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Inhibition of hepatic gluconeogenesis in type 2 diabetes by metformin: complementary role of nitric oxide

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Abstract

Metformin is the first-line treatment for type 2 diabetes mellitus. Type 2 diabetes mellitus is associated with decreased nitric oxide bioavailability, which has significant metabolic implications, including enhanced insulin secretion and peripheral glucose utilization. Similar to metformin, nitric oxide also inhibits hepatic glucose production, mainly by suppressing gluconeogenesis. This review explores the combined effects of metformin and nitric oxide on hepatic gluconeogenesis and proposes the potential of a hybrid metformin-nitric oxide drug for managing type 2 diabetes mellitus. Both metformin and nitric oxide inhibit gluconeogenesis through overlapping and distinct mechanisms. In hepatic gluconeogenesis, mitochondrial oxaloacetate is exported to the cytoplasm via various pathways, including the malate, direct, aspartate, and fumarate pathways. The effects of nitric oxide and metformin on the exportation of oxaloacetate are complementary; nitric oxide primarily inhibits the malate pathway, while metformin strongly inhibits the fumarate and aspartate pathways. Furthermore, metformin effectively blocks gluconeogenesis from lactate, glycerol, and glutamine, whereas nitric oxide mainly inhibits alanine-induced gluconeogenesis. Additionally, nitric oxide contributes to the adenosine monophosphate-activated protein kinase-dependent inhibition of gluconeogenesis induced by metformin. The combined use of metformin and nitric oxide offers the potential to mitigate common side effects. For example, lactic acidosis, a known side effect of metformin, is linked to nitric oxide deficiency, while the oxidative and nitrosative stress caused by nitric oxide could be counterbalanced by metformin's enhancement of glutathione. Metformin also amplifies nitric oxide-induced activation of adenosine monophosphate-activated protein kinase. In conclusion, a metformin-nitric oxide hybrid drug can benefit patients with type 2 diabetes mellitus by enhancing the inhibition of hepatic gluconeogenesis, decreasing the required dose of metformin for maintaining optimal glycemia, and lowering the incidence of metformin-associated lactic acidosis.

Key Words: glycemia; hepatocyte; hybrid; lactic acidosis; malate-aspartate shuttle; mitochondria; nitric oxide synthase; oxaloacetate; redox; tricarboxylic acid cycle

Introduction

Type 2 diabetes mellitus (T2DM) is characterized by carbohydrate, lipid, and protein metabolism dysregulation due to impaired insulin secretion and insulin resistance.[1] In 2021, approximately 483 million people were diagnosed with T2DM globally, representing 9.45% of the population,[2] and it remains a leading cause of mortality, claiming over a million lives each year.[3] Currently, metformin is the first-line standard treatment for T2DM and the most common glucose-lowering medication,[4] with more than 200 million patients using it daily.[4] Metformin decreases both basal and postprandial plasma glucose levels.[5] The glucose-lowering effects of metformin are mediated by increasing skeletal muscle glucose uptake and utilization,[6] lowering intestinal carbohydrate absorption,[6] and suppressing glucose production[6] mainly by inhibiting hepatic gluconeogenesis,[7,8] which is increased in T2DM.[1] The activities of cytoplasmic enzymes that participate in gluconeogenesis are increased in rats with diabetes.[9] Increased hepatic gluconeogenesis in T2DM is attributed to insulin resistance, insulin deficiency, hyperglucagonemia, increased glucagon sensitivity, and elevated substrate supply for gluconeogenesis.[1]

Animal studies indicate that nitric oxide (NO) supplementation has beneficial metabolic effects in T2DM by increasing insulin secretion[10,11] (via increasing insulin synthesis and exocytosis,[12] decreasing pancreatic islet oxidative stress,[13] and increasing pancreatic islet blood flow[10]) and by stimulating peripheral glucose utilization (via increasing insulin sensitivity[14] and increasing glucose uptake in skeletal muscle cells[14] and adipocytes[15]). This issue has been reviewed elsewhere.[16-18] Additionally, similar to metformin, NO inhibits hepatic glucose production mainly by inhibiting gluconeogenesis.[19,20] The mechanisms underlying this effect include the suppression of activities of cytoplasmic phosphoenolpyruvate carboxykinase (PEPCK1),[21,22] glucose 6-phosphatase (G6Pase),[23] and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)[24] by NO. Since both metformin and NO inhibit hepatic gluconeogenesis, a metformin-NO hybrid drug may offer superior glycemic control for patients with T2DM. Supporting this hypothesis, evidence shows that combination therapy with metformin plus nitrate nearly doubles the efficacy of reducing glycated hemoglobin levels and enhancing adenosine monophosphate-activated protein kinase (AMPK) activity, indicated by the phosphorylated-AMPK/AMPK ratio, in high-fat diet-fed mice

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compared to monotherapy with either agent alone.[25] Furthermore, in a rat model of T2DM, combination therapy with metformin and *L*-arginine (an NO precursor) reduces C-reactive protein levels by about 75%, which is higher than the effect observed by metformin alone (~50%) and *L*-arginine alone (~25%).[26] This review explores how combining metformin with NO-releasing agents can improve hepatic gluconeogenesis suppression, suggesting the potential efficacy of a metformin-NO hybrid drug to manage T2DM more effectively than metformin alone.

Search Strategy

In this narrative review, we conducted a literature search using the PubMed, Google Scholar, and Scopus databases to identify relevant studies on metformin and NO-releasing agents in hepatic gluconeogenesis. The keywords used included type 2 diabetes, gluconeogenesis, metformin, nitric oxide, nitric oxide synthase, and hybrid drug. In addition, the references cited in the retrieved articles were reviewed to identify further related studies. The included studies were primarily conducted on mammals, such as rodents and humans. Only articles written in English were considered.

Hepatic Gluconeogenesis: An Overview

Hepatic gluconeogenesis accounts for about 90% of glucose production during fasting.[27] Key precursors for this process include lactate,[28] glycerol,[28] all amino acids (except leucine and lysine, which are only degraded to acetyl-coenzyme A or acetoacetate),[29] and all intermediates of the tricarboxylic acid (TCA) cycle.[30] However, lactate, glycerol, alanine, and glutamine are the main precursors for hepatic gluconeogenesis.[28] Besides glycerol, which contributes to gluconeogenesis in later stages, other main gluconeogenesis precursors flow through oxaloacetate, indicating the crucial role of oxaloacetate as a metabolic intermediate in gluconeogenesis.[30]

Figure 1 illustrates the intricate nature of gluconeogenesis, where most reactions are largely the reverse of those in glycolysis, except for three irreversible glycolytic reactions. Reactions 1 [(glucose→glucose-6-phosphate (G6P)], 3 [fructose-6-phosphate (F6P)→fructose-1,6-bisphosphate (F1,6BP)], and 10 [phosphoenolpyruvate (PEP)→pyruvate)]. These reactions require the involvement of additional enzymes/mechanisms to be reversed, allowing the progression of hepatic gluconeogenesis.[29] The conversion of pyruvate to PEP, a critical step in gluconeogenesis, is particularly complex. It involves several enzymatic pathways that shuttle intermediates between the mitochondria and cytoplasm.[31]

Lactate, mainly produced in the skeletal muscle, enters the hepatocyte through monocarboxylate transporter 1.[28,32] Then, it is converted to pyruvate in a reaction catalyzed by lactate dehydrogenase (LDH), which reduces nicotinamide adenine dinucleotide (NAD^+) to nicotinamide adenine dinucleotide reduced form (NADH).[28] Alanine, mainly produced in the skeletal muscle, is transported into hepatocytes through sodium-coupled neutral amino acid transporters (SNAT) 2 and 4[33]; transamination of *L*-alanine by alanine aminotransferase then converts it to pyruvate.[34] Pyruvate enters the mitochondria by the mitochondrial pyruvate carrier[35] and is converted to oxaloacetate by pyruvate carboxylase in the mitochondrial matrix[28] (**Figure 1**).

Glutamine, another major precursor of gluconeogenesis, mainly derived from the skeletal muscle,[36] is transported into the hepatocyte via SNAT2, SNAT3, and SNAT5.[33] Then, it enters the mitochondria through the mitochondrial glutamine transporter.[37] Glutamine is converted to glutamate by glutaminase in mitochondria, consuming H_2O to produce ammonium (NH_4^+). Then, it is converted to α-ketoglutarate by glutamate dehydrogenase coupled with the reduction of NAD^+ to NADH and consuming H_2O to produce NH_4^+. α-Ketoglutarate enters the TCA cycle to produce oxaloacetate[28,30,38] (**Figure 1**).

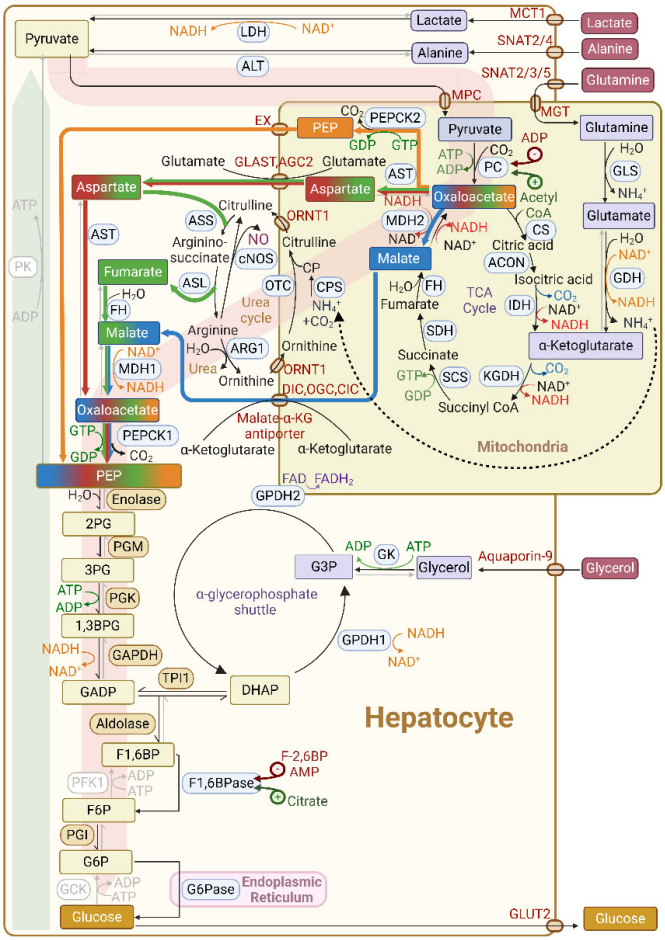

Figure 1 | An overview of hepatic gluconeogenesis.
The malate (blue arrows), direct (orange arrows), fumarate (green arrows), and aspartate (red arrows) pathways for exporting oxaloacetate from the mitochondrion to the cytoplasm. Transparent green and red arrows show the general direction of glycolysis and gluconeogenesis, respectively. In hepatic gluconeogenesis, most reactions are the reverse of those in glycolysis, except three irreversible reactions of glycolysis, reactions 1 (glucose→G6P), 3 (F6p→F1,6BP), and 10 (PEP→ pyruvate). Created with BioRender.com. 1,3BPG: 1,3-Bisphosphoglycerate; 2PG: 2-phosphoglycerate; 3PG: 3-phosphoglycerate; ACON: aconitase; ADP: adenosine diphosphate; AGC2: aspartate-glutamate carrier 2; ALT: alanine aminotransferase; AMP: adenosine monophosphate; ARG1: arginase; ASL: argininosuccinate lyase; ASS: argininosuccinate synthase; AST: aspartate aminotransferase; ATP: adenosine triphosphate; CIC: citrate carrier; CS: citrate synthase; CP: carbamoyl phosphate; CPS: carbamoyl phosphate synthetase; cNOS: constitutive nitric oxide synthase; DHAP: dihydroxyacetone phosphate; DIC: dicarboxylate carrier; EX: anion transporter; FAD: flavin adenine dinucleotide; FADH: flavin adenine dinucleotide (reduced form); FH: fumarase; F1,6BP: fructose 1,6-bisphosphate; F1,6BPase: fructose 1,6-bisphosphatase; F2,6B: fructose 2,6-bisphosphate; F6P: fructose 6-phosphate; G3P: glycerol 3-phosphate; G6P: glucose-6-phosphate; G6Pase: glucose 6-phosphatase; GADP: glyceraldehyde 3-phosphate; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; GCK: glucokinase; GDH: glutamate dehydrogenase; GDP: guanosine diphosphate; GK: glycerol kinase; GLAST: glutamate-aspartate antiporter; GLS: glutaminase; GPDH: glycerol 3-phosphate dehydrogenase; GTP: guanosine triphosphate; IDH: isocitrate dehydrogenase; KGDH: ketoglutarate dehydrogenase; LDH: lactate dehydrogenase; malate-α-KG antiporter: malate-α-ketoglutarate antiporter; MCT1: monocarboxylate transporter 1; MDH: malate dehydrogenase; MGT: mitochondrial glutamine transporter; MPC: mitochondrial pyruvate carrier; NAD^+: nicotinamide adenine dinucleotide; NADH: nicotinamide adenine dinucleotide (reduced form) ; OGC: 2-oxoglutarate carrier; ORNT1: mitochondrial ornithine transporter; OTC: ornithine transcarbamylase; PC: pyruvate carboxylase; PEP: phosphoenolpyruvate; PEPCK: phosphoenolpyruvate carboxykinase; PFK1: phosphofructokinase 1; PGI: phosphoglucose isomerase; PGK: phosphoglycerate kinase; PGM: phosphoglycerate mutase; PK: pyruvate kinase; SCS: succinyl coenzyme-A synthetase; SDH: succinate dehydrogenase; SNAT: sodium-coupled neutral amino acid transporters; TCA: tricarboxylic acid; TPI1: triosephosphate isomerase 1.

The mitochondria cannot directly transport oxaloacetate into the cytoplasm.[28] Thus, there are four primary pathways for its transport during gluconeogenesis[31] (**Figure 1**): malate, direct, aspartate, and fumarate. As shown in **Table 1**, the contribution of each pathway is determined by (1) the fasting stage and its effect on the mitochondrial NADH/NAD^+ ratio, (2) the gluconeogenesis substrate and the different enzymes it engages to provide the cytoplasmic NADH that GAPDH requires in the later stages of gluconeogenesis, and (3) accumulation of NH_4^+ in the hepatocytes that forces the urea cycle.[29,31,39]

Malate pathway

In the malate pathway, mitochondrial oxaloacetate is converted into malate coupled with the oxidation of NADH to NAD^+ by the mitochondrial malate dehydrogenase (MDH2).[29] The same enzyme contributes to the TCA cycle by driving the conversion of malate to oxaloacetate paired with the reduction of NAD^+ to NADH.[29] Mitochondrial malate is transported to the cytoplasm by dicarboxylate carrier, 2-oxoglutarate carrier, and citrate carrier.[40] Then, it is converted to oxaloacetate by cytoplasmic malate dehydrogenase (MDH1), providing NADH that GAPDH needs in the later steps of gluconeogenesis.[28,31] The malate pathway can be considered the main pathway in the earlier stages of fasting when the mitochondrial NADH/NAD^+ ratio is still high.[39] However, this pathway becomes limited during the later stages of fasting due to a decreased mitochondrial NADH/NAD^+ ratio because of NADH consumption by both the electron transport chain (ETC) and MDH2.[28,39] As a result, the malate-aspartate shuttle (MAS) becomes activated (see below) to replenish mitochondrial NADH[41] (**Figure 1**). This shift in metabolic activity alters MDH2, making it more efficient at converting malate back to oxaloacetate,[39,42] which hampers malate export. Five-day starvation decreases MDH1 activity by 16% in rat liver homogenates receiving mono, di, and TCA precursors.[9] In the malate pathway, MDH1 provides the cytoplasmic NADH, rendering the pathway independent of lactate and glycerol for providing NADH via NADH-producing enzymes of cytoplasm, LDH, and cytoplasmic glycerol 3-phosphate dehydrogenase (GPDH1). Thus, the malate pathway is considered substrate-independent[31] (**Table 1**).

In hepatocytes, the MAS in particular, and to a lesser extent, α-glycerophosphate maintain the redox balance between mitochondria and cytoplasm during gluconeogenesis.[41] MAS maintains a balance between mitochondrial and cytoplasmic NADH/NAD^+ ratio by modulating the flux through malate-oxaloacetate-aspartate between mitochondria and cytoplasm.[42] To transport cytoplasmic NADH to mitochondria, MAS recruits MDH1 to convert oxaloacetate to malate, paired with the oxidation of NADH to NAD^+, malate-α-ketoglutarate antiporter to transport malate into mitochondria, MDH2 to convert malate to oxaloacetate, paired with the reduction of NAD^+ to NADH, mitochondrial aspartate aminotransferase (AST) to convert oxaloacetate to aspartate, glutamate-aspartate antiporter to transport aspartate to the cytoplasm, and cytoplasmic AST to convert aspartate back to oxaloacetate[41,42] (**Figure 1**).

Direct pathway

In the direct pathway, oxaloacetate is directly converted to PEP by mitochondrial PEP carboxykinase (PEPCK2) at the cost of guanosine triphosphate (GTP) dephosphorylation to guanosine diphosphate.[28,31] PEP is transported to the cytoplasm by an anion transporter, exchanging PEP with another metabolite specific to each transporter[43] (**Figure 1**). The direct pathway is mainly used in the later stages of fasting when the mitochondrial NADH/NAD^+ ratio is further decreased. It depends on mitochondrial GTP, produced by succinyl coenzyme-A synthetase (SCS) during the TCA cycle.[39,44] This pathway contrasts with the malate pathway, which is more active in earlier stages of fasting when NADH levels are higher and the malate-aspartate shuttle is not as necessary.[39] In the later stages of fasting, the direct pathway compensates for the malate pathway's incapacitation in exporting oxaloacetate to the cytoplasm.[39] Unlike the other three pathways for exporting oxaloacetate, the direct pathway is MAS-independent, making it feasible to export oxaloacetate in the presence of GTP. Lactate is also required as a gluconeogenic substrate to provide the cytoplasmic NADH for GAPDH by LDH. Thus, the direct pathway compensates for the limitations of the malate pathway in exporting oxaloacetate in these conditions, making it both energy- and substrate-dependent since it relies on both mitochondrial GTP and lactate as a gluconeogenic substrate. This pathway ensures continued gluconeogenesis despite changes in mitochondrial redox states during fasting[28,31] (**Table 1**).

Aspartate pathway

In the aspartate pathway, mitochondrial oxaloacetate is converted to aspartate via mitochondrial AST.[40] Aspartate-glutamate carrier 2 transports aspartate to the cytoplasm in exchange of glutamate.[40] In the cytoplasm, AST converts aspartate to oxaloacetate[40] (**Figure 1**). The aspartate pathway contributes less to gluconeogenesis than the direct and the malate pathways.[9,39] In support of this notion, the activity of cytoplasmic AST was lower than MDH1 by 8-fold (23 *vs.* 198 μmol/min/g liver) and 4-fold (44 *vs.* 166 μmol/min/g liver) in the fed and fasting states, respectively.[9] Enzyme activities were measured during gluconeogenesis in the soluble fraction of rat liver homogenates supplied with mono-, di-, and TCA precursors.[9] The aspartate pathway is substrate-dependent and requires lactate as the substrate for gluconeogenesis to produce cytoplasmic NADH by LDH.[31] Regarding the absence of production of NADH and NH_4^+ in mitochondria through this pathway, activation of LDH is needed to provide the NADH that is required by GAPDH in later stages[29] (**Table 1**).

Fumarate pathway

In the fumarate pathway, mitochondrial oxaloacetate is converted to aspartate by AST and transported to the cytoplasm by aspartate-glutamate carrier 2. Cytoplasmic aspartate then contributes to the urea cycle, where argininosuccinate synthetase catalyzes the formation of argininosuccinate from aspartate and citrulline, consuming two adenosine triphosphate (ATP) equivalents. In the next step, argininosuccinate lyase uses argininosuccinate to produce fumarate and arginine. Fumarate is then converted to malate by cytoplasmic fumarase. MDH1 converts malate to oxaloacetate coupled with the reduction of NAD^+ to NADH in the cytoplasm[29,31,40] (**Figure 1**).

Table 1 | Comparison of pathways for exporting oxaloacetate from mitochondrion to cytoplasm during hepatic gluconeogenesis

Pathways	Contribution to fasting stages		Substrate dependency	NADH provision		Energy provision
	Early stage	Late stage		Mitochondrial	Cytoplasmic	
Malate	High	Low	None	TCA cycle	MDH1	Independent
Direct	Low	High	Lactate	Independent	LDH	SCS (GTP)
Aspartate	Low	Low	Lactate	Independent	LDH	Independent
Fumarate	Low	Low	Glutamine/AA	GDH/TCA cycle	MDH1	Independent

AA: Amino acid; GDH: glutamate dehydrogenase; GTP: guanosine triphosphate; LDH: lactate dehydrogenase; MDH1: cytoplasmic malate dehydrogenase; NADH: nicotinamide adenine dinucleotide (reduced form); SCS: succinyl coenzyme-A synthetase; TCA: tricarboxylic acid.

The fumarate pathway is the main pathway when glutamine and other amino acids are used as substrates for gluconeogenesis.[31] This pathway emphasizes the interaction of the urea cycle with gluconeogenesis and the dual role of glutamine, serving as a gluconeogenesis precursor and an NH_4^+ carrier.[31,45] Glutamate accepts free NH_4^+ derived from protein catabolism in different organs. Then, it is converted to glutamine, which the liver utilizes to convert the toxic NH_4^+ into urea through the urea cycle.[45] In the liver, other amino acids taken by hepatocytes are degraded to the TCA cycle intermediates, losing their amine group as NH_4^+. Their carbonic skeleton flows through the TCA cycle to attain oxaloacetate.[29] Toxic accumulation of NH_4^+ recruits the fumarate pathway, which forces aspartate to enter the urea cycle, converting NH_4^+ to urea through the urea cycle.[31] Also, glutamate dehydrogenase supplies NADH in this pathway.[30] Therefore, the substrate-dependency of the fumarate pathway is related to NH_4^+ accumulation and not cytoplasmic NADH-producing enzymes.[31] Supplementation of rat liver homogenates with di and TCA precursors in the presence of aspartate as the only source of amino acid showed the following results. The activity of fumarase is almost one-eighteenth of MDH1 in the fed state (198 *vs*. 10.9 µmol/min/g liver for MDH1 and fumarase, respectively) and one-eighth in fasting states (166 *vs*. 20 µmol/min/g liver for MDH1 and AST, respectively).[9] These findings indicate that except for when amino acids are the primary substrate for gluconeogenesis, the contribution of the fumarate pathway to gluconeogenesis is low (**Table 1**).

Except for the direct pathway, in which PEP is directly transported to the cytoplasm, within other pathways, the cytoplasmic oxaloacetate is converted to PEP by PEPCK1.[30] Cytoplasmic PEP is converted to 2-phosphoglycerate by enolase; 2-phosphoglycerate is further converted to 3-phosphoglycerate by phosphoglycerate mutase.[28] Phosphoglycerate kinase converts 3-phosphoglycerate into 1,3-bisphosphoglycerate, coupled with the conversion of ATP to adenosine diphosphate.[28] The NADH produced in the cytoplasm by MDH1 and LDH is used by GAPDH to transform 1,3-bisphosphoglycerate into glyceraldehyde 3-phosphate (GADP).[28] The reversible conversion of GADP into dihydroxyacetone phosphate (DHAP) is catalyzed by triose phosphate isomerase 1[28] (**Figure 1**).

Glycerol, produced from adipose tissue lipolysis, is transported into liver cells through aquaporin-9. Then, it is converted to glycerol 3-phosphate via glycerol kinase[46] and becomes DHAP by fluxing through the α-glycerophosphate shuttle. Depending on the mitochondrial redox state, the conversion of glycerol 3-phosphate to DHAP is catalyzed either by mitochondrial glycerol 3-phosphate dehydrogenase (GPDH2) or GPDH1.[30] GPDH2 is located in the inner mitochondrial membrane and pairs DHAP formation with the conversion of FAD to $FADH_2$ within the mitochondria. GPDH1 couples DHAP formation and the reduction of NAD^+ to NADH in cytoplasm.[30,47] Aldolase combines DHAP and GADP into F1,6BP, which becomes F6P by fructose 1,6-bisphosphatase (F1,6BPase) and is subsequently transformed into G6P by phosphoglucose isomerase. Finally, within the endoplasmic reticulum, G6P becomes glucose via G6Pase[28] and exits from hepatocytes by GLUT2[48] (**Figure 1**).

Nitric Oxide Synthesis in Hepatocytes

In the human body, NO is produced by (1) NO synthase (NOS)-dependent *L*-arginine pathway[49] and (2) nitrate-nitrite-NO pathway.[50,51] Almost 90% of the body's NO synthesis is related to the *L*-arginine-NO pathway, with the nitrate-nitrite-NO pathway responsible for the other 10%.[52] NO is produced from *L*-arginine by three isoforms of the enzyme NOS.[53] Two of them, i.e., endothelial NOS (eNOS) and neural NOS (nNOS), are constitutively expressed and are further activated following intracellular calcium rise.[53] The third isoform, inducible NOS (iNOS), is expressed following transcriptional activation by cytokines, such as interferon-gamma and lipopolysaccharide (LPS), interleukin-1, interleukin-2, and tumor necrosis factor-alpha.[54,55]

The *L*-arginine–NOS pathway of NO production is oxygen-dependent, whereas the nitrate-nitrite-NO pathway is further activated during hypoxia and acidosis.[56] Within the nitrate-nitrite-NO pathway, nitrate reduction by bacterial nitrate reductases in the oral cavity and gastrointestinal tract produces nitrite.[56] In addition, studies on germ-free mice displayed the presence of a mammalian nitrate reductase in different organs,[57] registering the colon as the site of the highest activity, followed by the stomach, kidney, small intestine, liver, heart, and lung.[57] NO oxidation through auto-oxidation in tissues or via ceruloplasmin in plasma also produces nitrite.[56] Nitrite is reduced to NO in blood and tissues by deoxyhemoglobin/myoglobin, cytoglobin, xanthine oxidoreductase, aldehyde oxidase, polyphenols, ascorbate, and protons.[56,58,59]

A study on the human liver using the citrulline assay, Western immunoblots, immunohistochemistry, and in situ hybridization shows the expression of eNOS and iNOS but not nNOS in hepatocytes.[60] eNOS is also expressed in the endothelium of hepatic arteries, terminal hepatic venules, sinusoids, and the biliary epithelium of the human liver.[60] iNOS is expressed in Kupffer cells of normal human liver tissue from patients undergoing partial hepatectomy for liver cancer.[61] In addition, studies on the livers of *iNOS* knockout ($iNOS^{-/-}$) mice indicated the *iNOS* expression in the liver Kupffer cells.[62-64] Hepatocyte iNOS is induced in response to Kupffer cell activation, reactive oxygen species generation, endotoxin, hepatic ischemia/reperfusion, and interleukin-1β.[65-67] iNOS-containing hepatocytes are mainly localized in the periportal zone of the liver acinus in humans.[60]

The expression of nNOS in the liver is controversial in animal studies; some studies have reported the expression and activation of nNOS in mice's primary isolated hepatocytes,[68,69] and others have shown no expression of nNOS in rat hepatocytes.[70] Immunohistochemistry of nNOS in rat liver revealed the presence of nNOS only in the liver endothelium of normal rats. In contrast, in type 1 diabetic and LPS-treated rats, nNOS presence was detected in hepatocytes, endothelium, and Kupffer cells.[71]

Given the inducible nature of iNOS and the lack of conclusive studies on the presence of nNOS in the liver, it appears that eNOS is the predominant NOS isoform in the liver and the main isoform contributing to glucose metabolism in the normal state.[60] In support of this notion, hyperinsulinemic-euglycemic clamp studies after a 24-hour fast during an insulin infusion in *eNOS* knockout (KO) and *nNOS* knockout mice demonstrated hepatic insulin resistance only in the *eNOS* knockout group.[72]

Effects of Metformin on Gluconeogenesis in Hepatocytes

Enterocytes uptake metformin through the apical membrane via plasma membrane monoamine transporter and organic cation transporter 3 (OCT3), then transport it through the basolateral membrane to the portal vein by OCT1.[73] The liver is the primary site of action for metformin,[7] and metformin uptake in hepatocytes is mainly through OCT1.[74] OCT3 and multidrug and toxin extrusion protein 1 transporter also contribute to hepatocyte metformin uptake.[47] Evaluation of metformin effects on patients with T2DM by a combination of isotope dilution, indirect calorimetry, bioimpedance, and tissue-balance techniques indicates that metformin decreases hepatic glucose production mainly through inhibition of gluconeogenesis.[8] The inhibitory effect of metformin on gluconeogenesis in type 2 diabetic patients has also been reported in a clinical trial using stable isotope tracers.[75]

As shown in **Figure 2**, the main mechanisms underlying the inhibitory effects of metformin on gluconeogenesis include (1) increasing the ratio of glutathione reductase (GR)-to-glutathione peroxidase (GPx) activities,[76] (2) inhibition of mitochondrial oxidative phosphorylation by (a) inhibition of complex I and complex IV of the ETC[76] and (b) inhibition of mitochondrial GPDH2.[76] These effects increase NADH/NAD$^+$ and AMP/ATP ratios; (3) induction of sirtuin silent information regulator 1 (SIRT1) and general control non-depressible 5 (GCN5) transcriptional regulators.[77] These mechanisms eventually inhibit liver gluconeogenesis by transcriptional, allosteric, substrate-specific, and redox-dependent mechanisms.[47]

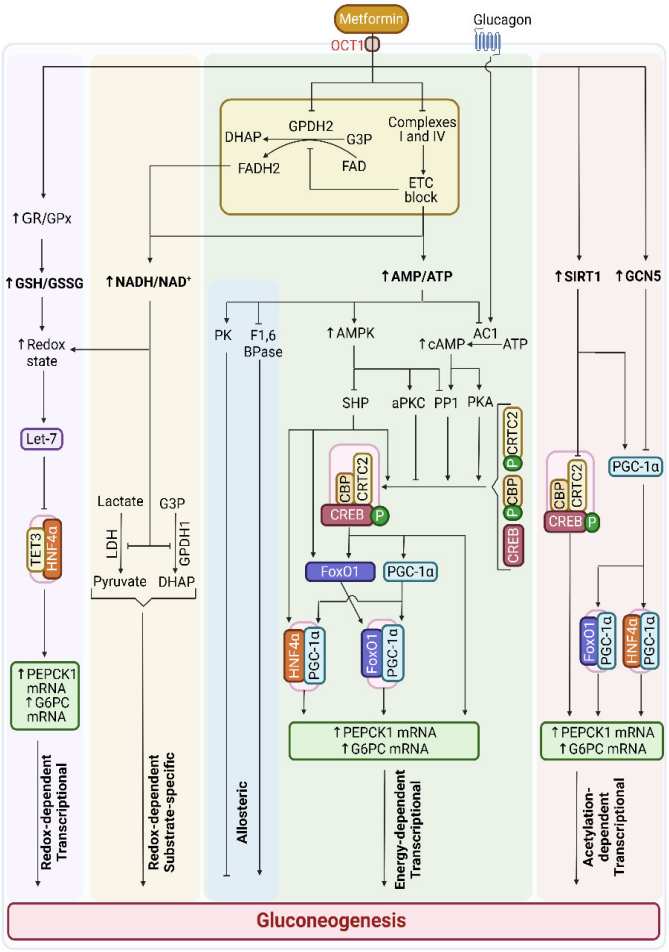

Figure 2 | Mechanisms underlying the inhibitory effect of metformin on hepatic gluconeogenesis.
Metformin inhibits hepatic gluconeogenesis by transcriptional, allosteric, substrate-specific, and redox-dependent mechanisms. Created with BioRender.com. AC1: Adenylyl cyclase 1; AMP: adenosine monophosphate; AMPK: AMP-activated protein kinase; aPKC: atypical protein kinase C; ATP: adenosine triphosphate; CBP: CREB binding protein; CREB: cAMP-response element binding protein; CRTC2: CREB-regulated transcription coactivator 2; DHAP: dihydroxyacetone phosphate; ETC: electron transport chain; F1,6BPase: fructose 1,6-bisphosphatase; FAD: flavin adenine dinucleotide; FADH: flavin adenine dinucleotide (reduced form); FoxO1: forkhead box protein O1; G3P: glycerol 3-phosphate; G6PC: glucose-6-phosphatase catalytic subunit; GCN5: general control non-depressible 5; GPDH: glycerol-3-phosphate dehydrogenase; GPx: glutathione peroxidase; GR: glutathione reductase; GSH: glutathione; GSSG: glutathione disulfide; HNF4α: hepatocyte nuclear factor 4 alpha; Let-7: lethal microRNA; LDH: lactate dehydrogenase; NAD$^+$: nicotinamide adenine dinucleotide; NADH: nicotinamide adenine dinucleotide hydrogen; OCT1: organic cation transporter; PEPCK1: cytoplasmic phosphoenolpyruvate carboxykinase; PGC-1α: peroxisome proliferator-activated receptor gamma (PPAR-γ) coactivator-1 alpha; PK: pyruvate kinase; PKA: protein kinase A; PP1: protein phosphatase 1; SHP: small heterodimer partner; SIRT1: sirtuin silent information regulator 1; TET3: tet methylcytosine dioxygenase 3.

Increasing the GR-to-GPx activity ratio

Metformin increases the GR/GPx activity ratio in HepG2 cells[78] and thus increases the ratio of reduced (GSH) to oxidized (GSSG) glutathione (GSH/GSSG).[79] Elevated GSH/GSSG ratio changes the cytoplasmic redox state. This increases the expression of lethal microRNA (*let-7*),[80] which binds tet methylcytosine dioxygenase 3 (TET3) mRNA and causes posttranscriptional inhibition of TET3 mRNA expression.[80] TET3 is a DNA demethylase[81] that binds to the P2 promoter of hepatocyte nuclear factor 4 alpha (*HNF4α*) and promotes its transcription.[82] HNF4α is a transcription factor that increases the mRNA expression of gluconeogenic enzymes PEPCK1 and G6Pase catalytic subunit (G6PC).[80] In addition to HNF4α, other transcription factors also regulate the expression of gluconeogenic enzymes, of which cyclic AMP (cAMP)-response element binding protein (CREB) and Forkhead box protein O1 (FoxO1) are affected by metformin.[83] Therefore, through a redox-dependent increase in let-7 microRNA, metformin decreases the expression of PEPCK1 and G6PC and inhibits hepatic gluconeogenesis by a redox-dependent transcriptional mechanism[84] (**Figure 2**).

Inhibition of mitochondrial oxidative phosphorylation

Metformin inhibits complex I of the mitochondrial respiratory chain, thereby blocking ETC.[85] Complex I accepts electrons from NADH and helps to create a proton gradient that serves as the driving force for ATP synthesis.[47,86] Metformin also inhibits complex IV, which catalyzes the final step of electron transfer, and fortifies ETC blockage.[76] Metformin-induced ETC block inhibits GPDH2 through decreasing electrons accepted by ubiquinone.[87] Metformin also directly inhibits GPDH2 noncompetitively in primary hepatocytes isolated from rats[84] (**Figure 2**). GPDH2 is an enzyme in the α-glycerophosphate shuttle[47] (**Figure 1**). Thus, metformin-induced inhibition of ETC[76] and GPDH2[84] increases NADH/NAD$^+$[76,84] and AMP/ATP[76] ratios.

In addition to providing redox-dependent inhibition of gluconeogenesis, increased NADH/NAD$^+$ ratio suppresses glucose formation from those substrates that their contribution to gluconeogenesis is paired with the reduction of NAD$^+$ to NADH.[76] Such substrates include lactate and glycerol, which are catalyzed by LDH and GPDH1.[76] Thus, metformin inhibits hepatic gluconeogenesis by a redox-dependent substrate-specific mechanism.[47] Metformin-induced increased NADH/NAD$^+$ ratio reverses the conversion of lactate to pyruvate catalyzed by LDH.[88] Lactate accumulation in hepatocytes results in the suppression of hepatocyte lactate uptake.[89] Increased lactate production and impaired hepatic lactate removal lead to lactic acidosis.[88] The absence of lactic acidosis in *OCT1*$^{-/-}$ mice treated with metformin supports the critical role of hepatocytes in metformin-associated lactic acidosis (MALA).[90]

Increased AMP/ATP ratio inhibits hepatic gluconeogenesis by three mechanisms: (1) allosteric activation of pyruvate kinase (PK)[91] and inhibition of F1,6BPase[92]; (2) inhibition of adenylyl cyclase 1 (AC1)[93]; and (3) stimulation of AMPK.[76] Metformin-induced elevated AMP levels allosterically inhibit F1,6BPase in cultured hepatocytes in mice.[94] In addition, the hypoglycemic effect of metformin is abolished in an AMP-independent *F1,6BPase* knockin mouse model.[92] The metformin-induced decrease in ATP concentration stimulates PK in the isolated rat hepatocytes perifused with dihydroxyacetone as the gluconeogenic substrate and thus inhibits hepatic gluconeogenesis.[91] Inhibition of F1,6BPase[47] and stimulation of PK[91] by metformin-induced elevated AMP/ATP ratio is known as allosteric inhibition of gluconeogenesis[47] (**Figure 2**).

Metformin counters glucagon-induced hepatic gluconeogenesis by inhibiting AC1[93] and activating AMPK.[95] Glucagon binds to its receptor, a G_S-protein-coupled receptor, in the hepatocyte membrane and activates AC1, which stimulates the conversion of

ATP to cAMP.[93] Elevated cAMP activates protein kinase A (PKA).[96] PKA mediates the phosphorylation of transcription factor, CREB as well as dephosphorylation of CREB-regulated transcription coactivator 2 (CRTC2) and CREB binding protein (CBP) to form the CREB-CBP-CRTC2 complex, which is a transcriptional and a coactivator complex.[97] Phosphorylated CREB of the CREB-CBP-CRTC2 complex binds the promoter of *PEPCK1* and *G6PC* genes and increases their expressions.[95] Phosphorylated CREB of the CREB-CBP-CRTC2 complex also binds the promoter of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (*PGC-1α*) and *FoxO1* genes and induces their expressions.[96] PGC-1α is a transcriptional coactivator,[98] which forms complexes with transcription factors FoxO1[96] and HNF4α[99] to bind the promoter of *PEPCK1* and *G6PC* genes and increase their expression.[97] Glucagon-induced increased cAMP also increases protein phosphatase 1 regulatory subunit 3C expression and enhances protein phosphatase 1 activity, further promoting gluconeogenesis[100] (**Figure 2**).

AMP-dependent AC1 inhibition in mouse hepatocytes and decreased cAMP levels block glucagon-induced expression of G6PC and PEPCK1,[93] an energy-dependent transcriptional inhibition of gluconeogenesis.[47] However, a study on AMPK-deficient mice showed no impairment in hepatocyte cAMP production by metformin.[101] In addition, in prediabetic individuals with hyperglucagonemia, the decremental effect of metformin on gluconeogenesis is decreased, debating the inhibitory effect of metformin on glucagon-stimulated gluconeogenesis in humans.[102]

AMPK activation induced by an increased ratio of AMP/ATP decreases protein phosphatase 1 regulatory subunit 3C expression in mouse hepatocytes,[100] activates atypical protein kinase C,[95] and inhibits small heterodimer partner (*SHP*) gene expression.[103] Inhibition of protein phosphatase 1, a regulator of hepatic gluconeogenesis,[104] decreases CRTC2 dephosphorylation,[100] while atypical protein kinase C phosphorylates CBP.[95] Downregulation of SHP, an orphan nuclear receptor, disrupts the CREB-CBP-CRTC2 complex[105] and suppresses FoxO1, HNF4α, and FoxA2 transcriptional factors; all AMPK-dependent mechanisms inhibit *PEPCK1* and *G6PC* gene promoters by an energy-dependent transcriptional mechanism[103] (**Figure 2**).

Induction of SIRT1 and GCN5 transcriptional regulators

Metformin treatment of T2DM mice stimulates SIRT1,[77] an NAD^+-dependent cellular energy sensor that deacetylates several substrates.[106] SIRT1 activation deacetylates CRTC2 and breaks the CREB-CBP-CRTC2 complex,[77] inhibiting gluconeogenesis. SIRT1 also deacetylates PGC-1α and induces the PGC-1α-mediated gluconeogenic genes transcription; however, this effect is inhibited by metformin-induced activation of GCN5, which acetylates and inhibits PGC-1α.[77] GCN5 (also known as lysine acetyltransferase 2A) is a histone acetyltransferase and lysine acetyltransferase.[107] Stimulation of SIRT1 and GCN5 counter the glucagon-induced cAMP-dependent CRTC2-mediated *PEPCK1* and *G6PC* gene expression[77] through an acetylation-dependent transcriptional inhibition of gluconeogenesis (**Figure 2**).

Effect of Nitric Oxide on Gluconeogenesis in Hepatocytes

NO inhibits hepatic gluconeogenesis. eNOS-derived NO,[22,108] iNOS-derived NO,[20,21] and NO donors, including *S*-nitroso-*N*-acetylpenicillamine,[19] 3-morpholinosydnonimine,[19] *S*-nitroso-glutathione,[19] sodium nitroprusside,[22] nitrate,[109] and nitrite[23] inhibit hepatic gluconeogenesis as demonstrated in type 2 diabetic rats,[23,109] type 2 diabetic mice,[108] and type 1 diabetic mice,[22] streptozotocin-induced diabetic mice,[22] isolated mice hepatocytes,[22,108] isolated rat hepatocytes,[19,21] and cultured rat hepatocytes.[20] NOS inhibitors, including N^{ω}-nitro-*L*-arginine methyl ester[108] and N^{G}-monomethyl-*L*-arginine,[110] restored the NO-induced inhibited gluconeogenesis in isolated mouse[108] and rat[110] hepatocytes. Considering the dominance of eNOS in hepatocytes[60,72] and the similar recovery of gluconeogenesis in the presence or absence of iNOS stimulators in isolated rat hepatocytes incubated with N^{G}-monomethyl-*L*-arginine,[110] it can be speculated that eNOS-derived NO is the main NOS-derived NO contributing to gluconeogenesis inhibition.[22,108]

Mechanisms underlying inhibitory effects of nitric oxide on hepatic gluconeogenesis

As shown in **Figure 3**, NO inhibits gluconeogenesis mainly through (1) cyclic guanosine monophosphate (cGMP)-dependent mechanisms[111-113] and (2) enzyme *S*-nitrosylation.[114] cGMP-dependent mechanisms include (a) inhibition of CREB phosphorylation,[111] (b) activation of AMPK,[112] and (c) activating glucokinase (GCK).[115,116] *S*-nitrosylation mechanisms include (a) GAPDH inhibition,[110,117] (b) TCA cycle inhibition,[114,118,119] and (c) ETC inhibition.[120-122]

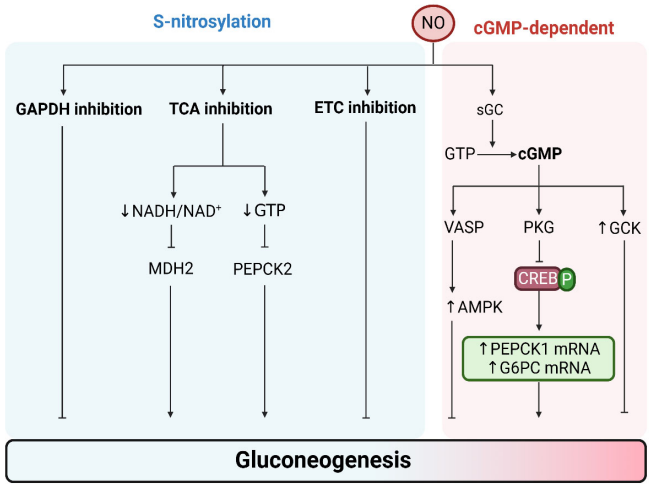

Figure 3 | Mechanisms underlying inhibitory effect of NO on hepatic gluconeogenesis.
NO inhibits hepatic gluconeogenesis via cGMP-dependent pathways and *S*-nitrosylation. Created with BioRender.com. AMPK: AMP-activated protein kinase; cGMP: cyclic guanosine monophosphate; CREB: cAMP-response element binding protein; ETC: electron transport chain; G6PC: glucose-6-phosphatase catalytic subunit; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; GCK: glucokinase; GTP: guanosine triphosphate; MDH2: mitochondrial malate dehydrogenase; NO: nitric oxide; PEPCK: phosphoenolpyruvate carboxykinase; PKG: cGMP-dependent protein kinase; sGC: soluble guanylate cyclase; TCA: tricarboxylic acid; VASP: vasodilator-stimulated phosphoprotein.

cGMP-dependent mechanisms

NO stimulates soluble guanylate cyclase, a heterodimer composed of two subunits, each containing catalytic and heme-binding domains. Both subunits are essential for enzyme activity. NO activates soluble guanylate cyclase through interaction with its heme component, leading to the conversion of GTP into cGMP in hepatocytes.[123,124] Subsequently, cGMP binds to allosteric sites in the regulatory domain of cGMP-dependent protein kinase (PKG),[125] enhancing its activity up to 10-fold.[126] The NO/cGMP/PKG signaling pathway initiates a cascade of reactions,[126] including the inhibition of phosphorylated CREB protein expression in hepatocytes[111] (**Figure 3**). As discussed earlier, phosphorylated CREB is a transcriptional factor and coactivator involved in glucagon-induced expression of gluconeogenic enzymes G6Pase and PEPCK1[97] (**Figure 2**). It has been shown that exogenous cGMP can inhibit basal, glucagon-stimulated[127] and epinephrine-stimulated[128] gluconeogenesis in rat liver.

cGMP also activates AMPK as indicated in nitrite-treated human liver cell line HepG2 cells and causes AMPK-dependent inhibition of gluconeogenic enzyme expressions.[112] cGMP-induced AMPK activation in hepatocytes is mediated by vasodilator-stimulated phosphoprotein (VASP), a protein associated with filamentous actin formation and plays a role in cell adhesion and motility.[129] In support, in hepatocytes of *VASP*$^{-/-}$ mice, AMPK phosphorylation was lower compared to wild-type mice.[129] AMPK mainly inhibits glucagon-induced gluconeogenesis.[130] According to experiments conducted in *eNOS* knockout mice,[22,108] it has been shown that eNOS-derived NO plays a critical role in AMPK-dependent inhibition of hepatic gluconeogenesis. cGMP also increases GCK activity, which converts glucose to G6P in the glycolysis pathway in isolated rat hepatocytes, thereby inhibiting gluconeogenesis[115,116] (**Figure 3**). Adding cGMP to the culture medium of rat hepatocytes increased GCK activity and *GCK* mRNA.[115] In rat hepatocytes, N$^{\omega}$-nitro-*L*-arginine methyl ester decreases cGMP levels, GCK activity, and G6P concentrations but increases glucose levels in rat hepatocytes; however, *L*-arginine, the precursor of NO, has opposite effects on these parameters.[113]

Enzyme S-nitrosylation

S-nitrosylation, a reversible post-translational regulation of enzymatic activity,[131] results from NO binding to a transition metal[132] or cysteine thiols of proteins.[114] *S*-nitrosylation mediates the post-translational regulation of nearly 1000 proteins.[133] In hepatocytes, eNOS-derived NO, which is the primary source of hepatic NO, stimulates protein *S*-nitrosylation[134] and protects against apoptosis[135] and ischemic complications,[136] and mediates the regulation of glucose metabolism.[119] Decreased *eNOS* expression due to a high-fat diet decreases protein *S*-nitrosylation in mice hepatocytes.[134] NO *S*-nitrosylates and inhibits GAPDH in isolated hepatocytes from rats injected with *Cryptosporidium parvum* to induce iNOS, thereby decreasing gluconeogenesis.[24] Exogenously delivered NO via *S*-nitroso-*N*-acetylpenicillamine and sodium nitroprusside inhibits GAPDH in isolated rat hepatocytes, as does endogenously synthesized NO through induction of NOS by a combination of cytokines and LPS.[110]

eNOS-derived NO also *S*-nitrosylates other enzymes involved in gluconeogenesis in mice liver,[114] including triose phosphate isomerase 1, aldolase, phosphoglycerate mutase, Phosphoglycerate kinase, and pyruvate carboxylase, as well as TCA enzymes (i.e., aconitase, isocitrate dehydrogenase, succinate dehydrogenase, and MDH2)[114] (**Figure 1**). Limited data on the regulatory consequence of *S*-nitrosylation of these enzymes are available,[114] and the overall effect of their *S*-nitrosylation on gluconeogenesis is controversial.[137] It is only assumed that NO inhibits rat liver mitochondrial isocitrate dehydrogenase via *S*-nitrosylation[118] and results in TCA cycle suppression.[137] However, the inhibitory effect of *S*-nitrosylation on other TCA cycle enzymes is speculated to be related to the redox-sensitive cysteine thiol groups of these proteins at their catalytic or regulatory sites.[138] NO inhibits mitochondrial aconitase in isolated rat hepatocytes exposed to NO gas or treated with a combination of cytokines and LPS to induce endogenous NO synthesis.[120] Although it has not been shown that NO *S*-nitrosylates aconitase in hepatocytes, the inhibitory effect of NO on pig heart aconitase via *S*-nitrosylation has been reported.[139] In addition, it has been shown that *S*-nitrosylated ketoglutarate dehydrogenase and SCS have lower activity in human pluripotent stem cell-derived neurons, leading to suppression of the TCA cycle.[140] Mitochondrial ketoglutarate dehydrogenase, isocitrate dehydrogenase, and SCS inhibition and the overall suppression of the TCA cycle decrease mitochondrial NADH[119] and GTP synthesis,[44] resulting in the inhibition of MDH2[119] and PEPCK2 within gluconeogenesis flux[44] (**Figure 3**).

NO inhibits mitochondrial NADH-ubiquinone oxidoreductase and succinate-ubiquinone oxidoreductase (complexes I and II of the mitochondrial ETC) in isolated rat hepatocytes exposed to NO gas and or treated with a combination of cytokines and LPS to induce endogenous NO synthesis.[120] Among different mechanisms suggested for the inhibition of complex I by NO, *S*-nitrosylation of critical thiols in the enzyme complex[141] is the most supported.[142] *S*-nitrosylation of bovine heart mitochondrial membrane proteins by *S*-nitroso-*N*-acetylpenicillamine[121] and MitoSNO1 (mitochondria-targeted NO donor and *S*-nitrosating agent)[143] showed lowered activities of complex I, and, to a lesser degree, complex II of the ETC.

NO derived from hepatic stellate cells of cytoglobin-deficient mice, lacking NO dioxygenase activity, which converts NO to nitrate, reversibly inhibits hepatocyte cytochrome c oxidase (complex IV of the ETC),[122] an effect that was restored by NO synthase inhibitor (*N*$^{\omega}$-nitro-*L*-arginine methyl ester) or NO scavenger (oxyhemoglobin), which displaces the binding of NO to cytochrome c oxidase.[122] Although NO-induced inhibition of complex IV is only described as direct binding of NO to cytochrome c oxidase in hepatocytes,[122] it is associated with *S*-nitrosylation of cysteine residues located on subunit II of the complex IV in porcine pulmonary artery endothelial cells.[144]

NO-induced inhibition of complex I, II,[120] and IV[122] suppresses the ETC, decreasing mitochondrial ATP.[119] Increased AMP/ATP ratio inhibits gluconeogenesis by countering glucagon-induced gluconeogenesis via AMPK activation[145] and AC1 inhibition[93] and by allosteric stimulation of PK [91] and inhibition of F1,6BPase[92] (**Figure 3**).

Comparison Between the Effects of Metformin and Nitric Oxide on Hepatic Gluconeogenesis

As mentioned above, mitochondria cannot transport oxaloacetate into the cytoplasm,[28] and this necessary step of gluconeogenesis is done through malate, direct, aspartate, and fumarate pathways.[31] Malate and direct pathways contribute the most to normal gluconeogenesis during the early and late stages of fasting, respectively.[39] Metformin increases the mitochondrial NADH/NAD$^+$ ratio in later stages of fasting (in favor of converting oxaloacetate to malate), in which the mitochondrial NADH/NAD$^+$ ratio is decreased.[39,76] In addition, metformin blocks the α-glycerophosphate shuttle (GPDH1 and GPDH2) and LDH, remaining MAS as the only means for providing cytoplasmic NADH, which is required for converting 1,3-bisphosphoglycerate to GADP by GAPDH during gluconeogenesis[42,76] (**Figure 1**). Both of these effects attenuate metformin's inhibitory effect on gluconeogenesis via the malate pathway. In support of this notion, metformin increases hepatic malate in type 2 diabetic mice and circulating malate in patients with T2DM.[146]

NO decreases the NADH/NAD$^+$ ratio, probably due to TCA cycle inhibition,[119] inhibits MDH2,[119] and forces the MAS so that malate is converted to oxaloacetate in the mitochondrion.[42] Collectively, these effects strongly inhibit gluconeogenesis via the malate pathway. Regarding the direct pathway, metformin inhibits the direct pathway of gluconeogenesis by increasing the mitochondrial NADH/NAD$^+$ ratio,[76] changing the activity of MDH2 in favor of converting oxaloacetate to malate and thereby decreasing the availability of oxaloacetate to the direct pathway of gluconeogenesis.[39] Furthermore, NO inhibits the TCA cycle and consequently decreases GTP production by SCS, which is required for the activity of PEPCK2[44] (**Figure 1**). The increase of the mitochondrial NADH/NAD$^+$ ratio by metformin can inhibit the fumarate and aspartate pathways.[47,119] Metformin increases the NADH/NAD$^+$ ratio and forces the direction of MAS to enter aspartate into the mitochondrion, thereby causing a stronger inhibitory effect on gluconeogenesis via fumarate and aspartate pathways than NO[28,29,31,42,76] (**Table 2**).

Table 2 | Comparison between metformin and NO in gluconeogenesis

	Metformin	NO
Oxaloacetate export		
Malate pathway	↑*	↓
Direct pathway	**	↓
Aspartate pathway	↓	↔
Fumarate pathway	↓	↔
Substrate-related gluconeogenesis		
Lactate	↓	↔
Alanine	↔	↓
Glutamine	↓	↓
Glycerol	↓	↔
Gluconeogenesis-related enzymes		
PEPCK1	↓	↓
GAPDH	↔	↓
G6Pase	↓	↓
F1,6BPase	↓	↓
PK	↑	↑
GCK	↔	↑

*Metformin increases the conversion of mitochondrial oxaloacetate to malate and thus oxaloacetate export; however, its overall effect on hepatic gluconeogenesis through the malate pathway is inhibitory. ** Preventing the compensatory increase. ↑: Increase; ↓: decrease; ↔: no change. F1,6BPase: Fructose 1,6-bisphosphatase; G6Pase: glucose 6-phosphatase; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; GCK: glucokinase; NO: nitric oxide; PEPCK1: cytoplasmic phosphoenolpyruvate carboxykinase; PK: pyruvate kinase.

Regarding gluconeogenic substrates, metformin increases the NADH/NAD^+ ratio, which affects LDH and GPDH activities and prevents lactate and glycerol utilization for gluconeogenesis.[47,76] In addition, glutamine contribution to gluconeogenesis can be suppressed by the metformin-induced increase of mitochondrial NADH/NAD^+ ratio.[28,31,76] Furthermore, NO decreases the NADH/NAD^+ ratio, which affects MDH2 activity[119] and inhibits alanine-induced gluconeogenesis.[28] The inhibition of TCA cycle key enzymes by NO can inhibit the glutamine contribution to gluconeogenesis[119] (**Figure 1**). To sum up, substrate-related effects of metformin and NO on inhibition of gluconeogenesis seem complementary, as metformin mainly inhibits lactate, glycerol, and glutamine-induced gluconeogenesis, whereas NO mainly inhibits alanine-induced gluconeogenesis.

Comparing the effects of metformin and NO on gluconeogenesis-related enzymes, both inhibit PEPCK1, G6Pase, and F1,6BPase and stimulate PK.[47,111,120] Unlike metformin, NO inhibits GAPDH[110,117] and activates GCK[115,116] (**Table 2**).

These findings suggest that the combination of metformin and NO could provide a more potent inhibition of gluconeogenesis compared to metformin alone. This enhanced effect is likely due to their complementary actions, including preventing oxaloacetate export, suppressing gluconeogenic enzymes, and inhibiting the utilization of gluconeogenic substrates (**Table 2**). However, this hypothesis requires empirical validation. Two studies have investigated the combined effects of metformin and NO donors on metabolic regulation. Cordero-Herrera et al.[25] reported that combination therapy with metformin (150 mg/kg/d) and nitrate (85 mg/kg/d) for 7 weeks in high-fat diet-fed mice reduced glycated hemoglobin by approximately 33%. This reduction was greater than that observed with the metformin effect alone (15%) or nitrate effect alone (12%), suggesting a synergic effect, as the combined effect exceeded the sum of the individual effects. Furthermore, the study demonstrated a synergic increase in AMPK phosphorylation and activation, with 140% increase in mice treated with both metformin and nitrate, compared to 66% and 60% increases with metformin and nitrate monotherapy, respectively. Similarly, Adeoye et al.[26] recently reported that combined treatment with metformin and *L*-arginine (100 mg/kg) for 6 weeks reduced serum C-reactive protein levels by approximately 75%. This effect was greater than the reductions achieved with metformin alone (~50%) or *L*-arginine alone (~25%), indicating an additive effect.

Clinical Applications of Nitric Oxide-Based Interventions

NO-based therapies have a long history in medicine, with glyceryl trinitrate (nitroglycerin) as a well-known example, used to manage angina pectoris (oral and intravenous forms),[147] anal fissure (ointment),[148] and pulmonary hypertension (nebulized).[149,150] Other notable NO-based therapies include isosorbide mononitrate and isosorbide dinitrate for chronic angina and heart failure,[147] nitroprusside for hypertensive crises,[147] sildenafil (Viagra), which inhibits phosphodiesterase V and amplifies NO signaling, for erectile dysfunction,[151] and inhaled NO for persistent pulmonary hypertension in infants.[152]

In line with our suggestion for the development of a metformin-NO hybrid drug, several efforts have been made to develop NO-donating and NO hybrid drugs for better managing of angina pectoris (e.g., nicorandil[147,153]), inflammation (NO-aspirin,[154] NO-naproxen,[155] NO-pravastatin,[156] NO-diclofenac,[157] and NO-paracetamol[158]), and diabetic neuropathy (NO-pregabalin[159]). In addition, in 2017, the Food and Drug Administration approved Latanoprostene Bunod, a NO-donating prostaglandin analog, for lowering intraocular pressure in patients with open-angle glaucoma or ocular hypertension.[160] In addition to NO, hybrid drugs incorporating other gasotransmitters, such as hydrogen sulfide, are being developed. A notable example is hydrogen sulfide-NO hybrid compounds (NOSH), such as NOSH-aspirin, which has shown promise in cancer therapy,[161] with reported anti-cancer effects in colon,[162] pancreas,[163] and breast cancers.[164] Moreover, NOSH-sulindac,[165] NOSH-naproxen,[166] and NOSH-aspirin[167,168] have been evaluated for managing inflammatory pain. Other areas of research for NO-based treatments include applications in cardiovascular diseases,[17,169,170] wound healing[171] and metabolic disorders.[16,18,170]

Developing a metformin-NO hybrid drug offers potential advantages for managing patients with T2DM. First, interactions between the effects of metformin and NO on hepatocytes may enhance their combined ability to inhibit gluconeogenesis. For instance, eNOS-derived NO contributes to AMPK-dependent inhibition of hepatic gluconeogenesis by metformin.[22,108] Additionally, metformin enhances the activation of AMPK by nitrate-derived NO in mouse hepatocytes.[25] Second, metformin is associated with lactic acidosis in approximately 10 cases per 100,000 patient-years, with mortality rates of 30–50%.[88] The therapeutic circulating concentration of metformin ranges from 0.5 to 3 mg/L,[172] but levels exceeding 4 mg/L increase the risk of MALA.[173] MALA is characterized by elevated plasma metformin (> 5 mg/L), lactate levels (> 5 mM), and blood pH < 7.35.[174] NO deficiency also plays a role in lactic acidosis.[175] Thus, a metformin-NO hybrid may mitigate MALA by reducing the required metformin dose and preventing NO deficiency-associated lactic acidosis. Notably, metformin is contraindicated in patients with severe renal dysfunction due to the risk of lactic acidosis.[7] The potential effect of NO in preventing MALA[175] could broaden the applicability of a metformin-NO hybrid in T2DM management, warranting further investigation in animal and human studies. Finally, while NO may exert oxidative and nitrosative stress,[176] metformin can elevate GSH,[76] potentially counteracting these effects. Elevated GSH also enhances NO-induced *S*-nitrosylation,[177] amplifying its inhibitory effect on gluconeogenesis.

Despite having potential advantages, several challenges must be addressed in developing a metformin-NO hybrid drug. First, NO is a short-lived free-radical gas, making compound stability and bioavailability critical considerations.[178] Second, combining the pharmacokinetic properties of metformin and NO requires precise formulation to avoid potential toxicity and ensure efficacy. Third, the effects of NO are dose-dependent. Physiological NO concentrations range from 100 pM to 5 nM,[179] with varying biological effects. For example, at concentrations between 1–30 nM, NO promotes cGMP-mediated processes; at 30–100 nM, it activates protein kinase B, promoting cell survival; and at 100–300 nM, NO stabilizes hypoxia-inducible factor 1, protecting against tissue injury. NO concentrations > 400 nM activate tumor suppressor protein P53, which mediates cell cycle arrest and apoptosis, and at concentrations > 500 nM and 1 μM, NO induces oxidative and nitrosative stress, respectively.[180] For developing a metformin-NO hybrid drug, key parameters such as the initial concentration of the NO donor, rate of release, decomposition and autoxidation rates, and half-life must be optimized.[181,182] Finally, metformin and NO have contrasting effects on glucose metabolism. For example, while metformin upregulates TCA cycle enzymes in hepatocytes,[146] NO inhibits these enzymes,[114,118,119] as well as pyruvate dehydrogenase.[119] This paradox is also observed in combined metformin and sodium-glucose-cotransporter-2 inhibitor treatments for T2DM.[146] Careful consideration of these factors will be crucial for developing a safe and effective metformin-NO hybrid drug.

Conclusions

Metformin is the most successful medication in treating T2DM[4] and exerts its antidiabetic effects primarily by inhibiting hepatic gluconeogenesis.[7,8] T2DM is associated with decreased eNOS-derived NO bioavailability[183] because of impaired NO synthesis from *L*-arginine,[184] downregulation of eNOS expression,[185,186] and posttranscriptional inhibition of eNOS.[187] T2DM decreases hepatic NO by decreasing eNOS phosphorylation[188] and increasing eNOS uncoupling.[189] NO is involved in the pathophysiology of T2DM,[190] and NO-based supplementations have been proposed to have beneficial metabolic effects in T2DM.[16-18] Similar to metformin, NO inhibits hepatic gluconeogenesis.[19,20] However, metformin and NO use shared, as well as unique mechanisms for inhibiting hepatic gluconeogenesis, suggesting that a metformin-NO hybrid drug may be more effective in inhibiting hepatic gluconeogenesis in patients with T2DM. First, the inhibitory effect of NO on hepatic gluconeogenesis via malate pathway is more effective than metformin. On the other hand, metformin exerts a stronger inhibitory effect on fumarate and aspartate pathways of gluconeogenesis than NO.[28,29,31,42,76] Second, substrate-related effects of metformin and NO on inhibition of gluconeogenesis seem complementary, as metformin mainly inhibits lactate, glycerol, and glutamine-induced gluconeogenesis, whereas NO mainly inhibits alanine-induced gluconeogenesis. Finally, both metformin and NO inhibit PEPCK1, G6Pase, and F1,6BPase and stimulate PK.[47,111,120] Unlike metformin, NO inhibits GAPDH[110,117] and activates GCK[115,116] (**Figure 4**).

Some limitations of this review need to be acknowledged to better direct future research and development efforts. First, there is a lack of extensive studies addressing the combined effects of metformin and NO in T2DM. In particular, randomized clinical trials are needed to assess the safety and efficacy of such interventions in humans. Second, much of the evidence supporting the development of a metformin-NO hybrid drug comes from animal models. These studies utilize different types and doses of NO-releasing agents for different durations, which complicates the direct translation of findings to clinical settings. Finally, while this review primarily focuses on the inhibition of gluconeogenesis as the basis for developing a metformin-NO hybrid drug, this compound may also influence other pathways of glucose metabolism, such as glycolysis,[91,94,119] the TCA,[118,119,140,146] and the ETC.[76,85,120,122] Both metformin and NO stimulate glycolysis: metformin achieves this through allosteric activation of phosphofructokinase 1[94] and PK,[91] while NO enhances glycolysis by increasing the AMP/ATP ratio,[119] leading to the allosteric activation of PK and GCK.[115,116] In addition, both metformin and NO inhibit complex I[85,120] and IV[76,122] of the ETC, while NO also inhibits complex II.[121,143] Further mechanistic studies are needed to determine how NO affects glucose metabolism.

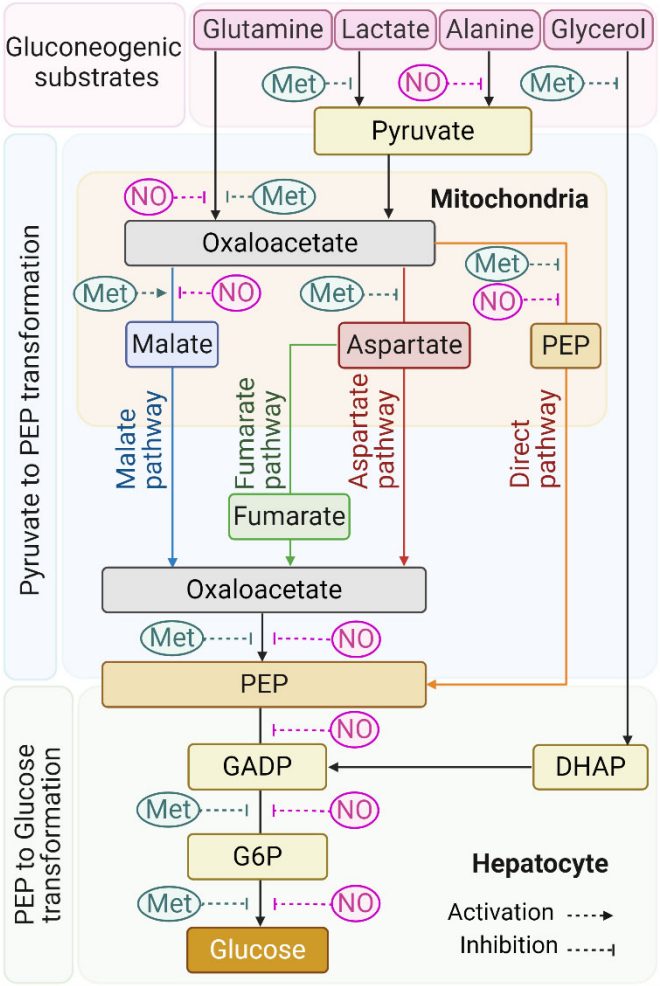

Figure 4 | **Effect of Met and NO on hepatic gluconeogenesis, indicating the site of combined actions.**
Created with BioRender.com. DHAP: Dihydroxyacetone phosphate; G6P: glucose-6-phosphate; GADP: glyceraldehyde 3-phosphate; Met: metformin; NO: nitric oxide; PEP: phosphoenolpyruvate.

In summary, a metformin-NO hybrid drug could offer more comprehensive glucose control for patients with T2DM, addressing both elevated gluconeogenesis and the risks associated with metformin therapy.

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Conflicts of interest: *KK holds patents related to NOSH-NSAIDs and has an equity interest in Avicenna Pharmaceuticals, Inc., which holds the license for this class of compounds. NOSH-NSAIDs were briefly mentioned in the section titled "Clinical applications of NO-based interventions." Other authors declare no actual or perceived conflicts of interest related to the contents of this article.*
Data availability statement: *No additional data are available.*

References

1. DeFronzo RA, Ferrannini E, Groop L, et al. Type 2 diabetes mellitus. *Nat Rev Dis Primers.* 2015;1:15019.
2. International Diabetes Fedration. IDF Diabetes Atlas, 10th ed. Brussels, Belgium: International Diabetes Federation, 2021. http://www.diabetesatlas.org.
3. Khan MAB, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of type 2 diabetes - Global Burden of Disease and Forecasted Trends. *J Epidemiol Glob Health.* 2020;10:107-111.
4. Schernthaner G, Schernthaner GH. The right place for metformin today. *Diabetes Res Clin Pract.* 2020;159:107946.
5. Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways: pharmacokinetics and pharmacodynamics. *Pharmacogenet Genomics.* 2012;22:820-827.
6. Ritter JM, Flower RJ, Henderson G, Loke YK, MacEwan D, Rang HP. *Rang & Dale's pharmacology.* 9th ed. Elsevier. 2019.
7. Chaudhary S, Kulkarni A. Metformin: past, present, and future. *Curr Diab Rep.* 2024;24:119-130.
8. Stumvoll M, Nurjhan N, Perriello G, Dailey G, Gerich JE. Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. *N Engl J Med.* 1995;333:550-554.
9. Shrago E, Lardy HA. Paths of carbon in gluconeogenesis and lipogenesis. II. Conversion of precursors to phosphoenolpyruvate in liver cytosol. *J Biol Chem.* 1966;241:663-668.
10. Nyström T, Ortsäter H, Huang Z, et al. Inorganic nitrite stimulates pancreatic islet blood flow and insulin secretion. *Free Radic Biol Med.* 2012;53:1017-1023.
11. Gheibi S, Bakhtiarzadeh F, Jeddi S, Farrokhfall K, Zardooz H, Ghasemi A. Nitrite increases glucose-stimulated insulin secretion and islet insulin content in obese type 2 diabetic male rats. *Nitric Oxide.* 2017;64:39-51.
12. Ghasemi A, Afzali H, Jeddi S. Effect of oral nitrite administration on gene expression of SNARE proteins involved in insulin secretion from pancreatic islets of male type 2 diabetic rats. *Biomed J.* 2022;45:387-395.
13. Ghasemi A, Gheibi S, Kashfi K, Jeddi S. Anti-oxidant effect of nitrite in the pancreatic islets of type 2 diabetic male rats. *Iran J Basic Med Sci.* 2023;26:420-428.
14. Jiang H, Torregrossa AC, Potts A, et al. Dietary nitrite improves insulin signaling through GLUT4 translocation. *Free Radic Biol Med.* 2014;67:51-57.
15. Khoo NK, Mo L, Zharikov S, et al. Nitrite augments glucose uptake in adipocytes through the protein kinase A-dependent stimulation of mitochondrial fusion. *Free Radic Biol Med.* 2014;70:45-53.
16. Lundberg JO, Carlström M, Weitzberg E. Metabolic effects of dietary nitrate in health and disease. *Cell Metab.* 2018;28:9-22.
17. Kapil V, Khambata RS, Jones DA, et al. The noncanonical pathway for in vivo nitric oxide generation: the nitrate-nitrite-nitric oxide pathway. *Pharmacol Rev.* 2020;72:692-766.
18. Ghasemi A, Jeddi S. Anti-obesity and anti-diabetic effects of nitrate and nitrite. *Nitric Oxide.* 2017;70:9-24.
19. Horton RA, Ceppi ED, Knowles RG, Titheradge MA. Inhibition of hepatic gluconeogenesis by nitric oxide: a comparison with endotoxic shock. *Biochem J.* 1994;299 (Pt 3):735-739.
20. Ceppi ED, Titheradge MA. The importance of nitric oxide in the cytokine-induced inhibition of glucose formation by cultured hepatocytes incubated with insulin, dexamethasone, and glucagon. *Arch Biochem Biophys.* 1998;349:167-174.
21. Horton RA, Knowles RG, Titheradge MA. Endotoxin causes reciprocal changes in hepatic nitric oxide synthesis, gluconeogenesis, and flux through phosphoenolpyruvate carboxykinase. *Biochem Biophys Res Commun.* 1994;204:659-665.
22. Abudukadier A, Fujita Y, Obara A, et al. Tetrahydrobiopterin has a glucose-lowering effect by suppressing hepatic gluconeogenesis in an endothelial nitric oxide synthase-dependent manner in diabetic mice. *Diabetes.* 2013;62:3033-3043.
23. Jeddi S, Gheibi S, Carlström M, Kashfi K, Ghasemi A. Long-term co-administration of sodium nitrite and sodium hydrosulfide inhibits hepatic gluconeogenesis in male type 2 diabetic rats: role of PI3K-Akt-eNOS pathway. *Life Sci.* 2021;265:118770.
24. Molina y Vedia L, McDonald B, Reep B, et al. Nitric oxide-induced S-nitrosylation of glyceraldehyde-3-phosphate dehydrogenase inhibits enzymatic activity and increases endogenous ADP-ribosylation. *J Biol Chem.* 1992;267:24929-24932.
25. Cordero-Herrera I, Guimarães DD, Moretti C, et al. Head-to-head comparison of inorganic nitrate and metformin in a mouse model of cardiometabolic disease. *Nitric Oxide.* 2020;97:48-56.
26. Adeoye SW, Dimeji IY, Jabba LH, et al. Synergistic effects of L-arginine and metformin on oxidative stress, inflammation and glucometabolic enzymes in diabetic rats. *Trends Appl Sci Res.* 2024;19:180-198.
27. Shah A, Wondisford FE. Gluconeogenesis flux in metabolic disease. *Annu Rev Nutr.* 2023;43:153-177.
28. Chandel NS. Carbohydrate metabolism. *Cold Spring Harb Perspect Biol.* 2021;13:a040568.
29. Voet D, Voet JG, Pratt CW. *Fundamentals of biochemistry: life at the molecular level.* 5th ed. Wiley. 2016.
30. Boron WF, Boulpaep EL. *Medical physiology.* 3rd ed. Elsevier Health Sciences. 2016.
31. Jungas RL, Halperin ML, Brosnan JT. Quantitative analysis of amino acid oxidation and related gluconeogenesis in humans. *Physiol Rev.* 1992;72:419-448.
32. Droździk M, Szeląg-Pieniek S, Grzegółkowska J, et al. Monocarboxylate transporter 1 (MCT1) in liver pathology. *Int J Mol Sci.* 2020;21:1606.
33. Mackenzie B, Erickson JD. Sodium-coupled neutral amino acid (System N/A) transporters of the SLC38 gene family. *Pflugers Arch.* 2004;447:784-795.
34. Adeva-Andany MM, Pérez-Felpete N, Fernández-Fernández C, Donapetry-García C, Pazos-García C. Liver glucose metabolism in humans. *Biosci Rep.* 2016;36:e00416.
35. Zangari J, Petrelli F, Maillot B, Martinou JC. The multifaceted pyruvate metabolism: role of the mitochondrial pyruvate carrier. *Biomolecules.* 2020;10:1068.
36. Paulusma CC, Lamers WH, Broer S, van de Graaf SFJ. Amino acid metabolism, transport and signalling in the liver revisited. *Biochem Pharmacol.* 2022;201:115074.
37. Yoo HC, Park SJ, Nam M, et al. A variant of SLC1A5 is a mitochondrial glutamine transporter for metabolic reprogramming in cancer cells. *Cell Metab.* 2020;31:267-283.e12.
38. Watford M. Glutamine and glutamate metabolism across the liver sinusoid. *J Nutr.* 2000;130:983S-987S.
39. Garber AJ, Hanson RW. The interrelationships of the various pathways forming gluconeogenic precursors in guinea pig liver mitochondria. *J Biol Chem.* 1971;246:589-598.
40. Holeček M. Roles of malate and aspartate in gluconeogenesis in various physiological and pathological states. *Metabolism.* 2023;145:155614.
41. Win S, Than TA, Kaplowitz N, et al. The central role of mitochondrial metabolism in hepatic steatosis. *Explor Dig Dis.* 2024;3:42-68.
42. Borst P. The malate-aspartate shuttle (Borst cycle): How it started and developed into a major metabolic pathway. *IUBMB Life.* 2020;72:2241-2259.
43. Stark R, Pasquel F, Turcu A, et al. Phosphoenolpyruvate cycling via mitochondrial phosphoenolpyruvate carboxykinase links anaplerosis and mitochondrial GTP with insulin secretion. *J Biol Chem.* 2009;284:26578-26590.
44. Stark R, Kibbey RG. The mitochondrial isoform of phosphoenolpyruvate carboxykinase (PEPCK-M) and glucose homeostasis: has it been overlooked? *Biochim Biophys Acta.* 2014;1840:1313-1330.
45. Barmore W, Azad F, Stone WL. Physiology, urea cycle. *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2025.
46. Rui L. Energy metabolism in the liver. *Compr Physiol.* 2014;4:177-197.
47. LaMoia TE, Shulman GI. Cellular and molecular mechanisms of metformin action. *Endocr Rev.* 2021;42:77-96.
48. Karim S, Adams DH, Lalor PF. Hepatic expression and cellular distribution of the glucose transporter family. *World J Gastroenterol.* 2012;18:6771-6781.
49. Palmer RM, Rees DD, Ashton DS, Moncada S. L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. *Biochem Biophys Res Commun.* 1988;153:1251-1256.

50. Benjamin N, O'Driscoll F, Dougall H, et al. Stomach NO synthesis. *Nature.* 1994;368:502.
51. Lundberg JO, Weitzberg E, Lundberg JM, Alving K. Intragastric nitric oxide production in humans: measurements in expelled air. *Gut.* 1994;35:1543-1546.
52. Ghasemi A. Quantitative aspects of nitric oxide production from nitrate and nitrite. *EXCLI J.* 2022;21:470-486.
53. Bredt DS. Endogenous nitric oxide synthesis: biological functions and pathophysiology. *Free Radic Res.* 1999;31:577-596.
54. Król M, Kepinska M. Human nitric oxide synthase-its functions, polymorphisms, and inhibitors in the context of inflammation, diabetes and cardiovascular diseases. *Int J Mol Sci.* 2020;22:56.
55. Gao J, Morrison DC, Parmely TJ, Russell SW, Murphy WJ. An interferon-gamma-activated site (GAS) is necessary for full expression of the mouse iNOS gene in response to interferon-gamma and lipopolysaccharide. *J Biol Chem.* 1997;272:1226-1230.
56. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov.* 2008;7:156-167.
57. Jansson EA, Huang L, Malkey R, et al. A mammalian functional nitrate reductase that regulates nitrite and nitric oxide homeostasis. *Nat Chem Biol.* 2008;4:411-417.
58. Li H, Hemann C, Abdelghany TM, El-Mahdy MA, Zweier JL. Characterization of the mechanism and magnitude of cytoglobin-mediated nitrite reduction and nitric oxide generation under anaerobic conditions. *J Biol Chem.* 2012;287:36623-36633.
59. Li H, Cui H, Kundu TK, Alzawahra W, Zweier JL. Nitric oxide production from nitrite occurs primarily in tissues not in the blood: critical role of xanthine oxidase and aldehyde oxidase. *J Biol Chem.* 2008;283:17855-17863.
60. McNaughton L, Puttagunta L, Martinez-Cuesta MA, et al. Distribution of nitric oxide synthase in normal and cirrhotic human liver. *Proc Natl Acad Sci U S A.* 2002;99:17161-17166.
61. Royer C, Steffan AM, Navas MC, Fuchs A, Jaeck D, Stoll-Keller F. A study of susceptibility of primary human Kupffer cells to hepatitis C virus. *J Hepatol.* 2003;38:250-256.
62. McKim SE, Gäbele E, Isayama F, et al. Inducible nitric oxide synthase is required in alcohol-induced liver injury: studies with knockout mice. *Gastroenterology.* 2003;125:1834-1844.
63. Nozaki Y, Fujita K, Wada K, et al. Deficiency of iNOS-derived NO accelerates lipid accumulation-independent liver fibrosis in non-alcoholic steatohepatitis mouse model. *BMC Gastroenterol.* 2015;15:42.
64. Rai RM, Lee FY, Rosen A, et al. Impaired liver regeneration in inducible nitric oxide synthasedeficient mice. *Proc Natl Acad Sci U S A.* 1998;95:13829-13834.
65. Tirmenstein MA, Nicholls-Grzemski FA, Schmittgen TD, Zakrajsek BA, Fariss MW. Characterization of nitric oxide production following isolation of rat hepatocytes. *Toxicol Sci.* 2000;53:56-62.
66. Hur GM, Ryu YS, Yun HY, et al. Hepatic ischemia/reperfusion in rats induces iNOS gene transcription by activation of NF-kappaB. *Biochem Biophys Res Commun.* 1999;261:917-922.
67. Kitade H, Sakitani K, Inoue K, et al. Interleukin 1 beta markedly stimulates nitric oxide formation in the absence of other cytokines or lipopolysaccharide in primary cultured rat hepatocytes but not in Kupffer cells. *Hepatology.* 1996;23:797-802.
68. Banerjee S, Melnyk SB, Krager KJ, et al. The neuronal nitric oxide synthase inhibitor NANT blocks acetaminophen toxicity and protein nitration in freshly isolated hepatocytes. *Free Radic Biol Med.* 2015;89:750-757.
69. Schild L, Jaroscakova I, Lendeckel U, Wolf G, Keilhoff G. Neuronal nitric oxide synthase controls enzyme activity pattern of mitochondria and lipid metabolism. *FASEB J.* 2006;20:145-147.
70. Wei CL, Khoo HE, Lee KH, Hon WM. Differential expression and localization of nitric oxide synthases in cirrhotic livers of bile duct-ligated rats. *Nitric Oxide.* 2002;7:91-102.
71. Villanueva C, Giulivi C. Subcellular and cellular locations of nitric oxide synthase isoforms as determinants of health and disease. *Free Radic Biol Med.* 2010;49:307-316.
72. Shankar RR, Wu Y, Shen HQ, Zhu JS, Baron AD. Mice with gene disruption of both endothelial and neuronal nitric oxide synthase exhibit insulin resistance. *Diabetes.* 2000;49:684-687.
73. Apostolova N, Iannantuoni F, Gruevska A, Muntane J, Rocha M, Victor VM. Mechanisms of action of metformin in type 2 diabetes: effects on mitochondria and leukocyte-endothelium interactions. *Redox Biol.* 2020;34:101517.
74. Wang DS, Jonker JW, Kato Y, Kusuhara H, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. *J Pharmacol Exp Ther.* 2002;302:510-515.
75. Dietsche KB, Magge SN, Dixon SA, et al. Glycemia and gluconeogenesis with metformin and liraglutide: a randomized trial in youth-onset type 2 diabetes. *J Clin Endocrinol Metab.* 2024;109:1361-1370.
76. Foretz M, Guigas B, Viollet B. Metformin: update on mechanisms of action and repurposing potential. *Nat Rev Endocrinol.* 2023;19:460-476.
77. Caton PW, Nayuni NK, Kieswich J, Khan NQ, Yaqoob MM, Corder R. Metformin suppresses hepatic gluconeogenesis through induction of SIRT1 and GCN5. *J Endocrinol.* 2010;205:97-106.
78. Ramachandran R, Saraswathy M. Up-regulation of nuclear related factor 2 (NRF2) and antioxidant responsive elements by metformin protects hepatocytes against the acetaminophen toxicity. *Toxicol Res.* 2014;3:350-358.
79. Ewis SA, Abdel-Rahman MS. Effect of metformin on glutathione and magnesium in normal and streptozotocin-induced diabetic rats. *J Appl Toxicol.* 1995;15:387-390.
80. Xie D, Chen F, Zhang Y, et al. Let-7 underlies metformin-induced inhibition of hepatic glucose production. *Proc Natl Acad Sci U S A.* 2022;119:e2122217119.
81. Wu X, Zhang Y. TET-mediated active DNA demethylation: mechanism, function and beyond. *Nat Rev Genet.* 2017;18:517-534.
82. Da L, Cao T, Sun X, et al. Hepatic TET3 contributes to type-2 diabetes by inducing the HNF4α fetal isoform. *Nat Commun.* 2020;11:342.
83. Oh KJ, Han HS, Kim MJ, Koo SH. CREB and FoxO1: two transcription factors for the regulation of hepatic gluconeogenesis. *BMB Rep.* 2013;46:567-574.
84. Madiraju AK, Erion DM, Rahimi Y, et al. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature.* 2014;510:542-546.
85. Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *Biochem J.* 2000;348 Pt 3:607-614.
86. Bridges HR, Jones AJ, Pollak MN, Hirst J. Effects of metformin and other biguanides on oxidative phosphorylation in mitochondria. *Biochem J.* 2014;462:475-487.
87. LaMoia TE, Butrico GM, Kalpage HA, et al. Metformin, phenformin, and galegine inhibit complex IV activity and reduce glycerol-derived gluconeogenesis. *Proc Natl Acad Sci U S A.* 2022;119:e2122287119.
88. DeFronzo R, Fleming GA, Chen K, Bicsak TA. Metformin-associated lactic acidosis: current perspectives on causes and risk. *Metabolism.* 2016;65:20-29.
89. Radziuk J, Zhang Z, Wiernsperger N, Pye S. Effects of metformin on lactate uptake and gluconeogenesis in the perfused rat liver. *Diabetes.* 1997;46:1406-1413.
90. Wang DS, Kusuhara H, Kato Y, Jonker JW, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in the lactic acidosis caused by metformin. *Mol Pharmacol.* 2003;63:844-848.
91. Argaud D, Roth H, Wiernsperger N, Leverve XM. Metformin decreases gluconeogenesis by enhancing the pyruvate kinase flux in isolated rat hepatocytes. *Eur J Biochem.* 1993;213:1341-1348.
92. Hunter RW, Hughey CC, Lantier L, et al. Metformin reduces liver glucose production by inhibition of fructose-1-6-bisphosphatase. *Nat Med.* 2018;24:1395-1406.
93. Miller RA, Chu Q, Xie J, Foretz M, Viollet B, Birnbaum MJ. Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. *Nature.* 2013;494:256-260.
94. Moonira T, Chachra SS, Ford BE, et al. Metformin lowers glucose 6-phosphate in hepatocytes by activation of glycolysis downstream of glucose phosphorylation. *J Biol Chem.* 2020;295:3330-3346.
95. He L, Sabet A, Djedjos S, et al. Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. *Cell.* 2009;137:635-646.
96. Ramatchandirin B, Pearah A, He L. Regulation of liver glucose and lipid metabolism by transcriptional factors and coactivators. *Life (Basel).* 2023;13:515.

97. Zhang X, Yang S, Chen J, Su Z. Unraveling the regulation of hepatic gluconeogenesis. *Front Endocrinol (Lausanne).* 2018;9:802.
98. Koo SH, Flechner L, Qi L, et al. The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. *Nature.* 2005;437:1109-1111.
99. Rhee J, Inoue Y, Yoon JC, et al. Regulation of hepatic fasting response by PPARgamma coactivator-1alpha (PGC-1): requirement for hepatocyte nuclear factor 4alpha in gluconeogenesis. *Proc Natl Acad Sci U S A.* 2003;100:4012-4017.
100. Ji X, Wang S, Tang H, et al. PPP1R3C mediates metformin-inhibited hepatic gluconeogenesis. *Metabolism.* 2019;98:62-75.
101. Foretz M, Hébrard S, Leclerc J, et al. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. *J Clin Invest.* 2010;120:2355-2369.
102. Konopka AR, Esponda RR, Robinson MM, et al. Hyperglucagonemia mitigates the effect of metformin on glucose production in prediabetes. *Cell Rep.* 2016;15:1394-1400.
103. Kim YD, Park KG, Lee YS, et al. Metformin inhibits hepatic gluconeogenesis through AMP-activated protein kinase-dependent regulation of the orphan nuclear receptor SHP. *Diabetes.* 2008;57:306-314.
104. Pelech S, Cohen P, Fisher MJ, Pogson CI, El-Maghrabi MR, Pilkis SJ. The protein phosphatases involved in cellular regulation. Glycolysis, gluconeogenesis and aromatic amino acid breakdown in rat liver. *Eur J Biochem.* 1984;145:39-49.
105. Lee JM, Seo WY, Song KH, et al. AMPK-dependent repression of hepatic gluconeogenesis via disruption of CREB.CRTC2 complex by orphan nuclear receptor small heterodimer partner. *J Biol Chem.* 2010;285:32182-32191.
106. Vassilopoulos A, Fritz KS, Petersen DR, Gius D. The human sirtuin family: evolutionary divergences and functions. *Hum Genomics.* 2011;5:485-496.
107. Haque ME, Jakaria M, Akther M, Cho DY, Kim IS, Choi DK. The GCN5: its biological functions and therapeutic potentials. *Clin Sci (Lond).* 2021;135:231-257.
108. Fujita Y, Hosokawa M, Fujimoto S, et al. Metformin suppresses hepatic gluconeogenesis and lowers fasting blood glucose levels through reactive nitrogen species in mice. *Diabetologia.* 2010;53:1472-1481.
109. Gheibi S, Jeddi S, Carlström M, Gholami H, Ghasemi A. Effects of long-term nitrate supplementation on carbohydrate metabolism, lipid profiles, oxidative stress, and inflammation in male obese type 2 diabetic rats. *Nitric Oxide.* 2018;75:27-41.
110. Stadler J, Barton D, Beil-Moeller H, et al. Hepatocyte nitric oxide biosynthesis inhibits glucose output and competes with urea synthesis for L-arginine. *Am J Physiol.* 1995;268:G183-188.
111. Lu M, Wang Y, Jiang Y, et al. Berberine inhibits gluconeogenesis in spontaneous diabetic rats by regulating the AKT/MAPK/NO/cGMP/PKG signaling pathway. *Mol Cell Biochem.* 2023;478:2013-2027.
112. Cordero-Herrera I, Kozyra M, Zhuge Z, et al. AMP-activated protein kinase activation and NADPH oxidase inhibition by inorganic nitrate and nitrite prevent liver steatosis. *Proc Natl Acad Sci U S A.* 2019;116:217-226.
113. Monti LD, Valsecchi G, Costa S, et al. Effects of endothelin-1 and nitric oxide on glucokinase activity in isolated rat hepatocytes. *Metabolism.* 2000;49:73-80.
114. Doulias PT, Tenopoulou M, Greene JL, Raju K, Ischiropoulos H. Nitric oxide regulates mitochondrial fatty acid metabolism through reversible protein S-nitrosylation. *Sci Signal.* 2013;6:rs1.
115. Spence JT, Koudelka AP. Effects of biotin upon the intracellular level of cGMP and the activity of glucokinase in cultured rat hepatocytes. *J Biol Chem.* 1984;259:6393-6396.
116. Spence JT, Merrill MJ, Pitot HC. Role of insulin, glucose, and cyclic GMP in the regulation of glucokinase in cultured hepatocytes. *J Biol Chem.* 1981;256:1598-1603.
117. Broniowska KA, Hogg N. Differential mechanisms of inhibition of glyceraldehyde-3-phosphate dehydrogenase by S-nitrosothiols and NO in cellular and cell-free conditions. *Am J Physiol Heart Circ Physiol.* 2010;299:H1212-1219.
118. Yang ES, Richter C, Chun JS, Huh TL, Kang SS, Park JW. Inactivation of NADP(+)-dependent isocitrate dehydrogenase by nitric oxide. *Free Radic Biol Med.* 2002;33:927-937.
119. Pappas G, Wilkinson ML, Gow AJ. Nitric oxide regulation of cellular metabolism: adaptive tuning of cellular energy. *Nitric Oxide.* 2023;131:8-17.
120. Stadler J, Billiar TR, Curran RD, Stuehr DJ, Ochoa JB, Simmons RL. Effect of exogenous and endogenous nitric oxide on mitochondrial respiration of rat hepatocytes. *Am J Physiol.* 1991;260:C910-916.
121. Dahm CC, Moore K, Murphy MP. Persistent S-nitrosation of complex I and other mitochondrial membrane proteins by S-nitrosothiols but not nitric oxide or peroxynitrite: implications for the interaction of nitric oxide with mitochondria. *J Biol Chem.* 2006;281:10056-10065.
122. Okina Y, Sato-Matsubara M, Kido Y, et al. Nitric oxide derived from cytoglobin-deficient hepatic stellate cells causes suppression of cytochrome c oxidase activity in hepatocytes. *Antioxid Redox Signal.* 2023;38:463-479.
123. Billiar TR, Curran RD, Harbrecht BG, et al. Association between synthesis and release of cGMP and nitric oxide biosynthesis by hepatocytes. *Am J Physiol.* 1992;262:C1077-1082.
124. McDonald LJ, Murad F. Nitric oxide and cGMP signaling. *Adv Pharmacol.* 1995;34:263-275.
125. García-Villafranca J, Guillén A, Castro J. Involvement of nitric oxide/cyclic GMP signaling pathway in the regulation of fatty acid metabolism in rat hepatocytes. *Biochem Pharmacol.* 2003;65:807-812.
126. Francis SH, Busch JL, Corbin JD, Sibley D. cGMP-dependent protein kinases and cGMP phosphodiesterases in nitric oxide and cGMP action. *Pharmacol Rev.* 2010;62:525-563.
127. Epand RM, Prosser C. Regulation of glucagon-stimulated production of glucose in rat liver by guanosine 3',5'-cyclic phosphate. *Can J Physiol Pharmacol.* 1976;54:834-837.
128. Fain JN, Tolbert ME, Pointer RH, Butcher FR, Arnold A. Cyclic nucleotides and gluconeogenesis by rat liver cells. *Metabolism.* 1975;24:395-407.
129. Tateya S, Rizzo-De Leon N, Handa P, et al. VASP increases hepatic fatty acid oxidation by activating AMPK in mice. *Diabetes.* 2013;62:1913-1922.
130. Johanns M, Hue L, Rider MH. AMPK inhibits liver gluconeogenesis: fact or fiction? *Biochem J.* 2023;480:105-125.
131. Aboalroub AA, Al Azzam KM. Protein S-nitrosylation: a chemical modification with ubiquitous biological activities. *Protein J.* 2024;43:639-655.
132. Mannick JB, Schonhoff CM. Nitrosylation: the next phosphorylation? *Arch Biochem Biophys.* 2002;408:1-6.
133. Wiseman DA, Thurmond DC. The good and bad effects of cysteine S-nitrosylation and tyrosine nitration upon insulin exocytosis: a balancing act. *Curr Diabetes Rev.* 2012;8:303-315.
134. Venetos NM, Stomberski CT, Qian Z, Premont RT, Stamler JS. Activation of hepatic acetyl-CoA carboxylase by S-nitrosylation in response to diet. *J Lipid Res.* 2024;65:100542.
135. Li J, Billiar TR. The anti-apoptotic actions of nitric oxide in hepatocytes. *Cell Death Differ.* 1999;6:952-955.
136. Lee HM, Choi JW, Choi MS. Role of nitric oxide and protein S-nitrosylation in ischemia-reperfusion injury. *Antioxidants (Basel).* 2021;11:57.
137. Nakamura T, Lipton SA. 'SNO'-storms compromise protein activity and mitochondrial metabolism in neurodegenerative disorders. *Trends Endocrinol Metab.* 2017;28:879-892.
138. Fatania HR, al-Nassar KE, Thomas N. Chemical modification of rat liver cytosolic NADP(+)-linked isocitrate dehydrogenase by N-ethylmaleimide. Evidence for essential sulphydryl groups. *FEBS Lett.* 1993;322:245-248.
139. Tórtora V, Quijano C, Freeman B, Radi R, Castro L. Mitochondrial aconitase reaction with nitric oxide, S-nitrosoglutathione, and peroxynitrite: mechanisms and relative contributions to aconitase inactivation. *Free Radic Biol Med.* 2007;42:1075-1088.
140. Doulias PT, Yang H, Andreyev AY, et al. S-Nitrosylation-mediated dysfunction of TCA cycle enzymes in synucleinopathy studied in postmortem human brains and hiPSC-derived neurons. *Cell Chem Biol.* 2023;30:965-975.e6.
141. Clementi E, Brown GC, Feelisch M, Moncada S. Persistent inhibition of cell respiration by nitric oxide: crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proc Natl Acad Sci U S A.* 1998;95:7631-7636.
142. Brown GC, Borutaite V. Inhibition of mitochondrial respiratory complex I by nitric oxide, peroxynitrite and S-nitrosothiols. *Biochim Biophys Acta.* 2004;1658:44-49.
143. Prime TA, Blaikie FH, Evans C, et al. A mitochondria-targeted S-nitrosothiol modulates respiration, nitrosates thiols, and protects against ischemia-reperfusion injury. *Proc Natl Acad Sci U S A.* 2009;106:10764-10769.

144. Zhang J, Jin B, Li L, Block ER, Patel JM. Nitric oxide-induced persistent inhibition and nitrosylation of active site cysteine residues of mitochondrial cytochrome-c oxidase in lung endothelial cells. *Am J Physiol Cell Physiol.* 2005;288:C840-849.
145. Viollet B, Guigas B, Leclerc J, et al. AMP-activated protein kinase in the regulation of hepatic energy metabolism: from physiology to therapeutic perspectives. *Acta Physiol (Oxf).* 2009;196:81-98.
146. Harada M, Adam J, Covic M, et al. Bidirectional modulation of TCA cycle metabolites and anaplerosis by metformin and its combination with SGLT2i. *Cardiovasc Diabetol.* 2024;23:199.
147. Miller MR, Megson IL. Recent developments in nitric oxide donor drugs. *Br J Pharmacol.* 2007;151:305-321.
148. Fenton C, Wellington K, Easthope SE. 0.4% nitroglycerin ointment : in the treatment of chronic anal fissure pain. *Drugs.* 2006;66:343-349.
149. Yurtseven N, Karaca P, Kaplan M, et al. Effect of nitroglycerin inhalation on patients with pulmonary hypertension undergoing mitral valve replacement surgery. *Anesthesiology.* 2003;99:855-858.
150. Goyal P, Kiran U, Chauhan S, Juneja R, Choudhary M. Efficacy of nitroglycerin inhalation in reducing pulmonary arterial hypertension in children with congenital heart disease. *Br J Anaesth.* 2006;97:208-214.
151. Sawatzky DA, Megson IL, Rossi AG. Sildenafil offers protection against NSAID-induced gastric injury. *Br J Pharmacol.* 2005;146:477-478.
152. Ghasemi A, Kashfi K. Nitric oxide: a brief history of discovery and timeline of its research. In: Ghasemi A, Kashfi K, Bahadoran Z, eds. *The role of nitric oxide in type 2 diabetes*. Bentham Science Publisher; 2022:27-38.
153. Simpson D, Wellington K. Nicorandil: a review of its use in the management of stable angina pectoris, including high-risk patients. *Drugs.* 2004;64:1941-1955.
154. Turnbull CM, Rossi AG, Megson IL. Therapeutic effects of nitric oxide-aspirin hybrid drugs. *Expert Opin Ther Targets.* 2006;10:911-922.
155. Amoruso A, Fresu LG, Dalli J, et al. Characterization of the anti-inflammatory properties of NCX 429, a dual-acting compound releasing nitric oxide and naproxen. *Life Sci.* 2015;126:28-36.
156. Amoruso A, Bardelli C, Fresu LG, et al. The nitric oxide-donating pravastatin, NCX 6550, inhibits cytokine release and NF-κB activation while enhancing PPARγ expression in human monocyte/macrophages. *Pharmacol Res.* 2010;62:391-399.
157. Bandarage UK, Chen L, Fang X, et al. Nitrosothiol esters of diclofenac: synthesis and pharmacological characterization as gastrointestinal-sparing prodrugs. *J Med Chem.* 2000;43:4005-4016.
158. Marshall M, Keeble J, Moore PK. Effect of a nitric oxide releasing derivative of paracetamol in a rat model of endotoxaemia. *Br J Pharmacol.* 2006;149:516-522.
159. Varani K, Vincenzi F, Targa M, et al. Repeated dosing with NCX1404, a nitric oxide-donating pregabalin, re-establishes normal nociceptive responses in mice with streptozotocin-induced painful diabetic neuropathy. *J Pharmacol Exp Ther.* 2016;357:240-247.
160. Addis VM, Miller-Ellis E. Latanoprostene bunod ophthalmic solution 0.024% in the treatment of open-angle glaucoma: design, development, and place in therapy. *Clin Ophthalmol.* 2018;12:2649-2657.
161. Kashfi K. Utility of nitric oxide and hydrogen sulfide-releasing chimeras as anticancer agents. *Redox Biol.* 2015;5:420.
162. Vannini F, Kodela R, Chattopadhyay M, Kashfi K. NOSH-aspirin inhibits colon cancer cell growth: effects of positional isomerism. *Redox Biol.* 2015;5:421.
163. Chattopadhyay M, Kodela R, Santiago G, Le TTC, Nath N, Kashfi K. NOSH-aspirin (NBS-1120) inhibits pancreatic cancer cell growth in a xenograft mouse model: modulation of FoxM1, p53, NF-κB, iNOS, caspase-3 and ROS. *Biochem Pharmacol.* 2020;176:113857.
164. Chattopadhyay M, Nath N, Kodela R, Metkar S, Soyemi SA, Kashfi K. NOSH-aspirin (NBS-1120) inhibits estrogen receptor negative breast cancer in vitro and in vivo by modulating redox-sensitive signaling pathways. *J Pharmacol Exp Ther.* 2025;392:100019.
165. Kashfi K, Chattopadhyay M, Kodela R. NOSH-sulindac (AVT-18A) is a novel nitric oxide- and hydrogen sulfide-releasing hybrid that is gastrointestinal safe and has potent anti-inflammatory, analgesic, antipyretic, anti-platelet, and anti-cancer properties. *Redox Biol.* 2015;6:287-296.
166. Chattopadhyay M, Kodela R, Duvalsaint PL, Kashfi K. Gastrointestinal safety, chemotherapeutic potential, and classic pharmacological profile of NOSH-naproxen (AVT-219) a dual NO- and H2S-releasing hybrid. *Pharmacol Res Perspect.* 2016;4:e00224.
167. Kodela R, Chattopadhyay M, Kashfi K. NOSH-aspirin: a novel nitric oxide-hydrogen sulfide-releasing hybrid: a new class of anti-inflammatory pharmaceuticals. *ACS Med Chem Lett.* 2012;3:257-262.
168. Fonseca MD, Cunha FQ, Kashfi K, Cunha TM. NOSH-aspirin (NBS-1120), a dual nitric oxide and hydrogen sulfide-releasing hybrid, reduces inflammatory pain. *Pharmacol Res Perspect.* 2015;3:e00133.
169. Lundberg JO, Carlström M, Larsen FJ, Weitzberg E. Roles of dietary inorganic nitrate in cardiovascular health and disease. *Cardiovasc Res.* 2011;89:525-532.
170. Omar SA, Webb AJ, Lundberg JO, Weitzberg E. Therapeutic effects of inorganic nitrate and nitrite in cardiovascular and metabolic diseases. *J Intern Med.* 2016;279:315-336.
171. Bahadoran Z, Mirmiran P, Hosseinpanah F, Kashfi K, Ghasemi A. Nitric oxide-based treatments improve wound healing associated with diabetes mellitus. *Med Gas Res.* 2025;15:23-35.
172. Calello DP, Liu KD, Wiegand TJ, et al. Extracorporeal treatment for metformin poisoning: systematic review and recommendations from the extracorporeal treatments in poisoning workgroup. *Crit Care Med.* 2015;43:1716-1730.
173. Vecchio S, Giampreti A, Petrolini VM, et al. Metformin accumulation: lactic acidosis and high plasmatic metformin levels in a retrospective case series of 66 patients on chronic therapy. *Clin Toxicol (Phila).* 2014;52:129-135.
174. Di Mauro S, Filippello A, Scamporrino A, Purrello F, Piro S, Malaguarnera R. Metformin: when should we fear lactic acidosis? *Int J Mol Sci.* 2022;23:8320.
175. El-Hattab AW, Emrick LT, Williamson KC, Craigen WJ, Scaglia F. The effect of citrulline and arginine supplementation on lactic acidemia in MELAS syndrome. *Meta Gene.* 2013;1:8-14.
176. Chen T, Pearce LL, Peterson J, Stoyanovsky D, Billiar TR. Glutathione depletion renders rat hepatocytes sensitive to nitric oxide donor-mediated toxicity. *Hepatology.* 2005;42:598-607.
177. Zheng H, Wu J, Jin Z, Yan LJ. Protein modifications as manifestations of hyperglycemic glucotoxicity in diabetes and its complications. *Biochem Insights.* 2016;9:1-9.
178. Thomas DD. Breathing new life into nitric oxide signaling: a brief overview of the interplay between oxygen and nitric oxide. *Redox Biol.* 2015;5:225-233.
179. Hall CN, Garthwaite J. What is the real physiological NO concentration in vivo? *Nitric Oxide.* 2009;21:92-103.
180. Thomas DD, Ridnour LA, Isenberg JS, et al. The chemical biology of nitric oxide: implications in cellular signaling. *Free Radic Biol Med.* 2008;45:18-31.
181. Schmidt K, Desch W, Klatt P, Kukovetz WR, Mayer B. Release of nitric oxide from donors with known half-life: a mathematical model for calculating nitric oxide concentrations in aerobic solutions. *Naunyn Schmiedebergs Arch Pharmacol.* 1997;355:457-462.
182. Ghasemi A, Jeddi S, Kashfi K. Brain glucose metabolism: role of nitric oxide. *Biochem Pharmacol.* 2025;232:116728.
183. Sansbury BE, Hill BG. Antiobesogenic role of endothelial nitric oxide synthase. *Vitam Horm.* 2014;96:323-346.
184. Avogaro A, Toffolo G, Kiwanuka E, de Kreutzenberg SV, Tessari P, Cobelli C. L-arginine-nitric oxide kinetics in normal and type 2 diabetic subjects: a stable-labelled 15N arginine approach. *Diabetes.* 2003;52:795-802.
185. Valerio A, Cardile A, Cozzi V, et al. TNF-alpha downregulates eNOS expression and mitochondrial biogenesis in fat and muscle of obese rodents. *J Clin Invest.* 2006;116:2791-2798.
186. Kraus RM, Houmard JA, Kraus WE, et al. Obesity, insulin resistance, and skeletal muscle nitric oxide synthase. *J Appl Physiol (1985).* 2012;113:758-765.
187. Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M. Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. *J Clin Invest.* 2001;108:1341-1348.
188. Sheldon RD, Laughlin MH, Rector RS. Reduced hepatic eNOS phosphorylation is associated with NAFLD and type 2 diabetes progression and is prevented by daily exercise in hyperphagic OLETF rats. *J Appl Physiol (1985).* 2014;116:1156-1164.
189. Elrod JW, Duranski MR, Langston W, et al. eNOS gene therapy exacerbates hepatic ischemia-reperfusion injury in diabetes: a role for eNOS uncoupling. *Circ Res.* 2006;99:78-85.
190. Bahadoran Z, Mirmiran P, Ghasemi A. Role of nitric oxide in insulin secretion and glucose metabolism. *Trends Endocrinol Metab.* 2020;31:118-130.