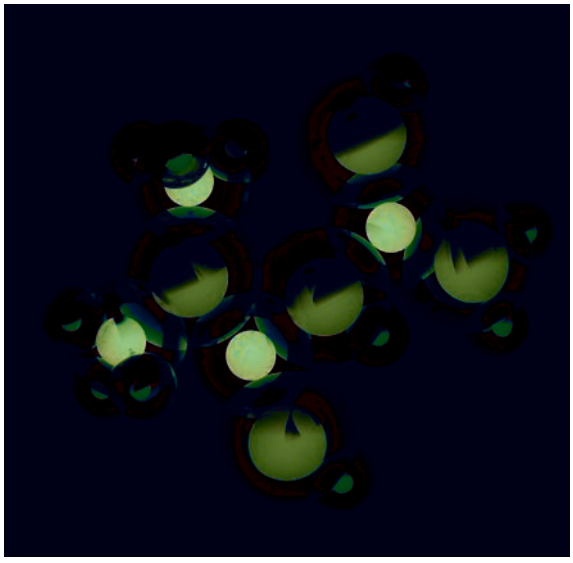
Dissecting the actions of widely used diabetes drugs

The biguanides, first discovered as glucose-lowering agents isolated from French lilac (*Galega officinalis*) plants, are one of the most effective first-line drugs to treat type 2 diabetes. Despite the wide use of the biguanides metformin and phenformin, their mechanism of action has been unclear. In a recent study, Miller *et al.*[1] describe a new mechanism through which metformin antagonizes the hormone glucagon, thus reducing fasting glucose concentrations. The authors' findings add further support to the notion that glucagon is a major player in the pathogenesis of diabetes. We asked three experts to comment on these results and on how they might be applied to develop new, more efficacious therapies to target diabetes.



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The mechanism of action of biguanides has remained elusive, despite their long-standing use to treat type 2 diabetes. Miller *et al.*[1] now may have finally resolved this issue with their work confirming earlier reports that biguanides, by preventing glucagon-stimulated rises in hepatic cyclic AMP (cAMP) concentrations, reduce the inappropriately high hepatic glucose production that is caused by glucagon-stimulated gluconeogenesis in hepatocytes. Increased cAMP concentrations activate protein kinase A (PKA), leading to phosphorylation of downstream target enzymes that mediate the glucagon-driven glucose production[2] that characterizes poorly controlled diabetes. Miller *et al.*[1] showed that biguanides block glucagon-stimulated PKA activation but they cannot block PKA activation caused by a cAMP analog, thus localizing the site of the inhibitory action to a glucagon-responsive site on the cAMP-producing enzyme adenylyl cyclase.

The authors also investigated the mechanism by which adenylyl cyclase is inhibited. Similarly to prior work on metformin[3], they found that phenformin blocks glucagon-stimulated increases in cAMP in mouse primary hepatocytes independently of AMP kinase (AMPK). The authors confirmed that biguanides increase hepatic AMP concentrations by inducing a mild energetic stress consistent with a reduced hepatic energy state. Thus, they propose that biguanide-induced hepatic AMP formation inhibits the production of cAMP that mediates glucagon-stimulated hepatic glucose overproduction and diabetic hyperglycemia.

The therapeutic implications of the work of Miller *et al.*[1] may transcend type 2 diabetes and apply to all forms of diabetes. Evidence accumulated over the past 50 years indicates that hyperglucagonemia is the *sine qua non* of the metabolic phenotype of diabetes[4]. In other words, hyperglycemia that meets the diagnostic criterion for diabetes is invariably accompanied by absolute

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Glucagon, a hormone counter-regulatory to insulin's actions, is a potent stimulator of hepatic glucose production. Blood concentrations of glucagon are elevated in diabetes, and glucagon is a major contributor to fasting hyperglycemia, a hallmark of diabetes[4]. Miller *et al.*[1] now make a direct mechanistic connection between the actions of metformin on mitochondrial energy charge and the glucagon receptor–cAMP–coupled signaling pathway. Elevated AMP abundance produced by the action of metformin on mitochondria inhibits glucagon receptor signaling by binding to and inhibiting adenylyl cyclase, thereby lowering cAMP concentrations, protein kinase A activity and gluconeogenesis. These findings are intriguing because they provide a new mechanism of action of metformin in the liver and provide further evidence that glucagon receptor signaling is diabetogenic.

The precise mechanism of action of metformin remains somewhat enigmatic. Metformin inhibits complex I (NADH quinone oxidoreductase) in the respiratory chain, reducing mitochondria energy charge, which results in a decrease in ATP and an increase in ADP and AMP. An earlier breakthrough study indicated that these elevated AMP concentrations led to the activation of the energy sensor AMPK[6]. Active AMPK stimulates fatty acid oxidation, impairs fat synthesis and thereby improves insulin sensitivity and reduces gluconeogenesis[6].

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or relative hyperglucagonemia. If all diabetic hyerglycemia is secondary to failure of alpha cells to turn off glucagon secretion appropriately when blood glucose rises and thereby reduce hepatic overproduction of glucose, then the hyperglycemic spikes that plague patients with even optimally controlled type 1 diabetes could also be a high-priority target. Moreover, it is now evident that the postprandial rise in blood glucose paradoxically stimulates a rise in glucagon[5], thereby inappropriately amplifying it.

Ligands binding to the 'P-site' of adenylyl cyclase to reduce cAMP formation would be predicted to be maximally effective in reducing glucagon-mediated hepatic cAMP overabundance and hyperglycemia if the insulin dosage is reduced so as to maximize the hyperglucagonemia and hyperglycemia, the targets for biguanides. However, a theoretical downside to this strategy would be loss of cAMP formation in response to adrenergic signaling. This would heighten risk of glucopenia in circumstances when glucose need is high or availability is decreased.

In summary, this new work identifies a third potential strategy for reducing glucagon-mediated diabetic hyperglycemia. The three strategies include suppressing glucagon-secreting alpha cells, blocking glucagon receptors and, now, inhibition of adenylate cyclase. The recognition of these independent therapeutic strategies should enhance initiatives to treat diabetic hyperglucagonemia, the long-unrecognized and untreated half of all forms of diabetes.

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Subsequently, metformin was shown to reduce glucose production in hepatocytes in the absence of AMPK[3], creating a quandary regarding whether there is a role for AMPK in the liver, now relieved somewhat by the findings of an AMPK-independent mechanism[1].

The new findings proposing an AMP-driven mechanism of metformin action independent of AMPK[1] prompt some speculations. A deficiency of cAMP resulting from metformin's actions on elevating AMP concentrations might also decrease gluconeogenesis by inactivating pyruvate dehydrogenase kinase, a cAMP-activated enzyme that inhibits glucose (pyruvate) oxidation by inhibiting the pyruvate dehydrogenase complex, thereby helping to reverse the glycolytic cycle from gluconeogenesis to glycolysis[7]. As metformin lowers energy charge and ATP concentrations, as well as raises AMP concentrations, a deficiency of ATP might be responsible for some of the effects observed. Because gluconeogenesis consumes energy, ATP deficiency might lower rates of glucose production *per se*. ATP is the substrate for adenylyl cyclase in the formation of cAMP, suggesting that limiting ATP could contribute to a reduction in cAMP formation, in addition to the direct inhibition of adenylyl cyclase by AMP. Importantly, these provocative findings in the liver by Miller *et al.*[1] further implicate the diabetogenic hormone glucagon as a major culprit in the pathogenesis of diabetes[4].

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For many years, metformin has been the drug of choice for the initial treatment of patients with newly diagnosed type 2 diabetes, despite the fact that we still do not have a clear picture of its mode of action. It has been known for some time that metformin inhibits mitochondrial complex 1, thereby reducing hepatic energy charge[1]. This led to the suggestion in 2001 that the drug works by activating AMPK in the cell[6]. Subsequent studies, however, showed that it can bring about its effect even in the absence of hepatic AMPK[3]. The recent data of Miller *et al.*[1] suggest a more compelling mechanism for metformin action, namely, that it increases the concentration of AMP in the hepatocyte, thereby increasing AMP binding to the 'P site' in adenylate cyclase, inhibiting its activity and as a result limiting glucagon's ability to raise cAMP concentration and cause excess glucose production.

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The conclusion by Miller *et al.*[1] that metformin's clinical benefit is dependent on its ability to inhibit glucagon action implies that glucagon is a major contributor to the dysregulation of glucose metabolism in individuals with type 2 diabetes. Clearly, plasma glucagon concentrations are either relatively (compared to the prevailing plasma glucose concentration) or absolutely elevated in type 2 diabetes, and this can lead to both impaired fasting glucose and abnormal glucose tolerance[8]. We now know that the efficacy of dipeptidyl peptidase 4 inhibitors and glucagon-like peptide 1 mimetics is in part attributable to their ability to reduce plasma glucagon levels[9]. More recently, glucagon receptor antagonists have proved efficacious in lowering hemoglobin Alc (a marker for blood glucose) in individuals with type 2 diabetes[10]. Notably, the diabetic phenotype resulting from streptozotocin treatment in mice can be completely abrogated by knocking out the glucagon receptor[4]. Therefore, there is currently little doubt that an excess of glucagon contributes substantially to the diabetic phenotype. It follows, then, that limiting its action with metformin would be expected to provide clear clinical benefit to people with diabetes, as would the development of new inhibitors of glucagon secretion or action.

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