**Metformin, metabolic stress, and mitochondria.** Focus on “A novel inverse relationship between metformin-triggered AMPK-SIRT1 signaling and p53 protein abundance in high glucose-exposed HepG2 cells”

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The p53 protein, best known as a tumor suppressor, participates in responses to cellular stresses, including DNA damage and hypoxia, but also determines metabolic substrate disposition. In cancer, p53 represses glycolysis while promoting mitochondrial oxidative phosphorylation via effects on gene transcription (3). Peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1), a member of a family of transcriptional coactivators that regulate the capacity for oxidative metabolism, interacts with and functions as a p53 transcriptional activator (17). PGC-1α-p53 interaction is promoted by transient glucose starvation (17). However, hepatic p53 protein abundance increases under conditions of nutrient excess (20).

AMP-activated protein kinase (AMPK) is also a cellular stress sensor. Its activity increases during glucose scarcity. ATP deprivation (usually due to impaired nutrient or oxygen delivery) and the antidiabetic drug metformin both activate AMPK (8, 18). AMPK activation suppresses anabolic processes (glycogen, protein and lipid synthesis) and gluconeogenesis (an energy-requiring process) to conserve ATP, while increasing mitochondrial carbohydrate and lipid oxidation to increase ATP production via the Krebs cycle and oxidative phosphorylation (Fig. 1). AMPK can cooperate with the fuel/NAD⁺-dependent sirtuin class of histone/protein deacetylases (SIRTs). Caloric restriction increases SIRT1 activity (9); conversely, both AMPK and SIRT1 are downregulated in the liver under conditions of nutrient excess [e.g., hyperglycemia, high-fat diet, obesity (4, 5)]. Nelson et al. (11) in this issue of *American Journal of Physiology-Cell Physiology* present novel data clarifying the mechanism of metformin action and highlight new insights into the interplay between p53, AMPK, and SIRT1 by investigating increased hepatic p53 protein expression and acetylation status in response to high glucose-induced changes in AMPK and SIRT1.

Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia partly through a mismatch between insulin resistance and insulin secretion. Insulin resistance impairs insulin-stimulated glucose uptake and suppression of hepatic glucose production (HGP). Antidiabetic effects of metformin are attributed to improved insulin (or insulin-like) actions, including decreased HGP. Metformin is believed to inhibit complex I of the electron transport chain (6), lowering the cellular energy charge \([([ATP]/0.5[ADP])/([ATP]+[ADP]+[AMP]))\) (Fig. 1). Although AMPK and its upstream kinase LKB1 may not be obligatory for metformin-induced decreases in HGP in mice (7), the metformin-induced lowering of energy charge is predicted to activate AMPK (21) (Fig. 1).

NADH generated via oxidation enters complex I of the electron transport chain to contribute to generation of the mitochondrial proton gradient necessary for ATP production. During oxidative phosphorylation, both reactive oxygen species (ROS) and ATP are generated (Fig. 1). Excess ROS impair respiratory chain function and cause mitochondrial and nuclear DNA damage. Various cellular stressors, including oxidative stress, can trigger p53 accumulation (1) (Fig. 1). Furthermore, p53 is deacetylated and repressed upon DNA damage or oxidative stress (10, 19).

Nelson and colleagues (11) employed HepG2 hepatoma cells exposed to high (25 mM) glucose as a metabolic stressor. Exposure to high, compared with low (5 mM), glucose increased ROS production. Metformin increased AMPK activity at both low glucose (LG) and high glucose (HG) and decreased ROS production in response to HG. With HG, AMPK knockdown increased ROS production and partially blunted metformin’s effect on ROS production, suggesting that part, but not all, of metformin’s action is linked to AMPK activation rather than to direct inhibition of complex I and lowering of the cellular energy charge. Metformin also dose dependently decreased p53 protein expression, suggesting that p53 expression in HepG2 cells under HG conditions is a consequence of oxidative stress. Metformin-induced decreases in p53 protein abundance with HG were dependent on AMPK activation: overexpression of a dominant negative AMPK [adenovirus (Ad)-DN-AMPK] in HepG2 cells both increased p53 abundance under basal conditions and prevented metformin-induced decreases in p53 protein abundance (Fig. 1). Furthermore, the decrease in p53 abundance caused by metformin was abolished by inhibition of murine double minute 2 (MDM2), a ubiquitin ligase that mediates p53 degradation.

To investigate potential reciprocity, the authors used nutlin-3 (an inhibitor of p53 degradation) to increase its expression. However, nutlin-3 did not affect ROS, implying that, although oxidative stress increases p53 protein expression, its expression alone does not affect ROS generation. While this might suggest that upregulation of p53 protein expression is peripheral to alleviation of oxidative stress induced by HG, the possibility remains that regulation of p53 activity (e.g., by posttranslational modifications or by binding proteins affecting its transcriptional activity) could be involved.

Humans express seven SIRTs (SIRT1–SIRT7) with distinct functions. SIRT1 counters cellular senescence and augments lifespan (9). Nutrient restriction increases SIRT1 expression;
conversely, high-fat feeding suppresses it (4). Sirt1-overexpressing mice are protected from high-fat diet-induced hepatic steatosis (14). The increased lifespan associated with caloric restriction in model organisms is accompanied by increased mitochondrial density and respiration. Nelson et al. (11) found that metformin treatment of HG-exposed HepG2 cells inhibited mitochondrial density and respiration. Nelson et al. (11) found that metformin treatment of HG-exposed HepG2 cells in mediated by increased AMPK activity (Fig. 1). The figure expands on potential mechanisms whereby these interactions could lead to changes in oxidative metabolism, highlighting the potential importance of acetylation-deacetylation mechanisms. 

In summary, the authors demonstrate that AMPK activation is required for SIRT1 activation and suppression of p53 expression by metformin in HepG2 cells (11). These findings suggest that reciprocal interactions exist between AMPK/SIRT1 activities and p53 protein abundance under conditions of nutrient excess which complement interactions in nutrient deprivation. However, a number of questions arise. Both SIRT1 and p53 augment oxidative metabolism, but metformin appears to activate SIRT1 and suppress p53 expression. What are the potential roles of other SIRTs, most notably SIRT3, in fine-tuning mitochondrial oxidative processes? Intriguingly, SIRT1 is linked to telomere biology and protection from DNA damage (13), which is consistent with the inverse relationship between SIRT1 and p53 in the current study. Importantly, if altered p53 protein abundance is accompanied by altered p53 transcriptional activity and modulation of glycolytic/oxidative flux—as inferred from changes in triglyceride accumulation—and occur in vivo in the normal liver as well as in HepG2 cells, the results could have implications for the pathogenesis and treatment of T2DM and other metabolic diseases with impaired mitochondrial function.

ACKNOWLEDGMENTS

We are very grateful to Dr. Paul Insel for constructive advice.

GRANTS

M. J. Holness and M. C. Sugden are supported in part by research grants from Diabetes UK (BDA:RD07/0003568 and BDA:RD08/0003665).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

M.C.S. drafted the manuscript; M.C.S. and M.J.H. edited and revised the manuscript; M.C.S. and M.J.H. approved the final version of the manuscript; M.J.H. prepared the figure.
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