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A western diet-induced mouse model reveals a possible mechanism by which metformin decreases obesity

Frank J. Gonzalez¹ and Changtao Jiang²

¹Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

²Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Peking University, and the Key Laboratory of Molecular Cardiovascular Science, Ministry of Education, Beijing, P. R. China

Metformin has been used for over 60 years for the treatment of type 2 diabetes and is still the first drug prescribed for this condition. Metformin treatment is also associated with weight loss. However, the mechanism of anti-obesity action is not fully understood. Some studies suggest that metformin acts at the level of the mitochondria, where it inhibits complex I of the respiratory chain and glycerol-3-phosphate dehydrogenase (GPD2) resulting in decreased hepatic gluconeogenesis [1, 2]. Other studies revealed that metformin alters the ATP/ADP ratio and activates the AMP-activated protein kinase (AMPK) in liver and skeletal muscle, resulting in multiple effects including increased fatty acid oxidation and decrease glucose uptake, and improving insulin sensitivity [3–5]. Metformin also inhibits the mammalian target of rapamycin complex 1 (mTORC1) [6], and targets endosomal Na⁺/H⁺ exchangers and the V-ATPase [2]. While most proposed mechanisms of action for metformin are liver centric, recent studies have focused on the possible effects of metformin in the intestine, since the drug is given orally at high doses and it increases glycolysis in intestinal epithelial cells [4]. Indeed, oral delivery of metformin produced far better metabolic benefits than intravenous delivery [7], and the intestinal concentrations of metformin are much higher than that found in the circulation [8]. A recent patient cohort study suggested that an oral extended-release metformin formulation exerts an improved glucose effect mainly by targeting the lower intestine than the more common immediate release metformin formulation [9]. The intestine has largely been overlooked as a potential target and a regulator of metabolism for metformin.

As noted above, in addition to its effect on glucose and insulin sensitivity, metformin use is associated with clinically significant weight loss [10]. The mechanism for this effect is also poorly understood. Schommers *et al.*, used a high-fat diet-induced model of obesity and glucose intolerance to explore the role of intestine in the metabolic effects of metformin [11]. Mice were fed a high-fat diet (HFD) either coincident with metformin (designated the HFD+Met group), or metformin was given after the mice had been on a HFD and developed obesity and glucose intolerance (designated the HFD+lateMet group). Metformin was administered by adding it to the diet at a dose of 500 mg/kg/day which is equivalent to the

highest dose prescribed to humans of 3 gms/day. While this is not the route that metformin is used in humans, it is the only reasonable means to dose mice with the drug, as bolus doses administered by gavage two or three times per day would be impractical, and it also mimics an oral extended-release formulation. Mice on the drug gained less weight or lost weight (when given to diet-induced obese mice) although the insulin sensitivity was modestly decreased in the HFD+lateMet group. Notably, the mice consumed similar amounts of food with and without metformin, although the animals on the drug drank more water. In addition, there was no difference in food adsorption and energy excretion in the intestine between the metformin-treated and vehicle-treated groups. Mice on metformin had higher energy expenditure, thus explaining the weight loss, and were even less active than the control group. Studies focused on reducing weight gain in HFD-fed mice have consistently revealed that browning or beiging of white adipose caused the observed increased energy expenditure [12–14]. This is most notable with the effects of fibroblast growth factor 21 (FGF21) on obesity that is due in large part on white adipose beiging [15, 16]. Modulation of intestinal farnesoid X receptor also leads to significant white adipose beiging promoting energy expenditure increase and weight loss [17, 18]. Unexpectedly, Schommers *et al.*, found no activation in brown adipose or white adipose beiging as revealed by analysis of uncoupling protein 1 (UCP1) protein in metformin-treated mice. However, this study could not exclude the possibility that metformin elevated the browning or beiging of adipose associated with UCP2 and UCP3, or with a creatine dephosphorylation-phosphorylation futile cycle controlled by creatine kinase (CK) [19].

The authors suggest that metformin decreased intestinal mitochondrial function as revealed by lower complex 1 activity, and this results in increased lactate production in the intestine of HFD-fed, metformin-treated mice as compared to HFD-fed, vehicle-treated mice early after drug treatment. However, there was a marked decrease in intestinal lactate at 3 weeks after continuous metformin feeding in both intestine and liver that was attributed to more efficient uptake of lactate in the intestinal epithelial cells. They also found that expression of the lactate transporter monocarboxylate transporter 1 (Mct-1; SLC16A1) was decreased in liver and other tissues possibly as an adaptive and protective response to increased systemic lactate. The increase in intestinal lactate after oral metformin exposure had previously been reported [20] and the authors suggest that this may be the reason for the gastrointestinal issues reported in patients under treatment. They propose that the increase in lactate in the portal venous blood leads to elevated hepatic glucose production in metformin-treated mouse liver resulting in increased energy consumption in a glucose futile cycle that accounts for the weight loss. The futile cycle of glycolysis is supported by increased conversion of glucose-1-¹³C to glucose-1,6-¹³C during metformin treatment, suggesting lactate production in the intestinal wall and its subsequent usage for gluconeogenesis in liver. This was further supported by the increase and decrease of lactate dehydrogenase in the intestine and liver, respectively. However, this result in contrast to other studies showing decreased hepatic gluconeogenesis after metformin treatment [21, 22] as Schommers *et al.*, noted. They attribute this to the route of administration used in their study; the earlier work used injection or gavage of bolus doses as compared to the feeding regimen used in their study. Nevertheless, despite increased hepatic gluconeogenesis, there was an improvement in glycemic control as revealed by lower serum HbA1c levels. The authors argue that in

humans, even under the bolus dosing, that the amount of metformin reaching the liver may not be at a sufficient intracellular concentration to cause metabolic effects such as inhibition of gluconeogenesis and thus their results might be translatable to humans. A recent pharmacokinetic study supports this view [9]. Also, microbiota-derived succinate was found to alleviate obesity and insulin sensitivity as the substrate for intestinal gluconeogenesis [23]. Similarly, it's unclear whether the metformin-increased intestinal lactate levels could enhance energy expenditure via the activation of intestinal gluconeogenesis.

The gut microbiota is a vital factor in modulating host metabolism and was shown to be involved in the development of metabolic diseases, including T2D and obesity [24]. Furthermore, recent studies in both mice and humans suggested that metformin treatment improved type 2 diabetes by remodeling the gut microbiota [25, 26]. An increased glucose-lactate-glucose futile cycle in the gut-liver axis was found in metformin-treated mice, however, whether it is induced by the modification of gut microbiota is still unknown.

This study makes a compelling case for the view that the effects of metformin, especially on weight loss, may reside in the intestine. However, the questions remain on the role of the metformin-mediated lactate-glucose futile cycle in the weight loss observed in this mouse model. Does the increased hepatic energy consumption account for the weight loss? As noted above, most mouse weight loss that occurs in the absence of decreased food intake and/or increased motor activity is the result of elevated brown and beige adipose fat burning. The proposed “futile cycle” would consume 4 mol of ATP/GTP per mol of glucose (or 2 mol of ATP/GTP per mol of lactate), equivalent to about 120 to 280 kJ per mol of glucose. In the Schommers *et al.*, study, lactate concentration in the portal vein increases from 2.5 to 3.9 mmol/L, while other venous blood shows no lactate increase. Assuming that this difference would reflect the futile cycle and the respective lactate would be transformed back to glucose in the liver, the amount of glucose channeled through the cycle can be calculated from the portal vein blood flow, which is about 3 ml/min for mice with an average weight of 29 g [27, 28] providing the additional 1.4 mmol/L lactate. This gives 6 mmol lactate and 12 mmol ATP per day for the cycle, corresponding to 0.4 – 0.8 kJ of additional energy expenditure per day. The futile cycle would thus account for about 1–2 % of daily energy consumption that might not account for the marked increase in total body energy expenditure and weight loss in mice treated with metformin? This answer to this conundrum requires additional experimentation.

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