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Control of gluconeogenesis by metformin: Does redox trump energy charge?

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Metformin is the most widely prescribed drug to lower glucose in type II diabetics, yet its mechanism of action remains controversial. A new study reveals that metformin inhibits mitochondrial glycerol-3-phosphate dehydrogenase, triggering reduction of the cytosolic NADH/NAD^+ pool and impaired utilization of redox-dependent substrates for gluconeogenesis (Madiraju et al., 2014).

Biguanides, including metformin, have been used for over 50 years to treat diabetes, and shown promise as cancer therapeutics. Central to metformin's effects is a dramatic lowering of hepatic glucose output, yet its precise mechanism of action has remained enigmatic. Metformin inhibits complex I of the electron transport chain, which was proposed to decrease the ATP/ADP ratio, shifting the equilibrium of the phosphoglycerate kinase reaction to disfavor glucose synthesis (Owen et al., 2000). Subsequently, it was suggested that metformin acts via the energy sensor AMP-activated protein kinase (AMPK)(Zhou et al., 2001). Although several studies have since indicated that metformin can function independently from AMPK (Foretz et al., 2010; Miller et al., 2013), recent reports have argued that AMPK is indeed required for some effects of the drug (Fullerton et al., 2013). In addition, metformin-induced AMP accumulation directly inhibits adenylate cyclase, blocking the induction of gluconeogenesis by glucagon (Miller et al., 2013). A new report now shows that metformin shifts the NADH/NAD^+ ratio in liver to inhibit glucose production independently of energy charge via a novel direct target, mitochondrial glycerol-3-phosphate dehydrogenase (mGPD)(Madiraju et al., 2014).

Mammalian tissues contain at least two pools of NADH and NAD^+, nucleo-cytosolic and mitochondrial. To study effects of metformin in the two compartments, Madiraju et al. measured hepatic lactate and pyruvate, which equilibrate with cytosolic NADH/NAD^+ (via lactate dehydrogenase), as well as beta-hydroxybutyrate and acetoacetate, which equilibrate

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with mitochondrial NADH/NAD^+(via beta-hydroxybutyrate dehydrogenase).Cytosolic NADH/NAD^+ ratio increased in the livers of metformin-treated animals while the mitochondrial NADH/NAD^+ ratio decreased. This is surprising given prior reports that biguanides increase both cytoplasmic and mitochondrial NADH/NAD^+ ratios, consistent with inhibition of complex I (Owen et al., 2000). Opposing shifts would not be expected to arise due to the activity of redox shuttles–biochemical reactions that transfer electrons from cytosolic NADH into the mitochondria – suggesting that shuttle systems themselves might be impaired (Madiraju et al., 2014). Accordingly, Madiraju et al. discovered that therapeutic concentrations of metformin inhibited a key enzyme in the glycerophosphate shuttle, mGPD, by ~50%. mGPD knockdown *in vivo* recapitulated the effects of metformin treatment and metformin had no further effect in these animals. It was concluded that that metformin works by halting the glycerophosphate shuttle, directly blocking gluconeogenesis from glycerol and preventing clearance of cytosolic NADH, leading to a higher NADH/NAD^+ ratio that impairs glucose production from lactate.

A central question raised by this work is whether flux through the glycerophosphate shuttle is high enough to cause the observed redox shifts. An alternative redox shuttle, the malate-aspartate shuttle, is operative in liver, although its activity is diminished during increased pyruvate carboxylate flux (i.e., gluconeogenesis)(Kunz and Davis, 1991). Even so, disruption of the malate-aspartate shuttle in mice lowers fasting glycemia, and increases the cytosolic NADH/NAD^+ ratio in the liver, whereas disrupting the glycerophosphate shuttle has no effect on glycemia (Saheki et al., 2007).Moreover, reliance on the malate-aspartate shuttle appears to be higher still in humans than in mice (Saheki et al., 2007). Inhibition of complex I might increase the importance of the glycerophosphate shuttle, since the malate-aspartate shuttle requires mitochondrial membrane potential. It is also unclear how much flux through shuttles is necessary during gluconeogenesis from lactate, since NADH produced by lactate dehydrogenase is subsequently consumed by GAPDH. This question is underscored by the finding that knocking down cGPD, an obligate component of the glycerophosphate shuttle, produces only a muted effect on redox status as compared to mGPD, and does not suppress glucose production. In considering mitochondrial redox status, even when triglycerides are used as the sole respiratory substrate, electrons donated by the glycerophosphate shuttle account for only ~0.5% of ATP production. Thus, the loss of these electrons would be unlikely to account for a measureable change in mitochondrial NADH/NAD^+ ratio.

To account for the cytosolic redox shift, we propose an alternative interpretation: the increase in cytosolic NADH may not reflect halting of glycerophosphate shuttle, but rather production of NADH by cGPD running in the opposite direction (see figure). The effects of metformin would then be blocked in the absence of cGPD, and depend on the presence of glycerol to generate glycerol-3-phosphate. The latter prediction might be related to the lack of metformin effects in mice with constitutively active acetyl-CoA carboxylase, since impaired fatty acid oxidation and enhanced synthesis would be expected to lower endogenous glycerol production (Fullerton et al., 2013).

Another critical question is whether mechanisms based on energy charge can be excluded. To address this, Madiraju et al. measured ATP, ADP, and AMP to argue that clinically

relevant concentrations of metformin do not affect energy charge, despite activating AMPK. In support, they cite data showing activation of AMPK in the absence of changes in AMP (Madiraju et al., 2014). However, Hardie and colleagues recently reported that even when it was impossible to detect an increase in cellular AMP, activation of AMPK still depended on AMP binding (Hawley et al., 2010). Therefore, the increase in phosphorylation of AMPK and its substrate ACC in the chronic *in vivo* studies in Madiraju et al. may be indicative of an AMP increase. Madiraju et al. also observed reduced phosphorylation of CREB, the major PKA substrate, in response to chronic metformin. Given the difficulties of detecting small changes in cAMP *in vivo*, the decrease in phospho-CREB likely reflects a reduction in cAMP level, possibly due to AMP-dependent inhibition of adenylate cyclase (Miller et al., 2013). Thus, the most harmonious interpretation of the Madiraju et al. data is that there are at least two complementary mechanisms of metformin action.

A final consideration is time dependency in the action of metformin. Metformin does not cross membranes efficiently, but concentrates slowly in cells and mitochondria by virtue of its positive charge interacting with polarized membranes; this is reflected by a lag to the onset of effects in many assays and increased efficacy of low doses after prolonged incubation (Davidoff, 1971; Miller et al., 2013; Owen et al., 2000). Dependence on membrane potential provides an attractive mechanism for metformin's effects to be self-limiting via inhibition of complex I, consistent with its excellent safety profile. Importantly, the *in vitro* experiments in Madiraju et al. that excluded a direct effect of metformin on complex I involved only acute treatment. It is also notable that while phenformin inhibits glucose production and complex I activity more effectively than does metformin, it does not appear to be more efficacious in inhibiting mGPD. Nevertheless, the observation of Madiraju et al. that mitochondrial NADH/NAD^+ ratio is oxidized by metformin is a key argument against the involvement of complex I inhibition. Importantly, Madiraju et al. administered metformin intravenously, which probably led to lower hepatic levels then when the drug is given orally, as done therapeutically or in previous studies where the opposite result was obtained (Owen et al., 2000).

Inhibition of mGPD is a new and potentially crucial piece of the puzzle as to how metformin exerts its beneficial effects on glucose homeostasis. A better understanding of how the most widely-prescribed glucose-lowering agent works could lead to improved outcomes for millions of diabetics worldwide.

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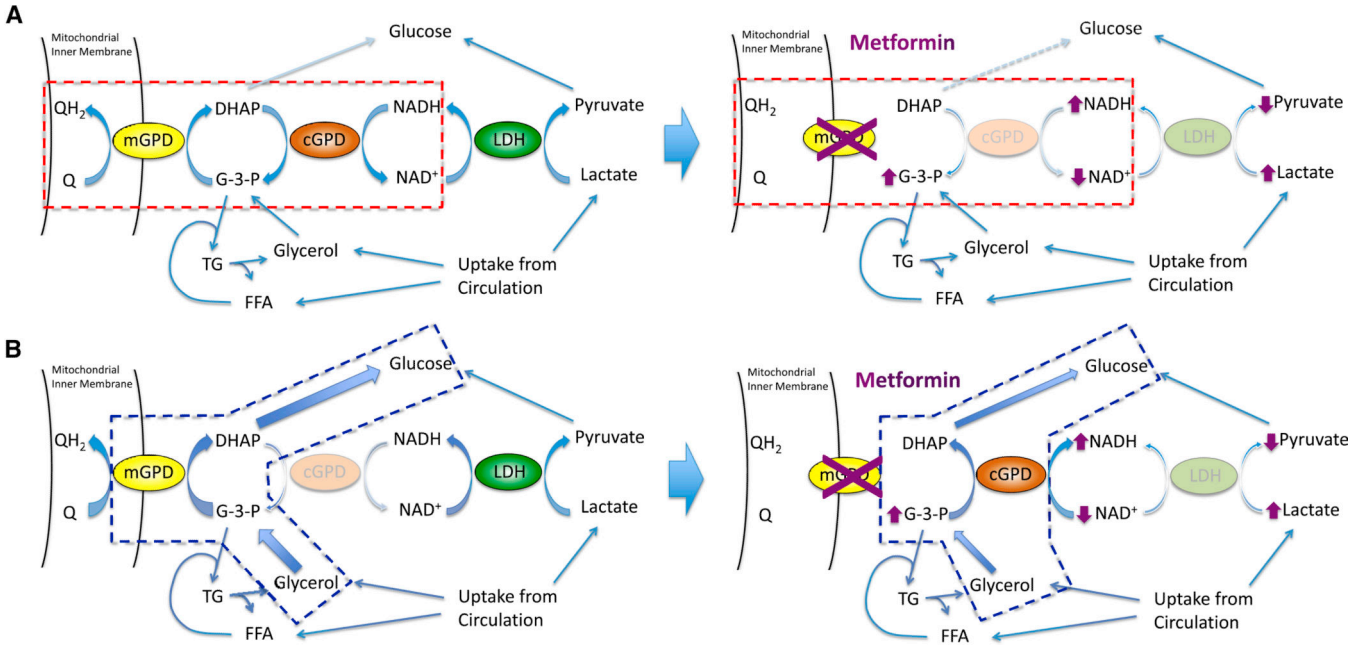

Figure.

Metformin inhibits mitochondrial Glycerol-3-phosphate dehydrogenase (mGPD), raising cytosolic NADH and blocking incorporation of lactate into glucose. A) If mGPD functions predominantly in the glycerophosphate shuttle (red box), inhibition by metformin will be expected to slow the removal of NADH, leading to an increase in the cytosolic NADH/NAD^+ ratio that feeds back on lactate dehydrogenase (LDH). B) If flux from glycerol to glucose is significant (blue box), inhibition of mGPD by metformin may lead to accumulation of glycerol-3-phosphate (G-3-P) such that oxidation to dihydroxyacetone phosphate (DHAP) by cGPD becomes favorable. Whereas mGPD catalyzes this reaction by donating electrons directly to the electron transport chain, cGPD would concomitantly produce NADH, increasing the cytosolic NADH/NAD^+ ratio, which would feed back on LDH. Note that the glycerophosphate shuttle catalyzes the net transfer of electrons from NADH to ubiquinone (Q) in the electron transport chain with regeneration of the intermediate dihydroxyacetone phosphate (DHAP) and G-3-P pools. Reverse flux through cGPD would not be expected in the absence of an external source of G-3-P or oxidation of the cytosolic NADH pool.