these metformin effects improve insulin signaling and sensitivity without much impact on the β cells but rather through modulation of insulin signaling in extrahepatic tissues.

Conclusions and Future Perspectives

Recently, the role of the intestine in metformin action has received increasing attention because intestinal metformin concentrations are 30–300 times higher than in circulation [16, 46]. High metformin concentrations inhibit oxidative phosphorylation in the mitochondria of intestinal epithelial cells, and increase glucose uptake from circulation or the intestine, leading to increased glucose utilization through glycolysis and overproduction of lactate. Subsequently, lactate is converted to glucose in hepatocytes at the cost of energy expenditure, creating a futile intestinal-liver cycle [50]. High metformin may also increase AMP to inhibit gluconeogenesis in intestinal epithelial cells. However, the extent of high metformin concentration on AMP and its regulation of adenylate cyclase or FBP-1 and the suppression of gluconeogenesis in intestinal epithelial cells is poorly understood and requires further investigation (see Outstanding Questions). High metformin can affect the functions of endocrine cells and microbiota composition in the intestine, therefore exerting antidiabetic effects by improving insulin sensitivity and reducing body weight. Altered microbiota composition decreases LPS production and LPS-initiated low-grade inflammation, which also leads to the improvement of insulin sensitivity. Since insulin sensitivity and mitochondrial function declines with age that contribute to the aging process,

and aging is associated with a chronic, low-grade inflammation termed “inflamm-aging” [103, 104], thus metformin improvement of insulin sensitivity and mitochondrial function may have an impact on the prolongation of the lifespan.

Our studies show that metformin activates AMPK by promoting the formation of the AMPKαβγ heterotrimeric complex in both *in vivo* and *in vitro* assays [33], which may lead to diverse responses in different tissues. This phenomenon may explain the pleiotropic and tissue-dependent effects of metformin; however, this remains to be dissected in detail. Our recent study showed that AMPKγ1 subunit plays a critical role in metformin-mediated activation of AMPK [105]. Since there are several glutamate residues within or around the CBS1 and CBS4 domains, it is possible that positively charged metformin can bind to the CBS1 and CBS4 domains. However, the exact metformin binding site(s) on AMPK subunits need to be determined in future studies.

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GLOSSARY

AMP-activated kinase (AMPK)
a master regulator in cellular and whole organism energy homeostasis. Functional AMPK is a heterotrimeric complex comprised of an α catalytic subunit and βγ non-catalytic subunits. Each subunit has multiple isoforms (α1, α2, β1, β2, γ1, γ2, γ3), and AMPK subunits are encoded by seven different genes. There are 12 heterotrimeric combinations of these subunits. AMPK subunits are unequally expressed in cells and the protein levels of AMPK subunits vary from tissue to tissue.

Chylomicron
monoacylglycerol, diacylglycerol, free fatty acids, and glycerol are reconverted to triacylglycerol and packaged with cholesterol and lipoproteins to form chylomicrons in intestinal epithelial cells for secretion to the lymphatic vessels.

Fibroblast growth factor 21 (FGF21)
a pleiotropic hormone. FGF21 is expressed in metabolically active organs and inhibits lipogenesis, activation of fatty acid oxidation, and regulation of glucose metabolism.

Glucagon-like peptide- 1 (GLP1)
secreted from L cells in the intestine upon nutrient, hormonal, and neural stimuli, and has an important role in the regulation of glucose homeostasis by increasing insulin release and inhibiting glucagon secretion.

Gluconeogenesis
an energy demanding process. When one molecule of glucose is generated from pyruvate or lactate requiring four molecules of ATP, two molecules of GTP, and two molecules of $NADH+H^+$.

Hepatic glucose production (HGP)
hepatocytes use pyruvate, lactate, amino acids, and glycerol to make glucose through gluconeogenesis.

Insulin resistance
the hallmark of T2D, results in increased HGP and decreased glucose utilization in extrahepatic tissues (muscle and adipose tissues), causing fasting hyperglycemia in patients with T2D.

Lactate
an important precursor for gluconeogenesis in the liver. Lactate is transported into hepatocytes by monocarboxylate transporter (MCT).

Liver kinase B1 (LKB1)
an upstream kinase for AMPKα subunit phosphorylation at T172.

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Box 1.

The absorption, uptake, and elimination of metformin

Metformin is absorbed by enterocytes in the proximal small intestine, with bioavailability of 40~80% depending on doses; increasing the dose leads to reduction of the absorption, suggesting a saturable absorption process. Plasma membrane monoamine transporter (PMAT) (Box 1, Table 1) may be the primary transporter involved because it is expressed on the apical membrane of polarized enterocytes and the uptake curve of metformin by PMAT is sigmoidal with a *Km* for metformin of 1.32 mM [17]. Organic cation transporter 3 (OCT3) is also expressed on the apical membrane and has a *Km* for metformin of 1.10 mM. OCT3 may have a role in metformin absorption because deletion of OCT3 leads to reduced metformin bioavailability [18]. OCT1 is expressed on the basolateral membrane of enterocytes and due to the bidirectional transport of OCTs, OCT1 transports metformin from enterocytes into the portal vein [19]. Several other transporters, such as carnitine/organic cation transporter 1 (OCTN1), serotonin transporter (SERT), and thiamine transporter 2 (THTR2) may also contribute to metformin absorption in the intestine; however, the importance of these transporters in metformin absorption remains to be determined.

Both OCT1 and OCT3 are expressed in hepatocyte membrane and knockout of these genes reduces metformin accumulation in the liver and its glucose lowering effects, suggesting that OCT1 and OCT3 are the primary metformin transporters for uptake in hepatocytes [18, 20]. Metformin is a good substrate for OCT1, with a *Km* of 1.47 mM [21]. Multidrug and toxin extrusion 1 (MATE1) is highly expressed in the liver and may excrete metformin from hepatocytes because inhibition of MATE1 activity increases metformin accumulation in the liver [22]. Whether significant amounts of metformin can be secreted into the biliary tract through MATE1 is unclear [12].

The majority of metformin clearance is through renal excretion into urine [16]. Metformin is a substrate for OCT2 and MATE1/2 expressed in the kidney, with OCT2 acting as the primary transporter for metformin uptake into renal epithelial cells with a *Km* of 0.99 mM [23]. MATE1 and MATE2 are responsible for the secretion of metformin from the tubule cells into the urine [24]. Metformin is a good substrate for both MATE1 and MATE2, with *Kms* of 0.23 and 1.05 mM, respectively [25, 26] and the loss of MATE1 leads to increased systemic metformin contents and causes metformin-induced lactic acidosis, indicating the importance of kidney function in metformin excretion.

Box 2.

Guanidine has biphasic effects on mitochondrial respiration

Mitochondria are the powerhouses of cells, driving the conversion of ADP to ATP using mitochondrial membrane potential generated through mitochondrial respiration (oxidative phosphorylation). In 1921, Meyerhof reported that 13 mM of guanidine inhibited oxygen uptake in dissected muscles by 10% [75]. Another study showed that higher concentration (20 mM) of guanidine inhibited oxygen consumption in isolated mitochondria from mouse liver and kidney by 50% and 90%, respectively [76]. However, during that period, lower guanidine concentrations were found to activate mitochondrial respiration and the activity of oxidative enzymes in mitochondria as well [77]. These data indicates that guanidine has biphasic effects on mitochondrial respiration, with low concentrations activating mitochondrial respiration and high concentrations inhibiting mitochondrial respiration.

Box 3.

Metformin Reduces Food Intake and Body Weight

With a molecular weight of 129 g/mol, metformin easily passes through the blood-brain barrier to influence the brain. Metformin decreases food intake and body weight in patients with T2D. In an animal study, intracerebroventricular administration of metformin suppresses orexigenic peptides, neuropeptide Y and agouti-related protein and therefore reduces food intake and body weight. This metformin effect is AMPK-dependent because inhibition of AMPK activity by compound C abolished this effect [100]. In contrast, we observed that metformin significantly increases serum ghrelin levels [15], which should stimulate appetite. However, metformin negated the effect of ghrelin-stimulated food intake by blocking AMPK-ACC-Raptor signaling in the hypothalamus [101]. Furthermore, metformin increases the expression of the leptin receptor gene in the arcuate nucleus to alleviate central leptin sensitivity and reduction of food consumption.

Growth differentiation factor 15 (GDF15) decreases food intake as its receptor is expressed in neurons of the area postrema that controls appetite. Metformin treatment chronically increases GDF15 level in the blood, and circulating GDF15 level is positively correlated with the amount of weight loss [102]. Thus, GDF15 could also be a target for metformin's beneficial effects on energy balance and body weight. Additionally, metformin can increase circulating GLP1 levels in patients with T2D [54] and elevated circulating GLP1 reduces food intake by inducing *cFos* in the paraventricular nucleus of hypothalamus. Notably, the suppression of HGP by metformin precedes the loss of body weight [15, 49]. Of clinical importance, the loss of body weight leads to the improvement of insulin sensitivity and facilitates further alleviation of hyperglycemia in T2D.

OUTSTANDING QUESTIONS

- What is the contribution of metformin-mediated inhibition of gluconeogenesis in intestinal epithelial cells to the control of systematic glucose metabolism?
- Will metformin improvement of insulin sensitivity and mitochondrial function have an impact on the aging process?
- Can metformin promote the formation of distinct combinational αβγ complex in different tissues?
- Are the glutamate residues within or around the CBS1 and CBS4 domains the binding sites of metformin in the AMPK complex?

HIGHLIGHTS

- Metformin improves hyperglycemia in diabetic patients mainly by suppressing liver glucose production and improving insulin sensitivity.
- Metformin can promote the formation of the AMPKαβγ heterotrimeric complex. This effect leads to a net increase in catalytic α subunit phosphorylation at T172 by augmenting phosphorylation by upstream kinase LKB1 along with hindering dephosphorylation by protein phosphatase.
- Due to high concentrations of metformin in the intestine, metformin increases glucose utilization through glycolysis and overproduction of lactate in intestinal epithelial cells, and lactate is converted to glucose in hepatocytes at the cost of energy expenditure, creating a futile intestinal-liver cycle.
- Metformin can change microbiota composition in the intestine, resulting in decreased serum LPS levels, reduced inflammation, and improved insulin sensitivity.
- Metformin has biphasic effects on mitochondrial respiration, with supra-pharmacological metformin concentrations inhibit mitochondrial respiration in the intestine, and pharmacological metformin concentration improves mitochondrial respiration and fatty acid oxidation in the liver and adipose tissue.
- Metformin reduces body weight by stimulating satiety and energy expenditure.

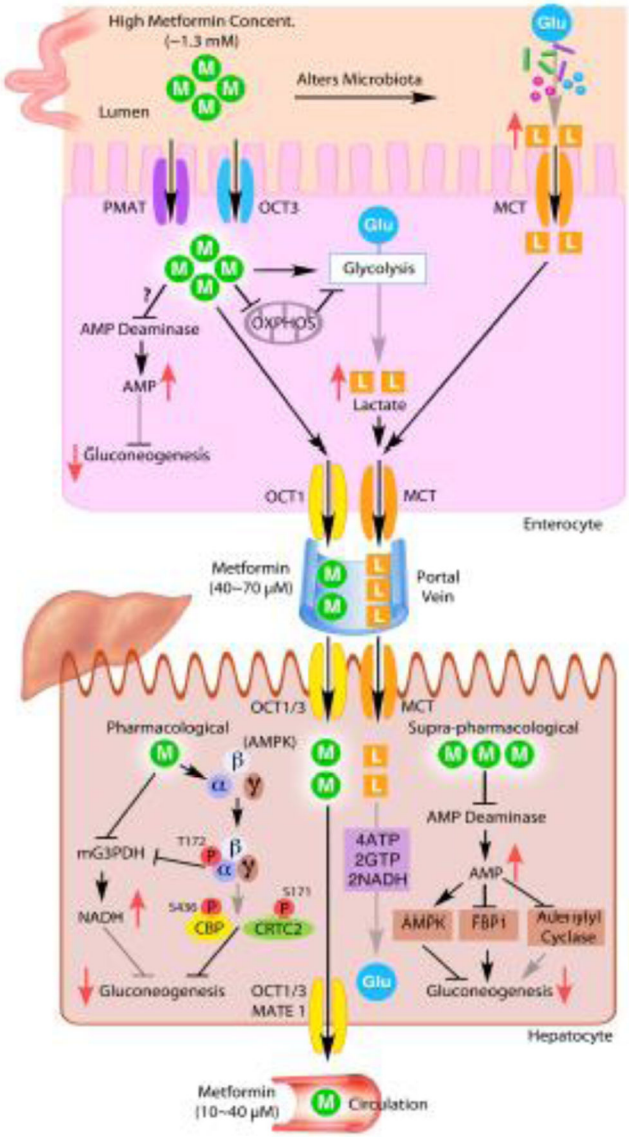

Figure 1. Metformin modulates glucose metabolism in the intestine and liver.

(Upper) In the intestine, metformin alters the composition and function of microbiota, leading to increased production of lactate from glycolysis; high metformin concentrations can inhibit oxidative phosphorylation in mitochondria of intestinal epithelial cells and increase glucose utilization through glycolysis and overproduction of lactate; high metformin concentration may inhibit gluconeogenesis by inactivating AMP deaminase in intestinal epithelial cells. (Lower) In the liver, pharmacological metformin concentration promotes the formation of the AMPKαβγ heterotrimeric complex and increases AMPKα phosphorylation at T172 and suppression of mG3PDH to reduce HGP. Supra-pharmacological metformin can inhibit the gluconeogenesis by increasing AMP, and AMP inhibits adenylate cyclase or FBP-1 to suppresses the HGP. Lactate generated from the intestine is used to make glucose in the liver through gluconeogenesis at the cost of cellular energy, generating a futile intestine-liver cycle. The black arrows indicate the direct effects,

and the shaded arrows indicate the indirect effects. CRTC2, creb-regulated transcriptional coactivator 2; FBP-1, fructose 1,6-bisphosphatase 1; mG3PDH, mitochondrial glycerol 3-phosphate dehydrogenase. *L*, lactate; *M*, metformin.

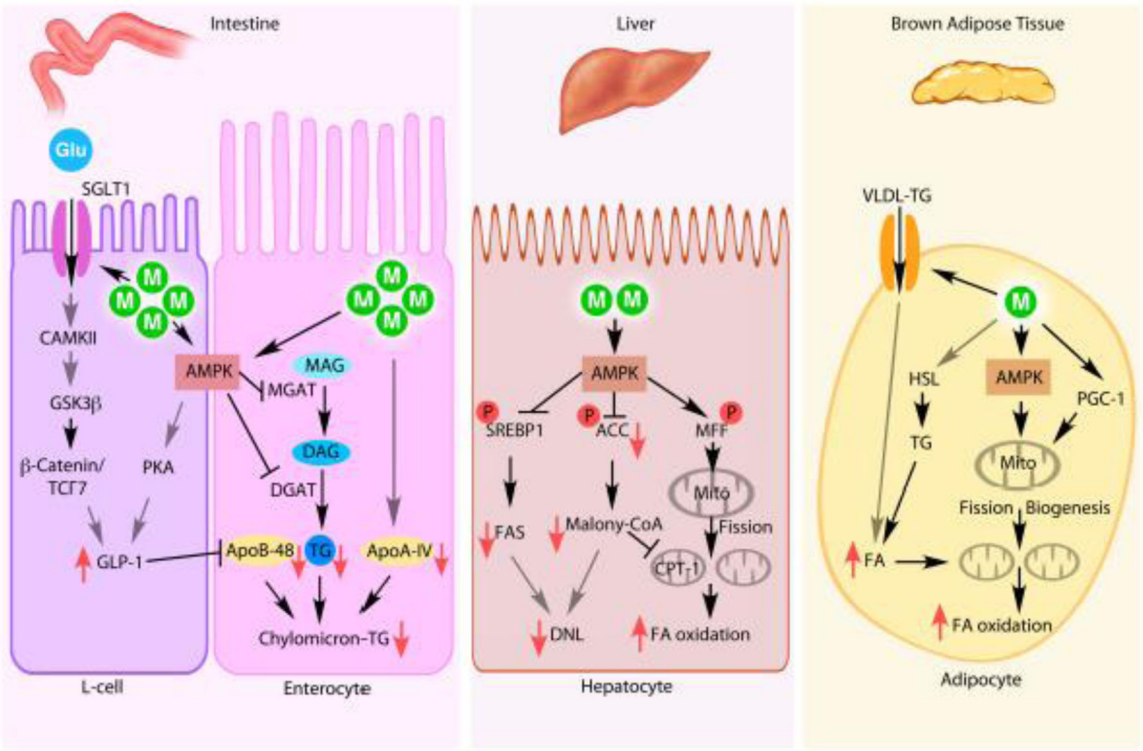

Figure 2. Metformin modulates lipid metabolism in the intestine, liver, and adipose tissue.

(Left) In the intestine, metformin stimulates GLP-1 expression and secretion through AMPK-dependent and -independent pathway in L cells. Metformin inhibits chylomicron assembly and secretion in enterocytes by reducing the levels of Apo-48, Apo-IV, and triglyceride synthesis. (Middle) In the liver, metformin-mediated activation of AMPK phosphorylates SREBP-1 and ACC to inhibit the de novo lipogenesis (DNL), disinhibits the activity of CPT-1; metformin also promotes the mitochondrial fission to increase mitochondria number; these metformin's effects lead to elevated fatty acid oxidation. (Right) In brown adipocytes, metformin stimulates fatty acid uptake and activates HSL to increase lipolysis. Metformin increases mitochondrial biogenesis by activating PGC-1 signaling. Activated AMPK also drives mitochondrial fission. Collectively, metformin augments fatty acid oxidation in brown adipose tissue. The black arrows indicate the direct effects, and the shaded arrows indicate the indirect effects. ACC1/2, acetyl-coenzyme A carboxylase 1/2; CAMKII, calcium/calmodulin-activated protein kinase II; CPT1, carnitine palmitoyltransferase 1; DAG, diacylglycerols; DGAT, diacylglycerol acyltransferase; FAS, fatty acid synthase; HSL, hormone-sensitive lipase; MAG, monoacylglycerols; MGAT, monoacylglycerol acyltransferase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1α; PKA, protein kinase A; SREBP1, sterol regulatory element binding protein 1; TG. triglycerides.

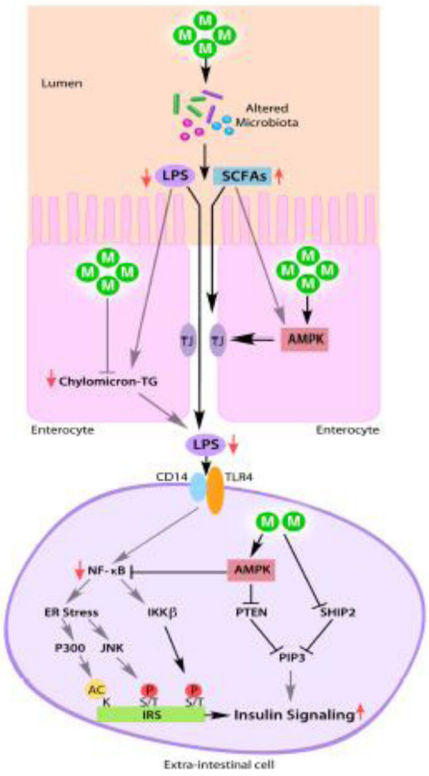

Figure 3. Metformin improves insulin sensitivity.

(Upper) In the intestine, metformin changes the composition of microbiota, resulting in decreased LPS production and increased SCFAs. SCFAs and metformin-mediated activation of AMPK strengths the intestinal barrier, along with decreased chylomicron generation, leading to reduction of LPS levels in the circulation. (Lower) In extra-intestinal cells, reduced LPS levels and its initiated low-grade of inflammation improves insulin signaling by reducing IRS acetylation and serine and threonine phosphorylation and by increasing PIP3 levels. The black arrows indicate the direct effects, and the shaded arrows indicate the indirect effects. ER stress, endoplasmic reticulum stress; IKKβ, IκB kinase β; JNK, c-jun N-terminal kinases; NF-κB, nuclear factor-kappa B; P300, E1A binding protein P300; PTEN, phosphatase and tensin homolog; TJ, tight junction; TLR4, toll like receptor 4; SHIP2, Src homology 2 domain containing inositol-5-phosphatase 2.

Box 1, Table 1.

Transporters involved in uptake and excretion of metformin

Type of cells	Uptake	Excretion
Enterocytes	PMAT	OCT1
	OCT3	
	SERT	
	THTR2	
	OCTN1	
Hepatocytes	OCT1	MATE1
	OCT3	
Renal tubule cells	OCT2	MATE1
		MATE2