

Commentary: lactate-induced glucose output is unchanged by metformin at a therape...

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A Commentary on

Lactate-Induced Glucose Output Is Unchanged by Metformin at a Therapeutic Concentration—A Mass Spectrometry Imaging Study of the Perfused Rat Liver

by Calza, G., Nyberg, E., Makinen, M., Soliymani, R., Cascone, A., Lindholm, D., et al. (2018). *Front. Pharmacol.* 9: 141. doi: 10.3389/fphar.2018.00141

[Calza et al. \(2018\)](#) challenge a widely accepted view ([Petersen et al., 2017](#)) that mitochondrial glycerophosphate dehydrogenase (mGPD, EC 1.1.5.3), a component of the glycerolphosphate shuttle (GPS), is the primary molecular target ([Madiraju et al., 2014](#)) for the antidiabetic drug metformin to lower plasma glucose in type 2 diabetic patients. The initial report was followed by a detailed preclinical investigation in which the hypothesis that the cellular redox state (i. e., the cellular $[NADH]/[NAD^+]$ ratio) increased by metformin's selective mGPD inhibition, explained decreased liver gluconeogenesis from lactate and glycerol whereas pyruvate or alanine as precursors were not affected ([Madiraju et al., 2018](#)). Already in 1969, [Krebs et al. \(1969\)](#) concluded from their experiments with the ethanol-perfused rat liver that the decrease of the $[free\ NAD^+]/[free\ NADH]$ ratio was responsible for the inhibition of gluconeogenesis from lactate and glycerol whereas pyruvate-driven formation of glucose was completely unaffected. Thus, the evidence for a precursor-selective inhibition of gluconeogenesis by the cellular redox ratio was presented 50 years ago and confirmed for metformin ([Madiraju et al., 2014](#) , [2018](#)). The crucial main point is that the claimed primary molecular target for the drug responsible for the ratio change is

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mGPD. Alternative opinions (e. g., [Hunter et al., 2018](#)) to explain reduced liver gluconeogenesis by metformin will not be discussed herein.

The main observations by [Madiraju et al. \(2014\)](#) were that metformin i. v. injections into fasting healthy rats with doses of 20 and 50 mg/kg led, within 30 min, to lowering of fasting plasma glucose (FPG) and endogenous glucose production (EGP), as measured by labeled glucose. Increased ratios in plasma and liver for [lactate]/[pyruvate] as well as decreased ratios of [β -hydroxybutyrate]/[acetoacetate] were seen. Similar observations were made upon chronic i. p. metformin dosing. Thus, acute or chronic metformin inhibits EGP, which is accompanied by changes in plasma and liver with increased [lactate]/[pyruvate] ratios as a proxy for the cytosolic [NADH]/[NAD⁺] redox state. These results in the preclinical models are contrary to the evidence from numerous studies in healthy non-diabetic humans, namely that neither acute nor chronic metformin lowers FPG ([Sulaiman and Johnson, 1977](#) ; [Bonora et al., 1984](#) ; [Nestler et al., 1994](#) ; [Sambol et al., 1996](#) ; [Chung et al., 2018](#) ; [Gormsen et al., 2018](#)) and even increases EGP ([Christensen et al., 2015](#) ; [Konopka et al., 2016](#)).

In order to explain the changed redox ratios the authors investigated the two main shuttles which can transfer cytosolic NADH to mitochondria, namely the malate-aspartate shuttle (MAS) and the GPS. They came to the conclusion that one component of the latter, mGPD, is responsible and a direct molecular target for metformin.

In the context of metformin's actions on mitochondria it is important to note that MAS, in contrast to GPS, can run in the reverse mode ([Vancura et al., 2018](#)) if NADH oxidation is impaired by inhibition of the electron transport chain and cytosolic redox ratios are thereby increased. GPS is a much simpler and faster system and consists of two enzymes: the cytosolic glycerol 3-phosphate dehydrogenase (cGPD, EC 1. 1. 1. 8) with NAD^+/NADH as co-factor [a K_m value around 30 μM for glycerophosphate (GP)] and mGPD with FAD as co-factor. The mGPD has a high K_m for GP (2 to 8 mM), is an important reactive oxygen species (ROS) producer ([Orr et al., 2012](#)), is stimulated by calcium and catalyzes the “ one-way street” reaction from GP to dihydroxyacetone phosphate. [Madiraju et al. \(2014\)](#) reported liver mitochondrial respiration in the presence and absence of 50 μM metformin by addition of pyruvate, malate, and glutamate, followed by rotenone. GP was then added and a 40% inhibition of the GP-stimulated oxygen consumption rate (OCR) was noticed. In figure 2 of their extended data dose responses of OCR with metformin present (between 0. 078 and 20 mM) on complex I OCR are shown. There was no inhibition up to 1. 25 mM. After addition of rotenone, succinate was added and a significant stimulation (about 40%) for all metformin concentrations was reported. As a crucial step of evidence, the authors investigated the activity of metformin on the kinetics of different preparations of mGPD, including an immunopurified recombinant human mGPD, rat mitochondrial lysates, immunoprecipitated purified rat mGPD and the distantly related purified *Pediococcus* sp. α -glycerophosphate oxidase. In all instances, a non-competitive inhibition was observed with K_i values of about 50 μM . The authors also performed *in silico*

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molecular docking simulations with a modified version of the crystal structure of α -glycerophosphate oxidase from *Streptococcus* sp. and suggested that metformin interacted with the FAD binding site.

To summarize: First, the observed acute inhibition of FPG in healthy rats by metformin in the preclinical model was puzzling as there was no parallel in any of the human studies. Second, we were wondering about the significant (40%) stimulation of succinate-driven OCR by metformin that has never been reported before. Third, high throughput screening for mGPD inhibitors with several thousands of chemicals yielded membrane permeable, high-affinity blockers such as iGP (STK017597, Vitas-M) but not metformin or related structures ([Orr et al., 2014](#)). Fourth, mGPD was not among the 41 computationally predicted biomolecular metformin targets ([Cuyas et al., 2018](#)). Consequently, we were searching for confirmations of the most important argument for the hypothesis that mGPD is a primary, direct molecular target of metformin but to our dismay, we did not find any. Instead, we discovered convincing proof that metformin does not inhibit mGPD. The first report by one of the laboratories with decades of experience with mGPD did not find any metformin inhibition up to 10 mM but, as a positive control, micromolar inhibition by iGP ([Pecinova et al., 2017](#)). The second report is a detailed study on the effects of metformin on the OCR of saponin-permeabilized ACR 549 cancer cells exposed to metformin. With complex I substrates, addition of metformin (1 mM) completely blocked OCR. With GP as substrate, metformin did not inhibit at all ([Gui et al., 2016](#)). The third article ([Al-Oanzi et al., 2017](#)) investigated concentrations of the

immediate intracellular precursor of free glucose in plasma, namely glucose-6-phosphate (G6P) and fructose-2, 6-bisphosphate (F26P) in isolated rat hepatocytes. When comparing metformin to iGP, the authors found that in the presence of an inhibitor of G6Pase, metformin (0. 1 to 0. 5 mM) dose-dependently decreased G6P but iGP had no effect even at 40 μ M. Metformin also reduced levels of F26P as proxy for fructose-6-phosphate in agreement with the report by [Hunter et al. \(2018\)](#).

Thus, the data by [Calza et al. \(2018\)](#) and the evidence presented above seriously challenge the “ primary, simple, and elegant” ([Ferrannini, 2014](#)) metformin target.

Author's Note

After submission of the manuscript two publications came to our attention, which support the notion that mGPD must be removed from the list of direct molecular metformin targets. [Alshawhi and Agius \(2018\)](#) report that metformin (0. 1-5 mM) does not inhibit mGPD in permeabilized rodent hepatocytes. [Li et al. \(2018\)](#) discovered that cytochrome C reduction, used as the activity reporter for mGPD in one of the assays by [Madiraju et al. \(2014\)](#), is inhibited by metformin in a concentration-dependent (0. 2-2. 5 mM) manner.

Author Contributions

HG and OL discussed the topic and wrote the paper.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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